A sustainable path towards water purification - an insight based on hydrophobic Deep Eutectic Solvents

Catarina Florindo

Dissertation presented to obtain the Ph.D degree in Engineering Sciences and Technology, Chemical Engineering

Instituto de Tecnologia Química e Biológica António Xavier | Universidade Nova de Lisboa

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I declare that the work presented in this thesis, except where otherwise stated, is based on my own research. The work was mainly performed in the Separation and Extraction Technologies Laboratory of the Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa between January 2015 and June 2016 and Instituto Superior Técnico, Universidade de Lisboa between July 2016 and January 2019, and supervised by Professor Doctor Isabel Marrucho (IST-UL and ITQB) and Doctor Luís C. Branco (REQUIMTE - FCT). Part of the results was also attained during visiting periods to Photochemistry and Supramolecular Chemistry Group, LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, and to the Tom Welton’s group, Department of Chemistry, Imperial college of London, United Kingdom.

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> *“Aqueles que passam por nós, não vão sós, não nos deixam sós. Deixam um pouco de si, levam um pouco de nós.”*
>
> *Antoine de Saint-Exupéry*

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Mateusz, o meu polaco preferido e também mais chato de sempre! 😊 Vou ter muitas saudades dos abracinhos, das frases mal construídas e das nossas cusquices…e também vou ter saudades das tuas birras. xD Como eu costumava dizer: és mesmo igual a mim..amiguinho!
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Este doutoramento preparou-me para enfrentar o mundo e escolher o meu caminho. Esta foi a minha segunda casa durante anos. Foi aqui que cresci pessoalmente e profissionalmente. Foi aqui que criei um novo eu. Foi aqui que ganhei asas e é agora que as consigo abrir para voar. O céu é o limite.

“Don’t stop when you are tired, stop when you are done”

MEMBERS OF THE JURY

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CONTENTS

A sustainable path towards water purification - an insight based on hydrophobic Deep Eutectic Solvents

CHAPTER 1 - DEVELOPMENT OF SUSTAINABLE ALTERNATIVE GREEN SOLVENTS

Chapter 1.1

CHAPTER 2 - A STEP TOWARDS HYDROPHOBIC DEEP EUTECTIC SOLVENTS

Chapter 2.1

Chapter 2.2

CHAPTER 3 - FROM CHARACTERIZATION TO WATER APPLICATION OF DEEP EUTECTIC SOLVENTS

Chapter 3.1

CHAPTER 4 - HYDROPHILIC VS. HYDROPHOBIC DEEP EUTECTIC SOLVENTS

CHAPTER 5 – FINAL REMARKS

ABSTRACT

One of the main concerns of modern society is to provide clean water for everyone. The occurrence of a wide diversity of micropollutants in aquatic ecosystems leads to serious public health and environmental problems. Thus, the development of sustainable and cost-effective alternatives for water purification technologies is nowadays an important challenge. Deep eutectic solvents (DESs) have been proposed as new alternatives to conventional solvents and even ionic liquids. The choice of low cost, naturally occurring compounds and the straightforward preparation are among the key features of these solvents.

This thesis focuses the development of novel designer solvents, and their thermophysical characterization, targeting the implementation of water purification technologies. The work starts with the development of new sustainable hydrophobic DESs, composed exclusively of natural compounds, including menthol and several fatty acids (Chapter 2.1 and 2.2). The application of hydrophobic DES for water purification was explored using two different approaches, liquid-liquid extraction (LLE) and supported DES as adsorbent materials, for the removal of three classes of major contaminants in aquatic environments: pesticides, plastic additive and pharmaceutical compounds (Chapter 3.1, 3.2 and 3.3).

The detailed knowledge of the properties of Hydrophobic DES is crucial for their implementation. In particular, polarity is a property that plays an important role in the extraction of multifunctional molecules. DES's polarity using solvatochromic probes were measured and hydrophilic and hydrophobic families of DES were compared for the first time (Chapter 4.1). Finally, novel hydrophobic DESs derived from a sodium salt of long chain fatty acids were also prepared and characterized and their water behaviour studied. Metalo DES-based hydrogels were observed in the presence of water (Chapter 4.2).

RESUMO

Uma das principais preocupações da sociedade moderna é desenvolver processos eficientes de tratamento de água de modo a fornecer água potável para todos. No entanto, a existência de uma grande diversidade de micropoluentes em ambientes aquáticos ocasiona graves problemas ambientais e de saúde pública. Assim, o desenvolvimento de alternativas sustentáveis para processos de purificação de água constitui um dos maiores desafios atuais. Os solventes eutécticos (SE) surgem como alternativas aos solventes convencionais e mesmo líquidos iónicos. A possibilidade da seleção de compostos naturais e de baixo custo, assim como a sua preparação fácil e rápida destacam-se como as principais vantagens destes solventes inovadores.

A presente tese apresenta o desenvolvimento de novos solventes sustentáveis, e a sua caracterização termofísica, visando a implementação de tecnologias sustentáveis de purificação de água. O trabalho apresentado inicia-se com o desenvolvimento de novos SE hidrofóbicos, exclusivamente constituídos por compostos naturais como o mentol e vários ácidos gordos (Capítulos 2.1 e 2.2). A aplicação de SE hidrofóbicos para a purificação de água foi também explorada, usando duas abordagens distintas; a extração líquido-líquido e a adsorção, para a remoção de três classes de contaminantes relevantes em ambientes aquáticos: pesticidas, aditivos e compostos farmacêuticos (Capítulos 3.1, 3.2 e 3.3).

O conhecimento detalhado das propriedades de SE hidrofóbicos é crucial para garantir o sucesso das extrações. Em particular, a polaridade é uma propriedade que desempenha um papel importante na extração de moléculas multifuncionais. A polaridade de vários SE usando sondas solvatocrómicas foi medida, e foram efetuadas comparações entre SE hidrofílicos e hidrofóbicos (Capítulo 4.1). Finalmente, foram também

desenhados e caracterizados novos SE hidrofóbicos derivados de um sal de sódio de diversos ácidos gordos de cadeia longa. O estudo do seu comportamento em água evidenciou a formação de metalo hidrogéis (Capítulo 4.2).

KEYWORDS

💧 Hydrophobic Deep Eutectic Solvents 💧 Eutectic Mixtures 💧 💧 Green Solvents 💧 Green Chemistry 💧 Water Purification 💧 💧 Micropollutants 💧

PALAVRAS-CHAVE

💧 Solventes Eutécticos Profundos Hidrofóbicos 💧 Misturas Eutécticas 💧 Solventes Verdes 💧 Química Verde 💧 💧 Purificação de água 💧 Micropoluentes 💧

PUBLICATIONS

Thesis publications (9)

C. Florindo, Leonardo Miakawa, Luís C. Branco, and Isabel M. Marrucho, Hydrophobic deep eutectic solvents as adsorbent materials for removal of micropollutants from water sources, *pending patent* (2019).

C. Florindo, Nathalie V. Monteiro, Bernardo D. Ribeiro, Luís C. Branco and Isabel M. Marrucho, Hydrophobic deep eutectic solvents for purification of water contaminated with bisphenol-A, Journal of Molecular Liquids, *in press,* (2019).

C. Florindo, Francine Lima, Luís C. Branco and Isabel M. Marrucho, Hydrophobic deep eutectic solvents: a circular approach to purify water contaminated with ciprofloxacin, ACS Sustainable Chem. Eng. (2019), 7, 14739-14746.

C. Florindo, Luís C. Branco, and Isabel M. Marrucho, In the quest for green solvents design: from hydrophilic to hydrophobic (deep) eutectic solvents, ChemSusChem (2019), 12, 1549.

C. Florindo, Lucas G. Celia-Silva, Luís F. G. Martins, Luís C. Branco, and Isabel M. Marrucho, Supramolecular hydrogel based on a sodium deep eutectic solvent, Chem. Commun. (2018), 54, 7527-7530.

C. Florindo, Leila Romero, Ignacio Rintoul, Luis Branco, and Isabel M. Marrucho, From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid-based Deep Eutectic Solvents, ACS Sustainable Chem. Eng. (2018), 6, 3888–3895.

C. Florindo, A. J. S. McIntosh, T. Welton, L. C. Branco, I. M. Marrucho, A closer look into deep eutectic solvents: exploring intermolecular interactions using solvatochromic probes, Phys. Chem. Chem. Phys. (2018), 20, 206-213.

C. Florindo, L. C. Branco, I.M. Marrucho, Development of hydrophobic deep eutectic solvents for extraction of pesticides from aqueous environments, Fluid Phase Equilibria (2017), 448,135-142.

B. D. Ribeiro, **C. Florindo**, L. Iff, M. A. Coelho, I. M. Marrucho, Novel Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents, ACS Sustainable Chem. Eng., (2015), 3 (10), 2469–2477.

Other publications (11)

C. Florindo, Lucas G. Celia-Silva, Luís F. G. Martins, Luís C. Branco and Isabel M. Marrucho, Novel Sodium salt-based Deep Eutectic Solvents: preparation and thermophysical characterization, *in preparation* (2019).

C. Florindo,* Filipa Lima,* Bernardo Dias Ribeiro, Isabel M. Marrucho, Deep Eutectic Solvents: Overcoming XXI Century Challenges, Current Opinion in Green and Sustainable Chemistry (2019), 31-36 (*equally contributing authors).

C. Florindo, M. Margarida Oliveira, L. C. Branco, I. M. Marrucho, Carbohydrates-based deep eutectic solvents: Thermophysical properties and rice straw dissolution, Journal of Molecular Liquids (2017), 247, 441-447.

C. Florindo, L. C. Tomé, I. M. Marrucho, Thermodynamic study of aggregation of Cholinium-based Perfluoroalkanoate Ionic Liquids, J. Chem. Eng. (2016), 61, 3979-3988.

D. Patinha, L. C. Tomé, **C. Florindo**, H. R. Soares, A. S. Coroadinha, I. M. Marrucho, New low-toxicity cholinium-based ionic liquids with perfluoroalkanoate anions for Aqueous Biphasic System implementation, ACS Sustainable Chem. Eng. (2016), 4 (5), 2670-2679.

C. Florindo, F. S. Oliveira, L.P.N. Rebelo, A. M. Fernandes, I. M. Marrucho, Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids, ACS Sustainable Chem. Eng. (2014), 2 (10), 2416-2425.

C. Florindo, A. Costa, L. Branco, C. Matos, S. Nunes, A. Matias, C. Duarte, L.P.N. Rebelo, I.M. Marrucho, Novel Organic Salts based on Fluoroquinolone drugs: synthesis, bioavailability and toxicological profiles, International Journal of Pharmaceuticals (2014), 469 (1), 179-189.

C. Florindo*, J. M. M. Araújo*, A. B. Pereiro, N. S. M. Vieira, A. A. Matias, C. M. M. Duarte, L.P.N. Rebelo, I.M. Marrucho, Cholinium-Based Ionic Liquids with Pharmaceutically Active Anions, RSC Advances (2014), 4 (53), 28126-28132 (*equally contributing authors).

L. Tomé, **C. Florindo**, C. S. R. Freire, L.P.N. Rebelo, I.M. Marrucho, Playing with ionic liquid mixtures to design engineered CO2 separation membranes, Physical Chemistry Chemical Physics (2014), 16 (32), 17172-17182.

T. Mourão, L. C. Tomé, **C. Florindo**, L. P. N. Rebelo, I. M. Marrucho, Understanding the Role of Cholinium Carboxylate Ionic Liquids in PEG-Based Aqueous Biphasic Systems, ACS Sustainable Chem. Eng. (2014), 2 (10), 2426-2434.

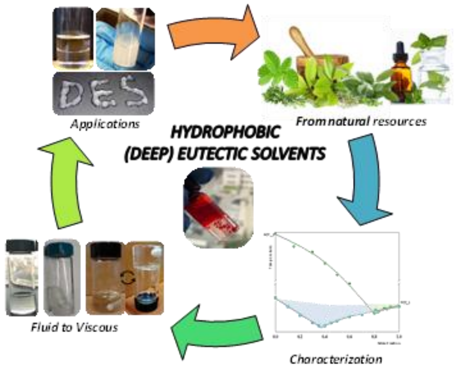
C. Florindo, J. M. M. Araújo, F. Alves, C. Matos, R. Ferraz, C. Prudêncio, J. P. Noronha, Ž. Petrovski, L. Branco, L.P.N. Rebelo, I.M. Marrucho, Evaluation of solubility and partition properties of ampicillin-based ionic liquids, International Journal of Pharmaceutics (2013), 456 (2) 553-559.

Thesis Layout

CHAPTER 1 - DEVELOPMENT OF SUSTAINABLE ALTERNATIVE GREEN SOLVENTS

Chapter 1.1

Introduction: state-of-the-art and thesis motivation

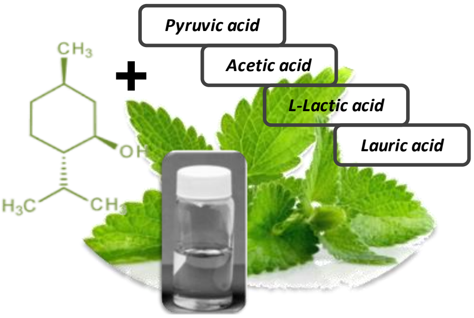


An integrated perspective on hydrophobic DESs, from its comparison with hydrophilic DESs to the most important applications are presented. The fundamental questions in this emerging field are also discussed.

CHAPTER 2 - A STEP TOWARDS HYDROPHOBIC DEEP EUTECTIC SOLVENTS

Chapter 2.1

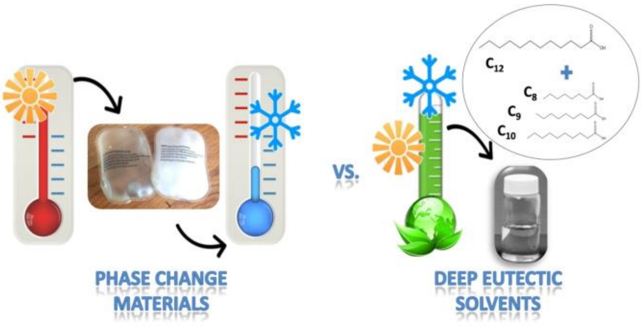
Novel menthol-based eutectic mixtures: hydrophobic low viscosity solvents



New cheap, biodegradable, all natural, hydrophobic and low viscous eutectic solvents from natural occurring resources were reported for the first time.

Chapter 2.2

From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid-based Deep Eutectic Solvents

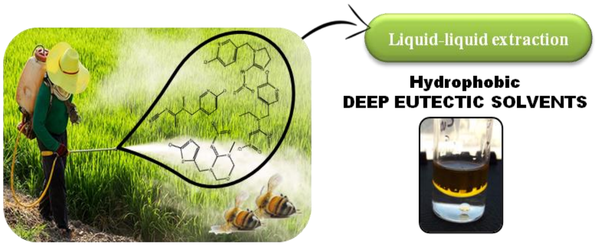


Hydrophobic, low viscous, all natural DESs exclusively based on fatty acids are proposed as sustainable solvents.

CHAPTER 3 - FROM CHARACTERIZATION TO WATER APPLICATION OF DEEP EUTECTIC SOLVENTS

Chapter 3.1

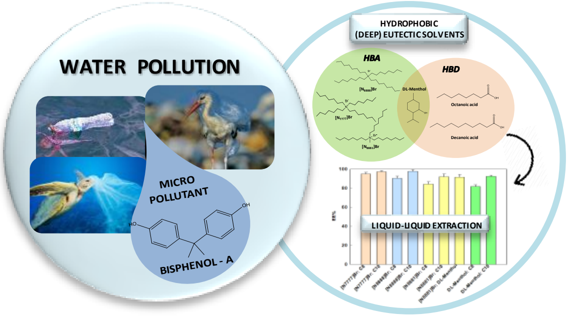
Development of Hydrophobic Deep Eutectic Solvents for Extraction of Pesticides from Aqueous Environments



Hydrophobic deep eutectic solvents are used in the removal of four pesticides neonicotinoids, imidacloprid, acetamiprid, nitenpyram and thiamethoxam, from aqueous solutions.

Chapter 3.2

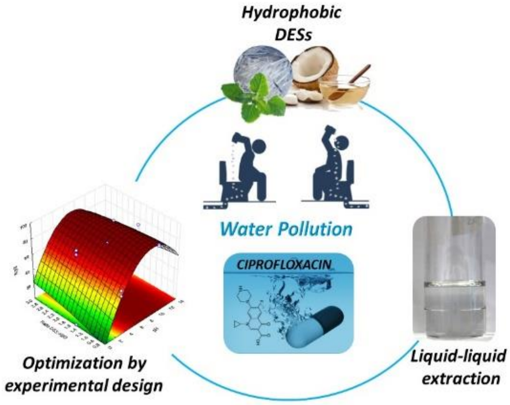
Task specific sustainable hydrophobic deep eutectic solvents for purification of water contaminated with Bisphenol-A



Neutral and ionic hydrophobic DESs are used in the extraction of Bisphenol-A, an endocrine disruptor micropollutant compound used as a plasticizer of water bottles, from aqueous environments.

Chapter 3.3

Removal of ciprofloxacin from water environments using task-specific hydrophobic deep eutectic solvents



Ciprofloxacin, one of the top ten high priority pharmaceutical compounds often detected in water environments, is efficiently removed from aqueous phases using task-specific hydrophobic DESs.

Chapter 3.4

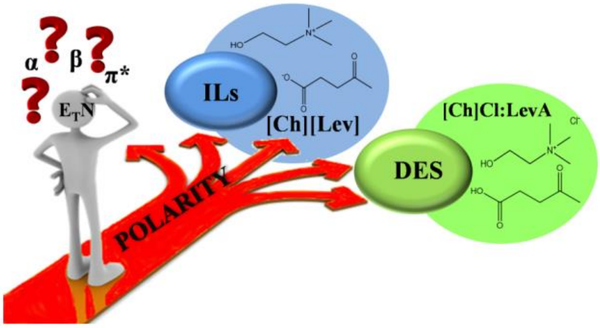
Hydrophobic deep eutectic solvents as adsorbent materials for removal of micropollutants from water sources

The easy preparation of hydrophobic DES adsorbent materials through their support in porous membranes enables the development of highly efficient and cost effective adsorption technology for the removal micropollutants from water.

CHAPTER 4 - HYDROPHILIC VS. HYDROPHOBIC DEEP EUTECTIC SOLVENTS

Chapter 4.1

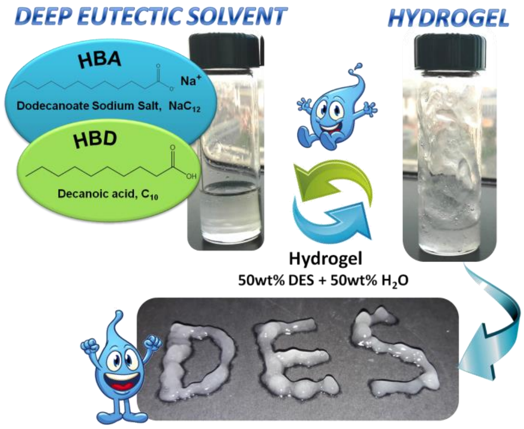
A closer look into Deep Eutectic Solvents: exploring intermolecular interactions using solvatochromic probes



Polarity plays an important role in solvation phenomena. The polarity of different families of DESs was studied through the solvatochromic responses of UV-vis absorption probes and the Kamlet-Taft α, β, π* and ETN parameters determined.

Chapter 4.2

Supramolecular hydrogel based on a sodium deep eutectic solvent



The introduction of metal containing components in hydrophobic DESs makes a game change in their hydrophilicity and allows for the formation of supramolecular hydrogel.

CHAPTER 5 – FINAL REMARKS

Chapter 5.1

Concluding Remarks and Outlook

The major conclusions, directions and future work in the DESs field are here highlighted.

A door to new possibilities and challenges for future research will be opened.

Thesis Guidelines

I decided to present the research developed during the time of my PhD project in an article-based thesis form, divided into five chapters, development of sustainable alternative green solvents, a step towards hydrophobic deep eutectic solvents, from characterization to water application of deep eutectic solvents, hydrophilic vs. hydrophobic deep eutectic solvents and final remarks, which are the main areas to which these thesis contributes. Each chapter is divided into several sub-chapters which contain the published articles. Chapter 1 introduces the general context of deep eutectic solvents as a new generation of sustainable solvents, and the main objectives of the thesis. All data presented in the following chapters (2 to 4) are entirely based on published (or submitted) scientific articles in peer reviewed journals, bearing in mind that they are not by their chronological order of publication, but rather build a comprehensive progress of the field. The thesis kick-off consisted of the development of novel hydrophobic DES, exploring and gathering evidences for the understanding their properties as well as their application as immiscible solvent for purification of water contaminated with several micropollutants. Finally, a comparison between hydrophilic and hydrophobic DES is presented, putting in evidence the design of DES containing both hydrophilic and hydrophobic parts and their behaviour in water.

Furthermore, in each article-based chapter is possible to find a brief review of the state of the art, materials and methods, results as well as their discussion and the main conclusions of the particular work. Moreover, since in each chapter of thesis, the figures, tables, symbols and abbreviations are properly identified and carefully described, a general list of them were not included in this thesis.

CHAPTER 1

DEVELOPMENT OF SUSTAINABLE ALTERNATIVE GREEN SOLVENTS

From solid to liquid state

CHAPTER 1.1

Introduction: state-of-the-art and thesis motivation

This chapter was adapted from an invited mini-review: **C. Florindo**, L. C. Branco, I. M. Marrucho, In the quest for green solvents design: from hydrophilic to hydrophobic (deep) eutectic solvents, ChemSusChem, 2019, 12, 1549 (invited to be a cover of the issue).

The author was involved in the literature review concerning the evolution of hydrophobic DES field as well as in the discussion and interpretation of results and the preparation of the review.

CONTENT

The following chapter contains essential information for understanding the thesis organization as well as the highlights, the framework, general concepts and motivation of the present thesis. The progressive development of the state of the art of deep eutectic solvents as new alternative solvents, in particular hydrophobic deep eutectic solvents as immiscible solvents for water purification applications is here detailed. In the end of this chapter, the main objectives and layout structure of this thesis are specified.

1. General context

In the increasingly critical field of sustainable chemistry, the search for new and better green solvents has become a top priority. Based on their biodegradability and low toxicity, as well as their potential to promote advantageous synthetic transformations, green solvents offer the promise of significantly reducing the hazardous effects of chemical processes on human health and the environment.

Solvents are ubiquitous in chemical processes, but they are also known to be one of the major contributors to industrial waste streams. By definition, solvents are liquid compounds that are able to dissolve, dilute or extract other compounds without modifying them chemically and without suffering chemical modifications themselves.[1] Nowadays, industry, research and academia employ substantial amounts of organic solvents in dissolution, chemical reactions, separation, purification and analytical processes.[2] Solvents are continuously used and can be found in almost every industrial sector, from cosmetics, pharmaceuticals and personal care products to paints, semiconductors, from biotechnology-based industries to iron and steel production. Consequently, solvents represent a huge economical market, reaching tenths to hundredths of million tons per year.[3] The contribution of Green Chemistry to modern industrial chemistry, together with the implementation of stricter regulations regarding solvent safety and human health issues, are changing the paradigm in solvents scenario.[4]

Today, alternative solvents are becoming key players in the development of sustainable chemical products and processes, reducing or even eliminating the use and formation of hazardous substances, while promoting the re-use and recycle of reagents, reaction media and catalysts.[5] In order for a solvent to be accepted as a green medium some important and strict requirements, such as sustainability, availability, toxicity, biodegradability, recyclability, and, of course, the price, have to be fulfilled.[6] In general, any cost-effective solvent that contribute to the optimization of the overall process, while simultaneously displaying low environmental and human health toxicity, is considered as an alternative solvent.[7]

The most well-known classes of alternative solvents currently used are water, supercritical fluids, bio-based solvents, polymers, switchable solvents, ionic liquids (ILs) and more recently - Deep Eutectic Solvents (DESs).[3] Interestingly, and as pointed out by Clark and Tavener,[8] these classes of alternative solvents have been proposed one after the one in order to impart alternative solvents with different properties, from water (highly polar and average volatility), to supercritical CO_2 (apolar), to ILs and DES (ionic, polar and non-polar, negligible volatility). Despite the similar properties of ILs and DESs, this last class has been finding its ubiquitous place in the alternative solvents market essentially due to the easiness of their preparation, just by heating and mixing or gridding, which is directly translated in low cost and high purity. This off-the-shelf character of DESs, combined with the easy tunability of their properties, through the suitable selection of the starting components, contributes for the recent booming interest in these solvents.

In 2003, Abbott and co-workers[9] defined a Deep Eutectic Solvent (DES) as a liquid compound that exhibits a melting point decrease superior to 100 ºC compared to its pure compounds. The first reported DES was a mixture of choline chloride and urea, with a melting temperature of 12 ºC, much lower than those of the starting materials, 302 ºC and 133 ºC, respectively.[10] After that, Abbott and co-workers[11] proposed a classification of DESs in four different types according to their constituents, as shown in Figure 1.

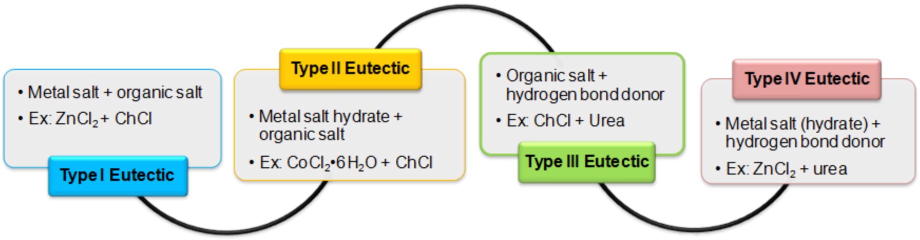


Figure 1. Four different types of DESs depending on the HBA and HBD, as proposed by Abbott.[11]

Type I, II and IV DES are composed of metal salts or metal salt hydrates combined with organic salts or other neutral compounds, while type III contains one organic salt, typically an ammonium halide with a chemical structure similar to those of Ionic Liquids (ILs), combined with a hydrogen bond donor. This largely explains why the majority of DESs reported in the literature belong to type III, in an effort to mimic ILs properties. The second component in type III DESs is usually selected from a wide range of natural compounds, such as organic acids, sugars, alcohols, aminoacids, among others, and consequently, type III is less toxic as well as less expensive compared to the other classes. Later on, Choi and co-workers,[12] introduced the so-called NADES (natural deep eutectic solvents), composed by choline chloride, natural carboxylic acids, sugars, aminoacids, and in some cases using water as a third component. Figure 2 summarizes typical combinations of HBDs and HBAs used in the formation of DESs, commonly reported in the literature.

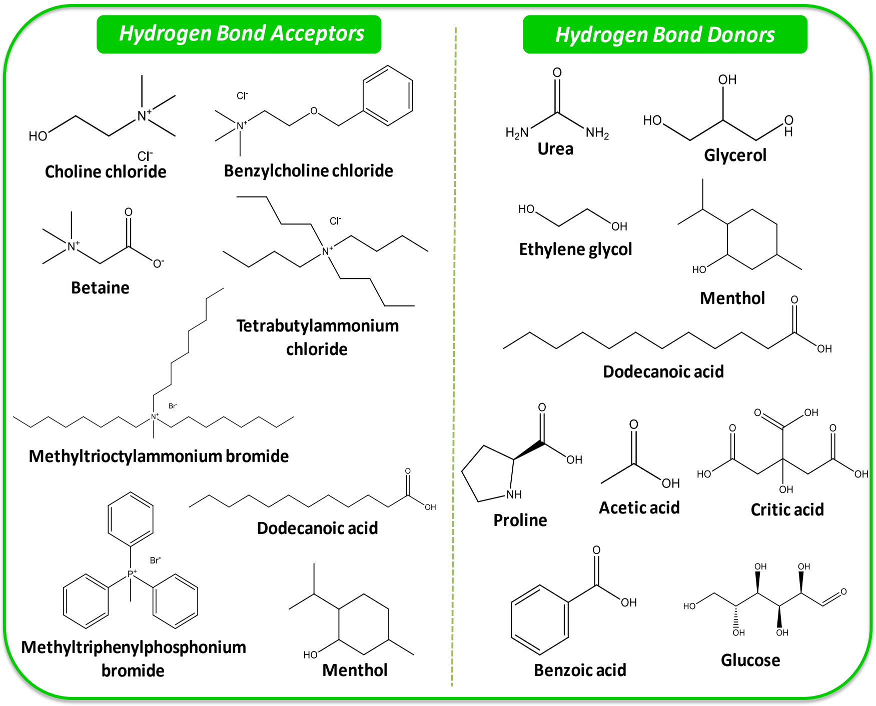


Figure 2. Chemical structures of HBDs and HBAs often used in DESs preparation.

One of the major advantages of DESs lies in their preparation method, which is straightforward, with 100% atom efficiency, by simply mixing HBDs and HBAs. Several strategies have been proposed for DESs preparation being the heating the preferred one, but others such as grinding or dissolution of the components in an appropriate solvent, typically water, and consequent evaporation, have also been reported.[10, 13] An important aspect to be emphasized is the low thermal stability of most natural compounds, rendering the formation of unwanted degradation products when the heating method is used.[13]

The idea of obtaining liquids just by mixing solid compounds with high melting points was very well accepted by the scientific community and thereafter the number of publications significantly increased. Nevertheless, few publications show fundamental studies concerning phase diagrams, properties and their interpretations, but rather applications on large diversity of fields, ranging from biological studies on DESs as secondary plant metabolites to biomass processing, also including extractants for water and traditional fuels and speciality solvents in pharmaceutical and cosmetic industries.[14-17] In Figure 3, a schematic representation of solid – liquid phase diagram of a binary eutectic mixture with a large melting point depression is presented. Since the magnitude of the melting point depression depends on the interactions between the components in the mixture, a DES is only obtained for a mixture with large deviation from ideality (usually by the establishment of a large network of hydrogen bonds or by the presence of charged compounds).

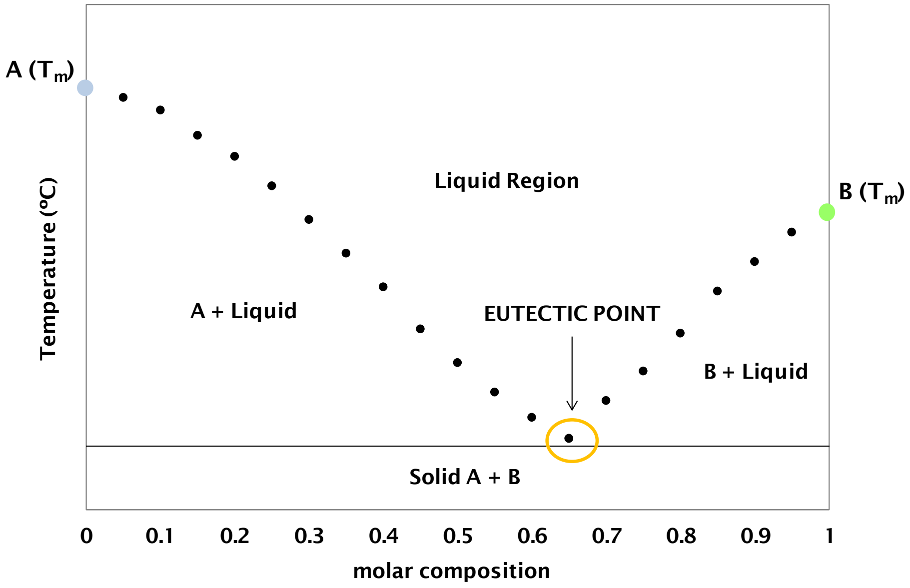


Figure 3. Schematic solid – liquid phase diagram showing simple binary eutectic behaviour.

The analysis of Figure 3 also allows to evaluate the different range of compositions and temperatures where a homogeneous liquid phase is available, showing that instead of working at one fixed temperature (typically room temperature) and/or one fixed composition (the eutectic mixture composition) a range of working temperatures and compositions can be selected to better suit DES properties to the envisaged application. This emphasizes the fact that DESs can only be confidently used as designer solvents if the solid-liquid phase diagram is available.

The large interest in DES urges the answers to fundamental questions about these solvents, like the nature of the interactions underpinning their formation, their bulk liquid structure, the role of water in DES formation and in the liquid structure, among others. The use of molecular dynamics, thermodynamic models and spectroscopic techniques to answer these and other questions have only shaded some light and deeper studies need to be pursuit. As more research is undertaken using DESs, the more obvious is the need of a uniform nomenclature.

2. Hydrophobic (Deep) Eutectic Solvents

Until 2015, the majority of DESs presented in the literature were hydrophilic in nature since they were composed of typically hydrophilic materials from renewable resources such as sugars, alcohols, acids and some organic salts. The full potential of hydrophobic (D)ES was only unleashed in 2015, with the seminal work of Kroon´s group where quaternary ammonium salts and decanoic acid were combined to prepare hydrophobic DESs. Marrucho’s group also reported hydrophobic eutectic mixtures composed by adequate combination of two neutral compounds, DL-menthol and carboxylic acids. The aim of both these two works was to develop hydrophobic phases which would be stable in contact with water. Figure 4 shows the evolution in the number of studies reporting hydrophobic (D)ESs compared to hydrophilic DESs.

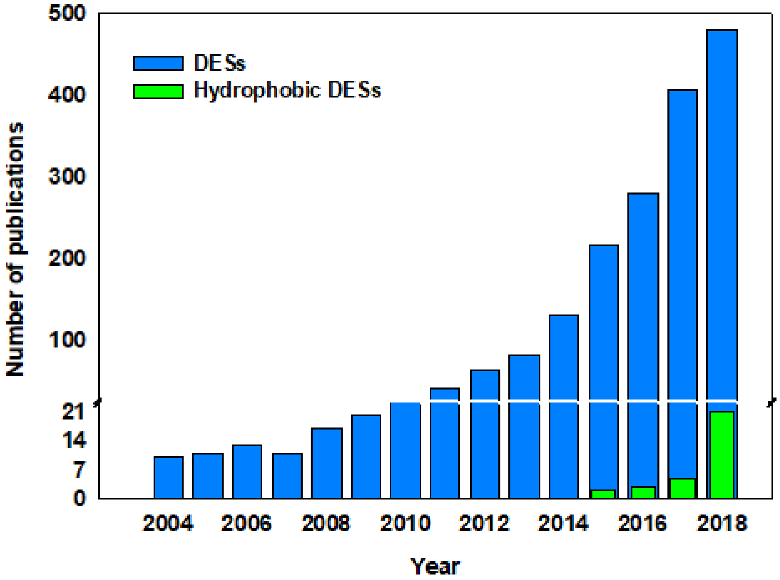


Figure 4. Evolution of the number of publications in the field of Deep Eutectic Solvents in general (blue) and Hydrophobic Deep Eutectic Solvents (green) during the last years, that contained “deep eutectic solvents” or “hydrophobic deep eutectic solvents” in their titles, keywords and abstracts as obtained from ISI web of knowledge. Data for 2018 include up to December.

As it can be observed from Figure 4, despite the interest of this novel class of DESs, a very limited number of hydrophobic (D)ESs has been proposed so far. The most reported hydrophobic (D)ESs are essentially composed of quaternary ammonium salts with long alkyl chains or hydrophobic natural compounds as HBA, combined with several hydrophobic HBD as carboxylic acids or alcohols with long alkyl chains, in contrast with hydrophilic DES, where combinations of choline chloride salt and other ammonium salts possessing small alkyl chains with several acids, amines, alcohols, aminoacids, among others, are the most studied. Figure 5 summarizes the most used HBDs and HBAs in the preparation of hydrophobic deep eutectic solvents. As it was mentioned before, the major difference in terms of chemical structures of the parent compounds, either ionic or neutral, lays in the presence of long alkyl chains and thus in the decreasing of the importance of the hydrophilic domains, such as charges of the salts, and hydrophilic groups, such as carboxylate and hydroxyl groups.

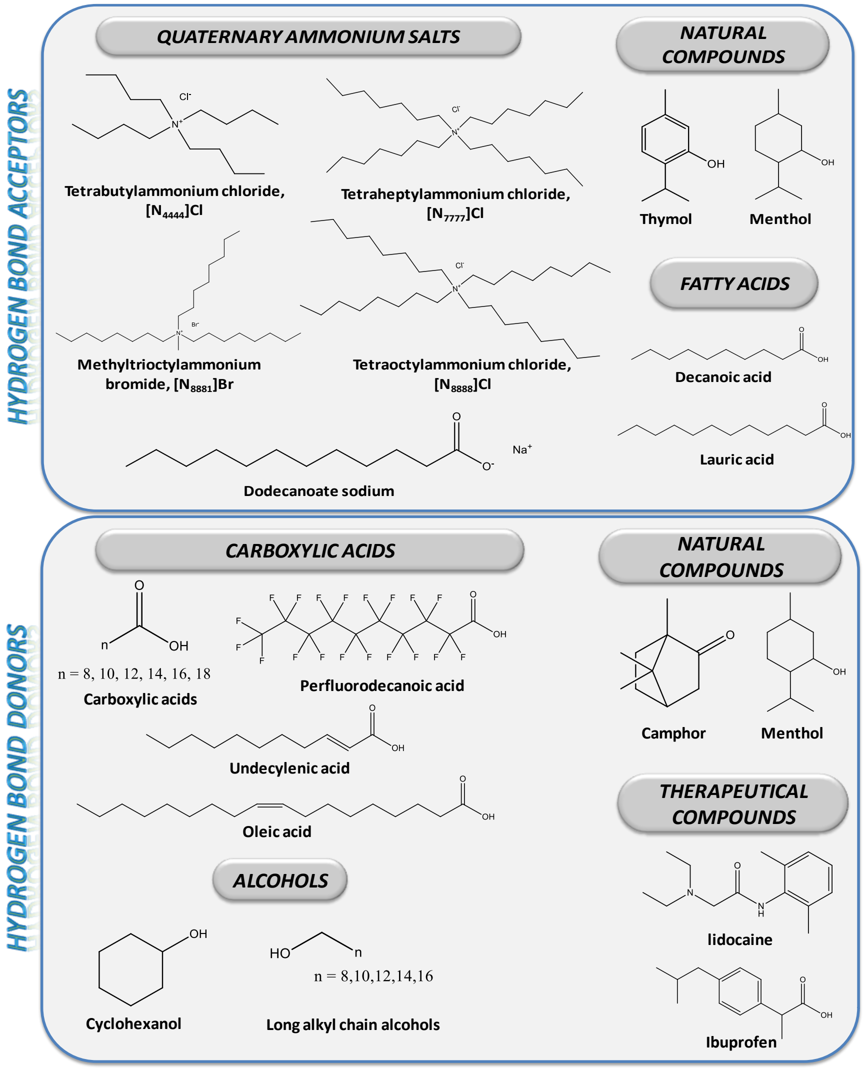


Figure 5. Typical HBDs and HBAs used in hydrophobic (D)ESs preparation.

The difference in the chemical structures of the parent compounds is responsible for the magnitude of the melting point depression. One of the differences between hydrophilic and hydrophobic (D)ESs is the fact that in the first class a deep depression in the melting point is typically obtained, unlike hydrophobic (D)ES where large and small depressions can be obtained. This difference in melting point depression behaviour is due to the presence of charged and polar moieties leading to extensive hydrogen bond interactions in hydrophilic DESs, whereas in hydrophobic (D)ES a large depression is only obtained in the presence of salts, even if their charges screened by the long hydrocarbon alkyl chains. Neutral hydrophobic (D)ESs are essentially eutectic mixtures, showing small depressions in the melting points, leading to highly fluid solvents, a very appealing property for some applications. Figure 6 shows a direct comparison of phase diagrams of two hydrophobic (D)ESs, one composed of dodecanoate sodium salt and decanoic acid[7] and the other containing L(-)-Menthol and decanoic acid.

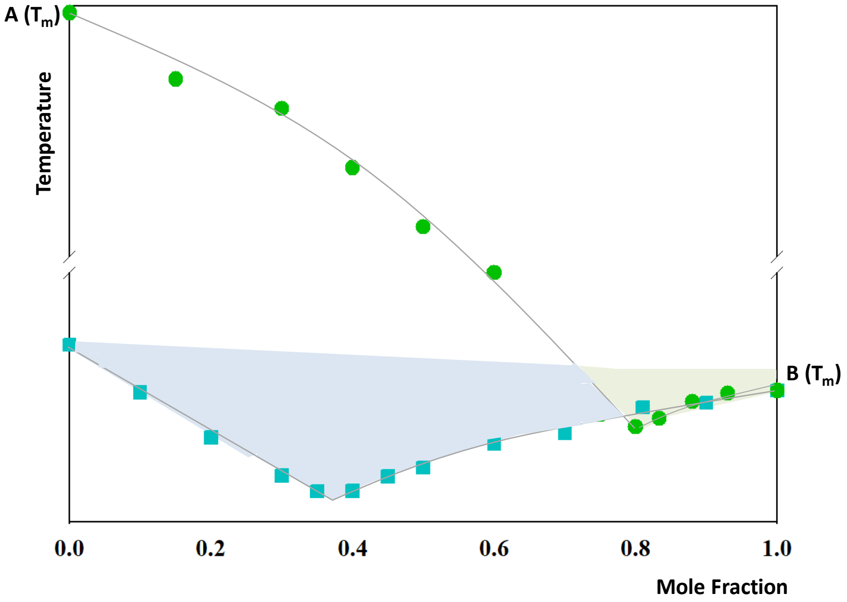


Figure 6. Comparison of solid-liquid phase diagram between a deep eutectic solvent composed of dodecanoate sodium salt and decanoic acid (● NaC_{12}: C_{10})[18] and an eutectic solvent composed of a natural compound, L-Menthol and decanoic acid (■ L(-)-Menthol: C_{10})[19].

Two distinct behaviors are depicted in Figure 6: hydrophobic deep eutectic solvent, due to the presence of dodecanoate sodium salt, or a hydrophobic eutectic solvent, when only neutral components are used. Since solid-liquid phase diagrams are not available for most DESs, it is difficult to discern when a deep depression of the melting points occurs. Although in literature all these compounds are designated by hydrophobic DESs, in this review we adopt the terminology hydrophobic (D)ESs in general, while DESs and ESs will be used when for those composed of salts or composed of neutral compounds, respectively.

Another important point that comes up from the observation of the phase diagrams in Figure 6 is the liquid region that is in fact available from the (D)ESs formation. The new temperature-composition surface where a liquid is attained due to (D)ESs formation depends on lower melting temperature of (D)ESs components and the temperature of the eutectic point. For example, for NaC_{12}: C_{10}, both these temperatures are close and thus a small liquid surface is obtained, while for L(-)-Menthol: C_{10}, since the difference in these temperatures is higher, a larger surface is available, as indicated in shadow in the text.

2.1. Development and preparation of Hydrophobic (D)ESs

Unlike hydrophilic DESs, the number of hydrophobic (D)ESs and ESs is quite limited. This is mainly due to the restricted number of cheap, readily available hydrophobic salts and other hydrophobic organic natural compounds that form eutectic solvents close or bellow room temperature. Just like hydrophilic DESs, hydrophobic DESs and ESs have also started to be studied from the application point of view, leaving similar fundamental questions unanswered.

A summary of the hydrophobic DESs and ESs reported in the literature is shown in Tables 1 (ammonium salts) and 2 (natural compounds).

Table 1. Summary of hydrophobic DESs reported in literature, composed of ammonium salts.

	HYDROPHOBIC DESs		Molar Ratio studied (HBA:HBD)	Temperature of Eutectic Point (ºC)	Reference
	HBA	**HBD**			
QUATERNARY AMMONIUM SALTS	Tetrabutylammonium Chloride **[N$_{4444}$]Cl**	Acetic acid*	1:1	—	20
		Levulinic acid*	1:2	—	20
		Hexanoic acid*	1:2	—	20
		Octanoic acid*	1:1, 1:2	—	20-22
		Decanoic acid*	1:1, 1:2	-11.95	20-25
		Lauric acid*	1:1, 1:2	—	20, 22
	Tetrabutylammonium Bromide **[N$_{4444}$]Br**	Acetic acid*	1:2	—	26
		Propionic acid*	1:2	—	26
		Acrylic acid*	1:2	—	26
		Butyric acid*	1:2	—	26
		Octanoic acid*	1:1, 1:2	0.58	22, 26
		Decanoic acid*	1:1, 1:2	16-17	22, 26
		Lauric acid*	1:1		22
		Oleic acid*	1:2	1.86	26
	Methyltrioctylammonium Chloride **[N$_{8881}$]Cl**	Hexanoic acid*	1:2	—	27
		Octanoic acid	1:2	—	21, 27
		Decanoic acid	1:2	-0.05	21, 23, 25, 27
		Lauric acid	1:2	—	27
		Myristic acid	1:1	—	27
		Palmitic acid	1:1	—	27
		cis-9-octadecenoic acid	1:2	—	27
		Ricinoleic acid	1:2	—	27
		Ethylene glycol*	1:2	—	28
		1-Propanol*	1:2	—	27-28
		1,3-Propanediol*	1:2	—	28
		Glycerol*	1:2	—	28
		1-Butanol*	1:2	—	27-28
		1,2-Butanediol*	1:2	—	28
		Hexyl alcohol*	1:2	—	27-28
		Capryl alcohol	1:2	—	27-28
		Decyl alcohol	1:2	—	27-28
		Dodecyl alcohol	1:2, 1:1	—	27-28
		1-Tetradecanol	1:2	—	27-28
		Hexadecanol	1:2	—	27
		Cyclohexanol*	1:2	—	27-28
		DL-Menthol	1:2	—	27-28

Methyltrioctylammonium Bromide **[N$_{8881}$]Br**	Decanoic acid	1:2	8.95	23, 25
Tetraheptylammonium Chloride **[N$_{7777}$]Cl**	Decanoic acid	1:2	-16.65	23, 29
	Lauric acid	1:2	—	29
	Oleic acid	1:2	—	29
	Ibuprofen	7:3	—	29
Tetraoctylammonium Chloride **[N$_{8888}$]Cl**	Decanoic acid	1:2, 1:1.5	1.95	23-25
	PerFluorodecanoic acid	1:2		24
Tetraoctylammonium Bromide **[N$_{8888}$]Br**	Decanoic Acid	1:2	8.95	23-25, 30
Dodecanoate sodium salt, **NaC$_{12}$**	Decanoic Acid **	All range	23	18

*unstable upon contact with water ** forms a hydrogel in contact with water

Table 2. Summary of hydrophobic ESs reported in literature, composed of neutral compounds.

	HYDROPHOBIC ESs		**Molar Ratio studied (HBA:HBD)**	**Temperature of Eutectic Point (ºC)**	**Reference**
	HBA	**HBD**			
NATURAL OR NEUTRAL COMPOUNDS	**DL-Menthol**	Acetic acid*	1:1	—	20, 31-32
		Butyric acid*	1:1	—	20, 32
		Pyruvic acid*	1:2	—	20, 31
		Lactic acid*	1:1, 1:2	—	31-32
		Levulinic acid*	1:1	—	20
		Propionic acid*	1:1	—	32
		Hexanoic acid*	1:1	—	20, 32
		Octanoic acid	2:1, 1:1, 1:2	-1.82	19-20, 22, 32
		Decanoic acid	2:1, 1:1, 1:2	8.86	19-20, 22, 29-30
		Lauric acid	2:1, 1:1, 1:2	21.23	19-20, 22, 29, 31-33
		Ibuprofen acid	3:1	—	16
		Phenylacetic acid*	1:1	—	32
		Mandelic acid*	1:1	—	32
	L(-)-Menthol	Octanoic acid	All range	-1.82	34, 29
		Decanoic acid	All range	8.86	
		Lauric acid	All range	21.23	
		Myristic acid	All range	26.62	
		Palmitic acid	All range	33.18	
		Stearic Acid	All range	37.88	
	Thymol	Octanoic Acid	All range	6.68	19
		Decanoic Acid	All range	18.86	19, 30, 35
		Lauric Acid	All range	24.83	19
		Myristic Acid	All range	38.16	19
		Palmitic Acid	All range	41.22	19
		Stearic Acid	All range	46.22	19
		Lidocaine	2:1	—	30
		Camphor	7:3, 3:2, 1:1	-44	35
		Undecylenic Acid	7:3, 3:2, 1:1, 1:2, 1:3, 1:4	7.5	35

FATTY ACIDS	**Decanoic acid**	Lidocaine	2:1, 3:1, 4:1	No melting, Tg = -66.15	36-37
	Lauric acid	Octanoic Acid	All range	9	38
		Nonanoic acid	All range	9	38
		Decanoic Acid	All range	18	38

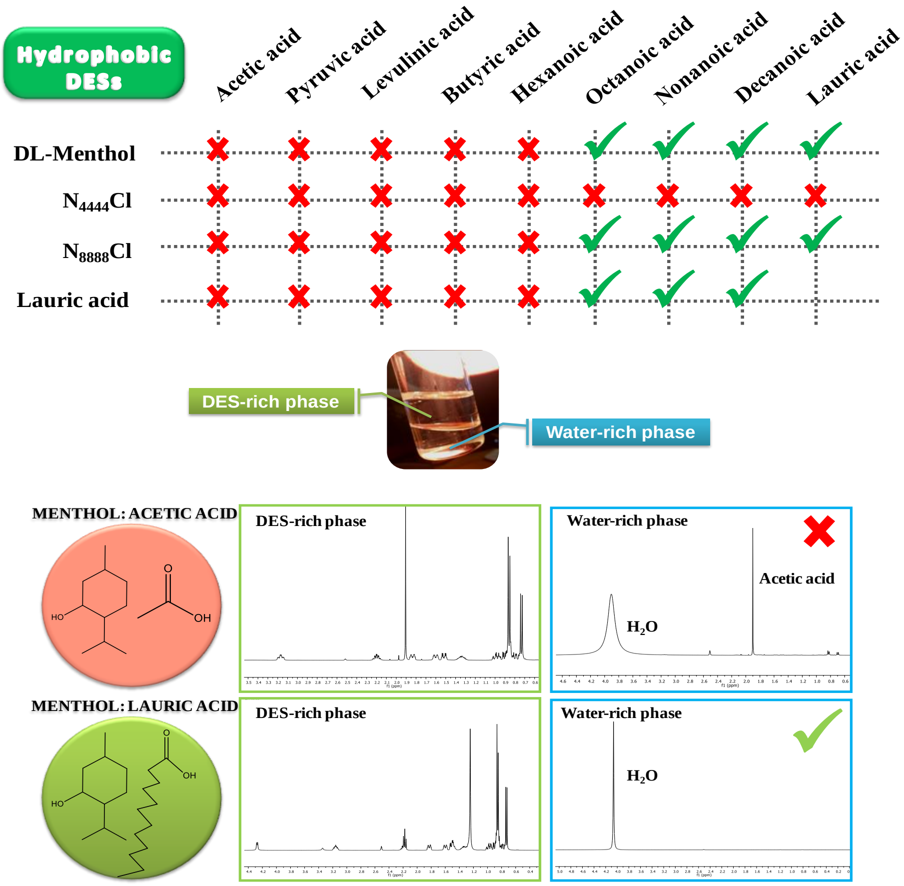
*unstable upon contact with water ** forms a hydrogel in contact with water

The first reported hydrophobic DES was based on quaternary ammonium salts with long alkyl chains and decanoic acid, where the hydrophobic character was confirmed by the study of the leaching of DES components to the water phase as well as their water uptake.[23] Following the same idea, several works using quaternary ammonium salts with long alkyl chains were then proposed, changing the HBD using different chemical structures, such as carboxylic acids and alcohols with different alkyl chains (C_2 to C_{18}) and with double bonds. Regarding the use of $[N_{4444}]Cl$ and $[N_{4444}]Br$ to prepare hydrophobic DESs, it is only highlighted the fact that hydrophobic DESs could be prepared using these compounds and that two phases are obtained when in contact with water. Their chemical stability will be discussed further ahead, and conclusions withdrawn.

As to what concerns hydrophobic ESs, Marrucho´s group[31] proposed the first hydrophobic ES based on natural renewable resources by combining DL-menthol and several natural carboxylic acids. In the same vein, Coutinho´s group[19] also contributed to increase the field of neutral hydrophobic solvents, proposing novel natural hydrophobic eutectic solvents based on terpenoids, L(-)-menthol and thymol combined with several carboxylic acids. Other hydrophobic ESs based on lidocaine and decanoic acid at several molar ratios were also proposed.[36] Other sustainable hydrophobic ESs composed of two compounds from the same family, long chain fatty acids, have also been proposed.[38] In these hydrophobic ESs, both acids can work as HBA and as HBD, and were inspired in phase change materials, but choosing the components carefully so that eutectic points lower than room temperature were obtained. In a subsequent work, Florindo et al.[18] presented a novel family of hydrophobic DES containing a sodium salt of a fatty acid, which acts as HBA, combined with another fatty acid, as HBD. Moreover, the authors found the formation of a hydrogel when contacting the sodium-based DES with water. The presence of the charged sodium atoms seems to be crucial for the formation of the hydrogel, since in the previous work where the reported DES was composed by two carboxylic acids with a long alkyl chain and the complete DES immiscibility with water was observed without gel formation. In the present case, instead of two mutually immiscible liquid phases, a hydrogel was obtained. The more water added, the more viscous the gel became.

Concerning the characterization of these hydrophobic systems, very few solid-liquid phase diagrams are available for hydrophobic DESs and ESs. In Tables 1 and 2, it is possible to see that phase diagrams are only available in the cases where the “all range” label is.

Regarding the development of hydrophobic (D)ESs, the selection of appropriate HBA and HBD is a key factor. Both Kroon's[23] and Marrucho’s[20] teams pointed out the relevance of the hydrophobicity of both components. A comprehensive study on the chemical and water stability of several hydrophobic (D)ESs, composed of either neutral or ionic compounds, was performed through their NMR analysis of the water phase. It was clear that the hydrophobicity of (D)ESs depends on the hydrophobicity of the individual components and that (D)ESs formed by one hydrophilic and one hydrophobic compound are not stable in water, since the hydrophilic component will leach to the water phase according to its water solubility.[20] Figure 7 illustrates the ^{1}H NMR spectra of ES-rich and water-rich phases of two different hydrophobic ESs, DL-menthol: acetic acid (1:1) and DL-menthol: lauric acid (2:1). Moreover, a matrix with the water stability of the studied hydrophobic (D)ESs, where ✓ indicates stable (D)ES and **X** unstable (D)ES in contact with water, is also presented.



Hydrophobic DESs	Acetic acid	Pyruvic acid	Levulinic acid	Butyric acid	Hexanoic acid	Octanoic acid	Nonanoic acid	Decanoic acid	Lauric acid
DL-Menthol	✗	✗	✗	✗	✗	✓	✓	✓	✓
$N_{4444}Cl$	✗	✗	✗	✗	✗	✗	✗	✗	✗
$N_{8888}Cl$	✗	✗	✗	✗	✗	✓	✓	✓	✓
Lauric acid	✗	✗	✗	✗	✗	✓	✓	✓	

Figure 7. NMR analysis of water stability of hydrophobic (D)ESs. Summary matrix illustrating the dependence of water stability from HBA and HBD (✓ stable and X unstable) (adapted from [9]).

2.2. Physicochemical Properties of Hydrophobic (D)ESs

Just like hydrophilic DESs, hydrophobic (D)ESs are also designer solvents, meaning that, different thermophysical properties can be obtained by judicious combination of individual components. In the next subsections, a systematic overview of some key properties, such as melting temperatures of the eutectic point, density, viscosity and solubility of water in hydrophobic

(D)ES will be compared and discussed. Although other properties, like vapour pressure and superficial tension, are slowly being published for hydrophilic DES, they are still not available for hydrophobic (D)ESs. Comparisons between representative elements of the two families of hydrophobic (D)ESs will be drawn in order to understand the influence of the chemical structure of each component in (D)ES properties. The selected hydrophobic (D)ESs are either neutral, composed of menthol and fatty acids or just fatty acids, or ionic, composed of sodium salt or quaternary ammonium salts with long alkyl chains. Since thermophysical properties are strongly affected by temperature and water content, only literature data at 25 ºC and water content bellow 1000 ppm were selected for this comparison.

2.2.1. Melting temperature of hydrophobic (D)ESs

The establishment of hydrogen bonds have been identified by several authors as the responsible interactions behind (D)ES formation.[11, 39] Hydrogen bonds are typically present in strongly non-ideal mixtures, leading to large melting point depressions at the eutectic composition. Figure 8 shows the melting points of several hydrophobic (D)ESs studied in the literature.

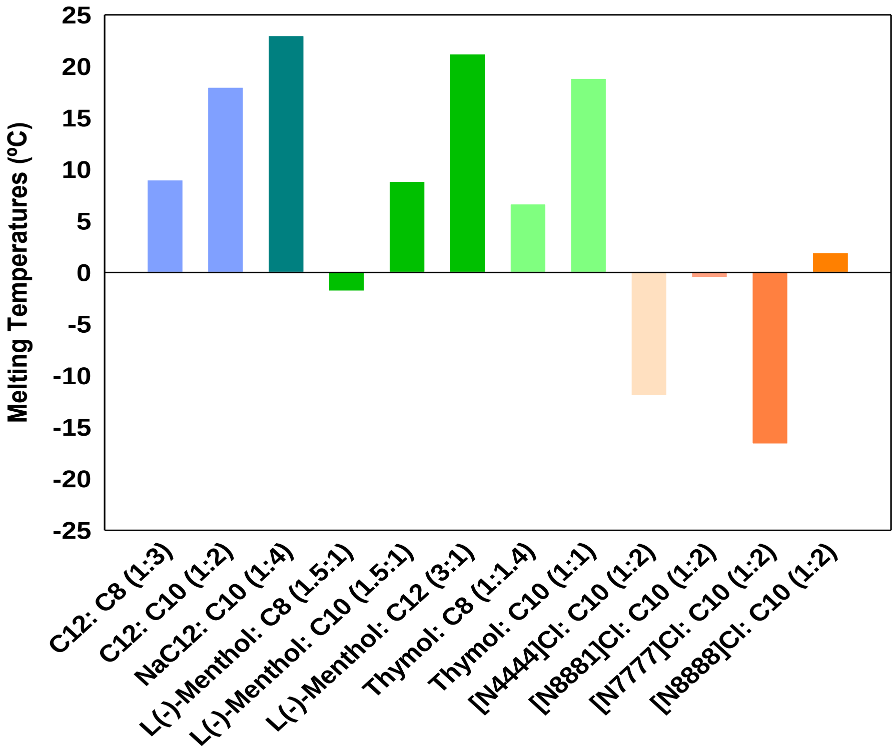


Figure 8. Comparison of the melting temperatures of different hydrophobic (D)ESs. Data taken from literature 19, 23, 31, 34, 38.

As expected, the melting temperature at the eutectic composition is quite diverse in hydrophobic (D)ESs. For all reported hydrophobic (D)ESs, melting points below 25 ºC were obtained, allowing their use as solvents or reaction media at room temperature. The lowest melting temperature was observed for [N_{7777}]Cl: C_{10}, -16.65 ºC. Important conclusions that also can be made from this Figure is the effect of the alkyl chain increase, present either in the HBA or in HBD, in the eutectic temperature. In general, for hydrophobic (D)ESs, an increase of the melting temperature with the alkyl chain of the acid component (HBD) can be observed. On the other hand, an opposite trend can be observed with the increase of the alkyl chains of the ammonium cation since the melting temperature decreases from [N_{4444}]Cl: C_{10} to [N_{7777}]Cl: C_{10}. However, an increase of melting temperature is observed from [N$_{7777}$]Cl: C$_{10}$ to [N$_{8888}$]Cl: C$_{10}$, indicating a discontinuity in this property that needs to be further studied. The comparison of the melting temperature of [N$_{8888}$]Cl with that of [N$_{8881}$]Cl: C$_{10}$ also leads to the same conclusion. Moreover, the replacement of dodecanoic acid in C$_{12}$: C$_{10}$ by its sodium salt leads to an increase in the eutectic melting temperature, which can probably be attributed to the presence of coulombic interactions. In conclusion, and just like hydrophilic DESs, the dimension of the liquid phase depends on the choice of components in hydrophobic (D)ESs.

2.2.2. Density and Viscosity of Hydrophobic (D)ESs

Density and viscosity are key properties of solvents since they greatly influence dissolution, reaction and separation processes, determining their viability. Figure 9 depicts density data of selected hydrophobic (D)ESs available in literature.

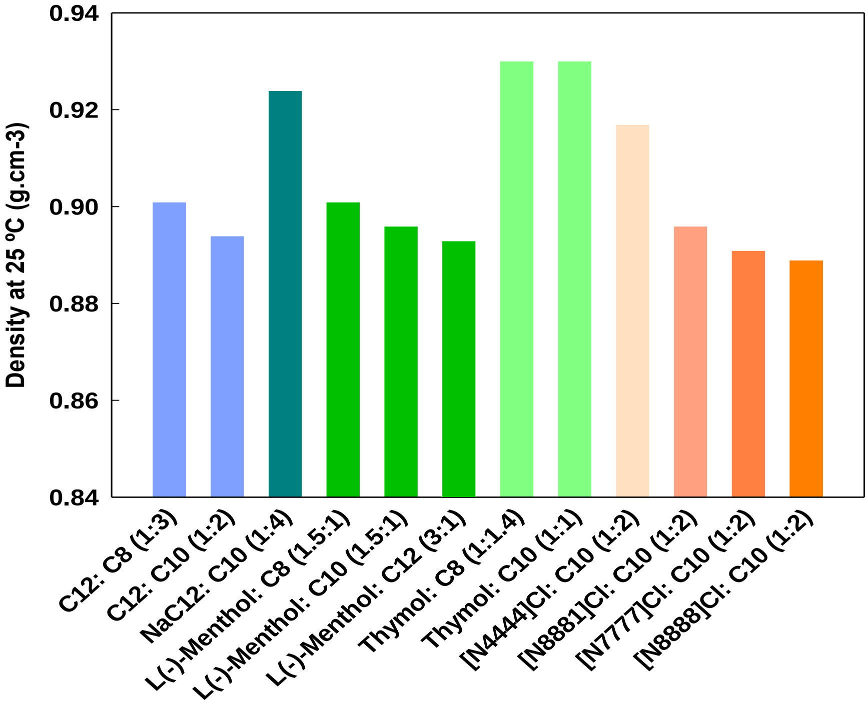


Figure 9. Comparison of density values at 25 ºC using different families of hydrophobic (D)ESs. Data taken from literature 19, 23, 31, 34, 38.

Contrary to hydrophilic DESs, hydrophobic (D)ESs exhibit similar and lower densities than water, varying between 0.889 and 0.93 depending on the components. The highest densities were observed for thymol's family (~0.93 g/cm3). For all the families of hydrophobic (D)ESs, density decreases with the increase of the alkyl chains, regardless if they are on the HBD or HBA. Moreover, a significant effect can be observed for DESs containing sodium salt as HBA instead of its homologous fatty acid, leading to a large increase in density values.

Figure 10 shows the viscosity data of commonly reported hydrophobic (D)ESs.

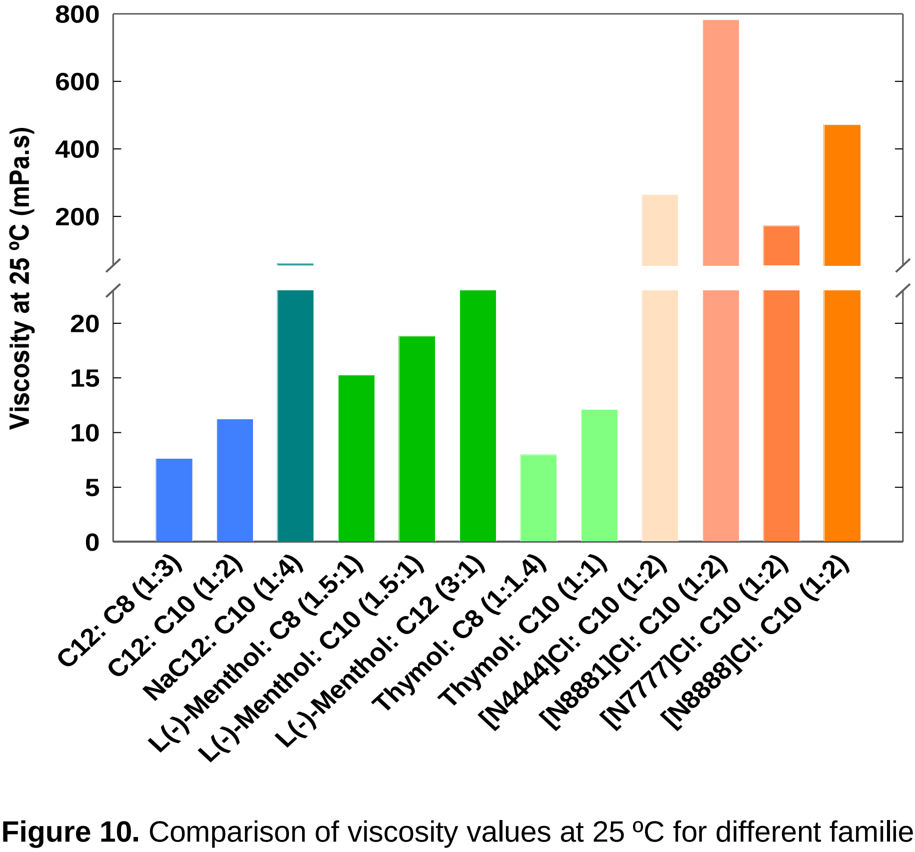


Figure 10. Comparison of viscosity values at 25 ºC for different families of hydrophobic (D)ESs. Data taken from literature 19, 23, 31, 34, 38.

Major changes in viscosity can be observed according to the hydrophobic (D)ESs components. Viscosity increases according to the chemical structure of HBA present in DESs as follows: fatty acids ≈ thymol < menthol < quaternary ammonium salts, the latter being the most viscous, probably due to the presence of coulombic forces in the quaternary ammonium salts. Another important point in quaternary ammonium salts, is the decrease in viscosity with increase of the alkyl chain from $[N_{4444}]Cl$ to $[N_{7777}]Cl$, due to the increase shielding of the localized charge in the nitrogen atom and the decrease of viscosity from $[N_{7777}]Cl$ to $[N_{8888}]Cl$, due to the bulkiness of the alkyl side chains. The shielding of the charge effect can be clearly seen when $[N_{8888}]Cl$ is compared to $[N_{8881}]Cl$. Noteworthy are the extraordinarily low viscosities (less than 20 mPa.s) of ES based on fatty acids and menthol, clearly overcoming one the major drawbacks of hydrophilic DES, where for example a viscosity of 859.45 mPa.s is attained for [Ch]Cl: urea (1:2).[40] The most fluid hydrophobic (D)ESs are C_{12}: C_8 (1:3) and Thymol: C_8 (1:1.4) with viscosities of 7.66 mPa.s and 8 mPa.s at 25 ºC, respectively, comparable to other organic hydrophobic solvents (viscosity of heptane ~ 0.4 mPa.s[41]).

2.2.3. Solubility of water in Hydrophobic (D)ESs

Considering the use of hydrophobic (D)ESs as extractants of solutes from aqueous phases, it is relevant to guaranty (D)ESs chemical integrity, ensuring that there is no contamination of the water phase as well as any loss of (D)ES. Usually, in the case of starting materials with very low water solubility, the final (D)ESs will also have a negligible solubility in water. In this section, only stable hydrophobic (D)ES are considered. On the other hand, it is also important to know the solubility of water in (D)ESs, since it drastically affects thermophysical properties. Figure 11 presents a comparison of water solubility (wt%) for different hydrophobic (D)ESs.

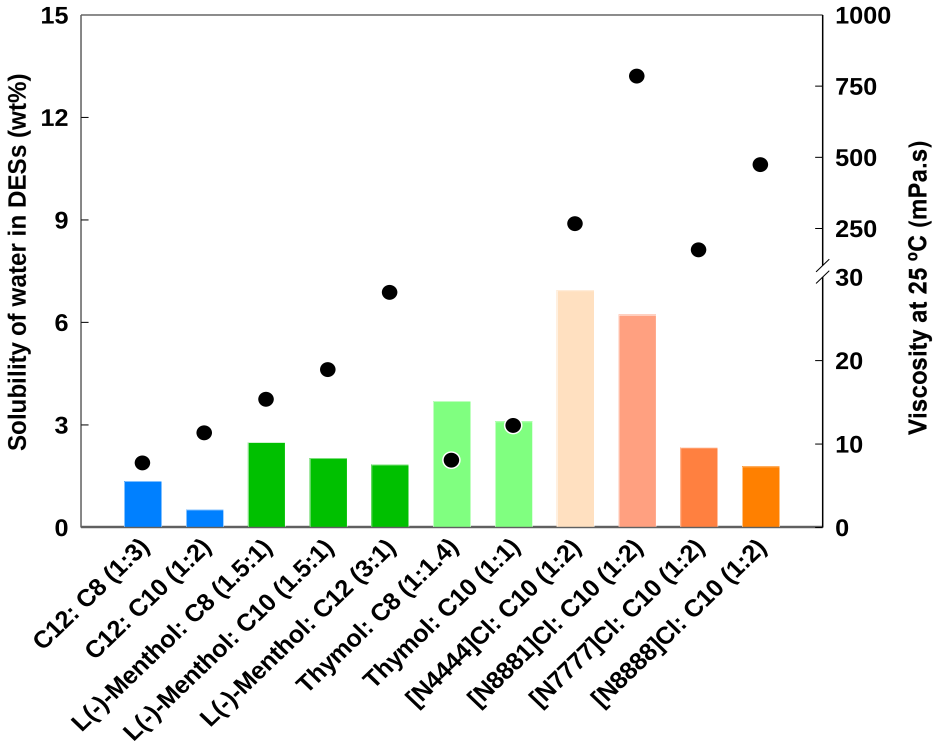


Figure 11. Comparison of water solubility (wt%) and viscosity for several hydrophobic (D)ESs at 25 ºC. Data taken from literature 19, 23, 31, 34, 38. Bars correspond to solubility of water in hydrophobic (D)ESs and points to viscosity data of pure hydrophobic (D)ESs.

It can be observed that hydrophobic (D)ESs have low water solubilities, lower than 7 wt%. The largest water solubility (6.94 wt%) was obtained for [N_{4444}]Cl: C_{10} (1:2), closely followed by [N_{8881}]Cl: C_{10} (1:2) (6.22 wt%). Again, this is probably due to the more available charge on the nitrogen atom of the ammonium cation owing to the presence of at least one short alkyl chain. This observation is in line with the recently reported hydrogel formation when NaC_{12}: C_{10} (1:4) is contacted with water, which was explained by the presence of the charged sodium atoms.[18] Overall, the water solubility can be ranked by family of hydrophobic (D)ESs according to following order: fatty acids < menthol < thymol < quaternary ammonium salts, where fatty acid-based ESs solubilize ≤ 5 wt% of water, menthol-based ESs less than 2.5 wt% of water, thymol-based DESs less than 3.7 wt% of water and finally long alkyl chains ammonium salts-based DESs solubilize between 2 to 7 wt% of water. Also, for all the families of hydrophobic (D)ESs, water solubility decreases with the increase of the HBD alkyl chain.

Curiously, the water solubility order for the 3 different selected families of hydrophobic (D)ESs matches the viscosity order, where, the tetralkylammonium salts-based DESs (the most viscous family of hydrophobic DES) is also the one with the highest water solubility, and the fatty-acids ESs family show the lowest water solubility and viscosity. Although hydrophobic (D)ESs the water solubility is small, the viscosity is less affected by this small water presence, probably because the hydrogen bonding network is not as important here as it is for hydrophilic DESs. In the case of ionic hydrophobic DESs, their high water solubility the formation of intermolecular crosslinked structures, usually involving the participation of water molecules, thus increasing the viscosity, sometimes forming hydrogels.[42]

2.3. Versatile applications of hydrophobic (D)ESs – From nature to green chemistry

Although some important information on hydrophobic (D)ESs from the fundamental points of view is still missing, the development of applications using these solvents is already a flourishing field. One advantage of hydrophobic (D)ESs is their water immiscibility, allowing their use in extractions and separations of compounds from aqueous phases. Although the field of hydrophobic (D)ESs is new, a fair amount of publications on their possible applications has been published. In Figure 12, main areas of application of hydrophobic (D)ESs, extraction of natural components from plants, CO_2 capture, organic synthesis and materials, and water purification, are represented.

Figure 12. Areas of significant impact and use of hydrophobic (D)ESs.

The first applications to be proposed for hydrophobic DESs focused on the use of long chain tetraalkylammonium bromide salts combined with long chain organic acids to extract volatile acids from aqueous solutions.[23] The potential of these hydrophobic DESs was proven and compared to the industrial standard compound (trioctylamine, TOA), with hydrophobic DESs showing superior extraction efficiencies. Ribeiro et al.[31] used all natural hydrophobic ESs, composed of menthol and naturally occurring acids (pyruvic acid, acetic acid, L-lactic acid and lauric acid), to illustrate their ability to extract four model biomolecules (caffeine, tryptophan, isophthalic acid and vanillin) from aqueous solutions. Van Osch et al.[36] proposed a hydrophobic ESs based on decanoic acid and lidocaine, for the removal of a wide range of metal ions from aqueous solutions. It was shown that all transition metal ions could be extracted with high distribution coefficients within 5 seconds, allowing the regeneration of the used ESs. Other authors[19] also reported the selective extraction and separation of Cu(II) from other transition metals in mildly acidic aqueous solutions using all natural hydrophobic ESs based on low-price and biodegradable terpenes and fatty acids. Furthermore, the used hydrophobic ESs could be successfully recovered and recycled using selected hydrophilic DESs. Persistent micropollutants, such as pesticides from neonicotinoids family,[20] Bisphenol-A,[38] a plasticizer present in water bottles and simultaneously acting as an endocrine disruptor, was successfully extracted using all natural hydrophobic ES, with extraction efficiencies around 70% and more than 90%, respectively. Recently, Dietz et al.[30] reported the selective separation of furfural and hydroxymethylfurfural from an aqueous solution using supported hydrophobic (D)ESs liquid membrane. Four different hydrophobic (D)ESs ([N_{8888}]Br: C_{10} acid, thymol: C_{10} acid, menthol: C_{10} acid and thymol: lidocaine) and the authors concluded that by tailoring hydrophobic DESs viscosity and pore size of the support, the recovery and separation of the compounds could be achieved. Makoś et al.[35] developed hydrophobic ESs based on thymol and (±)-camphor, decanoic and 10-undecylenic acids to isolate and enrich polycyclic aromatic hydrocarbons from aqueous samples using ultrasound-assisted dispersive liquid-liquid microextraction.

Another application with great potential is the use of hydrophobic (D)ESs for CO_2 capture. Dietz et al.[24] reported the use of PC-SAFT equation of state for modelling the CO_2 solubilities in several hydrophobic DESs. Afterwards, Zubeir al.[25] measured the solubility of CO_2 in hydrophobic DESs, where decanoic acid was combined with five different hydrophilic and hydrophobic quaternary ammonium salts, in the temperature range from 25 to 50 ºC and at CO_2 pressures up to 2 MPa. It was found that the CO_2 solubility was much higher in hydrophobic than in hydrophilic DES, but both could be repeatedly used as absorbing agents without losing absorption capacity.

Finally, another area in considerable expansion is extraction of secondary metabolites from plants using hydrophobic (D)ESs through microextraction techniques. Cao et al.[27] demonstrated the efficiency of hydrophobic DESs as designer solvents to extract polyprenyl acetates from Ginkgo biloba leaves. The authors proposed a ternary hydrophobic DESs, composed of methyl trioctylammonium chloride, capryl alcohol and octylic acid at a molar ratio of 1:2:3, as the most efficient extractant. The same team[28] also studied the extraction of artemisinin from Artemisia annua leaves. The hydrophobic DES based on methyltrioctylammonium chloride and 1-butanol at a molar ratio of 1:4 showed the highest extraction yield, and also higher efficiency than conventional organic solvent, petroleum ether. These studies illustrate the versatility and the properties' tunability of hydrophobic (D)ESs as green and safe extraction solvents for pharmaceutical applications. Finally, Křížeket al.[32] developed several hydrophobic ESs based on terpenes and natural organic acids to extract phytocannabinoids from raw cannabis plant material. The authors also proved that hydrophobic DES have superior performance in extraction efficiencies over other conventional solvents. For example, initial screening showed that ESs composed of a menthol: acetic acid (1:1) mixture had the greatest extraction efficiency with yields ranging from 118.6% to 132.6% compared to a methanol/chloroform mixture.

Finally, another area where these compounds have been widely applied is in organic synthesis and also in the development of novel materials. Boldrini et al., [28] reported the use of a hydrophobic eutectic solvent based on menthol and a naturally occurring carboxylic acid as an eco-friendly electrolyte medium in dye-sensitized solar cells. They concluded that the good performances, the higher voltage and the lower recombination resistances all suggest that hydrophobic eutectic mixtures can play an important role in improving the eco-compatible and sustainable character of liquid dye-sensitized solar cells.

3. Economic assessment of DESs

Since the price of solvents is a key issue for industry, whenever a new class of alternative solvents is proposed one of the most asked questions is how much will it cost? Up to this point, it can be affirmed that the success of DESs lays on its simplicity of preparation when compared with their parent compounds, organic salts and ionic liquids. Typically, quaternary ammonium and phosphonium salts are used to prepare DESs, which are cheaper than the iconic imidazolium-based ionic liquids. The simplicity of DESs preparation, typically just mixing and heating, and the absence of purification steps is a real advantage not only from the synthetic point of view but also from an economic point of view. The introduction of all neutral DESs, brought to the arena blue (green and cheap) DES, since the raw materials, fatty acids, terpene, alcohols etc., are now more environmentally friendly and cost-effective compounds. However, it should be kept in mind that these compounds will possibly have non-negligible vapour pressure, leading to evaporative losses.

The final comparison that needs to be made is with traditionally used organic solvents. In fact, solvents like toluene, methanol and chloroform are still cheaper than terpenes and organic acids. This is the reason why reuse and regeneration steps of alternative solvents always need to be studied, evaluated and included in the processes. If circular processes are developed, and green solvents regenerated, the process as a whole will be more economically and environmentally sustainable.

4. Framework and Objectives

The continuous presence of several micropollutants in the water sources of public water systems can be linked to a range of human and environmental health effects including cancer, birth defects, neurological and reproductive diseases, while decreasing the biodiversity. Although drinking and wastewater treatment plants use advanced technologies for micropollutants removal, none of these processes were specifically designed to remove all forms of pollution.

The main objective of this work is to study and develop new DES to be used in removal of several classes of micropollutants, namely pesticides, plastic additive and pharmaceutical compounds, from water environments. DESs present excellent solvating qualities, which can be tailored to guarantee the success of the proposed technologies. To achieve the goal, this thesis explores first the preparation and characterization of novel hydrophobic deep eutectic solvents, with the aim of making them attractive solvents for water applications. Furthermore, the work presented in this thesis also addresses the development of two different technologies, namely liquid-liquid extraction (LLE) and adsorption. In order to improve these technologies, already implemented in water treatment plants, and seeking a complete and efficient removal of micropollutants from aqueous streams, without contaminating the water bodies, properties tunability, micropollutants affinity and extraction performance of several families of hydrophobic DES were explored. Finally, to assure the complete sustainability of process using proposed technologies, the reuse and recycling of the hydrophobic DESs were investigated.

The results achieved in this thesis as well as the development and application of these innovative solvents are expected to open new doors to sustainability, pushing green chemistry to the deserved place in separation and purification processes.

5. Thesis outline

This thesis is organized in five blocks separated by sub-chapters. After a brief introduction on the evolution of the DES field and the development of hydrophobic DES, Chapter 2 is composed of sub-chapters 2.1 and 2.2, which describe the preparation and characterization of novel and sustainable hydrophobic eutectic solvents based on natural compounds. For the first time, solvents composed of DL-Menthol combining carboxylic acids and composed exclusively by fatty acids, possessing promising properties, such as very low viscosity and low water content, compared to the traditional solvents, were introduced in the field.

In Chapter 3, which includes sub-chapters 3.1, 3.2, 3.3 and 3.4, presents the development and application of techniques for cleaning aqueous solutions contaminated with several micropollutants ranging from pesticides, to plastic additive as well as active pharmaceutical ingredients. Particularly, liquid-liquid extraction (LLE) and adsorption processes were tested as well-known technologies combining several hydrophobic DESs were employed for the single-step extraction and concentration of four pesticides (acetamiprid, imidacloprid, thiamethoxam and nitenpyram), a plastic additive (Bisphenol-A), a nonsteroidal anti-inflammatory drugs (diclofenac) and finally a fluoroquinolone (ciprofloxacin). First, liquid-liquid extraction, an already well-exploited technique, was applied in order to understand the affinity of micropollutants, with different chemical structures and functionalities, to hydrophobic DES in terms of extraction efficiencies (chapters 3.1, 3.2 and 3.3). Following the excellent results and performances of hydrophobic DES and using the previous know-how acquired, a novel adsorption technique was also employed. It was shown that hydrophobic DESs can be supported in a porous filter and be used as adsorption material to successfully remove micropollutants from water (chapter 3.4). Moreover, the adsorption process here developed is a very interesting approach, since it is efficient and sustainable, due to the low quantity of solvent used, and the possible reuse for several cycles without losing efficiency.

Chapter 4 is divided in sub-chapters 4.1 and 4.2 and presents a comparison between hydrophilic and hydrophobic families of DES. Since these solvents are frequently proposed as green alternatives to traditional solvents, it is important to understand the differences between their physical properties. For example, polarity plays an important role in solvation phenomena and it is important to understand affinity for extractions. Therefore, studies through solvatochromic responses of UV-vis absorption probes for different families of DESs were conducted. On the other hand, Chapter 4.2 explores the design of novel DES composed of a sodium salt based on fatty acids which can be directly compared with those composed exclusively by fatty acids. The introduction of inorganic cations in DES brings some partial water solubility behaviour without compromising the DES chemical stability.

Finally, in Chapter 5, all results reported and discussed during the thesis are compiled and main conclusions are retreated. Novel directions and perspectives are presented, and future work highlighted. A door to new possibilities and challenges for future research is widely opened.

6. References

1. Pollet, P.; Davey, E. A.; Ureña-Benavides, E. E.; Eckert, C. A.; Liotta, C. L., Solvents for sustainable chemical processes. *Green Chemistry* 2014, *16* (3), 1034-1055.

2. Calvo-Flores, F. G.; Monteagudo-Arrebola, M. J.; Dobado, J. A.; Isac-García, J., Green and Bio-Based Solvents. *Topics in Current Chemistry* 2018, *376* (3), 18.

3. Clarke, C. J.; Tu, W.-C.; Levers, O.; Bröhl, A.; Hallett, J. P., Green and Sustainable Solvents in Chemical Processes. *Chemical Reviews* 2018, *118* (2), 747-800.

4. Warner, J. C.; Cannon, A. S.; Dye, K. M., Green chemistry. *Environmental Impact Assessment Review* 2004, *24* (7), 775-799.

5. Anastas, P.; Eghbali, N., Green Chemistry: Principles and Practice. *Chemical Society Reviews* 2010, *39* (1), 301-312.

6. Vanda, H.; Dai, Y.; Wilson, E. G.; Verpoorte, R.; Choi, Y. H., Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents. *Comptes Rendus Chimie* 2018, *21* (6), 628-638.

7. CHAPTER 1 Introduction to Solvents and Sustainable Chemistry. In *Sustainable Solvents: Perspectives from Research, Business and International Policy*, The Royal Society of Chemistry: 2017; pp 1-34.

8. Clark, J. H.; Tavener, S. J., Alternative Solvents: Shades of Green. *Organic Process Research & Development* 2007, *11* (1), 149-155.

9. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Tambyrajah, V., Novel solvent properties of choline chloride/urea mixtures. *Chemical Communications* 2003, (1), 70-71.

10. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *Journal of the American Chemical Society* 2004, *126* (29), 9142-9147.

11. Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep Eutectic Solvents (DESs) and Their Applications. *Chemical Reviews* 2014, *114* (21), 11060-11082.

12. Choi, Y. H.; van Spronsen, J.; Dai, Y.; Verberne, M.; Hollmann, F.; Arends, I. W. C. E.; Witkamp, G.-J.; Verpoorte, R., Are Natural Deep Eutectic Solvents the Missing Link in Understanding Cellular Metabolism and Physiology? *Plant Physiology* 2011, *156* (4), 1701-1705.

13. Florindo, C.; Oliveira, F. S.; Rebelo, L. P. N.; Fernandes, A. M.; Marrucho, I. M., Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids. *ACS Sustainable Chemistry & Engineering* 2014, *2* (10), 2416-2425.

14. Lima, F.; Gouvenaux, J.; Branco, L. C.; Silvestre, A. J. D.; Marrucho, I. M., Towards a sulfur clean fuel: Deep extraction of thiophene and dibenzothiophene using polyethylene glycol-based deep eutectic solvents. *Fuel* 2018, *234*, 414-421.

15. Grudniewska, A.; de Melo, E. M.; Chan, A.; Gniłka, R.; Boratyński, F.; Matharu, A. S., Enhanced Protein Extraction from Oilseed Cakes Using

Glycerol–Choline Chloride Deep Eutectic Solvents: A Biorefinery Approach. *ACS Sustainable Chemistry & Engineering* 2018, *6* (11), 15791-15800.

16. Duarte, A. R. C.; Ferreira, A. S. D.; Barreiros, S.; Cabrita, E.; Reis, R. L.; Paiva, A., A comparison between pure active pharmaceutical ingredients and therapeutic deep eutectic solvents: Solubility and permeability studies. *European Journal of Pharmaceutics and Biopharmaceutics* 2017, *114*, 296-304.

17. Jeong, K. M.; Ko, J.; Zhao, J.; Jin, Y.; Yoo, D. E.; Han, S. Y.; Lee, J., Multi-functioning deep eutectic solvents as extraction and storage media for bioactive natural products that are readily applicable to cosmetic products. *Journal of Cleaner Production* 2017, *151*, 87-95.

18. Florindo, C.; Celia-Silva, L. G.; Martins, L. F. G.; Branco, L. C.; Marrucho, I. M., Supramolecular hydrogel based on a sodium deep eutectic solvent. *Chemical Communications* 2018, *54* (54), 7527-7530.

19. Schaeffer, N.; Martins, M. A. R.; Neves, C. M. S. S.; Pinho, S. P.; Coutinho, J. A. P., Sustainable hydrophobic terpene-based eutectic solvents for the extraction and separation of metals. *Chemical Communications* 2018, *54* (58), 8104-8107.

20. Florindo, C.; Branco, L. C.; Marrucho, I. M., Development of hydrophobic deep eutectic solvents for extraction of pesticides from aqueous environments. *Fluid Phase Equilibria* 2017, *448*, 135-142.

21. Zhu, S.; Zhou, J.; Jia, H.; Zhang, H., Liquid–liquid microextraction of synthetic pigments in beverages using a hydrophobic deep eutectic solvent. *Food Chemistry* 2018, *243*, 351-356.

22. Ge, D.; Zhang, Y.; Dai, Y.; Yang, S., Air-assisted dispersive liquid–liquid microextraction based on a new hydrophobic deep eutectic solvent for the preconcentration of benzophenone-type UV filters from aqueous samples. *Journal of Separation Science* 2018, *41* (7), 1635-1643.

23. van Osch, D. J. G. P.; Zubeir, L. F.; van den Bruinhorst, A.; Rocha, M. A. A.; Kroon, M. C., Hydrophobic deep eutectic solvents as water-immiscible extractants. *Green Chemistry* 2015, *17* (9), 4518-4521.

24. Dietz, C. H. J. T.; van Osch, D. J. G. P.; Kroon, M. C.; Sadowski, G.; van Sint Annaland, M.; Gallucci, F.; Zubeir, L. F.; Held, C., PC-SAFT modeling of CO2 solubilities in hydrophobic deep eutectic solvents. *Fluid Phase Equilibria* 2017, *448*, 94-98.

25. Zubeir, L. F.; van Osch, D. J. G. P.; Rocha, M. A. A.; Banat, F.; Kroon, M. C., Carbon Dioxide Solubilities in Decanoic Acid-Based Hydrophobic Deep Eutectic Solvents. *Journal of Chemical & Engineering Data* 2018, *63* (4), 913-919.

26. Yousefi, S. M.; Shemirani, F.; Ghorbanian, S. A., Hydrophobic Deep Eutectic Solvents in Developing Microextraction Methods Based on Solidification of Floating Drop: Application to the Trace HPLC/FLD Determination of PAHs. *Chromatographia* 2018, *81* (8), 1201-1211.

27. Cao, J.; Yang, M.; Cao, F.; Wang, J.; Su, E., Tailor-made hydrophobic deep eutectic solvents for cleaner extraction of polyprenyl acetates from Ginkgo biloba leaves. *Journal of Cleaner Production* 2017, *152*, 399-405.

28. Cao, J.; Yang, M.; Cao, F.; Wang, J.; Su, E., Well-Designed Hydrophobic Deep Eutectic Solvents As Green and Efficient Media for the Extraction of Artemisinin from Artemisia annua Leaves. *ACS Sustainable Chemistry & Engineering* 2017, *5* (4), 3270-3278.

29. Tereshatov, E. E.; Boltoeva, M. Y.; Folden, C. M., First evidence of metal transfer into hydrophobic deep eutectic and low-transition-temperature mixtures: indium extraction from hydrochloric and oxalic acids. *Green Chemistry* 2016, *18* (17), 4616-4622.

30. Dietz, C. H. J. T.; Kroon, M. C.; Di Stefano, M.; van Sint Annaland, M.; Gallucci, F., Selective separation of furfural and hydroxymethylfurfural from an aqueous solution using a supported hydrophobic deep eutectic solvent liquid membrane. *Faraday Discussions* 2018, *206* (0), 77-92.

31. Ribeiro, B. D.; Florindo, C.; Iff, L. C.; Coelho, M. A. Z.; Marrucho, I. M., Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. *ACS Sustainable Chemistry & Engineering* 2015, *3* (10), 2469-2477.

32. Křížek, T.; Bursová, M.; Horsley, R.; Kuchař, M.; Tůma, P.; Čabala, R.; Hložek, T., Menthol-based hydrophobic deep eutectic solvents: Towards greener and efficient extraction of phytocannabinoids. *Journal of Cleaner Production* 2018, *193*, 391-396.

33. Verma, R.; Banerjee, T., Liquid–Liquid Extraction of Lower Alcohols Using Menthol-Based Hydrophobic Deep Eutectic Solvent: Experiments and COSMO-SAC Predictions. *Industrial & Engineering Chemistry Research* 2018, *57* (9), 3371-3381.

34. Martins, M. A. R.; Crespo, E. A.; Pontes, P. V. A.; Silva, L. P.; Bülow, M.; Maximo, G. J.; Batista, E. A. C.; Held, C.; Pinho, S. P.; Coutinho, J. A. P., Tunable Hydrophobic Eutectic Solvents Based on Terpenes and Monocarboxylic Acids. *ACS Sustainable Chemistry & Engineering* 2018, *6* (7), 8836-8846.

35. Makoś, P.; Przyjazny, A.; Boczkaj, G., Hydrophobic deep eutectic solvents as “green” extraction media for polycyclic aromatic hydrocarbons in aqueous samples. *Journal of Chromatography A* 2018.

36. van Osch, D. J. G. P.; Parmentier, D.; Dietz, C. H. J. T.; van den Bruinhorst, A.; Tuinier, R.; Kroon, M. C., Removal of alkali and transition metal ions from water with hydrophobic deep eutectic solvents. *Chemical Communications* 2016, *52* (80), 11987-11990.

37. Satlewal, A.; Agrawal, R.; Bhagia, S.; Sangoro, J.; Ragauskas, A. J., Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities. *Biotechnology Advances* 2018, *36* (8), 2032-2050.

38. Florindo, C.; Romero, L.; Rintoul, I.; Branco, L. C.; Marrucho, I. M., From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid–Based Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering* 2018, *6* (3), 3888-3895.

39. Florindo, C.; Lima, F.; Ribeiro, B. D.; Marrucho, I. M., Deep Eutectic Solvents: Overcoming XXI Century Challenges. *Current Opinion in Green and Sustainable Chemistry* 2018.

40. Shekaari, H.; Zafarani-Moattar, M. T.; Mohammadi, B., Thermophysical characterization of aqueous deep eutectic solvent (choline chloride/urea) solutions in full ranges of concentration at T=(293.15–323.15)K. *Journal of Molecular Liquids* 2017, *243*, 451-461.

41. Wang, Z.-F.; Wang, L.-S.; Fan, T.-B., Densities and Viscosities of Ternary Mixtures of Heptane, Octane, Nonane, and Hexyl Benzene from 293.15 K to 313.15 K. *Journal of Chemical & Engineering Data* 2007, *52* (5), 1866-1871.

42. Basak, S.; Nanda, J.; Banerjee, A., Multi-stimuli responsive self-healing metallo-hydrogels: tuning of the gel recovery property. *Chemical Communications* 2014, *50* (18), 2356-2359.

CHAPTER 2

A STEP TOWARDS HYDROPHOBIC DEEP EUTECTIC SOLVENTS

CHAPTER 2.1

Novel Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents

Adapted from: Bernardo D. Ribeiro, **C. Florindo**, Lucas C. Iff, Maria A. Z. Coelho, I. M. Marrucho, Novel Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents, ACS Sustainable Chemistry (2015), 3, 2469-2477.

Bernardo d. Ribeiro and Lucas C. Iff were involved in the synthesis of the new compounds as well as performed the biomolecules extraction experiments. The author contributed to the execution of the thermophysical properties and characterization experiments described in this chapter, as well as involved in the discussion and interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

Inspired by one of the major problems in pharmaceutical industry, we advantageously used the formation of eutectic mixtures to synthesize new solvents. The aim of this work is to identify low viscosity, cheap, biodegradable and hydrophobic eutectic solvents from natural resources. Consequently, novel eutectic mixtures based on DL-menthol and naturally occurring acids, namely pyruvic acid, acetic acid, L-lactic acid and lauric acid, were synthesized and are here reported for the first time. The obtained DL-menthol-based eutectic mixtures were analysed using NMR and FTIR spectroscopy in order to check their structures and purities and to confirm the interaction of the two compounds leading to the eutectic formation. Important solvent thermophysical properties, such as density and viscosity, of the prepared eutectic solvents with different water contents (dried and water-saturated) were measured. Finally, and taking advantage of their hydrophobic character, namely the formation of two phases with water at room temperature, four different biomolecules, caffeine, tryptophan, isophthalic acid and vanillin, were extracted and the extraction efficiencies of the prepared eutectic solvents compared.

1. Introduction

One of the major challenges in modern chemistry and chemical engineering is the formulation of new solvents, which simultaneously meet the Green Chemistry criteria and have the ability to dissolve as large as possible spectrum of solutes. Currently two major classes of solvents, ionic liquids (ILs) and eutectic mixtures,[1,2] are being developed and used by academia and industry. Regarding ILs, their commercial availability promoted the production of a large body of information concerning their thermophysical and transport properties, toxicity and phase equilibria, and consequently, new applications can now be properly evaluated. Conversely, eutectic mixtures, and in particular deep eutectic solvents, prepared from the combination of a salt or an ionic liquid with a neutral compound, are still in the infancy, lacking organization and properties systematization. Most of the eutectic mixtures proposed so far are based on materials from renewable resources, such as carboxylic acids, sugars, amines, polyols, etc, and although they are cheap and usually considered nontoxic and biodegradable, their inherent toxicity and biodegradability needs to be evaluated case by case. For example, it has been showed that choline chloride: glucose and choline chloride: glycerol deep eutectic solvents display low cytotoxicity, while choline chloride: oxalic acid possesses moderate cytotoxicity. Nevertheless, these three deep eutectic solvents were classified as ′readily biodegradable′ based on their high levels of mineralization. It has been showed that some deep eutectic solvents can be a good replacement for analogous ILs, due to the similarity between their properties and the fact that they can be advantageously prepared in a cheaper, quicker and easier way.[3] The preparation of deep eutectic solvents depends usually on the establishment of hydrogen bonds between the two compounds, one acting as hydrogen bond donor and the other as hydrogen bond acceptor. However, as in other fluids where hydrogen bonds are the dominant interactions, one of the major drawbacks of this class of solvents is their general high viscosity when compared to organic solvents and even sometimes to ILs or even their solid/pasty/gel-like state.

The main purpose of this work is to identify cheap and biodegradable solvents based on eutectic mixtures, which can be prepared from natural resources. A quick look into the eutectic solvents from natural resources that have been proposed in the literature, tells us that the vast majority, if not all, are hydrophilic or very hydrophilic. Consequently, the eutectic mixtures from renewable resources with a *certain* hydrophobic character, which displayed liquid-liquid equilibria with water, are in need. Due to its very small solubility in water (0.46 mg/ml at 25ºC),[4] menthol is a good candidate to prepare sustainable, cheap and hydrophobic solvents using the formation of eutectic mixtures approach. Menthol can be extracted from *Mentha* species, it is abundant and cheap (~€60/kg). Eutectic mixtures containing menthol have been reported in the pharmaceutical field. For example, menthol and borneol[5,6] and camphor[7] and *N*-Ethyl-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide[8] eutectic mixtures have been prepared and used as vehicles for transdermal drug delivery, while eutectic mixtures of menthol and testosterone[9], ibuprofen[10,11], lidocaine[12], ubiquinone[13], captopril[7], daidzein[5], fluconazole[6] have been used as analgesic, antimicrobial, antipruritic, anti-inflammatory and antitussive[14,15] compounds. Thermal properties of some of these eutectic mixtures have been researched: for example while L-menthol/testosterone 80:20 molar ratio and L-menthol/ubiquinone 70:30 molar ratio display a melting temperature (T_m) of 39.3 ºC[9] and 38.5 ºC[13], respectively, L-menthol/lidocaine 70:30 molar ratio has a lower T_m of 26 ºC[12], and L-menthol/ibuprofen 70:30 molar ratio even lower, 19 ºC[10]. These examples clearly illustrate the role of the second component on the melting temperature of these eutectics.

In this work, four eutectic mixtures composed of DL-menthol and different acids were prepared just by heating and mixing the pure compounds and studied, as listed in Table 1.

Table 1. Summary of different eutectic mixtures studied in this work using DL-menthol as hydrogen bond acceptor and different hydrogen bond donors.

Hydrogen Bond Donor	Molar ratio	Aspect
Acetic acid	1:1	Transparent liquid
Pyruvic acid	1:2	Transparent yellow liquid
Lactic acid	1:2	Transparent liquid
Lauric acid	2:1	Transparent liquid

The chemical structures of DL-menthol and the used organic acids are depicted in Figure 1.

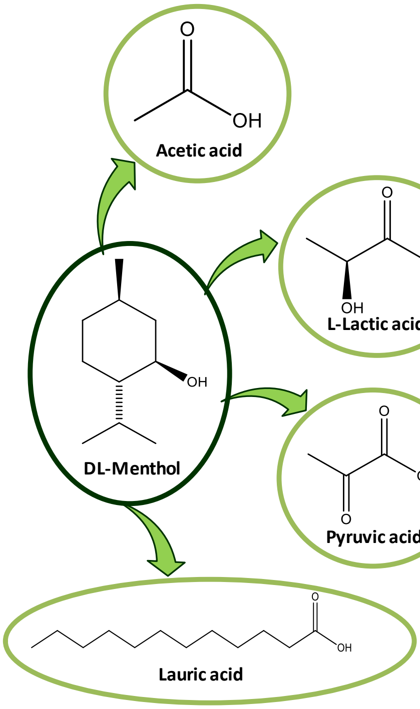


Figure 1. Chemical structure of the compounds used for the DL-menthol-based eutectic mixtures studied in this work.

In order to evaluate the real technological value of the prepared eutectic mixtures as solvents, relevant thermophysical properties, such as density and viscosity, were measured, within the temperature range indicated by the DSC and TGA studies and at different water contents, dried and water-saturated. Finally, and taking advantage of the hydrophobic character of these eutectics, and thus their two liquid phase formation in the presence of water at room temperature, the extraction efficiency of four different biomolecules, caffeine, tryptophan isophthalic acid and vanillin from the water phase to the eutectic mixture phase was evaluated in terms of their partition coefficients.

2. Experimental Section

2.1. Materials

DL-menthol (≥ 95% mass fraction purity), pyruvic acid (purity > 98%), acetic acid (purity ≥ 99.7%), lauric acid (purity > 98%), caffeine (purity 99%), vanillic acid (purity ≥ 97%, tetracycline (purity ≥ 98%) and tryptophan (purity ≥ 98%) were purchased from Sigma-Aldrich and used as received. L-lactic acid solution (81 wt % in water) was supplied by Fluka. The water used was ultrapure, double distilled, passed through a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

2.2. Methodologies

2.2.1. Preparation Methodology of DESs

DL-menthol-based eutectic mixtures were prepared by adding different hydrogen bond donors (polyethylene glycol, monoethylene glycol, cholesterol, pyruvic acid, acetic acid, L-lactic acid, butyric acid, hexanoic acid and lauric acid) to DL-menthol in different molar ratios. The mixture of the two compounds was then heated up to 50 ºC for 15 min and then cooled down slowly until it reaches the room temperature. Some of these eutectic mixtures yielded either pasty compounds or liquids with melting points higher than that of DL-menthol. Others also did not yield an eutectic mixture (such as in the case when glycols were used) and thus other ratios were attempted without success. Consequently, these eutectic mixtures were discarded. Only four eutectic mixtures of DL-menthol and different acids were studied in this work, as listed in Table 1. For the preparation of the dried samples, the DL-menthol-based eutectic mixtures were maintained for at least four days in a schlenk under high vacuum (circa 10^{-1} Pa) at room temperature. For the water saturated samples, binary mixtures containing water and the eutectic mixture were prepared, vigorously shaken and left to equilibrate for a day. Samples of the eutectic mixture phase were carefully taken with a syringe. The amount of water present in each dried and saturated sample was measured, in order to infer their relative hygroscopicity, and thus hydrophobicity. The water content was determined by Karl Fisher titration (model Metrohm 831 Karl Fisher coulometer). After preparation of eutectic mixtures, proton and carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) and Fourier Transform Infrared Spectroscopy (FTIR) were used to check the eutectic mixtures structures and purities and to confirm the interaction between two compounds leading to the eutectic formation.

2.2.2. NMR Measurements

All the experiments were carried out on a Bruker AVANCE 400 spectrometer operated at room temperature with 16 and 500 scans for the ^{1}H NMR and ^{13}C NMR spectra, respectively. The eutectic mixtures samples were prepared on 5 mm NMR tubes by weight approximately 30 mg of eutectic mixture and then adding 0.5 mL of deuterated chloroform ($CDCl_3$). The homogeneity of the sample was assured by vortex mixing.

2.2.3. FTIR Measurements

FTIR measurements were carried out using a Brüker IFS66/S FTIR spectrometer (Brüker Daltonics, MA, USA) with a single reflection ATR cell (DuraDisk, equipped with a diamond crystal). The data relative to spectral region were recorded between 4000 cm^{-1} and 600 cm^{-1} at room temperature. For each sample, 290 scans were recorded at a spectral resolution of 4 cm^{-1} and five replica spectra were collected in order to evaluate reproducibility (OPUS v5.0).

2.2.4. Thermophysical Properties

A DSC (Differential Scanning Calorimeter TA Instrument Model DSC Q200) and a TGA (TA instrument Model TGA Q50) were used to measure the thermal properties of the prepared menthol-based eutectic mixtures. All solid-liquid phase transitions were determined in a range of temperatures from 193.15 K at 373.15 K, with a heating and cooling rate of 5 ºC.min^{-1}. Condensation in the DSC furnace was prevented by using dry nitrogen as a purge gas with a flow rate of 50 mL/min. Indium (T_{melting} = 429.76 K) was used as standard compound for calibration. Samples of each mixture, typically 3 to 15 mg, were transferred to the aluminium DSC pan, which was then hermetically sealed in order to prevent vaporization. The uncertainty in the melting point temperature obtained by calculation of the standard deviation of several consecutive measurements for the same sample is better than ± 1 K.

The TGA was used for measuring the decomposition temperature of the eutectic mixtures, using also nitrogen at a flow rate of 60 ml·min^{-1}. Samples were placed inside of aluminium pans and heated to 600 ºC at a rate of 10 ºC·min^{-1} until complete thermal degradation was achieved.

Measurements of viscosity and density of the eutectic mixtures were carried out in the temperature range from 293.15 K up to 353.15 K and at atmospheric pressure, using an Anton Paar (model SVM 3000) automated rotational Stabinger viscometer-densimeter. The temperature uncertainty is ± 0.02 K. The relative uncertainty of the dynamic viscosity is ± 0.35 %, and the absolute uncertainty of the density is ± 0.0005 g·cm^{-3}. In order to evaluate the effect of water on the density and viscosity of the prepared eutectic mixtures two different scenarios were studied: dried and water-saturated. The eutectic mixtures were simultaneously sampled for the water content determination and the density and viscosity measurements.

2.2.5. Biomolecules Extraction

Aqueous solutions of different biomolecules, namely caffeine, tryptophan and vanillin, with a concentration of 0.2 g dm^{-3}, and isophthalic acid with 0.1 g dm^{-3} were prepared. Then, 1 ml of these solutions was put into contact with equal amount of each one of the eutectic mixtures and stirred for 1 min at 3000 rpm, at room temperature and pressure conditions, to ensure that the equilibrium of the biomolecules between the two phases as reached. Afterwards, the flasks were let to separate the phases for 24h, so that complete separation of the two phases was attained. Samples of the aqueous phase were taken, and the quantification of the biomolecules was carried out by UV-VIS spectroscopy using a SHIMADZU UV-1700, Pharma-Spec spectrophotometer, at wavelength of 278 nm for tryptophan and 273 nm for other compounds, by means of a calibration curve previously established for each compound. At least three samples of each individual aqueous phase were quantified, and the deviations evaluated.

3. Results and Discussion

Eutectic mixtures containing menthol have already been reported in the literature[5-13], but in a pharmaceutical context. In this work, eutectic mixtures of DL-menthol and organic acids were prepared and important solvent properties, such as density and viscosity, was measured in order to evaluate their feasibility as extraction solvents. Their performance as extraction solvent for several model biomolecules was also evaluated. For this purpose, different proportions of several natural organic acids were mixed with DL-menthol and those which are liquid at room temperature are listed in Table 1 and their chemical structures depicted in Figure 1.

Eutectic mixtures of DL-menthol and other compounds, such as polyethylene glycol, monoethyleneglycol and cholesterol, were also prepared by yielded either pasty compounds or liquids with melting points higher than that of menthol. Consequently, these eutectic mixtures were discarded. In Figure 2, an illustration of the formation of an eutectic mixture, in the case DL-menthol and lauric acid which are both solids and when they are heated become liquid mixture. After, when water is added, two phases are formed.

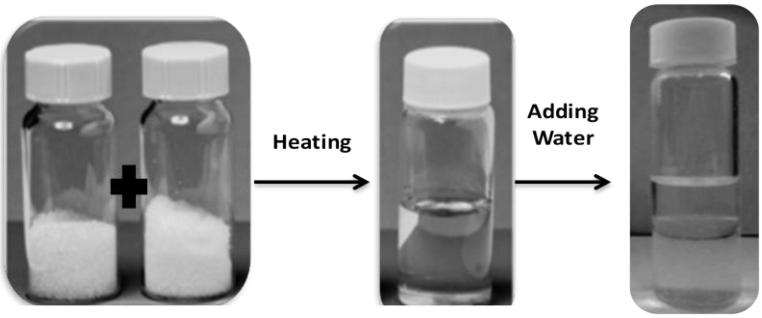


Figure 2. Illustration of the formation of an eutectic mixture and its reaction with water. From left to right: DL-menthol + Lauric acid (both solids), DL-menthol: lauric acid eutectic mixture and eutectic mixture after mixing with water.

3.1. Thermal Properties

The thermal behavior of the eutectic mixtures was studied using TGA and DSC. The TGA was used to acquire the eutectic mixtures decomposition temperature (T_{dec}), which is one of the most important properties of these compounds, especially for their application as new, alternative solvents so that optimization of the working conditions below this temperature can be achieved. These temperatures determined for the studied eutectic mixtures are summarized in Table S1, present in Supplementary Information. It can be observed that the decomposition temperature of all the prepared eutectic mixtures is above 200 ºC. Note that in the case of DL-menthol: pyruvic acid the eutectic mixture is more stable than the separate compounds.

Differential scanning calorimetry is another one of the most widely used methods of thermal analysis, measuring the temperature and energy associated with a range of thermal events including melting, crystallization and glass transitions.[16] Morrison *et al*[17] have used this technique to characterize urea–choline chloride and malonic acid–choline chloride eutectic systems. In the Figure S12, DSC graphics of pure DL-menthol and the eutectic mixtures prepared in this work are presented. It can be observed that DL-menthol presents two melting points, at 27 ºC and 34 ºC. Corvis *et al*[18] explained that DL-menthol possesses at least two polymorphs α and β. On the other hand, X-ray diffraction studies concluded that DL-menthol is a racemic mixed crystal, where the hydrogen atom of the hydroxyl group is shared by two independent menthol entities. Probably, this unusual crystal conformation, together with the presence of different polymorphs, promoted the existence of asymmetric chains in liquid menthol-based eutectic mixtures, which are responsible for the peaks shown in Figure S13.

3.2. FTIR analysis

The mechanism of the formation of a eutectic mixture differs according to the compounds used. In deep eutectic solvents based on choline chloride, sugars and organic acids, the establishment of hydrogen bonds between the two compounds, one acting as hydrogen bond donor and the other as hydrogen bond acceptor, is responsible for the eutectic mixture formation. However, *Nashiretty* and *Panchagnula*[19], who studied the effect of menthol in eutectic mixtures of ceramide and cholesterol, reported that menthol interacts with both the lipid alkyl tails and the polar head groups of mixture, reducing their melting temperature, and breaking the inter- and intra-lamellar hydrogen bonding network.

To this end, it is important to establish the mechanism through which the DL-menthol interacts with the second compound for the four eutectic mixtures prepared in this work. For that purpose, FTIR experiments were performed for the neat DL-menthol, each of the acids and the eutectic mixtures. The overlapping FTIR spectra obtained for the DL-menthol: acetic acid eutectic mixture and the respective components are presented in Figure 3 as an example and the other FTIRs are presented in Figures S9-S11 in the Supplementary Information.

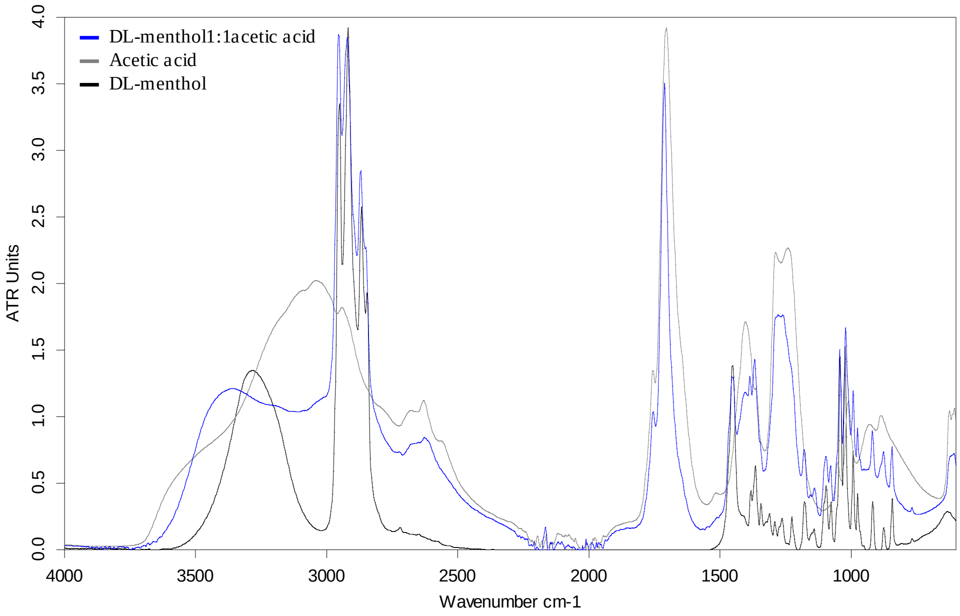


Figure 3. FTIR spectra of the pure DL-menthol, acetic acid and the eutectic mixture of DL-menthol and acetic acid (1:1 molar ratio).

All the hydrogen bond donors used in these eutectic mixtures have a carboxylic acid group in their structure, that presents a representative band (ketone or carbonyl group) around 1700 cm^{-1} in spectra. On the other hand, in the FTIR spectra of the hydrogen bond acceptor, DL-menthol, only one representative band corresponding to the hydroxyl group, at about 3300 cm^{-1}, can be observed. Additionally, FTIR measurements were also used to confirm the formation of a new compound, through the formation of hydrogen bonds, between the DL-menthol and the hydrogen bond donors. FTIR spectra data of the eutectic mixtures show the intermolecular hydrogen-bond interaction between the hydrogen bond donors and DL-menthol mostly in the carboxyl group region. It can be observed in Figure 3, that for the DL-menthol: acetic acid eutectic mixture, the carbonyl band from the HBD was originally located at low wavenumber values (~1692 cm^{-1}) and it broadens and shifts to highest values (~1735 cm^{-1}) in the eutectic mixture. This is a strong indication of a new hydrogen bond formation, thus proving that a new compound was formed. This evidence can be further confirmed through the physical state of the compounds, for example, the eutectic mixture composed by DL-menthol and lauric acid: initially, the physical state of both these compounds is solid and after DES formation a liquid is obtained.

3.3. Thermophysical Properties

Density and viscosity are important properties of any solvent since they influence the mass transport phenomena, there by affecting their suitability for particular applications. As mentioned before, green solvents such as ILs, deep eutectic solvents and polyethylene glycols oligomers, are usually quite viscous fluids when compared to organic solvents and for that reason they have to be used in solutions with water or other solvents thus hampering their application when a hydrophobic solvent is required. As we can see in Table 2, the water content in the saturated eutectic mixtures is quite low comparing to similar eutectic mixtures produced using cholinium chloride instead of DL-menthol.

Table 2. Molecular weight (MW) and water content, in mass percentage (wt %), of the dried and water-saturated DL-menthol-based eutectic mixtures studied in this work.

Eutectic Mixtures	**MW (g.mol^{-1})**	**Water content (wt %)**	
		Dried	**Saturated**
DL-menthol: acetic acid	108.16	0.429	1.567
DL-menthol: pyruvic acid	110.79	1.023	1.268
DL-menthol: lactic acid	112.14	0.153	1.634
DL-menthol: lauric acid	170.95	0.276	1.237

For example, Florindo *et al*.[3] reported the water content of water saturated deep eutectic solvents composed of a salt, cholinium chloride, and several organic di-acids, with values between 14-19 wt %, and monoacids, such as levulinic acid with water content near 10 wt %. Consequently, the hydrophobicity of the DL-menthol-based eutectic mixtures is much higher than those produced from salts, probably also due to the presence of charges in these last ones.

The experimental density results for the dried and water-saturated (Table 2) samples of the four prepared eutectic mixtures as a function of temperature are plotted in Figure 4.

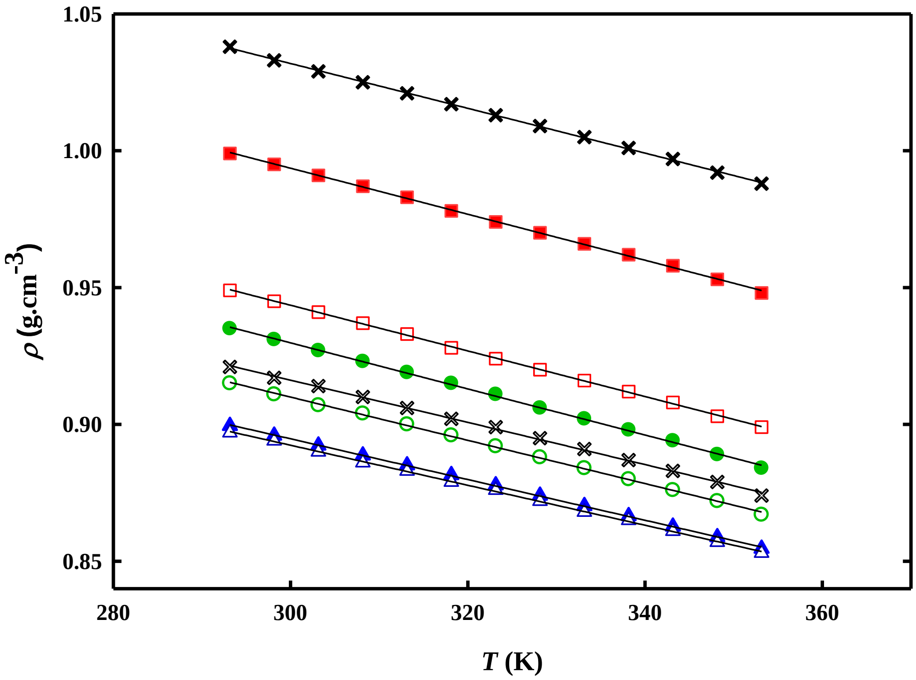


Figure 4. Experimental densities (*ρ*) of the dried and water-saturated eutectic mixtures as function of temperature: DL-menthol: acetic acid (●), DL-menthol: pyruvic acid (■), DL-menthol: lactic acid (×), DL-menthol: lauric acid (▲). The filled symbols correspond to the dried samples and the empty symbols correspond to the water-saturated samples. The lines represent the fitted data calculated using equation (1).

The density data are presented in Tables S2 in the Supplementary Information. It is very difficult to extract conclusions regarding the relationship between the chemical structure of the acids present in the eutectic mixtures and the respective thermophysical properties since different molar ratios were used. Only in the case of the eutectic mixtures containing pyruvic and L-lactic acids, both derived from propanoic acid, the same molar ratio (1:2) was used. However, even then for these DES, a straightforward comparison cannot be clearly established. Several explanations can be put forward, such as the possibility of dimers formation in L-lactic acid and the conformational effects between the two carboxyl groups in the pyruvic acid, to account for this behaviour.[20]

It can be observed that generally the presence of water increases the density of the eutectic mixtures. The only exception is the DL-menthol: lactic acid where a small decrease was observed. However, the effect of water in much more pronounced for the eutectic mixtures containing pyruvic and lactic acids, which can be probably linked to presence of substituent groups which are able to interact with water via hydrogen bonding. The densities of the eutectic mixtures containing the linear acids, acetic and lauric acids are less affected by the water presence. In fact, dried and saturated DL-menthol: pyruvic acid present a very small difference in the water content which is however reflected in the density of the two mixtures.

The density decreases linearly with temperature for all dried and water saturated samples, in the whole temperature range studied. A linear equation was used to express the correlation with the temperature:

$$\rho = a + bT \qquad \text{(eq. 1)}$$

where ρ corresponds to density in $g \cdot cm^{-3}$, T is the temperature in K and a and b are the fitting parameters. The adjustable parameters (a and b) were determined from the fitting of the experimental density data, from the equation 1 and are presented in Table 3, as well as the thermal expansion coefficients (α_p) were determined and are presented in Table S4 in the Supplementary information.

Table 3. Fitted values of parameters, *a* and *b,* by equation (**1**) and standard deviation *σ*, for the density of dried and water-saturated eutectic mixtures as a function of temperature.

Eutectic Mixtures	***a*** **(g·cm^{-3})**		***b*** **(10^{-4}) (g·cm^{-3}·K)**		**σ***	
	Dried	***Sat.***	***Dried***	***Sat.***	***Dried***	***Sat.***
DL-menthol: acetic acid	1.183	1.147	-0.8418	-0.7901	4.7 E-04	4.2E-04
DL-menthol: pyruvic acid	1.246	1.194	-0.8407	-0.8341	4.1 E-04	2.8E-04
DL-menthol: lactic acid	1.278	1.149	-0.8187	-0.7749	3.2 E -04	2.5E-04
DL-menthol: lauric acid	1.112	1.118	-0.7308	-0.7442	3.3 E -04	3.3 E -04

*$\sigma = \left(\frac{\sum(\rho_i^{exp} - \rho_i^{cal})^2}{n-v}\right)^{0.5}$ where *n* is the number of experimental points, *v* the number of adjustable parameters.

The thermal expansion coefficient values obtained for the eutectic mixtures vary between 7.891 to 8.998 x 10^4 K^{-1} in the case of the dried samples, and in general are up to 2 – 6 % higher for the water-saturated samples. The highest thermal expansion coefficients were observed for the DL-menthol: acetic acid, whereas the DL-menthol: lactic acid displayed the lowest ones.

The molar volumes (V_m) of dried and water-saturated eutectic mixtures studied in this work were calculated from the density results and are presented in Table S5 in the Supplementary Information. Molar volumes were determined for a range of temperatures from 293.15 K and 353.15 K, and were obtained from the following equation:

$$V_m = \frac{M}{\rho} \qquad \text{(eq. 2)}$$

where M corresponds to the molar mass in g·mol^{-1} and ρ is the density in g·cm^{-3}. The molar volumes for the water-saturated eutectic mixtures containing pyruvic and lactic acid are higher than those for the dried mixtures, probably due to the water present. The molar volumes increase in the following order: DL-menthol: lactic acid < DL-menthol: pyruvic acid < DL-menthol: acetic acid < DL-menthol: lauric acid. Usually, the molar volumes are in agreement with the molar mass of each compound, which does not happen in this study, since the eutectic mixture with the highest molar mass does not exhibit the highest molar volume. This can be probably be explained by chemical and structural differences in hydrogen bond donors (here the presence of dimmers might play an important role), the different proportions between DL-menthol and hydrogen bond donors and the different amounts of water. The molar volume of the dried and saturated DL-menthol: lauric acid eutectic mixture do not significantly differ from each other, due to minimal difference in their water content. As for the dried and saturated DL-menthol: acetic acid mixture the molar volumes, they are also very similar in agreement to what was observed for their density values.

The experimental viscosity data for the dried and the water-saturated samples of eutectic mixtures, as a function of temperature, are depicted in Figure 5 and reported in Table S3 in Supplementary Information.

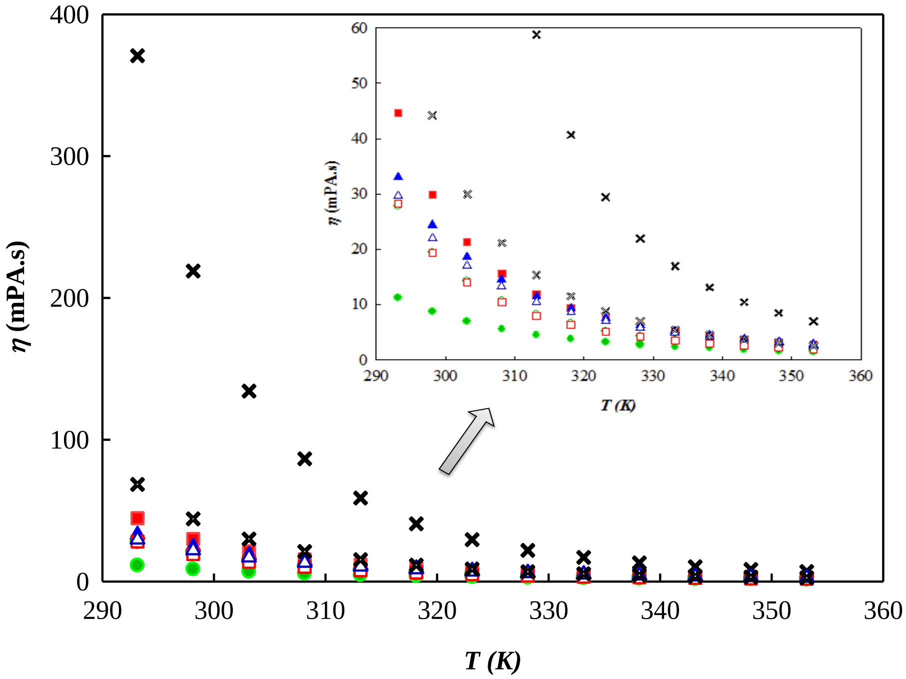


Figure 5. Experimental viscosities (*η*) of the dried and water-saturated eutectic mixtures as function of temperature: DL-menthol: acetic acid (●), DL-menthol: pyruvic acid (■), DL-menthol: lactic acid (✕), DL-menthol: lauric acid (▲). The filled symbols correspond to the dried samples and the empty symbols correspond to the water-saturated samples. The lines represent the fitted data calculated using equation (2).

The viscosities for the dried samples of the eutectic mixtures increase in the following order: DL-menthol: acetic acid < DL-menthol: lauric acid < DL-menthol: pyruvic acid < DL-menthol: lactic acid. It can be observed that despite the similar chemical structure of the pyruvic and lactic acids and the fact that they are in same proportion in the eutectic mixtures they display very different viscosity values. In fact, DL-menthol: lactic acid mixtures viscosity is one order of magnitude higher than that of the other three eutectic mixtures studied in this work. Regarding the water content, two distinct types of behaviours can be observed, independently of the DL-menthol: carboxylic acid proportions: for the eutectic mixtures containing lauric and acetic acids, the presence of water increases the viscosity, while for the other two mixtures, the opposite behaviour is observed, in the whole temperature range. These facts clearly illustrate the great variety of behaviours that these eutectic mixtures in particular display, which cannot be *a priori* predicted. The lack of systematization of the eutectic mixtures and deep eutectic mixtures thermophysical properties is probably due to the large variety of compounds that are possible to prepare, leading to compounds which properties are not possible to compare.

The experimental viscosity values were fitted as a function of temperature, using the Vogel-Fulcher-Tammann (VFT) model using the following equation:

$$\ln \eta = \mathrm{A}_{\eta} + \frac{\mathrm{B}_{\eta}}{(\mathrm{T}-\mathrm{C}_{\eta})} \qquad \text{(eq. 3)}$$

where η is the viscosity in mPa·s, T is the temperature in K, and A_η, B_η, and C_η are adjustable parameters. The adjustable parameters were determined from the fitting of the experimental values and are listed in Table 4.

Table 4. Fitted parameters of VFT model given by Equation 3 and respective correlation coefficient (R^2) for dried and water-saturated eutectic mixtures.

		A_η (mPa.s)	B_η (K)	C_η (K)	R^2
DL-menthol: acetic acid	Dried	-3.213	595.666	187.422	1
	Saturated	-3.689	677.581	196.432	0.9999
DL-menthol: pyruvic acid	Dried	-2.423	464.436	218.436	0.9999
	Saturated	-2.782	476.198	215.341	1
DL-menthol: lactic acid	Dried	-3.381	745.522	213.049	1
	Saturated	-3.902	725.375	203.865	1
DL-menthol: lauric acid	Dried	-3.599	815.874	178.123	0.9999
	Saturated	-3.883	887.449	171.033	0.9999

The activation energy was calculated based on the viscosity dependence with temperature as follows:

$$E_a = R.\frac{\partial(\ln \eta)}{\partial\left(\frac{1}{T}\right)} = R.\left(\frac{B_\eta}{\left(\frac{C_\eta^2}{T^2}-\frac{2C_\eta}{T}+1\right)}\right) \qquad \text{(eq. 4)}$$

where η is the viscosity, T is the temperature, B_η and C_η are the adjustable parameters obtained from equation 3 and R is the universal gas constant. The values of the activation energies for the four prepared eutectic mixtures are presented in Table 5.

Table 5. Activation energy values (E_a (KJ/mol)) for dried and water-saturated eutectic mixtures as a function of temperature, determined by equation 4.

***T* (K)**	**DL-menthol: acetic acid**		**DL-menthol: pyruvic acid**		**DL-menthol: lactic acid**		**DL-menthol: lauric acid**	
	Dried	**Sat.**	**Dried**	**Sat.**	**Dried**	**Sat.**	**Dried**	**Sat.**
293.15	38.1	51.7	59.5	56.2	83.0	65.0	44.1	42.5
298.15	35.9	48.4	54.0	51.3	76.1	60.3	41.9	40.6
303.15	33.9	45.5	49.5	47.2	70.2	56.3	39.9	38.8
308.15	32.3	42.9	45.6	43.6	65.0	52.7	38.1	37.3
313.15	30.7	40.6	42.2	40.6	60.7	49.5	36.5	35.8
318.15	29.3	38.5	39.3	37.9	56.8	46.7	35.1	34.6
323.15	28.1	36.6	36.8	35.6	53.4	44.3	33.7	33.3
328.15	26.9	34.9	34.5	33.5	50.4	42.0	32.5	32.2
333.15	25.9	33.4	32.6	31.7	47.7	40.1	31.3	31.2
338.15	24.9	32.1	30.8	30.1	45.3	38.2	30.3	30.2
343.15	24.1	30.8	29.2	28.5	43.1	36.6	29.3	29.3
348.15	23.2	29.7	27.8	27.2	41.2	35.1	28.4	28.5
353.15	22.5	28.6	26.5	26.0	39.4	33.8	27.6	27.8

The use of equation 4 to describe the temperature behaviour of viscosity allows the discussion of the energy barrier of a fluid to shear stress, E_a, values. The higher the E_a, the more difficult it is for the molecules/aggregates to move past each other. This can be a direct consequence of the size or entanglement of the molecules/aggregates and/or the presence of stronger interactions within the fluid. Regarding the size of the hydrogen bond donors and using the calculated molar volume calculated in Table S6, it can be observed that this parameter does not greatly influence the viscosity, since DL-menthol: lauric acid has a large V_m and it is not the most viscous mixture. On the other hand, it can be observed that the mixtures containing lactic acid are the most viscous, which clearly indicates the relevant role of the hydroxyl substituent group in the establishment of hydrogen bonds, thus increasing the viscosity. In the case of the pyruvic acid, the carboxyl group is not as good hydrogen bond donor as the hydroxyl, and thus its capacity to establish hydrogen bonds in much smaller than that of lactic acid. Both the two eutectic mixtures containing linear non substituted carboxylic acids, lauric and acetic acids, have the smallest E_a values, in agreement with their small viscosity. Only in this case, the differences in V_m accounts for the differences in E_a and thus, in viscosity.

The E_a values obtained for water saturated samples are similar to each other, indicating that the presence of water, even in different amounts, and in mixtures with different molar ratios, leads an easier and similar flux of the different molecules/aggregates.

3.4. Biomolecules Partition

Four model biomolecules, namely caffeine, tetracycline, tryptophan and vanillic acid, were used to illustrate the usage of the proposed eutectic mixtures as extraction solvents of biomolecules from aqueous solutions. Since the proposed eutectic mixtures are all partially miscible with water, no addition of salting out agents, such as salts, polymers etc, is necessary to achieve a two-phase system. The partition coefficient of each biomolecule *i*, K_i, was calculated as the ratio of their concentration in both the eutectic mixture-rich phase ($C_{i,EM}$) and in the water-rich phase ($C_{i,AQ}$), according to the following equation:

$$K_i = \frac{C_{i_{EM}}}{C_{i_{AQ}}} \qquad \text{(eq. 5)}$$

The results obtained for the four biomolecules tested are presented in Table 6.

Table 6. Partition coefficients, K_i, of the different biomolecules between DL-menthol-based eutectic mixtures and aqueous solutions. The pKa of the biomolecules and their water solubility is also shown.

Eutectic Mixtures	**pH**	**Caffeine**	**Tryptophan**	**Vanillin**	**Isophthalic Acid**
DL-menthol: acetic acid	1.91	1.81 ± 0.01	9.05 ± 0.33	4.74 ± 0.03	8.52 ± 0.23
DL-menthol: lactic acid	1.18	37.3 ± 0.09	14.76 ± 0.17	3.03 ± 0.15	3.31 ± 0.12
DL-menthol: lactic acid	1.47	1.16 ± 0.01	2.85 ± 0.04	6.65 ± 0.04	12.94 ± 0.10
DL-menthol: lauric acid	3.74	1.09 ± 0.01	5.23 ± 0.12	7.02 ± 0.01	1.96 ± 0.03
pKa*		14.0	2.38; 9.39	7.4	3.7; 4.6
Water Solubility* (mg/mL) at 25 ºC		16	11.4	10	0.13

*http://pubchem.ncbi.nlm.nih.gov/

It was not possible to evaluate partition data using DL-menthol: pyruvic acid due to its absorption in the same range of in UV-Visible spectra as the four molecules.

Although caffeine presents a higher affinity for aqueous phase, as ascertained by its octanol-water partition coefficient (*K_{ow})* equal to 0.85[21], it is possible to successfully extract this solute to the organic solvents. For example, the extraction of caffeine from water using chloroform reached partition coefficients between 18.7 to 22.8[22], while using aqueous biphasic systems (ABS) composed of imidazolium-based ILs, such as [C_7H_7mim]Cl, [Amim]Cl, [C_4mim]Cl and [C_2mim]Cl, and K_3PO_4[21], partition coefficients higher than 120. ABS using [C_4mim][DCA] and lysine were also explored[23] for the extraction of caffeine. However, the partition coefficient obtained are much lower (K_i = 5.8) than those obtained with K_3PO_4. Louros *et al*[24] also reported the partition of caffeine using ABS containing a phosphonium ionic liquid [P_{4441}][$MeSO_4$] and K_3PO_4, resulting in K_i = 4.75. In the present case, only when DL-menthol/lauric acid 2:1 eutectic mixture was used as extractant, the obtained partition coefficient was higher than 1, indicating a more favorable partition towards the eutectic mixture phase.

Tryptophan was another model biomolecule chosen, since it is one of the most hydrophobic aminoacids. Nevertheless, tryptophan possesses a polar –NH group allowing the establishment of hydrogen bonds, depending on the solvent.[24] The partition of two tryptophan analogues, N-methylindole and 3-methylindole, in a cyclohexane/water system, was addressed by *Winle* and *White*[25], achieving K_i = 290 and 19.1, respectively, at 25 ºC. These results are consistent with the hydrophobic nature of tryptophan. The results obtained in this work for the partition of tryptophan between an aqueous phase and each one of the prepared eutectic mixtures are higher than those obtained for caffeine. Again, no difference in the partition coefficient was observed when DL-menthol/acetic acid or DL-menthol/lactic acid were used, but for the DL-menthol/lauric acid 2:1 eutectic mixture a partition coefficients of 14.5 was obtained. Tomé *et al*[26] used hydrophobic ILs to extract tryptophan from aqueous solutions and obtained partition coefficients of 4.5 and 7.8 for $[C_2mim][NTf_2]$ and $[C_8mim][BF_4]$, respectively. Neves *et al.*[27] and Ventura *et al.*[28] also studied the tryptophan partition in ABS containing imidazolium-based IL and K_3PO_4, obtaining large partition coefficients of K_i = 124 and K_i = 59 for the [Amim]Cl and $[C_2mim]Cl$ ILs, respectively. Louros *et al.*[23] also reported the partition of tryptophan (K_i = 9.0) in ABS based on $[P_{4441}][MeSO_4]$ and K_3PO_4 while Passos *et al.*[29] $[N_{4444}]Cl$ and potassium citrate achieved the best result (K_i = 67).

In the case of vanillic acid, with K_{ow} = 16.3 indicating its more hydrophilic character[30], the highest K_i (5.72) was obtained with DL-menthol/lactic acid 1:1 eutectic mixture probably due to larger number of hydrogen bonds that can be promoted between the solute and the eutectic mixture. Cláudio *et al*[31] studied the extraction of a similar compound, vanillin using a ABS containing imidazolium-based IL and K_3PO_4, reaching partition coefficients larger than 44 with $[C_7H_7mim]Cl$, $[C_4mim]Cl$ and $[C_6mim]Cl$.

Isophthalic acid possesses very low water solubility (130 mg/L) and was chosen due to that characteristic. Buch *et al*[32] tested a extraction of isophthalic acid from soil samples using dichloromethane, ethyl acetate, acetonitrile, ethanol and propanol, and observed a larger affinity for ethyl acetate and propanol, being 80 % of the compound removed from soil. Feng *et al*[33] studied the partition of this compound in a system containing propyl acetate and water and obtained a partition coefficient of 40 at 30 ºC. These data indicate that isophthalic acid has medium polarity which confirms results obtained using menthol-based eutectic mixtures, with acetic and lactic acids with higher coefficients partition.

4. Conclusions

In this work, new green solvents based on eutectic mixtures with components from renewable resources are presented and studied. These new solvents are eutectic mixtures composed of DL-menthol and several organic acids in different proportions. Contrary to Deep Eutectic Solvents, these new eutectic solvents presented low viscosities (5-100 cP) and densities lower than water, as can be evaluated by the density and viscosity values, from 293.15 K up to 353.15 K and at atmospheric pressure, independently of the water content. A large diversity of behaviors in terms of the measured thermophysical properties can be observed when the different organic acids, proportions between DL-menthol and hydrogen bond donor and water contents are used making their understanding and thus prediction very difficult. Despite the fact that these eutectic mixtures are composed of naturally occurring hydrophilic acids usually also used in the preparation of Deep Eutectic Solvents, they are immiscible with water, which confers them a privilege place among the green solvents, which are usually very hydrophilic. Using the hydrophobic character of these eutectic mixtures partitions of selected solvents were performed to illustrate their utility in separation systems.

5. Acknowledgements

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6. Supplementary Information

6.1. Characterization of Eutectic Mixtures

1H-NMR Spectras of hydrophobic eutectic mixtures

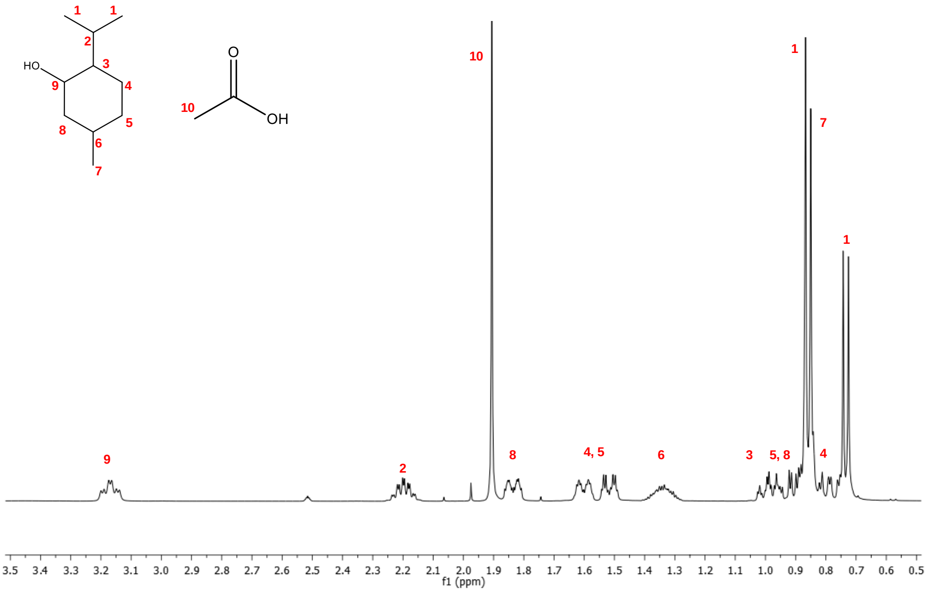

Figure S1. ^{1}H NMR spectra of DL-menthol: acetic acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

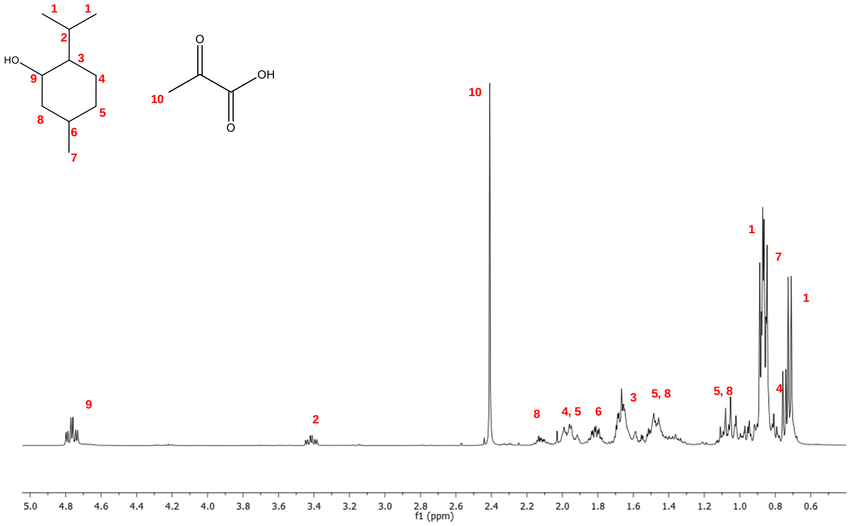

Figure S2. ^{1}H NMR spectra of DL-menthol: pyruvic acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

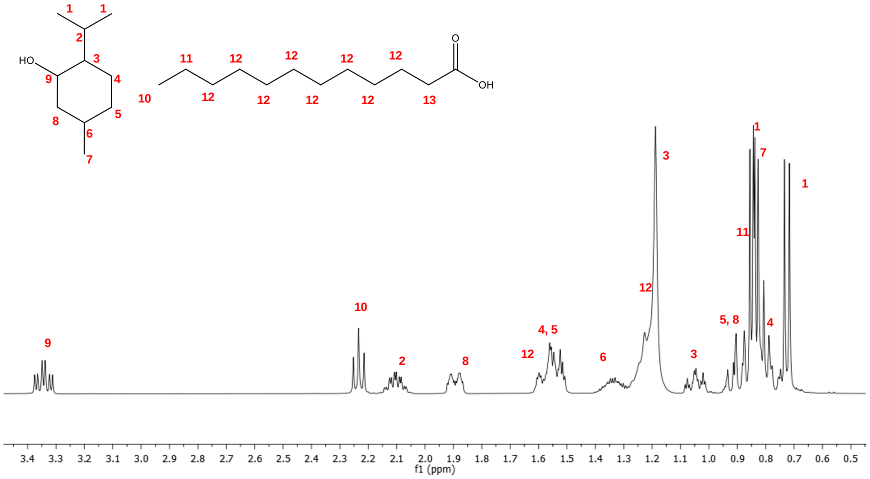

Figure S3. ^{1}H NMR spectra of DL-menthol: lauric acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

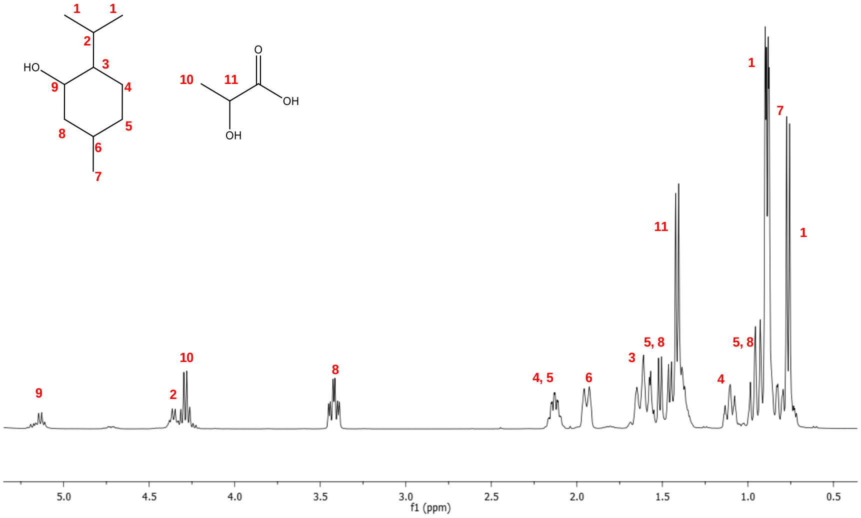

Figure S4. ^{1}H NMR spectra of DL-menthol: lactic acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

^{13}C-NMR Spectra of hydrophobic eutectic mixtures

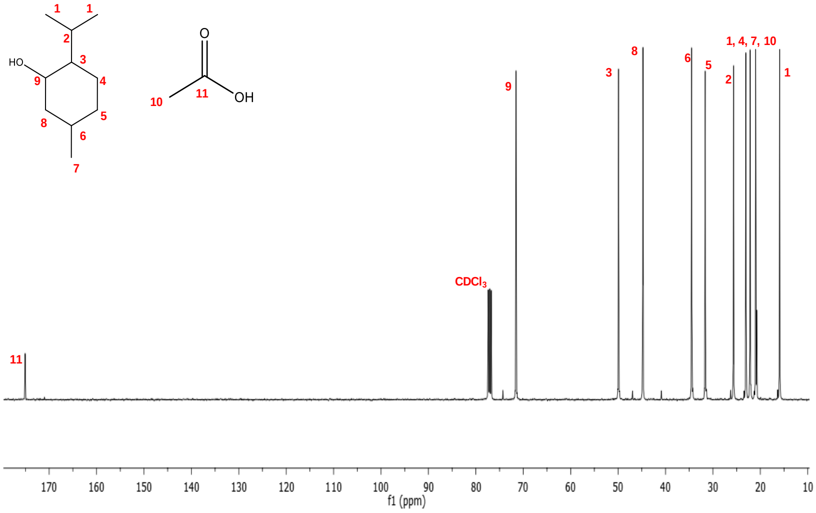

Figure S5. ^{13}C NMR spectra of DL-menthol: acetic acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

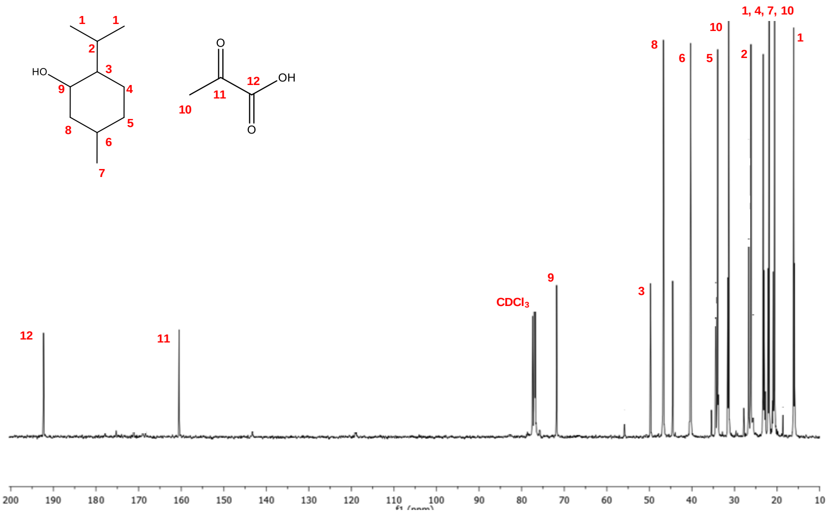

Figure S6. ^{13}C NMR spectra of DL-menthol: pyruvic acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

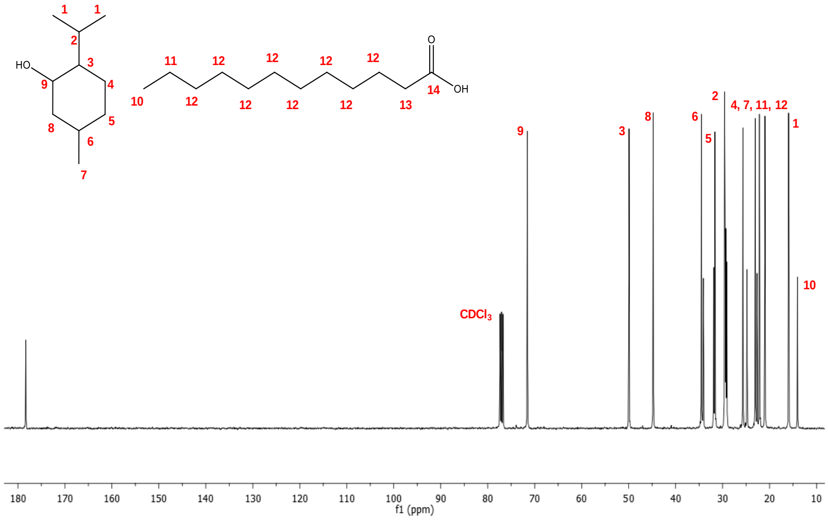

Figure S7. ^{13}C NMR spectra of DL-menthol: lauric acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

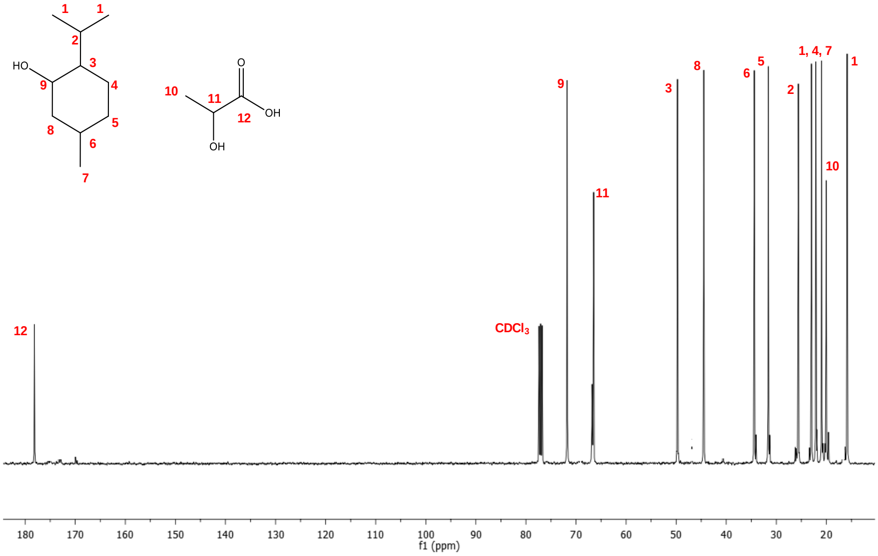

Figure S8. ^{13}C NMR spectra of DL-menthol: lactic acid eutectic mixture in $CDCl_3$. The structure and numbering of both compounds is also depicted.

FTIR spectra of eutectic mixtures

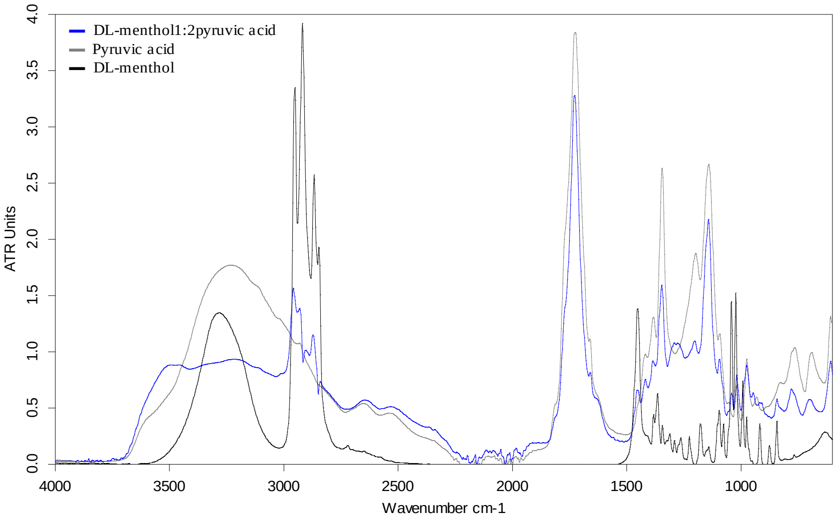

Figure S9. FTIR spectra of the pure DL-menthol, pyruvic acid and the eutectic mixture of DL-menthol and pyruvic acid (1:2 molar ratio).

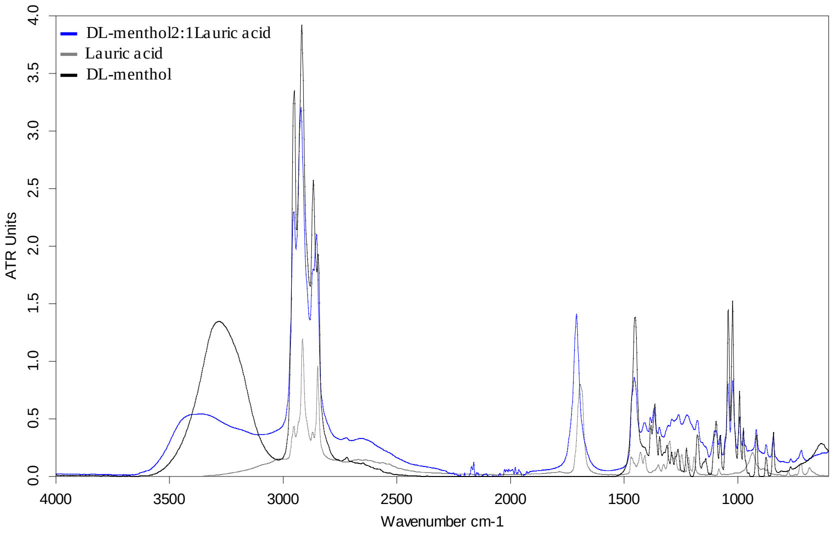

Figure S10. FTIR spectra of the pure DL-menthol, lauric acid and the eutectic mixture of DL-menthol and lauric acid (2:1 molar ratio).

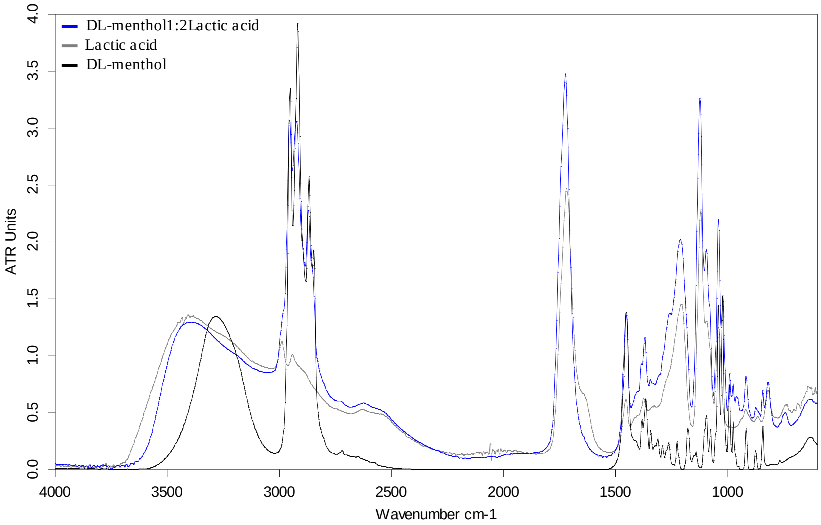

Figure S11. FTIR spectra of the pure DL-menthol, lactic acid and the eutectic mixture of DL-menthol and lactic acid (1:2 molar ratio).

Thermal properties of eutectic mixtures

TGA analysis

Table S1. Decomposition temperatures (T_{dec}) for the eutectic mixtures studied in this work.

Pure compounds	T_{dec} **(ºC)**
DL-menthol	168.95
Acetic acid	—*
Pyruvic acid	164.48
Lactic acid	229.46
Lauric acid	292.34
Eutectic Mixtures	T_{dec} **(ºC)**
DL-menthol: acetic acid	200.79
DL-menthol: pyruvic acid	218.50
DL-menthol: lactic acid	228.89
DL-menthol: lauric acid	231.49

*Acetic acid has a boiling point at 118 °C, thus was not possible to determine its decomposition temperature by TGA measurements.

DSC Analysis

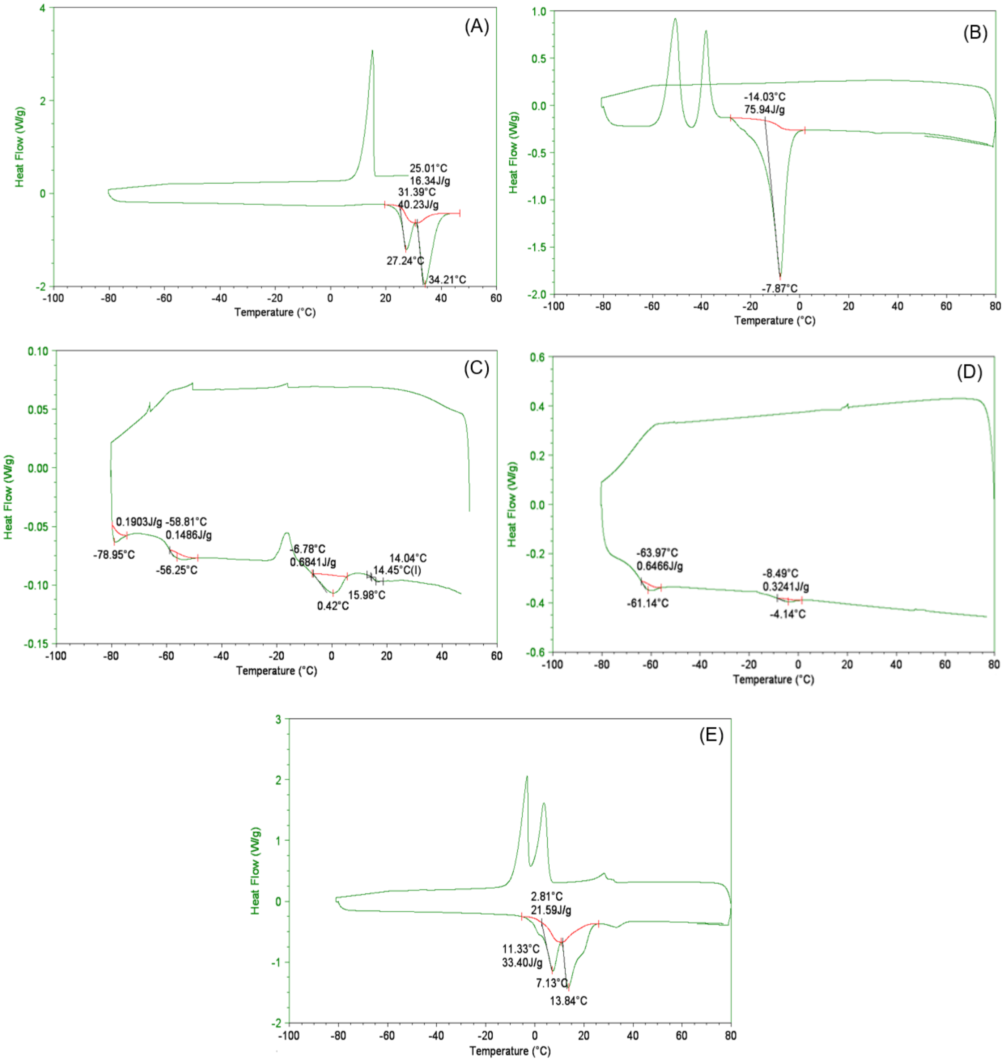

Figure S12. DL-menthol-based eutectic mixtures: (A) pure DL-menthol; (B) DL-menthol: Acetic acid (50%); (C) DL-menthol: Pyruvic Acid (50%); (D) DL-menthol: Lactic acid (66.7%); (E) DL-menthol: Lauric Acid (66.7%).

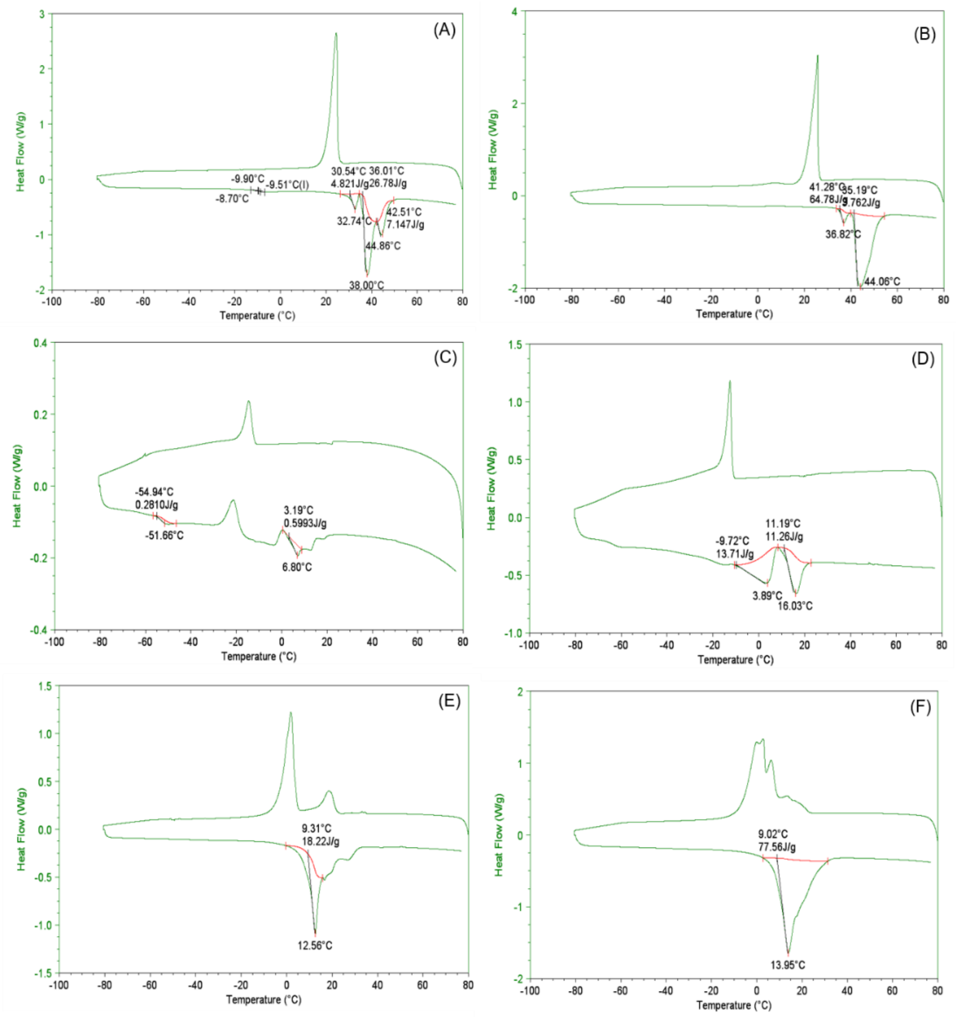

Figure S13. Effect of menthol isomers in eutectic mixtures: (A) L-Menthol; (B) D-Menthol; (C) L-Menthol: Lactic acid; (D) D-Menthol: Lactic acid; (E) L-Menthol: Lauric Acid; (F) D-Menthol: Lauric Acid).

Thermophysical properties of eutectic mixtures

Table S2. Experimental densities (ρ (g·cm^{-3})) of dried and water-saturated samples of the eutectic mixtures as a function of temperature.

T (K)	DL-menthol: acetic acid		DL-menthol: pyruvic acid		DL-menthol: lactic acid		DL-menthol: lauric acid	
	Dried	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*
293.15	0.935	0.915	0.999	0.949	1.038	0.921	0.897	0.8995
298.15	0.931	0.911	0.995	0.945	1.033	0.917	0.894	0.8959
303.15	0.927	0.907	0.991	0.941	1.029	0.914	0.890	0.8923
308.15	0.923	0.904	0.987	0.937	1.025	0.910	0.886	0.8887
313.15	0.919	0.900	0.983	0.933	1.021	0.906	0.883	0.8851
318.15	0.915	0.896	0.978	0.928	1.017	0.902	0.879	0.8815
323.15	0.911	0.892	0.974	0.924	1.013	0.899	0.876	0.8778
328.15	0.906	0.888	0.970	0.920	1.009	0.895	0.872	0.8740
333.15	0.902	0.884	0.966	0.916	1.005	0.891	0.868	0.8702
338.15	0.898	0.880	0.962	0.912	1.001	0.887	0.865	0.8665
343.15	0.894	0.876	0.958	0.908	0.997	0.883	0.861	0.8627
348.15	0.889	0.872	0.953	0.903	0.992	0.879	0.857	0.8588
353.15	0.884	0.867	0.948	0.899	0.988	0.874	0.853	0.8546

Table S3. Experimental viscosities (η (mPa·s)) of dried and water-saturated eutectic mixtures as a function of temperature.

T **(K)**	**DL-menthol: acetic acid**		**DL-menthol: pyruvic acid**		**DL-menthol: lactic acid**		**DL-menthol: lauric acid**	
	Dried	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*
293.15	11.296	27.751	44.637	28.288	370.860	68.421	33.058	29.689
298.15	8.691	19.454	29.951	19.383	218.927	44.181	24.417	22.076
303.15	6.904	14.258	21.241	13.992	134.253	30.006	18.630	16.957
308.15	5.590	10.752	15.667	10.469	86.529	21.155	14.513	13.286
313.15	4.564	8.234	11.882	7.993	58.840	15.385	11.453	10.527
318.15	3.843	6.561	9.360	6.379	40.711	11.533	9.293	8.587
323.15	3.251	5.277	7.511	5.151	29.467	8.849	7.610	7.057
328.15	2.782	4.310	6.144	4.239	21.951	6.938	6.315	5.873
333.15	2.391	3.522	5.034	3.492	16.954	5.467	5.226	4.872
338.15	2.095	3.000	4.321	3.006	13.119	4.506	4.507	4.201
343.15	1.843	2.548	3.700	2.582	10.460	3.716	3.862	3.599
348.15	1.632	2.183	3.203	2.242	8.487	3.104	3.342	3.107
353.15	1.464	1.866	2.744	1.941	7.013	2.569	2.859	2.650

Table S4. Thermal expansion coefficients (α_p) determined for dried and water-saturated menthol-based eutectic mixtures. The isobaric thermal expansion coefficient (α_p) was calculated through $\alpha_p = -\frac{1}{\rho}\left(\frac{\delta\rho}{\delta T}\right)_p = -\left(\frac{\delta \ln\rho}{\delta T}\right)_p$, where ρ is the density in g·cm^{-3}, T is the temperature in K and p is the pressure in MPa.

T (K)	$\alpha_p \times 10^4$ (K^{-1})							
	DL-menthol: acetic acid		**DL-menthol: pyruvic acid**		**DL-menthol: lactic acid**		**DL-menthol: lauric acid**	
	Dried	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*
293.15	8.998	8.631	8.412	8.787	7.891	8.410	8.144	8.270
298.15	9.039	8.669	8.448	8.825	7.922	8.445	8.177	8.305
303.15	9.080	8.706	8.484	8.865	7.954	8.481	8.211	8.339
308.15	9.121	8.744	8.520	8.904	7.986	8.517	8.245	8.374
313.15	9.163	8.783	8.556	8.944	8.018	8.554	8.279	8.409
318.15	9.205	8.822	8.593	8.984	8.050	8.590	8.313	8.445
323.15	9.248	8.861	8.630	9.025	8.082	8.627	8.348	8.481
328.15	9.291	8.900	8.668	9.065	8.115	8.665	8.383	8.517
333.15	9.334	8.940	8.705	9.107	8.148	8.702	8.418	8.553
338.15	9.378	8.980	8.743	9.148	8.182	8.740	8.454	8.590
343.15	9.422	9.021	8.782	9.190	8.215	8.779	8.490	8.627
348.15	9.467	9.061	8.821	9.233	8.249	8.818	8.526	8.665
353.15	9.512	9.103	8.860	9.276	8.283	8.857	8.562	8.702

Table S5. Values of Molar Volume (V_m) of eutectic mixtures as a function of temperature.

T (K)	DL-menthol: acetic acid		DL-menthol: pyruvic acid		DL-menthol: lactic acid		DL-menthol: lauric acid	
	Dried	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*	*Dried*	*Saturated*
293.15	115.67	118.20	110.90	116.75	108.03	121.75	190.58	190.05
298.15	116.17	118.72	111.35	117.24	108.56	186.35	191.21	190.81
303.15	116.67	119.24	111.80	117.74	108.98	187.11	192.07	191.58
308.15	117.18	119.64	112.25	118.24	109.40	187.89	192.94	192.36
313.15	117.69	120.17	112.71	118.75	109.83	188.65	193.60	193.14
318.15	118.20	120.71	113.29	119.39	110.27	189.44	194.48	193.93
323.15	118.72	121.25	113.75	119.91	110.70	190.24	195.14	194.74
328.15	119.38	121.80	114.22	120.43	111.14	191.07	196.04	195.59
333.15	119.91	122.35	114.69	120.95	111.58	191.91	196.94	196.44
338.15	120.44	122.90	115.17	121.48	112.03	192.76	197.63	197.28
343.15	120.98	123.46	115.65	122.02	112.48	193.64	198.54	198.15
348.15	121.66	124.03	116.26	122.69	113.04	194.56	199.47	199.05
353.15	122.35	124.75	116.87	123.24	113.50	195.55	200.41	200.03

7. References

1. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R.L.; Duarte, A.R.C. Natural Deep Eutectic Solvents – Solvents for the 21st Century. *ACS Sustainable Chem. Eng.* 2014, *2* (5), 1063-1071.

2. Rogers, R.D.; Seddon, K.R.; Volkov, S. *Green Industrial Applications of Ionic Liquids*; Springer Netherlands, 2002.

3. Florindo, C.; Oliveira, F.S.; Rebelo, L.P.N; Fernandes, A.M.; Marrucho, I.M. Insights into the Synthesis and Properties of Deep Eutectic Solvents based on Cholinium Chloride and Carboxylic Acids. *ACS Sustainable Chem. Eng*. 2014, *2* (10), 2416-2425.

4. BASF, L-Menthol Pharma and L-menthol flakes Pharma. Technical information. http://www.pharma-ingredients.basf.com/Statements/Technical%20Informations/EN/Pharma%20Solutions/03_130101e_L-Menthol%20Pharma.pdf January 2013.

5. Shen, Q.; Li, X.; Li, W.; Zhao, X. Enhanced Intestinal Absorption of Daidzein by Borneol/Menthol Eutectic Mixture and Microemulsion. *AAPS PharmSciTech* 2011, *12* (4), 1044-1049.

6. Liu, J.; Fu, S.; Wei, N.; Hou, Y.; Zhang, X.; Cui, H. The effects of combined menthol and borneol on fluconazole permeation through the cornea ex vivo. *Eur. J. Pharm*. 2012, *688*, 1-5.

7. Gohel, M.C.; Nagori, S.A. Resolving Issues of Content Uniformity and Low Permeability Using Eutectic Blend of Camphor and Menthol*. Indian J. Pharm. Sci.* 2009, *71* (6), 622-629.

8. Tuntarawongsa, S.; Phaechamud, T. Menthol, Borneol, Camphor and WS-3 Eutectic Mixture. *Adv. Mat. Res.* 2012, *506*, 355-358.

9. Kaplun-Frischoff, Y.; Touitou, E. Testosterone Skin Permeation Enhancement by Menthol through Formation of Eutectic with Drug and Interaction with Skin Lipids. *J. Pharm Sci.* 1997, *86* (12), 1394-1399.

10. Stott, P.W.; Williams, A.C.; Barry, B.W. Transdermal delivery from eutectic systems: enhanced permeation of a model drug, ibuprofen. *J. Control. Release* 1998, *50*, 297–308.

11. Yong, C.S.; Jung, S.H.; Rhee, J.-D.; Choi, H.-G.; Lee, B.-J.; Kim, D.-C.; Choi, Y.W.; Kim, C.-K. Improved Solubility and In Vitro Dissolution of

Ibuprofen from Poloxamer Gel Using Eutectic Mixture with Menthol. *Drug Delivery* 2003, *10*, 179–183.

12. Kang, L.; Jun, H.W.; McCall, J.W. Physicochemical studies of lidocaine–menthol binary systems for enhanced membrane transport. *Int. J. Pharm.* 2000, *206*, 35-42.

13. Nazzal, S.; Smalyukh, I.I.; Lavrentovich, O.D.; Khan, M.A. Preparation and in vitro characterization of a eutectic based semisolid self-nanoemulsified drug delivery system (SNEDDS) of ubiquinone: mechanism and progress of emulsion formation. *Int. J. Pharm.* 2002, *235*, 247–265.

14. Kamatou, G.P.P.; Vermaak, I.; Viljoen, A.M.; Lawrence, B.M. Menthol: A simple monoterpene with remarkable biological properties. *Phytochem.* 2013, *96*, 15-25.

15. Patel, T.; Ishiuji, Y.; Yosipovitch, G. Menthol: A refreshing look at this ancient compound. *J. Am. Acad. Dermatol*. 2007, *57*(5), 873-878.

16. Craig, D.Q.M.; Reading, M. *Thermal Analysis of Pharmaceuticals*. CRC Press, Boca Raton, 2007.

17. Morrison, H.G.; Sun, C.C.; Neervannan, S. Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles. *Int. J. Pharm.* 2009, *378*, 136–139.

18. Corvis, Y.; Négrier, P.; Massip, S.; Leger, J.-M.; Espeau, P. Insights into the crystal structure, polymorphism and thermal behavior of menthol optical isomers and racemates. *CrystEngComm* 2012, *14*, 7055–7064.

19. Narishetty, S.T.K.; Panchagnula, R. Effect of l-menthol and 1,8-cineole on phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. *J. Control. Rel.* 2005, *102*, 59–70.

20. Marstokk, K.M.; Møllendal, H. Microwave Spectrum, Conformation, Barrier to Internal Rotation and Dipole Moment of Pyruvic Acid. *J. Mol. Struct.* 1974, *20*, 257-267.

21. Freire, M.G.; Neves, C.M.S.S.; Marrucho, I.M.; Lopes, J.N.C.; Rebelo, L.P.N.; Coutinho, J.A.P. High-performance extraction of alkaloids using aqueous two-phase systems with ionic liquids. *Green Chem.* 2010, *12*, 1715-1718.

22. Hu, X.; Wan, X.; Bal, R.; Yang, H. Theobromine and Caffeine Recovery with Solvent Extraction. *Sep. Sci. Technol.* 2003, *38*, 3609-3624.

23. Domínguez-Pérez, M.; Tomé, L.I.N.; Freire, M.G.; Marrucho, I.M.; Cabeza, O.; Coutinho, J.A.P. (Extraction of biomolecules using) aqueous biphasic systems formed by ionic liquids and aminoacids. *Sep. Purif. Technol.* 2010, *72,* 85-91.

24. Louros, C.L.S.; Cláudio, A.F.M.; Neves, C.M.S.S.; Freire, M.G.; Marrucho, I.M.; Pauly, J.; Coutinho, J.A.P. Extraction of Biomolecules Using Phosphonium-Based Ionic Liquids + K3PO4 Aqueous Biphasic Systems*. Int. J. Mol. Sci.* 2010, *11*, 1777-1791.

25. Winley, W.C.; White, S.H. Partitioning of Tryptophan Side-Chain Analogs between Water and Cyclohexane. *Biochem.* 1992, *31*, 12813-12818.

26. Tomé, L.I.N.; Catambas, V.R.; Teles, A.R.R.; Freire, M.G.; Marrucho, I.M.; Coutinho, J.A.P. Tryptophan extraction using hydrophobic ionic liquids. *Sep. Purif. Technol.* 2010, *72,* 167-173.

27. Neves, C.M.S.S.; Ventura, S.P.M.; Freire, M.G.; Marrucho, I.M.; Coutinho, J.A.P. Evaluation of Cation Influence on the Formation and Extraction Capability of Ionic-Liquid-Based Aqueous Biphasic Systems. *J. Phys. Chem. B* 2009, *113*, 5194–5199.

28. Ventura, S.P.M.; Neves, C.M.S.S.; Freire, M.G.; Marrucho, I.M.; Oliveira, J.; Coutinho, J.A.P. Evaluation of Anion Influence on the Formation and Extraction Capacity of Ionic-Liquid-Based Aqueous Biphasic Systems. *J. Phys. Chem. B* 2009, *113*, 9304–9310.

29. Passos, H.; Ferreira, A.R.; Cláudio, A.F.M.; Coutinho, J.A.P.; Freire, M.G. Characterization of aqueous biphasic systems composed of ionic liquids and a citrate-based biodegradable salt. *Biochem. Eng. J.* 2012, *67*, 68-76.

30. Noubigh, A.; Mgaidi, A.; Abderrabba, M. Temperature Effect on the Distribution of Some Phenolic Compounds: An Experimental Measurement of 1-Octanol/Water Partition Coefficients. *J. Chem. Eng. Data* 2010, *55*, 488-491.

31. Cláudio, A.F.M.; Freire, M.G.; Freire, C.S.R.; Silvestre, A.J.D.; Coutinho, J.A.P. Extraction of vanillin using ionic-liquid-based aqueous two-phase systems. *Sep. Purif. Technol.* 2010, *75*, 39-47.

32. Buch, A.; Sternberg, R.; Meunier, D.; Rodier, C.; Laurent, C.; Raulin, F.; Vidal-Madjar, C. Solvent extraction of organic molecules of exobiological interest for in situ analysis of the Martian soil. *J. Chromat. A* 2003, *999*, 165–174.

33. Feng, L.; Wang, L.; Peng, G.; Guo, X.; Li, X. Solubility of Isophthalic Acid in Propyl Acetate and Partition Coefficient between Propyl Acetate and Water. *J. Chem. Eng. Data* 2010, *55*, 500–503.

CHAPTER 2.2

From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid-based Deep Eutectic Solvents

Adapted from: **C. Florindo**, L. Romero, I. Rintoul, L. C. Branco and I. M. Marrucho, From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid–Based Deep Eutectic Solvents, ACS Sustainable Chemistry & Engineering 2018 6 (3), 3888-3895 (selected for cover – Volume 6 Number 3).

L. Romero was involved in the synthesis and screening of DESs. The author contributed to the execution of all characterization and properties experiments described in this chapter as well as involved in the discussion and interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

Inspired by the use of fatty acids in development of low temperature latent heat storage materials, novel low viscous and hydrophobic deep eutectic solvents (DESs) based exclusively on fatty acids are herein proposed as sustainable solvents. Three DESs were prepared by exclusively combining fatty acids, namely octanoic acid (C_8), nonanoic acid (C_9), decanoic acid (C_{10}) and dodecanoic acid (C_{12}), which can simultaneously act as hydrogen bond donors and acceptors. The obtained fatty acid-based DESs were analyzed in order to check their structures, purities and proportions. Water stability was also carefully evaluated through ^{1}H NMR. Fatty-acid DESs melting point's diagrams were determined by visual observation. Good agreement was obtained between the experimental eutectic point and that predicted by considering an ideal system of two individually melting compounds. Important solvent thermophysical properties, such as density and viscosity of the dried and water-saturated DESs were measured. Finally, the removal of bisphenol-A, a persistent micropollutant present in aqueous environments illustrates the potential of binary and ternary fatty acid-based DESs as extraction solvents. All prepared DESs showed good ability to extract bisphenol-A from water with extraction efficiencies up to 92%.

1. Introduction

Developing a cost effective and environmentally benign solvent is of vital importance in chemical industry. The demand for environmentally benign, less toxic, biodegradable, natural and low-cost solvents has slowly encouraged the development of novel alternative solvents. Over the past decade, some green solvents have emerged as harmless solvents, namely supercritical fluids, bio-based solvents, ionic liquids (ILs) and more recently deep eutectic solvents (DESs).[1-3] Eutectic mixtures are a well-known class of multicomponent solid systems, presenting melting point temperature depressions relative to their parent compounds. However, the high melting points of many eutectic mixtures hamper their application as green solvents at room temperature. Eutectics solvents that are liquid around room temperature have been scarcely reported in the literature until the beginning of the 21st century.[4] Only in 2003, Abbott et al. proposed eutectic mixtures as a versatile and sustainable platform of tunable solvents. In fact, these authors showed that if a salt or a solid ionic liquid is used in the mixture, a large melting point depression can be obtained, thus coining the designation Deep Eutectic Solvents (DESs). These solvents have been emerging as a new generation of solvents with a great potential for a variety of applications, such as in electrochemistry and metal extraction area,[5] nanotechnology,[6] stabilization of DNA,[7] materials chemistry,[8] catalysis,[9] organic synthesis,[10] among others.

DESs have been introduced as alternatives to ionic liquids, overcoming their main disadvantages, such as high cost and toxicity, while keeping their negligible vapour pressure, easy tunability and excellent solvation properties.[11-12] Although less explored, mixtures of two neutral natural compounds can also yield liquids at room temperature. Despite the fact that melting point depressions of neutral eutectic mixtures are not as deep as when ionic compounds are used, they have also adopted the unifying DESs designation, since they can also be used as solvents. Choi and co-workers[13] have been reporting a large number of stable natural DESs, either based on charged compounds or neutral natural compounds, which are liquid at room temperature. One of the main advantages of DESs is their very simple synthetic process, which consists on mixing different proportions of two or more components, until a liquid with a melting point lower than the starting materials is obtained.[14] Generally, DESs are often simply prepared from binary mixtures of hydrogen bond acceptors (HBA), such as quaternary ammonium salts combined with a hydrogen bond donor (HBD), such as an aminoacid,[15] a sugar,[16] an alcohol[17] or a carboxylic acid.[18] Recently, other non-ionic DESs have been reported, for example eutectic mixtures composed of dimethylurea combined with citric acid, mannose or L-tartaric acid.[19-20]

This field has greatly evolved in the last couple of years, showing a great diversity of starting compounds that form liquids at room temperature when combined in certain molar ratio,[21] thus providing a powerful tool to control the properties of DESs. Recently, the lack of chemical stability of hydrophilic DESs based on hydrophilic ammonium salts when in contact with water motivated the development of hydrophobic DESs as green substitutes of conventional organic solvents.[22-23] Diverse starting compounds have been used in hydrophobic DESs preparation, ranging from long chain quaternary ammonium halides[23] to hydrophobic natural compounds, such as menthol[24] and long alkyl chain fatty acids.[22-23]

In this work, new natural hydrophobic DESs, composed exclusively of fatty acids, are developed. Long hydrocarbon chain fatty acids eutectics have been largely explored as phase change materials (PCMs), for low temperature latent heat storage.[25] Their importance is essentially related to their good chemical stability, smaller volume change during phase transition and high latent heat of fusion and melting temperature in the desired operating range.[26] The phase change temperatures of fatty acids eutectics can be easily adjusted by mixing fatty acids with diverse alkyl chain lengths in suitable proportions while keeping non-reactivity, recyclability, non-corrosiveness, low cost and the non-toxicity advantages of fatty acids.[27] To the best of our knowledge, no study addressed yet the preparation and use of green solvents using fatty acids PCMs starting materials. In this perspective, the goal of the present contribution is to use the same class of materials that can be modulated for heat storage applications to develop novel sustainable hydrophobic DESs to be used in water purification technologies. For that purpose, dodecanoic acid (C_{12} acid) was combined with octanoic acid (C_8 acid), nonanoic acid (C_9 acid) and decanoic acid (C_{10} acid) to prepare new hydrophobic sustainable solvents. The use of acids with hydrocarbon chains smaller than C_8 was discarded since it leads to chemically unstable DES when in contact with water, due to the leaching of the hydrophilic acids to the aqueous phase.[23,25] Bisphenol A (BPA), a synthetic organic chemical used as a efficient plasticizer in the processing of a number of plastics,[28] was chosen to illustrate the use of the prepared DES in water treatment. Despite its small concentrations in water streams, BPA has been recognized as a persistent micropollutant due to its important effect as an endocrine disruptor, leading to altered immune functions, imbalanced hormone ratios, reproductive disorders, diabetes, cardiovascular disease and many alterations in children.[29-30] Binary and ternary fatty acids based DESs were evaluated as possible extractors of BPA from water media.

2. Experimental Section

2.1. Materials

All fatty acids, namely octanoic acid (C_8) (purity ≥ 98%), nonanoic acid (C_9) (purity ≥ 98%), decanoic acid (C_{10}) (purity ≥ 98%) and dodecanoic acid (C_{12}) (purity ≥ 98%) were purchased from Sigma-Aldrich. All materials were used without further purification. In Figure 1, the chemical structures and respective acronyms of the eutectic solvents used in this work are presented. The water used was ultrapure; double distilled, passed through a reverse osmosis system and further treated with Milli-Q plus water purification apparatus.

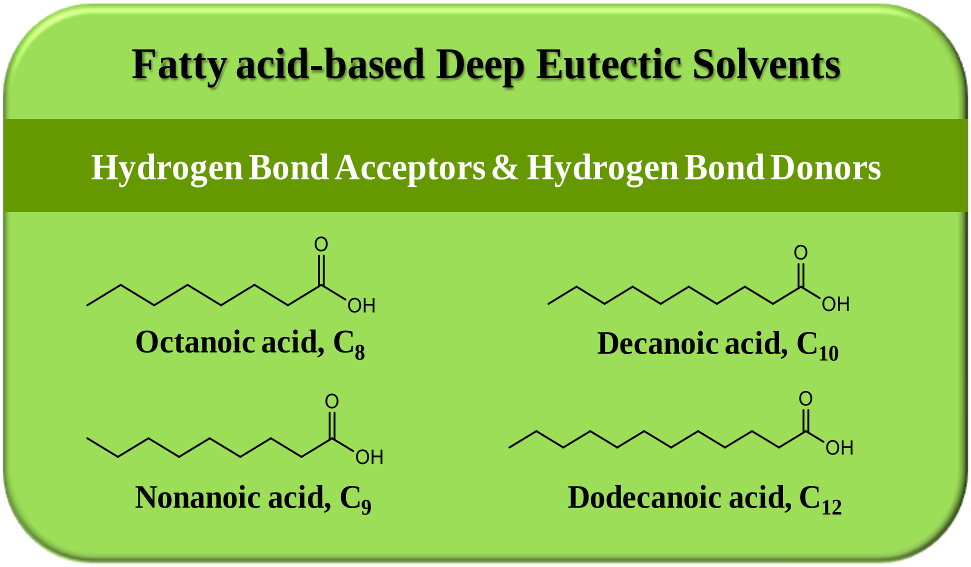

Figure 1. Chemical structures of the compounds used for the fatty acid-based deep eutectic solvents studied in this work.

2.2. Apparatuses and procedures

2.2.1. Preparation Methodology

Mixtures of several carboxylic acids with long alkyl chains were prepared by adding different fatty acids, which can simultaneously act as hydrogen bond donors and acceptors, in a sealed glass vial at 40 °C until a homogeneous clear solution was formed. After the determination of the solid-liquid phase diagrams, proton and carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) were used to check the eutectic mixtures structures and purities (presented in *Supporting Information* (SI)). As it can be seen, all the peaks have been attributed DES components, and no extra peaks were found in the ^{1}H and ^{13}C NMR spectra indicating that no side reactions occurred and these DES are pure.

2.2.2. Solid-Liquid Phase diagrams determination

Mixtures of different fatty acids (HBA and HBD) were prepared in the whole composition range, allowing the measurement of the solid-liquid phase diagrams of these new DESs. The (*T, x*) phase diagrams were measured in a glass flask using a visual methodology, at atmospheric pressure, under constant stirring. The eutectic systems compositions were determined by weighting, at room temperature, all components within ± 10^{-4} g using an analytical balance model AX223 from Ohaus.

Briefly, mixtures in varying molar proportions of the HBD and HBA were prepared into several glass flasks, as previously described. Then, the prepared eutectic mixtures with different compositions were heated in an oil bath under stirring using a heating plate until the complete melting of the mixtures was observed. After this first heating cycle, the temperature was turned off and during the cooling cycle, temperatures corresponding to the first crystal appearance were recorded. The temperature was measured with a Pt100 probe with a precision of ± 0.1 ºC. For all the eutectic mixtures compositions, the melting occurs over a range of temperatures in between the melting temperatures of the starting compounds. The above procedure was repeated to obtain sufficient data to construct the phase diagrams of each eutectic solvent system.

2.2.3. Hydrophobicity analysis

In order to infer about the DESs hydrophobicity, and consequently quantify the water content in dried and saturated new eutectic solvents, mixtures containing both components (water and the eutectic mixtures) were prepared, vigorously shaken for 30min and left to settle for 24h. Samples of each phase were carefully taken with a syringe and the amount of water present in each sample was quantified. The water content present in both phases for the investigated eutectic solvents was determined by Karl Fischer titration (Metrohm 870 KF Titrino Plus) and the results are presented in Table 1.

Table 1. Summary of compositions, and water content of dried and water-saturated fatty acid-based DESs.

Deep Eutectic Solvents (HBA + HBD)		**Mole ratio**	**Water content (wt %)**	
			Dried	**Saturated**
Dodecanoic acid (C_{12})	**Octanoic acid, (C_8)**	1:3	0.004	1.353
	Nonanoic acid, (C_9)	1:3	0.006	0.843
	Decanoic acid, (C_{10})	1:2	0.019	0.523

2.2.4. Liquid-Liquid Extraction of Bisphenol A

Aqueous solutions of the micropollutant Bisphenol-A with a concentration of 0.05 g dm^{-3} of concentration was prepared. Although micropollutants are present in real water samples in low concentrations, ranging from ng/L to several µg/L, the higher concentration of bisphenol A in the starting aqueous solution used in this work, allowed the use of a simple and direct detection technique, UV-vis spectroscopic technique. On the other hand, the use of concentrated solutions of BPA guarantees that there is no saturation of BPA in the coexisting phases when envisaging the use of the proposed technology in real water samples. Afterwards, 2 mL of this solution was put into contact with equal amount of each one of the eutectic mixtures and stirred at 300 rpm, at room temperature and pressure conditions, to maximize mass transfer. The flasks were then left for phase separation for 24h, so that complete separation of the two phases and consequently the equilibration of pollutant between the two phases were achieved. Samples of the aqueous phase were taken and the quantification of the pollutant was carried out by

UV-vis spectroscopy using a SHIMADZU UV-1700, Pharma-Spec spectrophotometer, at wavelength of 276 nm for Bisphenol A, by means of a calibration curve previously established. Each value of the extraction efficiency is the average of three measurements.

2.2.5. Thermophysical Properties

Measurements of viscosity and density of the DESs were carried out in the temperature range from 20 up to 80 ºC and at atmospheric pressure, using an Anton Paar (model SVM 3000) automated rotational Stabinger viscometer-densimeter. The temperature uncertainty is ±0.01ºC. The relative uncertainty of the dynamic viscosity is ± 0.25 %, and the absolute uncertainty of the density is ± 0.0005 $g\cdot cm^{-3}$. DESs were simultaneously sampled for the water content quantification and the density and viscosity measurements.

2.2.6. NMR Measurements

All the experiments were carried out on a Bruker AVANCE 400 spectrometer operated at room temperature with 16 and 500 scans for the ^{1}H NMR and ^{13}C NMR spectra, respectively. The eutectic mixtures samples were prepared on 5 mm NMR tubes by weight approximately 30 mg of eutectic mixture and then adding 0.5 mL of deuterated dimethylsulfoxide (DMSO-d6). The homogeneity of the sample was assured by vortex mixing.

3. Results and Discussion

The experimental (*T*, *x*) solid-liquid phase diagram of mixtures of C_{12} acid with C_8, C_9 and C_{10} acids are presented in Figure 2.

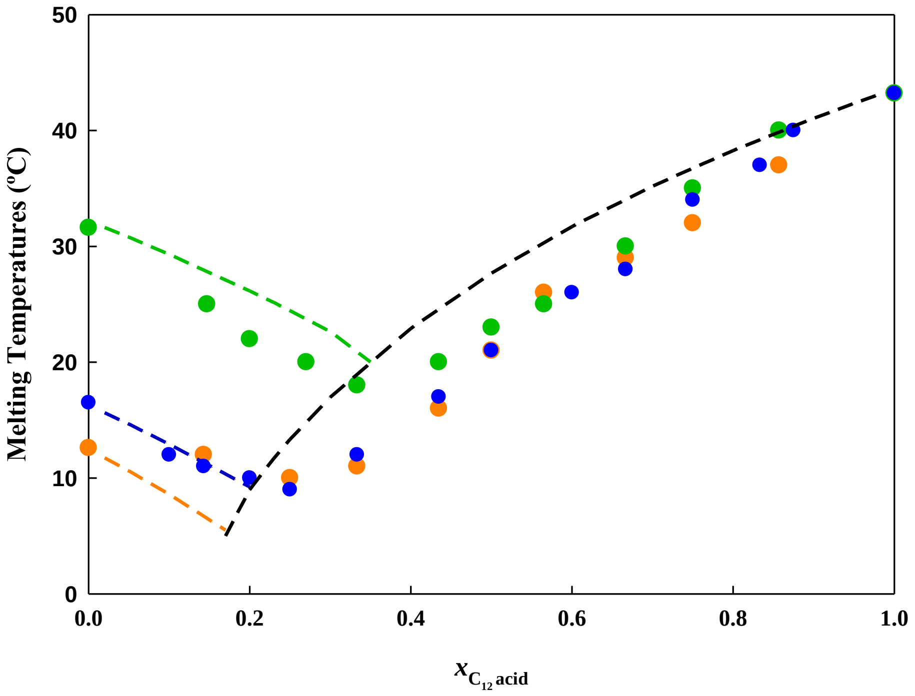

Figure 2. Experimental (dots) and predicted (lines) solid-liquid phase diagrams for the fatty acids-based DES studied in this work (▬ C_{12} acid ● C_8:C_{12}; ● C_9:C_{12}; ● C_{10}:C_{12}).

It can be observed that the eutectic points of C_8:C_{12} and C_9:C_{12} eutectic mixtures occur approximately at 0.25 mole fraction of C_{12} acid, yielding an eutectic point at C_8:C_{12} and C_9:C_{12} molar proportions of (3:1), while for the C_{10}:C_{12} eutectic mixture, the eutectic point happens at 0.33 mole fraction of C_{12} acid, yielding an eutectic point at C_{10}:C_{12} molar proportion of (2:1). The melting temperatures of the eutectic mixtures are at 9, 9 and 18 ºC for C_8:C_{12}, C_9:C_{12} and C_{10}:C_{12} fatty acids DESs, respectively, validating their use as solvents at room temperature. The melting temperatures and molar mass (MM) of fatty acid-based DESs and starting materials used in this work are presented at Table 2.

Table 2. Melting temperatures and molar mass (MM) of fatty acid-based DESs and starting materials used in this work.

		MM (g/mol)	Melting Temperature (ºC)
Fatty acids	**C_{12} acid**	200.32	43.2*
	C_{10} acid	172.26	31.6*
	C_9 acid	158.23	12.6*
	C_8 acid	144.21	16.7*
Fatty acid-based DESs	**C_8:C_{12} (3:1)**	162.91	9.00
	C_9:C_{12} (3:1)	168.75	9.00
	C_{10}:C_{12} 2:1)	179.28	18.0

*Note: values were obtained from Sigma Aldrich datasheet.

It can be observed that the melting point depressions of these new neutral eutectic solvents are not as large as when ionic compounds are used, 178ºC for example in the case of choline chloride and urea.[31] However, a considerable lowering of the melting point, more than 10ºC, is observed when compared to the starting materials.

The prediction of the melting temperature of DESs is of great value, since it is often reported in the literature that a large amount of prepared DESs are solid at room temperature. Binary DES can be regard as a binary solid-liquid equilibria system, where the two components can be independently crystallized, that is, they are not miscible in the solid state, and thus no co-crystals are formed. A simplified phase equilibrium equation for a solid solute, designated by subscript 2 that partly dissolves in a liquid solvent, at a temperature T and a pressure P can be written as:

$$x_2^l \gamma_2^l = exp\left[\left(\frac{\Delta H_2^{sl}}{RT_{m2}}\right)\left(\frac{T\text{-}T_{m2}}{T}\right)\right] \qquad \text{(eq. 1)}$$

where x_2^l is the mole fraction of the component 2 in the liquid phase (solubility), γ_2^l is the activity coefficient of the component 2 in the liquid phase, T_{m2} and ΔH_2^{sl} are the melting temperature (K) and melting enthalpy ($J \cdot mol^{-1}$) of the pure component 2, T is the melting temperature (K) of the mixture and R is the ideal gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$).

This equation takes into account that the difference between the heat capacities in the liquid and the solid state is small compared to the difference in enthalpies. Considering that the DESs here under study can be treated as ideal mixtures, the activity coefficient is set equal to unit. The use of equation (1) to predict the melting temperatures of the here studied DESs can be evaluated in Figure 2. It can be observed that equation (1) was capable of predicting both, the temperature and the composition of the eutectic solvents for the three studied fatty acid mixtures. Differences in temperature of 4, 30 and 9 in percentage and in composition of 5, 7 and 2 in percentage for the C_8:C_{12}, C_9:C_{12}, C_{10}:C_{12} were obtained. However, for mixtures with a composition of C_{12} higher than that of the eutectic mixture, a poor description of the melting temperatures was obtained and thus for the full description of the entire phase diagram, an activity coefficient model needs to be used. Other authors have successfully used equations of state to describe the highly non-ideal behaviour of (T, x) solid-liquid phase diagrams of DESs containing ammonium salts and fatty acids.[32]

In Figure 3, the melting temperatures of the 3 binary DESs prepared in this work are compared with those of other eutectic mixtures based on fatty acids with alkyl chains varying from C_{10} to C_{18}, used to develop PCMs, have melting temperatures between 15–45ºC.[33]

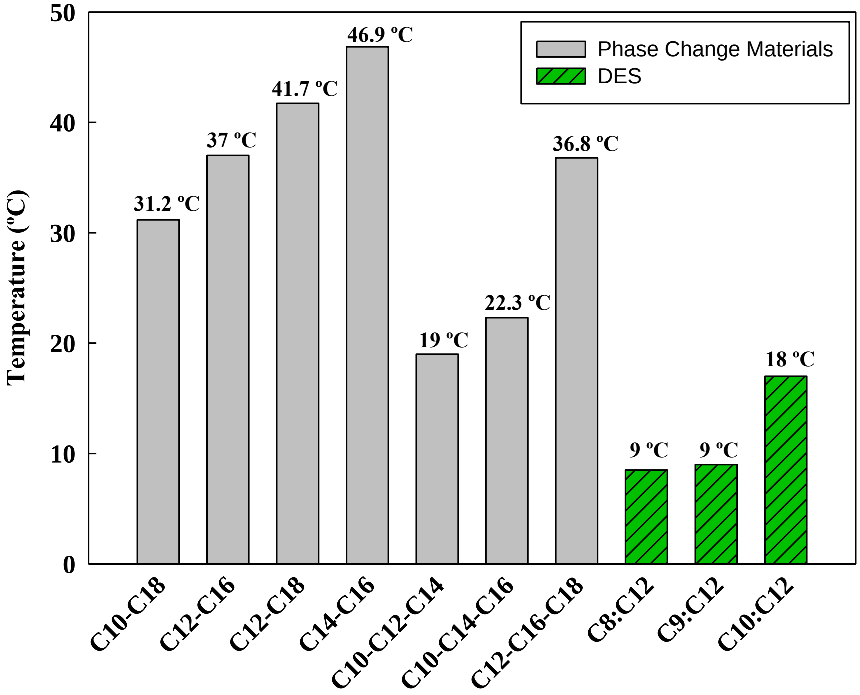

Figure 3. Comparison of melting temperatures of DESs studied in this work with literature values for other fatty acids used as phase change materials. The literature values are from refs [34] and [15].

It can be clearly observed that the melting temperatures of fatty acids eutectic mixtures can be tuned by the careful choice of the length of the fatty acids alkyl chains and their relative proportion in the mixture. The use of fatty acids with chains longer than C_{12} leads to eutectic mixtures with high melting temperatures, which are here not classified as DESs, since they are not liquid at room temperature. Despite the small difference in the chain length between the two fatty acids used to prepare the eutectic mixtures, the differences in the pKa´s of the two acids enables fatty acid with the longest alkyl chain to act as HBD, while that with the smallest chain acts as HBA.[33, 35] The hydrophobic character of the studied DESs was confirmed by the very low water contents of their dried and water saturated forms presented in Table 1. As expected, the longer the fatty acids chains are the higher hydrophobicity of resulting DESs. For example, for the C_8:C_{12} water contents of 0.004 and 1.353 wt% were measured for the dried and the saturated forms, while for C_{10}:C_{12} water contents of 0.019 and 0.523 wt% were obtained. These new fatty acid-based DESs are the most hydrophobic reported so far in the open literature, since they present the smallest water solubility. For example, Kroon's group[36] published the water content of dried (0.02 to 0.07 wt %) and water-saturated (7 to 20 wt%) hydrophobic DESs composed of lidocaine and decanoic acid. In another publication,[22] hydrophobic DESs composed of quaternary ammonium salts and decanoic acid, values of 0.4 to 0.8 wt% for dried samples and 2 to 7 wt% for water saturated samples were reported. Ribeiro et al. [24] presented hydrophobic DESs based on DL-menthol and several carboxylic acids, with water contents of 0.1 to 1 wt% and 1.2 to 1.6 wt% for dried and saturated samples, respectively. More recently, Atkin's group[37] presented new long chains ammonium salts and urea based DESs with water contents around 0.6 wt%. In conclusion, the hydrophobicities of the new fatty acid-based DESs here reported are much higher than those published before.

NMR spectra before and after mixing DESs with water were also registered (see the SI, Figure S4-12). NMR analysis of dried and water saturated DESs showed no chemical reaction or dissociation of the fatty acids constituents and confirmed their chemical stability in presence of water. In particular, the relative proportions of each one of the constituents is kept in the three prepared DESs, even after contacting with water. This fact means that none of the DESs components leaches to the water phase (SI, Figure S10-12).

Thermophysical properties, such as density and viscosity, are also important properties of any solvent since they influence the mass transport phenomena, there by affecting their suitability for particular applications. The density and viscosity data of these new fatty acid-based DESs, dried and water-saturated, were obtained within the range of 20 to 80 ºC at ambient pressure and can be found in SI, and are depicted in Figures 4 and 5, respectively. For all the samples, either in the dried or water saturated form, the density decreases linearly with temperature in the whole temperature range studied and thus equation (2) was used to describe the density temperature dependence:

$$\rho = a + bT \qquad \text{(eq. 2)}$$

where ρ corresponds to density in g·cm^{-3}, T is the temperature in K and a and b are the fitting parameters. The experimental density results for the dried and saturated eutectic mixtures as a function of temperature are presented in Table S2. The adjustable parameters (a and b) were determined from the fitting of the experimental density data to equation 2 and are presented in Table S3. From Figure 4, it can be observed that the densities at 25 ºC are within the range of 894 to 901 kg·m^{-3}.

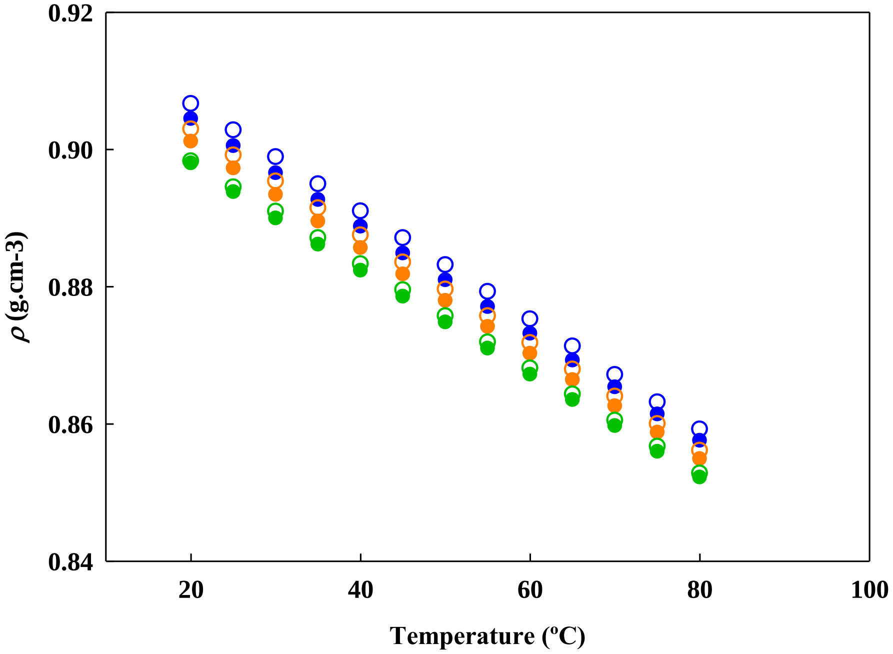

Figure 4. Experimental densities of the studied dried (filled) and water saturated (empty) fatty acid-based DESs (● C_8 acid: C_{12} acid; ● C_9 acid: C_{12} acid; ● C_{10} acid: C_{12} acid) as function of temperature.

These values are lower than the densities found for most hydrophilic DESs (around 1150 kg.m^{-3})[38-39] and also for the reported hydrophobic DESs (889 to 942 kg.m^{-3})[22] and can be explained by the low densities of the starting fatty acids. As expected, the presence of water increases the density of the fatty acids DESs. However, due to the high hydrophobicity of these new DESs, their water content is very small, and thus only a very small effect of water was observed in the DESs density. The same trend was observed for the density of dried and water saturated DESs: C_8:C_{12} DES < C_9:C_{12} DES < C_{10}:C_{12} DES. This trend shows that as the alkyl chain of acid increases, the density of the DESs decreases in a linear manner.

The experimental viscosity values were fitted as a function of temperature, using the Vogel-Fulcher-Tammann (VFT) model:

$$ln\,\eta = A_\eta + \frac{B_\eta}{(T\text{-}C_\eta)} \qquad \text{(eq. 3)}$$

where η is the viscosity in mPa·s, T is the temperature in ºC, and A_η, B_η, and C_η are adjustable parameters. The experimental viscosity results for the dried and saturated DESs as a function of temperature are presented in Table S4 and plotted in Figure 5.

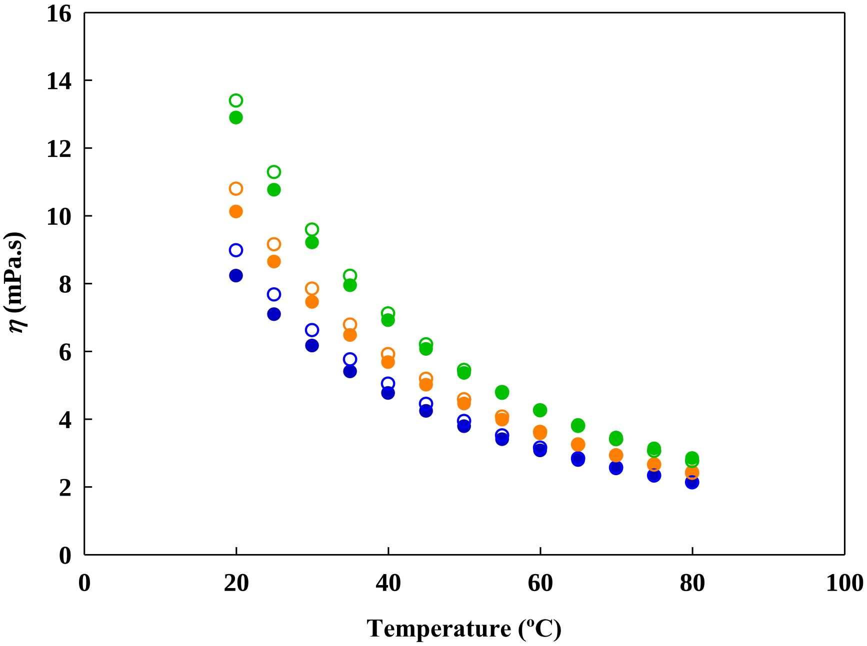

Figure 5. Experimental viscosities of the new dried (filled) and water saturated (empty) fatty acid-based DESs (● C_8 acid: C_{12} acid; ● C_9 acid: C_{12} acid; ● C_{10} acid: C_{12} acid) as function of temperature.

The adjustable parameters of VFT model were determined from the fitting of the experimental values with temperature and are listed in Table S5. The obtained viscosities are quite low, between 7 and 11 mPa·s at 25 ºC, for a very low water content, compared to other hydrophobic low viscous DESs reported in the literature, such as those based on DL-menthol and others containing several quaternary ammonium salts, which typically vary from 11 to 50 mPa.s[24] and 173 to 783 mPa.s[22], respectively.

As expected, an exponential decrease of viscosity with increasing temperature was observed for all samples of DESs. Again and due to the high hydrophobic character of these DESs, the viscosity values of the dried and water saturated fatty acid-based DESs are very similar. Moreover, the viscosity is strongly affected by the HBD alkyl chain of fatty acids since the shorter the alkyl chains of the fatty acid, the lower the DES viscosity, according to the following the order C_8:C_{12} DES < C_9:C_{12} DES < C_{10}:C_{12} DES.

The activation energy was calculated based on the viscosity dependence with temperature as follows:

$$E_a=R\left(\frac{B_\eta}{\left(\frac{C_\eta^2}{T^2}-\frac{2C_\eta}{T}+1\right)}\right) \qquad \text{(eq. 4)}$$

where η is the viscosity, T is the temperature, B_η and C_η are the adjustable parameters obtained from equation 4 and R is the universal gas constant. The values of the activation energies for the prepared eutectic solvents are presented in Table S6.

The use of equation 4 allows the discussion of the energy barrier of a fluid to shear stress, *Ea*, values. The higher the *Ea*, the more difficult it is for the molecules/aggregates to move past each other. This can be a direct consequence of the size or entanglement of the molecules/aggregates and/or the presence of stronger interactions within the fluid. The same trend observed in viscosity values was also observed for the *Ea* of dried and water saturated DESs: C_8:C_{12} DES < C_9:C_{12} DES < C_{10}:C_{12} DES. This trend shows that as the alkyl chain of acid increases, the *Ea* of the DES increases in a linear manner. All fatty acid-based DESs containing linear non substituted carboxylic acids have low *Ea* values (22 to 25 KJ/mol), in agreement with their small viscosity. The *Ea* values obtained for water saturated DESs are similar to each other, indicating that the presence of water, even in different amounts, and in mixtures with different molar ratios, leads an easier and similar flux of the different molecules/aggregates.

Taking into account the results obtained for the prepared fatty acid-based DESs, their performance in the extraction of a persistent micropollutant, BPA, from water was studied. The one step-extraction efficiencies, EE, were calculated using equation 5, from the BPA concentration in the water phase before, $C_{BPA,0}^{aq}$, and after the extraction, $C_{BPA,1}^{aq}$:

$$\% \, EE = \frac{C_{BPA,0}^{aq} - C_{BPA,1}^{aq}}{C_{BPA,0}^{aq}} \times 100 \qquad \text{(eq. 5)}$$

The one-step extraction efficiencies of BPA using the proposed binary fatty acid-based DESs are summarized in Table 3 and plotted in Figure 6.

Table 3. Extraction efficiencies (% EE) of bisphenol-A using binary and ternary fatty acid-based DESs (stirring speed = 300 rpm, ratio DES/Water = 1:1, temperature = 25 ºC, mixing time = 15 min).

Fatty acid-based DESs		Extraction Efficiencies (EE%)
Binary DESs	**C_8: C_{12} (3:1)**	76.04 ± 1.13
	C_9: C_{12} (3:1)	88.32 ± 0.23
	C_{10}: C_{12} (2:1)	81.81 ± 0.34
Ternary DESs	**C_8:C_9:C_{12} (1:1:1)**	85.49 ± 0.86
	C_8:C_9:C_{12} (1:2:1)	84.53 ± 0.43
	C_8:C_9:C_{12} (2:1:1)	82.34 ± 1.10
	C_8:C_9:C_{12} (3:1:1)	79.42 ± 0.54
	C_8:C_9:C_{12} (3:2:1)	80.32 ± 0.78
	C_9:C_{10}:C_{12} (1:1:1)	87.65 ± 1.06
	C_9:C_{10}:C_{12} (1:2:1)	87.81 ± 0.67
	C_9:C_{10}:C_{12} (2:1:1)	89.01 ± 0.72
	C_9:C_{10}:C_{12} (2:2:1)	89.06 ± 0.34
	C_9:C_{10}:C_{12} (3:1:1)	91.52 ± 0.41
	C_9:C_{10}:C_{12} (3:2:1)	90.50 ± 0.57
	C_8:C_{10}:C_{12} (1:1:1)	82.77 ± 1.03
	C_8:C_{10}:C_{12} (2:1:1)	79.45 ± 0.46
	C_8:C_{10}:C_{12} (3:1:1)	77.75 ± 0.72
	C_8:C_{10}:C_{12} (3:2:1)	79.62 ± 0.58

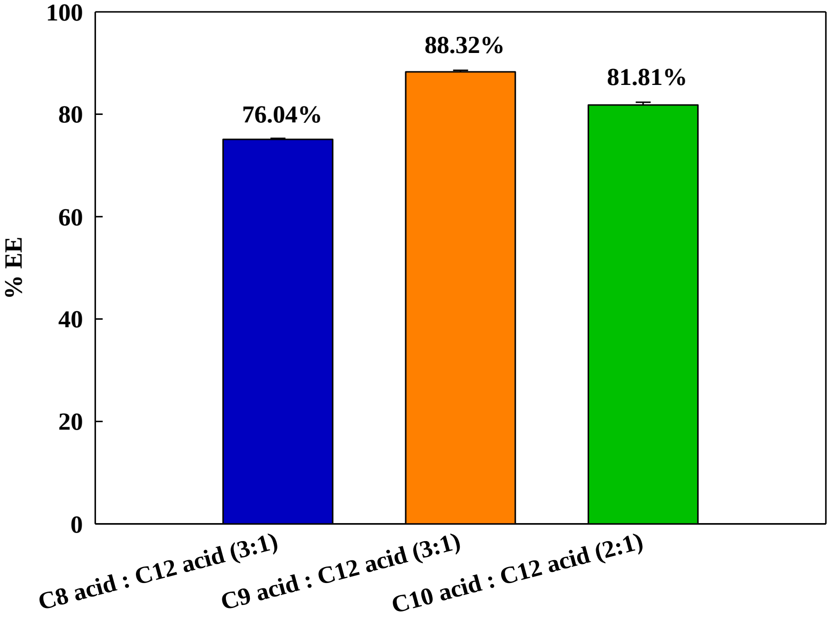

Figure 6. One-step extraction efficiencies (% EE) of Bisphenol A using the fatty acid-based DESs developed in this work (stirring speed = 300 rpm, ratio DES/Water = 1:1, temperature = 25 ºC, mixing time = 15 min).

The studied hydrophobic DESs showed very high 1-step extraction efficiencies (up to 92%) for BPA. Moreover, the obtained extraction efficiencies can be ordered according to the following sequence: C_8:C_{12} DES < C_{10}:C_{12} DES < C_9:C_{12} DES, showing no clear trend between the % EE and the hydrophobicity of the DESs. However, this might be linked to the fact that C_{10}:C_{12} DES has a different molar ratio (2:1) than the other two DESs (3:1).

Since DESs can be prepared from two or more components and consequently their properties can be tailored by changing the number of HBDs, ternary DESs were also prepared by addition of a second HBD to C_8:C_{12}, C_9:C_{12} and C_{10}:C_{12}, aiming at achieving higher extraction efficiencies. In this way, three families of ternary DESs were prepared by the addition of a third component (octanoic, nonanoic and decanoic acids) to the binary DESs studied so far. The extraction of BPA from aqueous media was carried out and obtained EE% are also listed in Table 3 and presented in Figure S1 of the SI. In Figures S2, S3 and S4 a direct comparison of the EE% of BPA for each binary DESs with the corresponding ternary DESs is illustrated. Overall, the EE% obtained for the ternary DESs are between 79 and 91%, indicating that addition of a third component in the binary DESs is indeed capable to tuning the extraction efficiencies of BPA. Furthermore, the EE% obtained for the binary DESs C_8:C_{12} (76.04%) is the lowest, showing that a higher hydrophobicity is favourable in the extraction of BPA. In other words, the addition of either C_9 to form C_8:C_9:C_{12} or C_{10} to form C_8:C_{10}:C_{12} enhance the EE% of BPA. This can be seen from the EE% obtained for the binary DESs C_8:C_{12} and the ternary DES C_8:C_9:C_{12} (3:1:1) and C_8:C_9:C_{12} (3:2:1), with values of 79.42% and 80.32%, respectively, with those obtained for C_8:C_{10}:C_{12} (3:1:1) and C_8:C_{10}:C_{12} (3:2:1) with values of 77.75% and 79.62%, respectively. On the other hand, it can also observed that a decrease in the proportion of C_8 in the ternary DESs leads to higher extraction efficiencies, with EE% of 79.42%, 82.34% and 85.49% for C_8:C_9:C_{12} (3:1:1), C_8:C_9:C_{12} (2:1:1) and C_8:C_9:C_{12} (1:1:1), respectively. The same trend can be observed for the ternary DES C_8:C_{10}:C_{12} with EE% of 77.75%, 79.45% and 82.77% for C_8:C_{10}:C_{12} (3:1:1), C_8:C_{10}:C_{12} (2:1:1) and C_8:C_{10}:C_{12} (1:1:1), respectively. Moreover, comparing the EE% of the C_8:C_9:C_{12} and C_8:C_{10}:C_{12} ternary DES prepared in the same proportions, it can be concluded that the addition of C_9 as a third component provides an higher increase of the EE% than the addition of C_{10}. However, all these extraction efficiencies are still lower than that obtained for the binary DES C_9:C_{12}, with a value of 88.32%. Nevertheless, it is still possible to further tune this EE% by the addition of C_{10}, by the maximum EE% of 91.42% obtained for C_9:C_{10}:C_{12} (3:1:1). In fact, all the ternary DES C_9:C_{10}:C_{12} yield higher efficiencies than the two other ternary DES families.

Passos, et al.[29] also studied the Bisphenol A extraction from aqueous media using in aqueous biphasic systems composed of 15 wt% of K_3PO_4 + 25 wt%

of imidazolium or ammonium-based ionic liquid + 60 wt% of aqueous phase, obtaining extraction efficiencies that reached 100% of extraction, in most systems, in one single step. Nevertheless, the cross contamination of the water phase is not negligible, since the composition of the salt rich phase in equilibrium with the ionic liquid rich phase is 22.00 wt % of K_3PO_4 and 25.00 wt % of ionic liquid, thus leading to an additional environmental problems. On the other hand, the fatty acid-based DESs here proposed are all immiscible with water, not requiring the addition of salting out agents, making them more economic and environmentally friendly. Since the proposed DESs present very low solubility in water, minimal cross contamination of the aqueous phase occurs, as can be seen by NMR spectra. It should be remarked that in this work the recovery of BPA from DESs has not been addressed, however it can be easily recovered from the DESs phase, by the addition of water as anti-solvent and changes in pH, which lead to the pollutant precipitation and allow the DES reuse with minimal losses, as previously reported.[40-41]

It was demonstrated that the alternative process herein proposed is greener, safer, nontoxic and more economical than ionic liquid-based aqueous biphasic systems and it illustrates the potential of the fatty acids DES solvents in the removal of persistent pollutants from water environments.

4. Conclusions

In this work, new low viscous hydrophobic DES containing only fatty acids, that can act as hydrogen bond donors and acceptors simultaneously, are presented and studied for the first time. These new solvents are eutectic mixtures composed of several fatty acids, namely octanoic, nonanoic, decanoic and dodecanoic acid, in different proportions. These hydrophobic solvents were designed to be chemically stable when in contact with water environments, which was proved using NMR spectroscopy.

Densities and viscosities were studied in the range of temperatures from 20 ºC to 80 ºC, for dried and water saturated DESs. Contrary to common deep eutectic solvents, these new eutectic solvents presented the lowest viscosities ever obtained (2 to 14 mPa.s) for this class of solvents and densities lower than water, independently of the water content.

These new fatty acids based DESs have a privileged place among the green solvents, since they are immiscible in water and still have low viscosity. In addition, taking advantage of their hydrophobic character and the low viscosity, which allows quick and efficient mass transfer between the two phases in equilibrium, these DESs were used to extract one micropollutant, Bisphenol-A, from water, illustrating one of the major concern of our society, clean water. Very high 1-step extraction efficiencies (around 92%) obtained for binary and ternary fatty acid-based DESs, showing that it is possible to tune the extraction efficiencies by introducing new components into the DESs.

5. Acknowledgements

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6. Supplementary Information

6.1. Phase Diagram Determination

Table S1. Experimental solid-liquid equilibria data of fatty acid-based DESs at atmospheric pressure.

x_{acid}	T_m / ºC	x_{acid}	T_m / ºC	x_{acid}	T_m / ºC
Octanoic acid		**Nonanoic acid**		**Decanoic acid**	
0.000	16.50	0.000	12.60	0.000	31.60
0.100	12.00	0.143	12.00	0.147	25.00
0.143	11.00	0.250	9.00	0.200	22.00
0.200	10.00	0.333	12.00	0.270	20.00
0.250	9.00	0.435	15.00	0.333	18.00
0.333	12.00	0.500	21.00	0.435	20.00
0.435	17.00	0.565	26.00	0.500	23.00
0.500	21.00	0.667	29.00	0.565	25.00
0.600	26.00	0.750	32.00	0.667	30.00
0.667	28.00	0.857	37.00	0.750	35.00
0.750	34.00	1.000	43.20	0.857	40.00
0.833	37.00			1.000	43.20
0.875	40.00				
1.000	43.20				

6.2. Thermophysical Characterization

Table S2. Experimental densities of the studied dried and saturated DESs as function of temperature.

	Density (ρ / kg·m-3)					
	Dried			Saturated		
T **(ºC)**	**C_8:C_{12}**	**C_9:C_{12}**	**C_{10}:C_{12}**	**C_8:C_{12}**	**C_9:C_{12}**	**C_{10}:C_{12}**
20	904	901	898	907	903	898
25	901	897	894	903	899	895
30	897	893	889	899	895	891
35	893	890	886	895	891	887
40	889	886	882	891	888	883
45	885	882	879	887	884	880
50	881	878	875	883	880	876
55	877	874	871	879	876	872
60	873	870	867	875	872	868
65	869	866	864	871	868	864
70	865	863	860	867	864	861
75	861	859	856	863	860	857
80	858	855	852	859	856	853

Table S3. Parameters, *a* and *b,* of Equation (2) and respective correlation coefficient (R^2), describing temperature dependence of density of the studied DESs.

	DESs	***a*** **(g·cm^{-3})**	***b*** **(x10^{-4}) (g·cm^{-3}·K)**	**R^2**
Dried	**C_8: C_{12}**	1.133	-7.812	1
	C_9: C_{12}	1.127	-7.703	1
	C_{10}: C_{12}	1.120	-7.592	0.999
Saturated	**C_8: C_{12}**	1.139	-7.915	1
	C_9: C_{12}	1.133	-7.824	1
	C_{10}: C_{12}	1.121	-7.585	1

Table S4. Experimental values of the dynamic viscosity of the studied dried and saturated DESs as function of temperature.

	Viscosity (η / mPa·s)					
	Dried			Saturated		
T **(°C)**	C_8:C_{12}	C_9:C_{12}	C_{10}:C_{12}	C_8:C_{12}	C_9:C_{12}	C_{10}:C_{12}
20	8.223	10.115	12.886	8.969	10.787	13.387
25	7.085	8.636	10.756	7.668	9.148	11.280
30	6.158	7.443	9.199	6.613	7.836	9.579
35	5.394	6.470	7.939	5.749	6.775	8.217
40	4.759	5.669	6.909	5.033	5.904	7.107
45	4.227	5.003	6.059	4.435	5.178	6.196
50	3.777	4.444	5.349	3.932	4.571	5.439
55	3.393	3.971	4.753	3.507	4.063	4.786
60	3.063	3.567	4.247	3.143	3.613	4.246
65	2.779	3.221	3.815	2.831	3.241	3.786
70	2.531	2.921	3.443	2.560	2.921	3.392
75	2.314	2.660	3.121	2.325	2.644	3.051
80	2.124	2.432	2.839	2.119	2.401	2.752

Table S5. Fitted parameters of VFT model given by Equation (3) and respective correlation coefficient (R^2) for DESs.

		Parameters			
	DESs	A_η **(mPa.s)**	B_η **(K)**	C_η **(K)**	R^2
Dried	C_8: C_{12}	-3.075	880.4	123.2	1
	C_9: C_{12}	-2.999	870.8	129.2	1
	C_{10}: C_{12}	-2.809	824.7	139.2	1
Saturated	C_8: C_{12}	-3.561	1033	113.6	1
	C_9: C_{12}	-3.594	1068	114.3	1
	C_{10}: C_{12}	-3.773	1159	111.2	1

Table S6. Activation energy values (E_a (KJ/mol)) for dried and saturated DESs as a function of temperature, determined by equation (4).

	Ea (KJ/mol)					
	Dried			Saturated		
***T* (ºC)**	**C_8:C_{12}**	**C_9:C_{12}**	**C_{10}:C_{12}**	**C_8:C_{12}**	**C_9:C_{12}**	**C_{10}:C_{12}**
20	21.8	23.2	24.9	22.9	23.9	25.0
25	21.3	22.6	24.1	22.4	23.4	24.5
30	20.8	22.0	23.5	22.0	22.9	24.0
35	20.3	21.5	22.8	21.6	22.4	23.6
40	19.9	21.0	22.2	21.2	22.0	23.2
45	19.5	20.5	21.7	20.8	21.6	22.8
50	19.1	20.1	21.2	20.4	21.3	22.4
55	18.8	19.7	20.7	20.1	20.9	22.0
60	18.4	19.3	20.2	19.8	20.6	21.7
65	18.1	19.0	19.8	19.5	20.3	21.4
70	17.8	18.6	19.4	19.2	20.0	21.1
75	17.5	18.3	19.0	18.9	19.7	20.8
80	17.3	18.0	18.7	18.7	19.4	20.5

6.3. Liquid-liquid Extractions

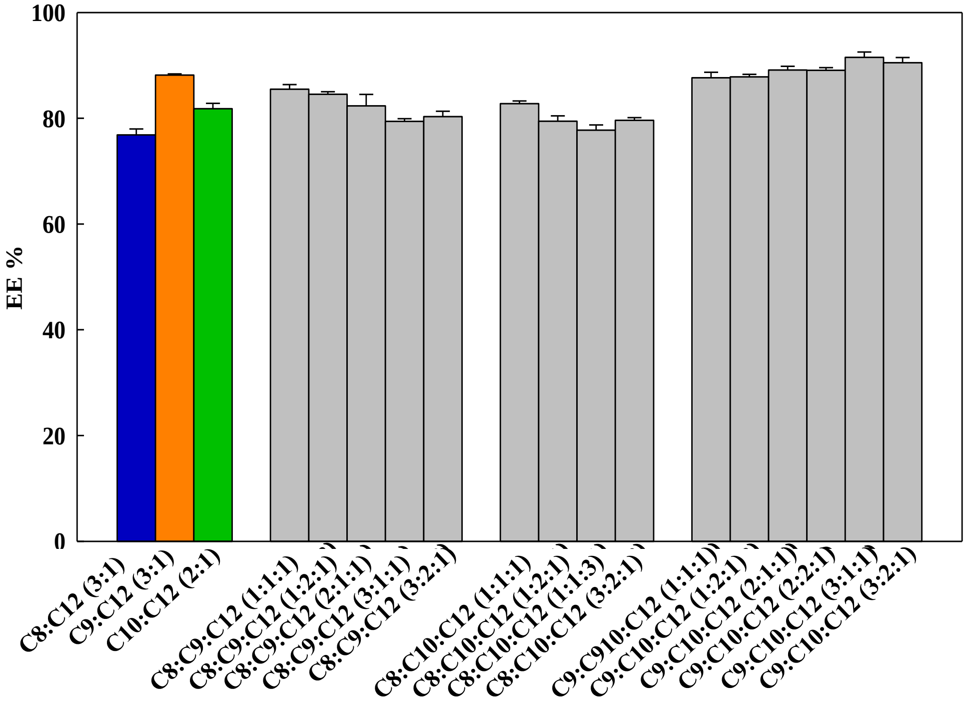


Figure S1. Comparison of one-step extraction efficiencies (% EE) of Bisphenol A using binary and ternary fatty acid-based DESs developed in this work (stirring speed = 300 rpm, ratio DES/Water = 1:1, temperature = 25 ºC, mixing time = 15 min).

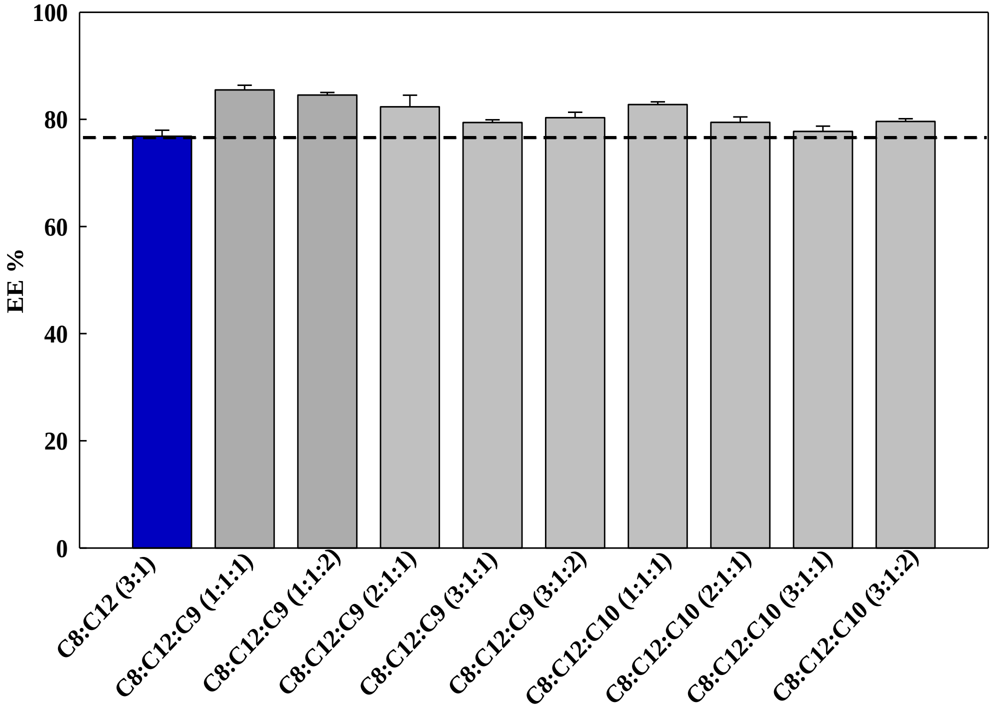


Figure S2. Comparison of the extraction efficiencies (EE%) of one-step extraction of Bisphenol A of the binary fatty acid-based DES (C_8:C_{12}) with the corresponding ternary DESs (stirring speed = 300 rpm, ratio DES/Water = 1:1, temperature = 25 ºC, mixing time = 15 min).

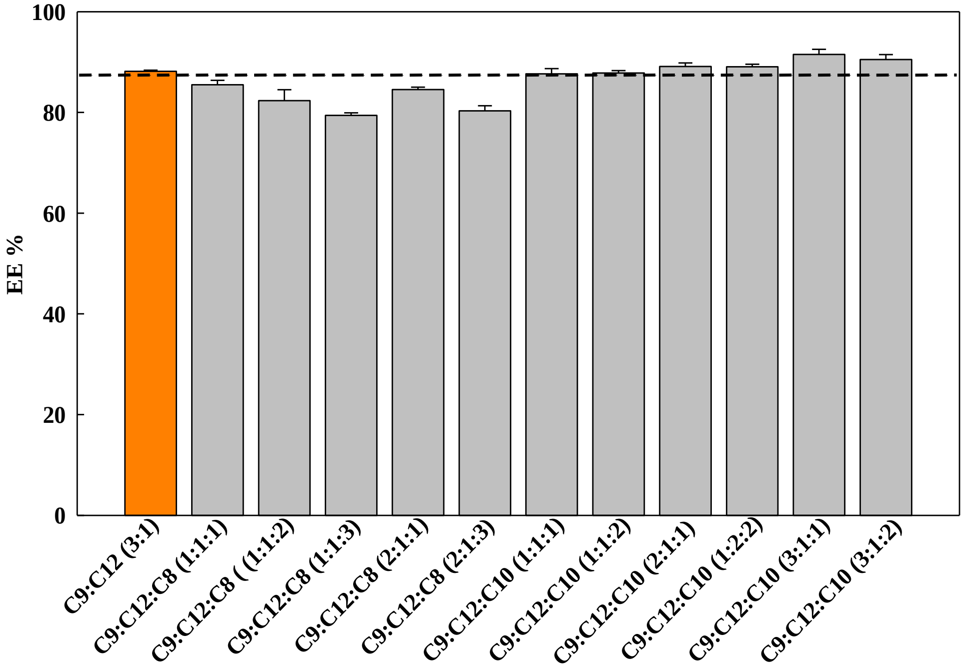


Figure S3. Comparison of the extraction efficiencies (EE%) of one-step extraction of Bisphenol A of the binary fatty acid-based DES (C_9:C_{12}) with the corresponding ternary DESs (stirring speed = 300 rpm, ratio DES/Water = 1:1, temperature = 25 ºC, mixing time = 15 min).

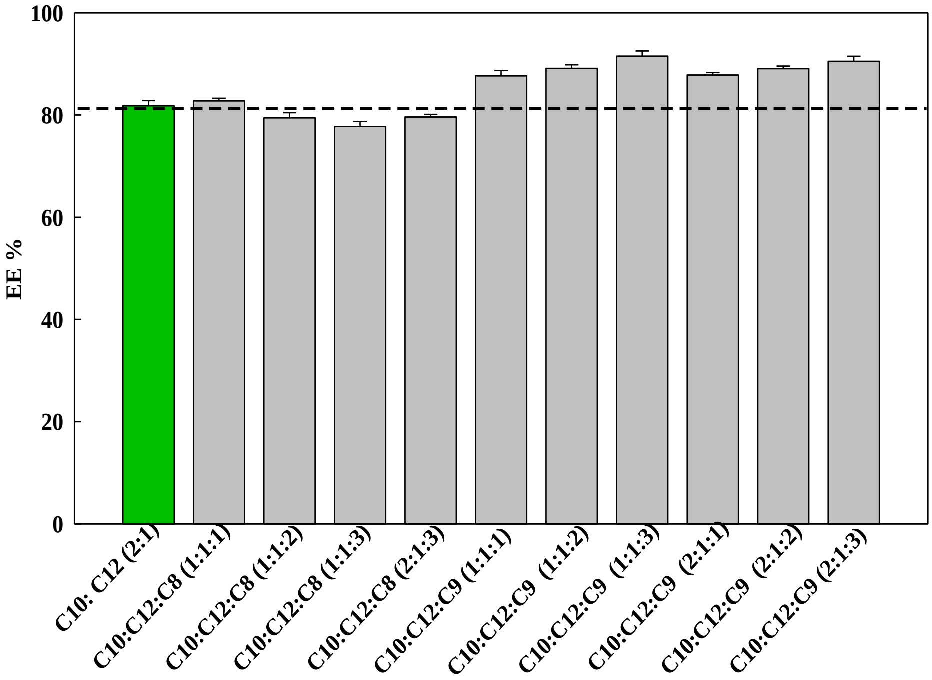


Figure S4. Comparison of the extraction efficiencies (EE%) of one-step extraction of Bisphenol A of the binary fatty acid-based DES (C_{10}:C_{12}) with the corresponding ternary DESs (stirring speed = 300 rpm, ratio DES/Water = 1:1, temperature = 25 ºC, mixing time = 15 min).

6.4. Characterization of DES using NMR spectroscopy

C_8 acid: C_{12} acid Eutectic Solvent

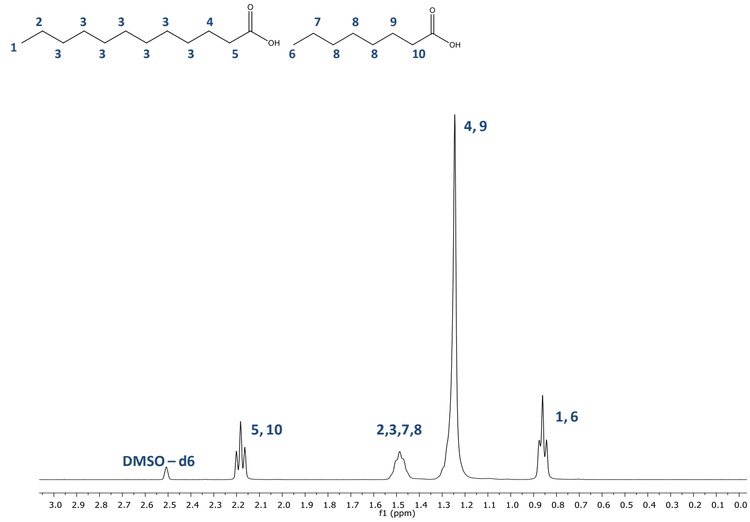


Figure S5. ^{1}H NMR spectra of C_8 acid: C_{12} acid eutectic solvent in DMSO. The structure and numbering of both compounds is also depicted.

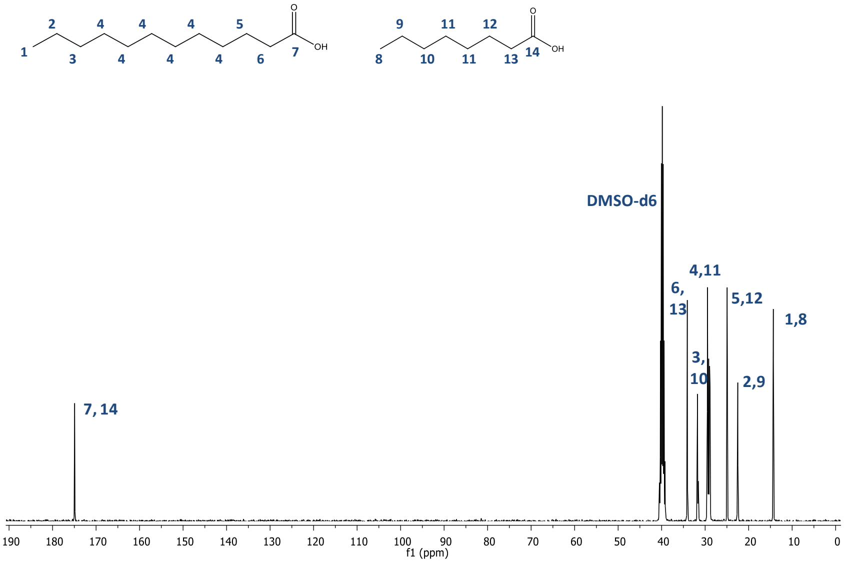


Figure S6. ^{13}C NMR spectra of C_8 acid: C_{12} acid eutectic solvent in DMSO. The structure and numbering of both compounds is also depicted.

C_9 acid: C_{12} acid Eutectic Solvent

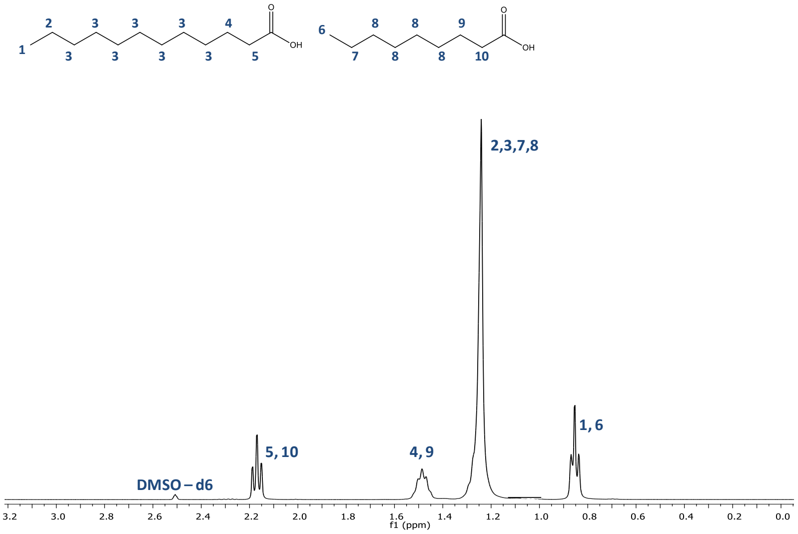


Figure S7 - ^1H NMR spectra of C_9 acid: C_{12} acid eutectic solvent in DMSO. The structure and numbering of both compounds is also depicted.

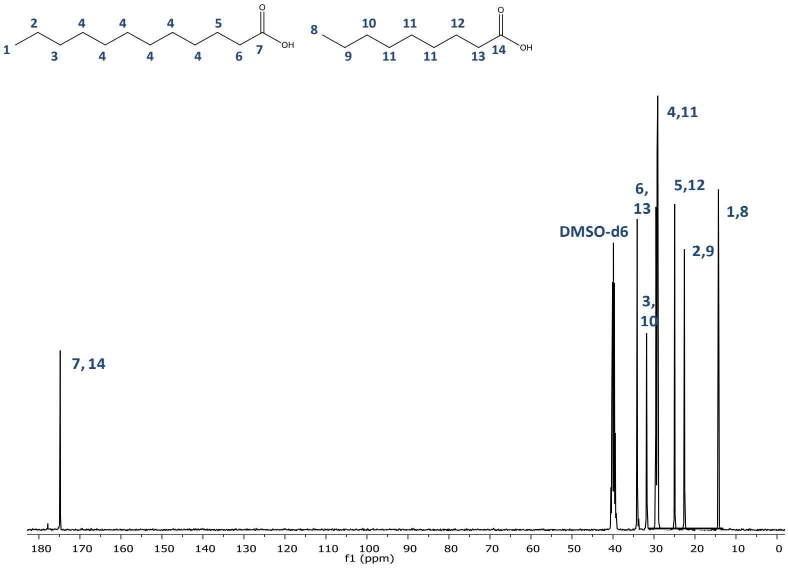


Figure S8 – ^{13}C NMR spectra of C_9 acid: C_{12} acid eutectic solvent in DMSO. The structure and numbering of both compounds is also depicted.

C_{10} acid: C_{12} acid Eutectic Solvent

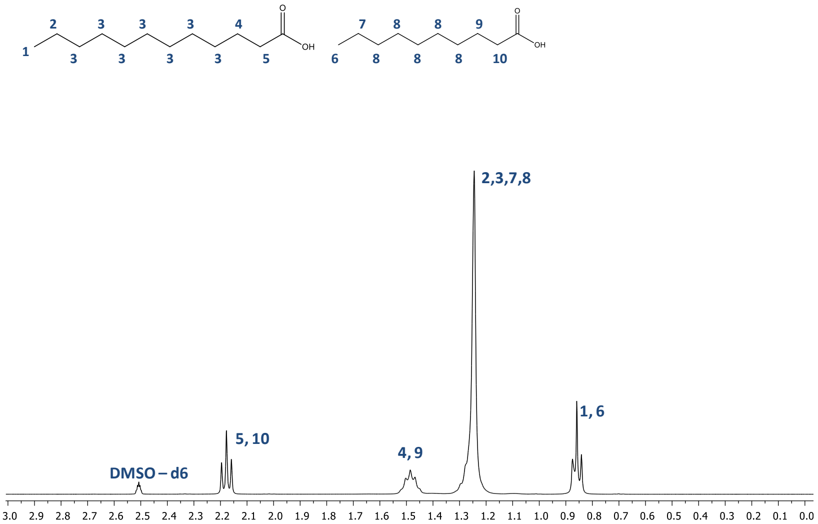


Figure S9 - ^{1}H NMR spectra of C_{10} acid: C_{12} acid eutectic solvent in DMSO. The structure and numbering of both compounds is also depicted.

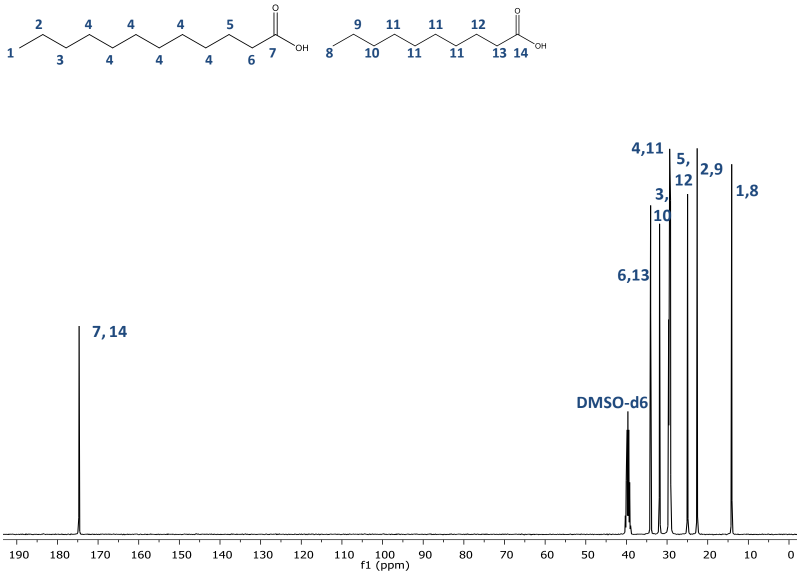


Figure S10 – ^{13}C NMR spectra of C_{10} acid: C_{12} acid eutectic solvent in DMSO. The structure and numbering of both compounds is also depicted.

SI5: NMR spectra of the water -rich phase after the extraction experiments using DES

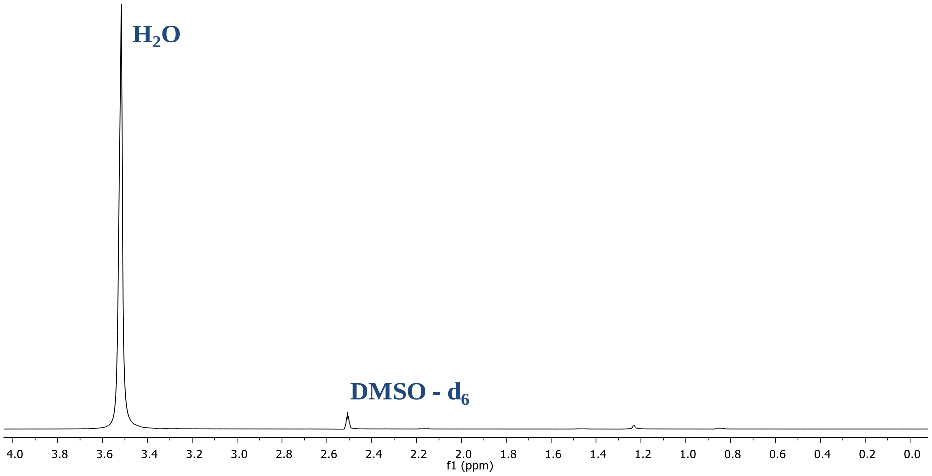


Figure S11 – ^{1}H NMR spectra of water- rich phase of LLE using C_8 acid: C_{12} acid eutectic solvent dissolved in DMSO.

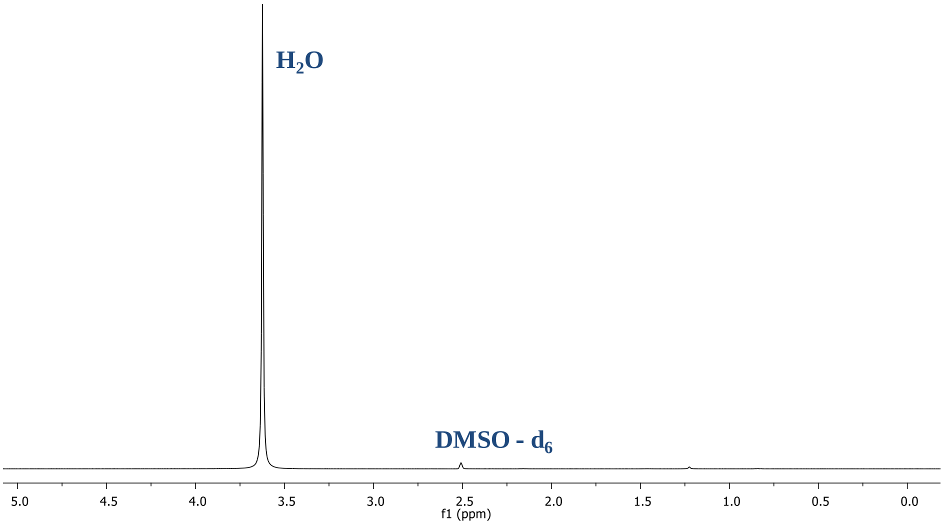


Figure S12 – ^{1}H NMR spectra of water- rich phase of LLE using C_9 acid: C_{12} acid eutectic solvent dissolved in DMSO.

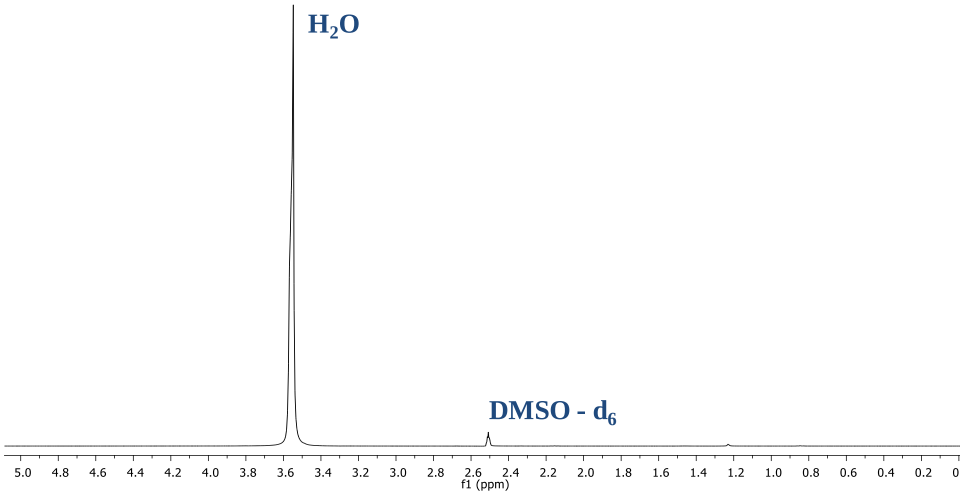


Figure S13 – ^{1}H NMR spectra of water- rich phase of LLE using C_{10} acid: C_{12} acid eutectic solvent dissolved in DMSO.

7. References

1. Wasserscheid, P., *Handbook of Green Chemistry: Ionic liquids. Vol. 6 : Green solvents*. Wiley-VCH-Verlag: 2010.

2. Anastas, P. T.; Li, C. J., *Green Solvents: Reactions in Water*. Wiley: 2014.

3. Anastas, P. T.; Leitner, W.; Jessop, P. G., *Green Solvents: Supercritical Solvents*. Wiley: 2014.

4. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R. L.; Duarte, A. R. C., Natural Deep Eutectic Solvents – Solvents for the 21st Century. *ACS Sustainable Chemistry & Engineering* 2014, *2* (5), 1063-1071.

5. Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep Eutectic Solvents (DESs) and Their Applications. *Chemical Reviews* 2014, *114* (21), 11060-11082.

6. Abo-Hamad, A.; Hayyan, M.; AlSaadi, M. A.; Hashim, M. A., Potential applications of deep eutectic solvents in nanotechnology. *Chemical Engineering Journal* 2015, *273* (Supplement C), 551-567.

7. Zhao, H., DNA stability in ionic liquids and deep eutectic solvents. *Journal of Chemical Technology & Biotechnology* 2015, *90* (1), 19-25.

8. del Monte, F.; Carriazo, D.; Serrano, M. C.; Gutiérrez, M. C.; Ferrer, M. L., Deep Eutectic Solvents in Polymerizations: A Greener Alternative to Conventional Syntheses. *ChemSusChem* 2014, *7* (4), 999-1009.

9. García-Álvarez, J., Deep Eutectic Mixtures: Promising Sustainable Solvents for Metal-Catalysed and Metal-Mediated Organic Reactions. *European Journal of Inorganic Chemistry* 2015, *2015* (31), 5147-5157.

10. Vidal, C.; García-Álvarez, J.; Hernán-Gómez, A.; Kennedy, A. R.; Hevia, E., Exploiting Deep Eutectic Solvents and Organolithium Reagent Partnerships: Chemoselective Ultrafast Addition to Imines and Quinolines Under Aerobic Ambient Temperature Conditions. *Angewandte Chemie International Edition* 2016, *55* (52), 16145-16148.

11. Chapter 7 Room-Temperature Ionic Liquids and Eutectic Mixtures. In *Alternative Solvents for Green Chemistry (2)*, The Royal Society of Chemistry: 2013; pp 175-209.

12. Li, X.; Row, K. H., Development of deep eutectic solvents applied in extraction and separation. *Journal of Separation Science* 2016, *39* (18), 3505-3520.

13. Choi, Y. H.; van Spronsen, J.; Dai, Y.; Verberne, M.; Hollmann, F.; Arends, I. W. C. E.; Witkamp, G.-J.; Verpoorte, R., Are Natural Deep Eutectic Solvents the Missing Link in Understanding Cellular Metabolism and Physiology? *Plant Physiology* 2011, *156* (4), 1701-1705.

14. Zhang, Q.; De Oliveira Vigier, K.; Royer, S.; Jerome, F., Deep eutectic solvents: syntheses, properties and applications. *Chemical Society Reviews* 2012, *41* (21), 7108-7146.

15. Mjalli, F. S.; Al-Hajri, R.; Al-Muhtaseb, A. a.; Ahmed, O.; Nagaraju, M., Novel amino acid-based ionic liquid analogues: neutral hydroxylic and sulfur-containing amino acids. *Asia-Pacific Journal of Chemical Engineering* 2016, *11* (5), 683-694.

16. Hayyan, A.; Mjalli, F. S.; AlNashef, I. M.; Al-Wahaibi, T.; Al-Wahaibi, Y. M.; Hashim, M. A., Fruit sugar-based deep eutectic solvents and their physical properties. *Thermochimica Acta* 2012, *541*, 70-75.

17. Abbott, A. P.; Harris, R. C.; Ryder, K. S.; D'Agostino, C.; Gladden, L. F.; Mantle, M. D., Glycerol eutectics as sustainable solvent systems. *Green Chemistry* 2011, *13* (1), 82-90.

18. Florindo, C.; Oliveira, F. S.; Rebelo, L. P. N.; Fernandes, A. M.; Marrucho, I. M., Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids. *ACS Sustainable Chemistry & Engineering* 2014, *2* (10), 2416-2425.

19. Imperato, G.; Eibler, E.; Niedermaier, J.; Konig, B., Low-melting sugar-urea-salt mixtures as solvents for Diels-Alder reactions. *Chemical Communications* 2005, (9), 1170-1172.

20. Liu, P.; Hao, J.-W.; Mo, L.-P.; Zhang, Z.-H., Recent advances in the application of deep eutectic solvents as sustainable media as well as catalysts in organic reactions. *RSC Advances* 2015, *5* (60), 48675-48704.

21. García, G.; Aparicio, S.; Ullah, R.; Atilhan, M., Deep Eutectic Solvents: Physicochemical Properties and Gas Separation Applications. *Energy & Fuels* 2015, *29* (4), 2616-2644.

22. van Osch, D. J. G. P.; Zubeir, L. F.; van den Bruinhorst, A.; Rocha, M. A. A.; Kroon, M. C., Hydrophobic deep eutectic solvents as water-immiscible extractants. *Green Chemistry* 2015, *17* (9), 4518-4521.

23. Florindo, C.; Branco, L. C.; Marrucho, I. M., Development of hydrophobic deep eutectic solvents for extraction of pesticides from aqueous environments. *Fluid Phase Equilibria* 2017, *448* (Supplement C), 135-142.

24. Ribeiro, B. D.; Florindo, C.; Iff, L. C.; Coelho, M. A. Z.; Marrucho, I. M., Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. *ACS Sustainable Chemistry & Engineering* 2015, *3* (10), 2469-2477.

25. Zhang, Z.; Yuan, Y.; Zhang, N.; Cao, X., Thermophysical Properties of Some Fatty Acids/Surfactants as Phase Change Slurries for Thermal Energy Storage. *Journal of Chemical & Engineering Data* 2015, *60* (8), 2495-2501.

26. Sharma, A.; Tyagi, V. V.; Chen, C. R.; Buddhi, D., Review on thermal energy storage with phase change materials and applications. *Renewable and Sustainable Energy Reviews* 2009, *13* (2), 318-345.

27. Farid, M. M.; Khudhair, A. M.; Razack, S. A. K.; Al-Hallaj, S., A review on phase change energy storage: materials and applications. *Energy Conversion and Management* 2004, *45* (9), 1597-1615.

28. Richardson, S. D.; Ternes, T. A., Water Analysis: Emerging Contaminants and Current Issues. *Analytical Chemistry* 2014, *86* (6), 2813-2848.

29. Passos, H.; Sousa, A. C. A.; Pastorinho, M. R.; Nogueira, A. J. A.; Rebelo, L. P. N.; Coutinho, J. A. P.; Freire, M. G., Ionic-liquid-based aqueous biphasic systems for improved detection of bisphenol A in human fluids. *Analytical Methods* 2012, *4* (9), 2664-2667.

30. Tickner, J. A., Science of problems, science of solutions or both? A case example of bisphenol A. *Journal of Epidemiology and Community Health* 2011, *65* (8), 649-650.

31. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *Journal of the American Chemical Society* 2004, *126* (29), 9142-9147.

32. Crespo, E. A.; Silva, L. P.; Martins, M. A. R.; Fernandez, L.; Ortega, J.; Ferreira, O.; Sadowski, G.; Held, C.; Pinho, S. P.; Coutinho, J. A. P., Characterization and Modeling of the Liquid Phase of Deep Eutectic Solvents Based on Fatty Acids/Alcohols and Choline Chloride. *Industrial & Engineering Chemistry Research* 2017, *56* (42), 12192-12202.

33. Mehling, H.; Cabeza, L. F., Solid-liquid phase change materials. In *Heat and cold storage with PCM: An up to date introduction into basics and applications*, Springer Berlin Heidelberg: Berlin, Heidelberg, 2008; pp 11-55.

34. Tang, F.; Su, D.; Tang, Y.; Fang, G., Synthesis and thermal properties of fatty acid eutectics and diatomite composites as shape-stabilized phase change materials with enhanced thermal conductivity. *Solar Energy Materials and Solar Cells* 2015, *141*, 218-224.

35. Kenar, J. A., Carbonate phase change materials. Google Patents: 2013.

36. van Osch, D. J. G. P.; Parmentier, D.; Dietz, C. H. J. T.; van den Bruinhorst, A.; Tuinier, R.; Kroon, M. C., Removal of alkali and transition metal ions from water with hydrophobic deep eutectic solvents. *Chemical Communications* 2016, *52* (80), 11987-11990.

37. Chen, Z.; Greaves, T. L.; Warr, G. G.; Atkin, R., Mixing cations with different alkyl chain lengths markedly depresses the melting point in deep eutectic solvents formed from alkylammonium bromide salts and urea. *Chemical Communications* 2017, *53* (15), 2375-2377.

38. Xie, Y.; Dong, H.; Zhang, S.; Lu, X.; Ji, X., Effect of Water on the Density, Viscosity, and CO2 Solubility in Choline Chloride/Urea. *Journal of Chemical & Engineering Data* 2014, *59* (11), 3344-3352.

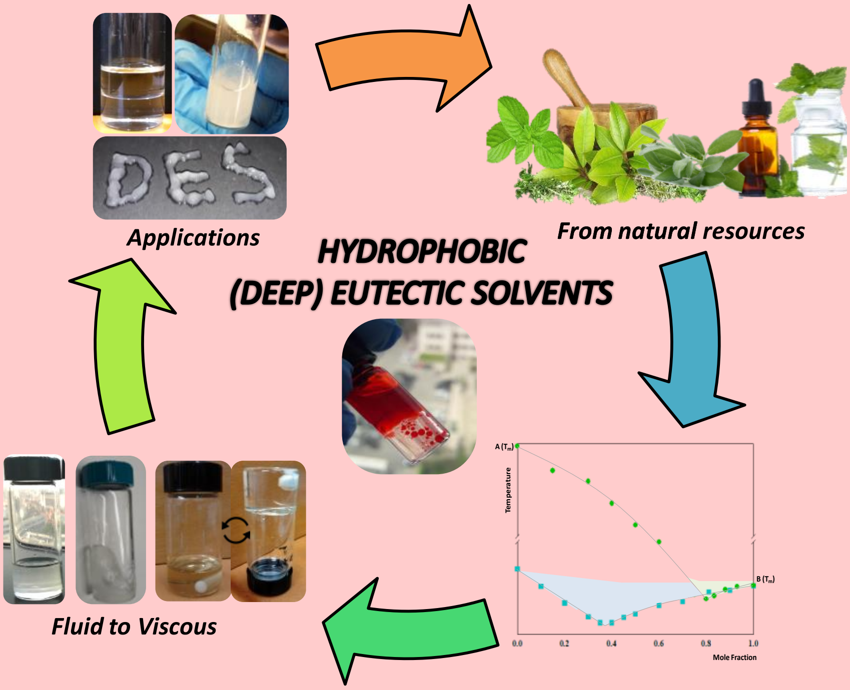
39. Leron, R. B.; Soriano, A. N.; Li, M.-H., Densities and refractive indices of the deep eutectic solvents (choline chloride+ethylene glycol or glycerol) and their aqueous mixtures at the temperature ranging from 298.15 to 333.15K. *Journal of the Taiwan Institute of Chemical Engineers* 2012, *43* (4), 551-557.

40. Almeida, H. F. D.; Freire, M. G.; Marrucho, I. M., Improved extraction of fluoroquinolones with recyclable ionic-liquid-based aqueous biphasic systems. *Green Chemistry* 2016, *18* (9), 2717-2725.

41. Almeida, H. F. D.; Freire, M. G.; Marrucho, I. M., Improved monitoring of aqueous samples by the preconcentration of active pharmaceutical ingredients using ionic-liquid-based systems. *Green Chemistry* 2017, *19* (19), 4651-4659.

CHAPTER 3

FROM CHARACTERIZATION TO WATER APPLICATION OF HYDROPHOBIC DEEP EUTECTIC SOLVENTS


Applications
DES
From natural resources
HYDROPHOBIC
(DEEP) EUTECTIC SOLVENTS
A (T_m)
Temperature
B (T_m)
0.0
0.2
0.4
0.6
0.8
1.0
Mole Fraction
Fluid to Viscous

CHAPTER 3.1

Development of Hydrophobic Deep Eutectic Solvents for Extraction of Pesticides from Aqueous Environments

Adapted from: **C. Florindo**, L. C. Branco, I. M. Marrucho, Development of Hydrophobic Deep Eutectic Solvents for Extraction of Pesticides from Aqueous Environments, Fluid Phase Equilibria (2017), 448, 135-142.

The author contributed to the planning and execution of all the experiments described in this chapter, as well as involved in the discussion and interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

Wastewater treatment plants do not properly address the removal of emerging micropollutants, such as pesticides, and thus these compounds contaminate water sources of public drinking water systems. In this context, this work focuses on the development of hydrophobic deep eutectic solvents (DESs), as cheap extractants for the removal of four neonicotinoids, Imidacloprid, Acetamiprid, Nitenpyram and Thiamethoxam, from diluted aqueous solutions. In particular, two different families of DESs, one based on natural neutral ingredients (DL-Menthol and natural organic acids) and the other based on quaternary ammonium salts and organic acids were prepared and their water stability carefully studied through ^{1}H NMR. Only the chemically stable DESs were selected to be used as solvents in the extraction of the four neonicotinoids so that no contamination of the water cycle is attained, while reuse of the DES is possible. The final results were compared with those obtained for liquid-liquid extraction using hydrophobic imidazolium-based ionic liquids as solvents.

1. Introduction

One of the major problems of modern society is to be able to provide clean water to everyone, or in another words, to develop efficient wastewater treatment processes and simultaneously reduce the hazardousness of the current pollutants present in different kinds of wastewater as a result of domestic, industrial and agricultural water activities.[1] Micropollutants have been emerging as a new class of pollutants since, despite their low concentrations, ranging from ng/L to µg/L, their presence can be linked to a several negative effects on the health of animals and humans, namely short-term and long-term toxicity, endocrine disrupting effects and antibiotic resistance of microorganisms.[2-4] Due to their trace concentrations in surface water, their detection has been hampered by the detection limits of the most used analytical techniques and only recent advances on these non-specific techniques allowed their detection.[3] Heavy metals, pharmaceuticals, personal care products, dyes and pesticides[5] are all classified as micropollutants.

According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent pollutants are pesticides.[6-7] In order to ensure environmental and health safety, the EU issued a list of priority substances under the European Water Framework Directive.[8] Pesticides are a unique group of synthetically chemicals designed to fight pests into the environment and consequently improve agricultural production. These compounds are commonly toxic for living organisms and recalcitrant, being toxic agents with persistent bioaccumulative effects.[9] The use of pesticides also constitutes a risk for water quality in agricultural areas due to the fact that these components may pass through the soil and subsoil and pollute surface waters and groundwater.[10-11] In particular, neonicotinoids have been among the most popular and widely used pesticides around the world.[12-13] Neonicotinoids are generation of nicotine-related insecticides that present simultaneously high target specificity to insects and a relatively low risk for non-target mammalian species and the environment, combined with versatile application methods.[14] However, in 2012, some insecticides belonging to group of neonicotinoids showed high risks for bees, and immediately some were banned from the US market as well as in some countries of EU, more precisely Germany, Italy and France. Environmental Protection Agency (EPA) is carrying out an exhaustive risk assessment of all neonicotinoids, which should be completed by 2018. Meanwhile, tens of millions of hectares of farmland are still treated with neonicotinoids each year. Although conventional Drinking Water Treatment Plant (DWTP) multistep treatment processes use advanced technologies as adsorption, membrane filtration, ozonation and chlorination, none was specifically designed to remove pesticides and thus are not effective.[15-16] Modifications of these processes and the inclusion of new ones, advanced oxidation, biological degradation, photocatalytic degradation, have been proposed to achieve the efficient removal of pesticides from water sources.[17-18] Despite the relative advantages and disadvantages of each one of these processes, adsorption is still the most widely used technology for micropollutants removal at wastewater treatment plants, not only due to its easy design and operation, but also the moderate/high removal of many types of pollutants.[19] Due to its mechanical stability, high adsorption capacity and fast adsorption rate, activated carbon is the most common adsorbent used in wastewater treatment[20] and its performance in the removal of pesticides from aqueous streams has been evaluated.[10] Nevertheless, the wide diversity of pesticides, with different chemical functionalities and structures, is a true challenge for this non-specific process.[21-22] As a result, alternative techniques and materials for large-scale use in water decontamination processes have been considered. Among them, Ionic Liquids (ILs) have been used as task specific solvents or in the preparation of task specific materials for the effective extraction of pesticides. Although variety of extraction strategies, such as aqueous biphasic systems,[23] liquid membranes[24-25] and adsorption,[26,27] have been tested, ILs's high cost and difficult purification, and thus recycling, hinder their application in wastewater treatment.

Deep eutectic solvents (DESs) were introduced as analogues and alternative green solvents to the conventional ILs, with the advantage of easy preparation with high purities and low cost.[28-29] By definition, DESs result from the establishment of specific interactions, mainly hydrogen bonds, between two compounds, rendering a new chemical entity with a melting point lower than that of the initial compounds. Most of the DESs proposed so far in the open literature have an hydrophilic character and thus are unstable in water,[30-31] leading to the separation of both components. In our previous work,[32] DL-Menthol and several natural acids were used to prepare hydrophobic DES for the extraction of biomolecules from aqueous media.

Kroon's group[33] also reported the preparation of hydrophobic DES using several quaternary ammonium salts and decanoic acid and their use in the recovery of volatile fatty acids from diluted aqueous solutions. Hydrophobic DESs based on lidocaine and decanoic acid in various proportions were also proposed by Kroon's group for the removal of metal ions from non-buffered water.[34] These two groups showed the hydrophobicity of DES by mixing them with water, and observing the distinct formation of two phases in equilibrium.

In this work, we give another step in the development and understanding of hydrophobic DESs behavior in water so that they can be used in the extraction and concentration of solutes, in particular neonicotinoids, from dilute aqueous solutions. For that purpose, two different families of hydrophobic DES were selected: one based on DL-Menthol and the other based on tetrabutylammonium chloride salt. Both these hydrogen bond acceptors (HBAs) were combined with acids with different alkyl chain lengths and functionalities, which will act as hydrogen bond donors (HBDs), in several proportions, so that a liquid DES at room temperature could be obtained. The chemical stability of the prepared hydrophobic DES was evaluated and only the water stable DESs were tested in the extraction of four neonicotinoids. The re-use of the DES was evaluated. The attained results were compared with those obtained when hydrophobic ILs were used.

2. Experimental Section

2.1. Materials

DL-menthol (purity ≥ 95%), tetrabutylammonium chloride ($N_{4444}Cl$) (purity ≥ 97%), acetic acid (purity ≥ 99.7%), pyruvic acid (purity ≥ 98%), levulinic acid (purity ≥ 99%), butyric acid (purity ≥ 98%), hexanoic acid (purity ≥ 98%) octanoic acid (purity ≥ 98%), decanoic acid (purity ≥ 98%) and dodecanoic acid (purity > 98%) were purchased from Sigma-Aldrich and used as received in the preparation of hydrophobic deep eutectic solvents. Chemical structures of DESs and their respective acronyms are depicted in Figure 1.

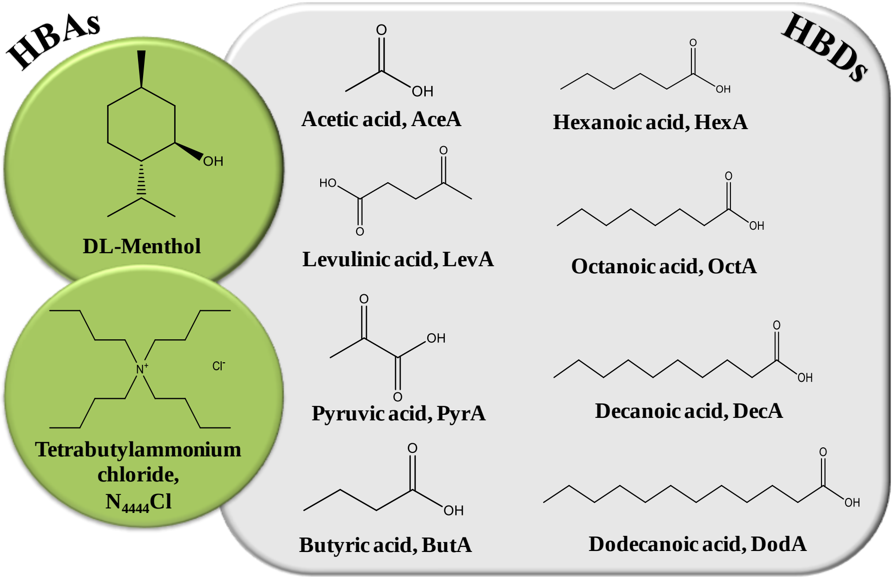


Figure 1. Chemical structures and respective acronyms of deep eutectic solvents studied in this work.

Ionic liquids, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_2MIM][NTf_2]$), 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_4MIM][NTf_2]$) and 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_6MIM][NTf_2]$) were purchased from Iolitec with mass fraction purities more than 0.98, and were used as supplied.

All pesticides used namely Imidacloprid, Thiamethoxam, Acetamiprid and Nitenpyram (all ≥ 99 % mass fraction purity) were purchased from Sigma Aldrich and used with no further purification. Chemical structure and the respective acronym of the neonicotinoids used in this work are presented in Figure 2. The aqueous solutions were prepared using high purity water (Milli-Q water) with a specific conductance <0.1 mS/cm.

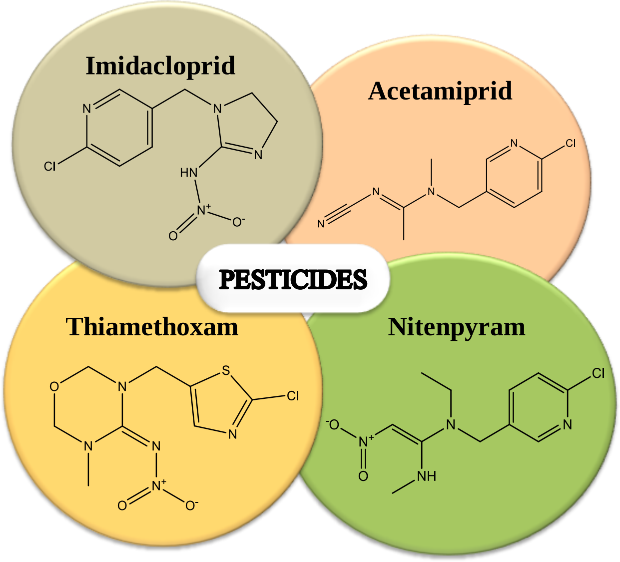


Figure 2. Chemical structures of pesticides studied in this work.

2.2. Methods

2.2.1. Preparation of DESs

All DESs were prepared according to the methodology proposed in our previous work,[32] by mixing the two components (HBA and HBD) in a jacketed glass vessel with a mechanical stirrer, at 350 rpm and 353.15 K until a homogeneous liquid appeared and no solid is observed. DESs tested in this work were prepared with the following molar ratios: DL-Menthol: Acetic acid (1:1), DL-Menthol: Levulinic acid (1:1), DL-Menthol: Pyruvic acid (1:2), DL-Menthol: Butyric acid (1:1), DL-Menthol: Hexanoic acid (1:1), DL-Menthol: Octanoic acid (1:1), DL-menthol: Decanoic acid (1:1), DL-menthol: Dodecanoic acid (2:1), $N_{4444}Cl$: Acetic acid (1:1), $N_{4444}Cl$: Levulinic acid (1:2), $N_{4444}Cl$: Hexanoic acid (1:2), $N_{4444}Cl$: Octanoic acid (1:2), $N_{4444}Cl$: Decanoic acid (1:2). In Figure 1, the chemical structures of all DESs prepared in this work, and their respective acronyms are depicted.

In the preparation of DESs, an analytical high-precision balance with an uncertainty of ± 10^{-5} g, by weighing known masses of each component into bottles with caps, was used. The HBAs (DL-Menthol and tetrabutylammonium chloride, $N_{4444}Cl$) were first dried in a high vacuum pump at 40 °C for at least four days, while the carboxylic acids were used without any further purification.

2.2.2. NMR Measurements

^{1}H NMR and ^{13}C NMR spectra were carried out on a Bruker AVANCE 400 spectrometer operated at room temperature with 16 and 500 scans, respectively. DESs samples were prepared on 5 mm NMR tubes using approximately 30 mg of DES and then adding 0.5 mL of deuterated dimethyl sulfoxide (DMSO-d6), and then the sample was homogenised by vortex mixing.

2.2.3. Liquid-liquid extraction procedure

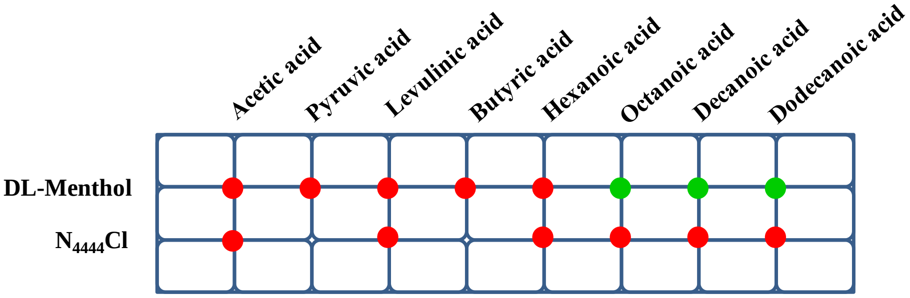
Liquid-liquid extractions were carried out using the prepared hydrophobic DESs as extractants at room temperature. Stock aqueous solutions of pesticides in water were previously prepared. Usually micropollutants are present in water resources at low concentrations, ranging from a ng/L to several µg/L.[10] The relatively higher concentrations of pesticides (0.025 g/L) in the starting aqueous solutions were used in this work, allow the use of a simple detection technique, UV-vis spectroscopic technique, and simultaneously guarantee that the proposed DES's reuse procedure will work for larger number of cycles. A calibration curve was previously established for each one of the pesticides in Milli-Q water (for all curves $R^2 > 0.997$). The pH of the aqueous solutions of each pesticide and each DESs were measured, indicating pH ≈ 7.00 and pH ≈ 5.00, respectively, meaning that all the pesticides are in their protonated form throughout these experiments. An equal mass of each one of the aqueous solutions of pesticides and the prepared hydrophobic DESs was put in contact and vigorously stirred, during

4h at room temperature, and then left to settle for 24h, to ensure complete separation of phases. Subsequently, the both top (DES-rich phase) and bottom (water-rich phase) phases of phases were carefully separated by means of a needled syringe and UV-vis spectroscopy was used to monitor the pesticide concentration. DES-rich phases were very concentrated and thus several dilutions were needed for their analysis through UV-vis. Furthermore, to eliminate possible interferences of DESs and their interaction with pesticides in the quantification, the DES-rich phase was not quantified. Thus, the only phase that could be straight analyzed was water-rich phase of the LLE, since it just contained the residues of pesticides and consequently their characteristic peaks could be easily followed. In order to evaluate the reuse of the DES in the subsequent cycles, after phase separation, the upper or DES-rich phase was exposed to fresh water with pesticides, in the same mass ratio (1:1) for the following extraction cycle. This procedure was repeated four times.

3. Results and Discussion

3.1. Stability of DES in water

As mentioned previously, this work addresses the extraction of micropollutants, in particular four neonicotinoids, imidacloprid, acetamiprid, nitenpyram and thiamethoxam, using hydrophobic DES. Inspired by our previous work on hydrophobic DESs,[32] where DL-menthol and several organic acids are used, and the work conducted by van Osch et al.[33] where hydrophobic DES based on quaternary ammonium salts were described, a matrix of hydrogen bond donors and acceptors to be tested as DES starting materials was set up. These two families of hydrophobic DES are highly fluid compounds at room temperature with viscosities ranging between 11 to 300 mPa.s, clearly overcoming one the major drawbacks of hydrophilic DES, their high viscosities.[32-33] The matrix of all the DESs studied in this work, a total of 15, is presented in Figure 3.



	Acetic acid	Pyruvic acid	Levulinic acid	Butyric acid	Hexanoic acid	Octanoic acid	Decanoic acid	Dodecanoic acid
DL-Menthol	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢
$N_{4444}Cl$	🔴		🔴		🔴	🔴	🔴	🔴

Figure 3. Matrix of deep eutectic solvents studied in this work, based on two hydrogen bond acceptors, DL-Menthol or $N_{4444}Cl$, and different carboxylic acids, as hydrogen bond donors (🔴 – not stable in water and 🟢 – stable in water).

This matrix provides two different types of hydrophobic DES, according to the HBA: charged (based on ammonium salts) and non-charged (based on DL-Menthol). The organic acids were chosen in such a way that a wide variety of acids was included, from small chain acids, such as acetic acid, to long chain acids, such as dodecanoic acid, diacids, such as pyruvic acid, and with substituent groups, such as levulinic acid, where a ketone group is introduced. These hydrophobic DESs based on DL-Menthol and $N_{4444}Cl$ have already been reported and characterized by NMR and FTIR.[32-33] In the present study, some new DESs from the same families but with longer alkyl chain lengths were synthesized and the same characterization, using ^{1}H and ^{13}C NMR, was performed, in order to confirm their structures and final purities, as shown in Figures S1-S6 (presented in *Supplementary Information*).

Despite the fact that the quaternary ammonium- and DL-Menthol-based DESs are non-expensive solvents, it is important to reduce the amount of extractant used while keeping good extraction efficiencies. Therefore, some preliminary tests with different mass phase ratios were investigated. It was verified that the extraction efficiencies increase with the amount of DESs used and thus, the mass ratio of 1:1 of DESs to aqueous solutions of pesticides was used.

Different results were observed when different DES were used as extractants: the characteristic peaks of the pesticides become deformed or changed completely their characteristic wavelength, putting forward the hypothesis the chemical environment of the water phase changed during the extraction. In order to further investigate this fact, ^{1}H NMR studies of both phases were carried out for all the extraction systems. In Figure 4, three examples of ^{1}H NMR spectra of both phases for the systems containing three DESs, DL-Menthol: Acetic acid, DL-Menthol: Dodecanoic acid and $N_{4444}Cl$: Octanoic acid, are presented.

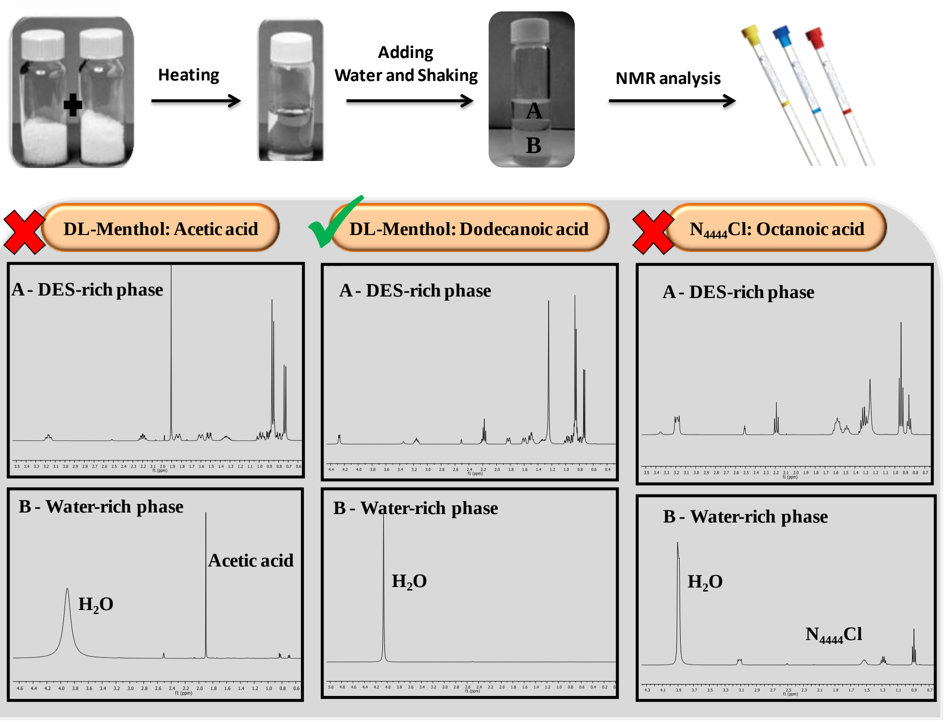


Figure 4. NMR analysis of DES- and water-rich phases after liquid-liquid extraction technique for three selected DESs.

It can clearly be seen in the ^{1}H NMR spectra of the water-rich phase that, when a DES composed of an hydrophilic HBD, such as in the case of DL-Menthol: Acetic acid, is contacted with an aqueous solution, the final aqueous solution, after separation of the phases, is contaminated with the HBD, acetic acid in this case. On the other hand, in the case of $N_{4444}Cl$: Octanoic acid DES, it is the hydrophilic HBA, $N_{4444}Cl$, that leaches to the water-rich phase, as it can be observed in the ^{1}H NMR spectra. However, no traces of the DL-Menthol, in the case DL-Menthol: acetic acid DES, or octanoic acid, in the case of $N_{4444}Cl$: Octanoic acid DES, could be observed in the respective spectra. Also, comparing the ^{1}H NMR of DES before and after being contacted with water, a change in the peaks of hydrophilic compound, namely a decrease in intensity, is found, indicating that the initial proportion between the HBD: HBA is kept after water contact. It can thus be concluded that in the DES formed by one hydrophilic and one hydrophobic compound, such as the case of DL-Menthol: acetic acid (1:1), DL-Menthol: pyruvic acid (1:2), DL-Menthol: hexanoic acid (1:1), DL-Menthol: levulinic acid (1:1), $N_{4444}Cl$: hexanoic acid (1:2), $N_{4444}Cl$: octanoic acid (1:2), $N_{4444}Cl$: decanoic acid (1:2) and $N_{4444}Cl$: dodecanoic acid (1:2), or two hydrophilic compounds, such as $N_{4444}Cl$: acetic acid (1:1) and $N_{4444}Cl$: levulinic acid (1:1), the hydrogen bond(s) responsible for the DES formation are partially broken and the compound(s) solubilise into the water phase according to their individual water solubility. This can also be concluded by comparing the ^{1}H NMR of the DESs without water and analyzing the water-rich phase after mixing with water, where a decrease in the intensity, and consequently on the number of protons (that is proportional to the area peak), of the HBD is observed. Finally, analysing the DL-Menthol: dodecanoic acid DES ^{1}H NMR water-rich phase spectra, no peak other than water can be observed. The ^{1}H NMR spectra for some other examples, including DL-Menthol: acetic acid (1:1), DL-Menthol: levulinic acid (1:1), DL-Menthol: butyric acid (1:1), DL-Menthol: octanoic acid (1:1), DL-Menthol: dodecanoic acid (2:1), $N_{4444}Cl$: octanoic acid (1:2), $N_{4444}Cl$: decanoic acid (1:2) DESs are presented in Table S1 to S7 in *Supplementary Information.*

These facts allow the conclusion that only when the two components of DESs are hydrophobic, water stable DESs is attained. Note, however, that although this does not prevent the use of DES composed of one hydrophilic component in extractions schemes, it contaminates the water solutions and increases the amount of extractant needed since re-utilization and recycling are not feasible. Nevertheless, it should be taken into account that the leaching of the hydrophilic compound is proportional to its hydrophilicity: when DL-Menthol: acetic acid DES is considered, a loss of around 90 % of the acid to the water-rich phase is observed, which decreases to around 20 % for DL-Menthol: butyric acid and finally no loss is observed when DL-Menthol: octanoic acid DES is used. Despite the amount of the hydrophilic component leached to the water-rich phase, the existence of a DES-rich phase throughout the experience can clearly be observed.

The penetration of water molecules in the DESs structure and the disruption of the strong hydrogen bonding pattern among the DESs constituents has already been proposed by other authors.[30, 35] Molecular dynamics studies on hydrophilic choline chloride-based DESs indicate that the addition of water results in an increase of the hydrogen bonding between the HBD and water molecules, at the expense of those among the DES constituents.[36] Also, molecular dynamics studies on the addition of water to Reline (cholinium chloride: urea (1:2)), indicate that the number of urea-urea hydrogen bonds increases with the water content up to 25 %, when the two components of DES become individually highly hydrated with water.[37] For the hydrophobic and water stable DES presented in this work, although no peaks were observed in the water-rich phase for the DESs constituted by two hydrophobic compounds, such as the case of DL-Menthol: octanoic acid, DL-Menthol: decanoic acid and DL-Menthol: dodecanoic acid, it does not mean that the DESs structure remains intact. Further studies are needed to enable a full understanding of effect of water on hydrophobic DES.

3.2. Extractions with hydrophobic DES

Coming back to the extraction of pesticides from aqueous solutions, and as it was mentioned before, all hydrophobic DESs proposed in Figure 3 may be used as extractants, but the contamination of the water phase with the DES hydrophilic constituents as well as the non-recyclability and re-usability of these solvents needs to be taken into account. Consequently, in this work, only the 3 hydrophobic water stable DES, which have constituents are both hydrophobic, DL-Menthol: octanoic acid, DL-Menthol: decanoic acid and DL-Menthol: dodecanoic acid, were used in the removal of neonicotinoids from aqueous solutions. The extraction efficiencies, EE, were calculated from the pesticides (PES) concentration in the water-rich phase before, $C_{PEST,0}^{aq}$, and after the extraction, $C_{PEST,1}^{aq}$:

$$\% \, EE = \frac{\mathrm{C}_{\mathrm{PEST},0}^{\mathrm{aq}} - \mathrm{C}_{\mathrm{PEST},1}^{\mathrm{aq}}}{\mathrm{C}_{\mathrm{PEST},0}^{\mathrm{aq}}} \text{ x } 100 \qquad \text{(eq. 1)}$$

The extraction efficiencies for the selected pesticides using the proposed DESs are plotted in Figure 5 and listed in Table S8 of *Supplementary Information*. Also in Table S9 is presented the distribution ratio, i.e. the ratio of the concentration of pesticides in the DES or IL phase related to the concentration in the aqueous phase.

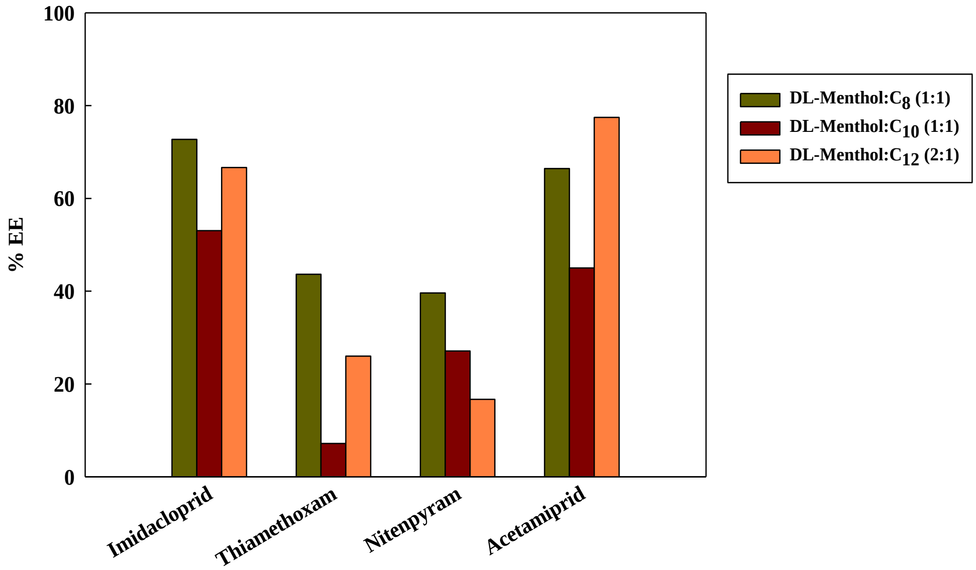


Figure 5. Extraction efficiencies of the studied pesticides obtained using different DESs based on DL-Menthol as HBA and different carboxylic acids as HBDs.

It can be observed that in general the extraction efficiencies of the studied four pesticides using different DES follow the order: Imidacloprid (~80 % EE) > Acetamiprid (~75 % EE) > Thiamethoxam (~40% EE) > Nitenpyram (~35 % EE). Despite the chemical diversity of these four pesticides, it is interesting to note that their % EE follow the inverse order of their water solubility, listed in Table 1, indicating that the pesticides hydrophobicity is the key property ruling their extraction from aqueous environments. Also, to be remarked that no correlation can be established between the extractions efficiency of the studied pesticides from water and the octanol-water partition coefficient (K_{ow}).

Table 1. Physical-chemical parameters, molecular weight (MW), acid dissociation constant (pKa), octanol-water partition coefficient (log K_{ow}), melting point and solubility in water of pesticides studied in this work, adapted from Bonmatin et al.[12].

	MW (g/mol)	λ_{max} (nm)	Pka	Log K_{ow}	Melting Point (ºC)	Solubility in water (g/L)
Imidacloprid	255.7	269	1.56	0.57	144	0.61 at 20 °C
Thiamethoxam	291.7	249	0.41	-0.13	139	4.10 at 25 ºC
Nitenpyram	270.7	271	3.1	-0.64	84	840 at 20 °C
Acetamiprid	222.7	245	0.7	0.8	99	2.95 at 25°C

The obtained extraction efficiencies for each pesticide can be ordered according to the following sequence of HBD: for Imidacloprid and Thiamethoxam: $C_8 > C_{12} > C_{10}$; for Nitenpyram: $C_8 > C_{10} > C_{12}$ and for Acetamiprid: $C_{12} > C_8 > C_{10}$. This means that, surprisingly, there is no clear trend between the % EE of the pesticides and the increase of hydrophobicity of the DESs, here given by the alkyl chain length of the HBD. DL-Menthol: octanoic acid is the best extractant for 3 of the studied pesticides, Imidacloprid, Thiamethoxam and Nitenpyram, while for Acetamiprid the best extraction efficiencies were attained using DL-Menthol: dodecanoic acid, followed up very closely by DL-Menthol: octanoic acid. In conclusion, it can be affirmed that DL-Menthol: octanoic acid is generally the most suitable extractant for removal of pesticides from aqueous environments.

In order to further explore the role of the hydrophobic character of the extractant, and since DESs can be considered as low cost and environmentally acceptable alternatives to conventional ILs, we decided to evaluate the extraction of the four pesticides with three hydrophobic ILs, $[C_2MIM][NTf_2]$, $[C_4MIM][NTf_2]$, and $[C_6MIM][NTf_2]$. The experimental data relative to extraction efficiencies of the four pesticides using imidazolium-based ILs are plotted in Figure 6 and listed in Table S8 of *Supplementary Information*.

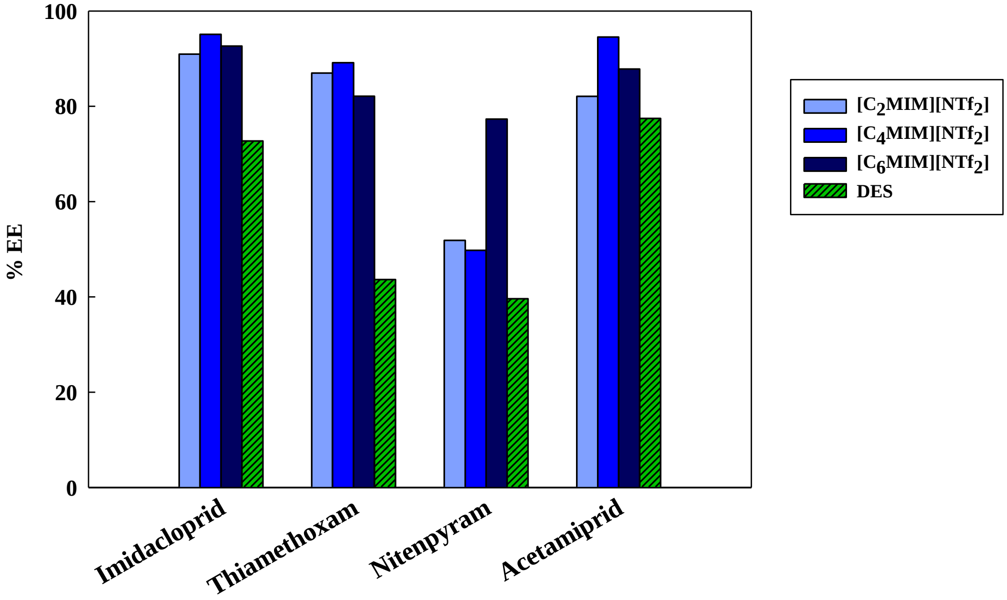


Figure 6. Extraction efficiencies obtained using imidazolium-based ILs with different alkyl chains and comparison with the best extraction using DES.

Very high extraction efficiencies, around 90 %, were obtained for all the pesticides, although a somewhat lower value was obtained for Nitenpyram, in agreement with the extraction efficiency trend obtained for the tested DES and the pesticides's water solubility. Interestingly, no remarkable influence of the alkyl chain length of the imidazolium cation in the extraction efficiencies can be observed, with the exception of Nitenpyram, where the most hydrophobic IL ($[C_6MIM][NTf_2]$) displays the higher extraction efficiencies. In Figure 6, a comparison between the relative performance of ILs and DES in the extraction of these four pesticides is also established. The DES's data represented in Figure 6 are the best results obtained for each one of the pesticides, independently of the DES used. It can be observed that the extraction results obtained for the DES were not much lower than those obtained when ILs were used. It should be taken into account that these DES can be further fine tuned for the extraction of pesticides, probably leading to more promising results. Nevertheless, in the case of acetamiprid, the use of a DES is nearly as appealing as the use of ILs.

In order to further optimize the extractions of the four studied neonicotinoids, the reuse of DES is very important, as far as economic and environmental viability of the process are concerned. Consequently, the reutilization of the DESs in the extraction of Imidacloprid in four consecutive cycles, with a mass ratio aqueous solution of pesticide/DES of 1, was attempted. In Figure 7, the extraction efficiency throughout these cycles is presented.

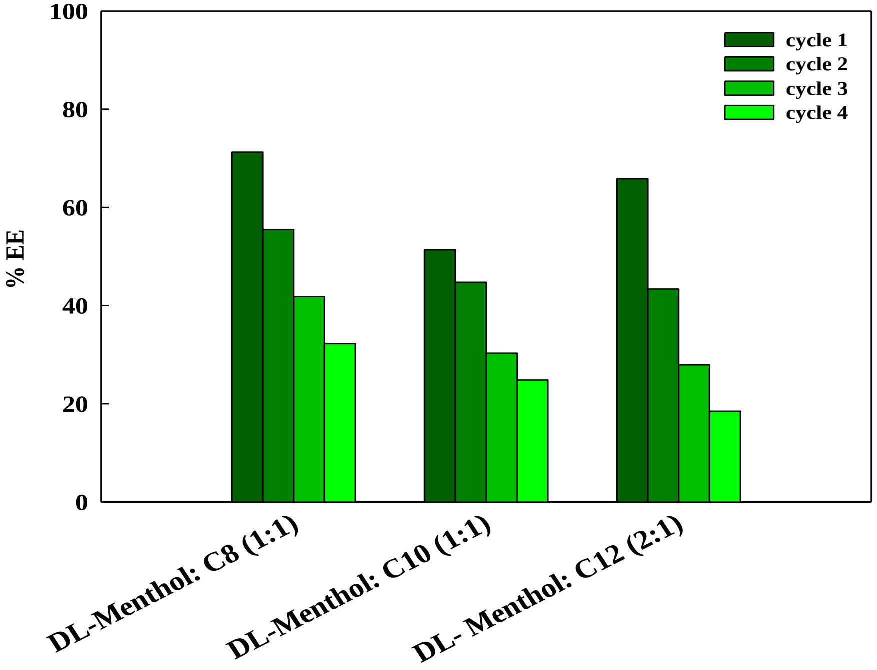


Figure 7. Extraction efficiency (% EE) of Imidacloprid using the DL-Menthol: Octanoic acid, DL-Menthol: Decanoic acid and DL-Menthol: Dodecanoic acid DESs in consecutive cycles.

It can be seen that, as the number of cycles of DES use increases, the extraction capability of DES decreases. The explanation is because as the DES starts to have pesticides dissolved, it is more difficult to dissolve the same amount of pesticides as when fresh DES is used. Nevertheless, DL-Menthol: C_8 presents the best extraction efficiencies, 75 % in the first cycle decreasing to 33 % in the fourth cycle for imidacloprid extraction.

4. Conclusions

In this work, the use of hydrophobic DESs in the extraction of four neonicotinoids, Imidacloprid, Acetamiprid, Nitenpyram and Thiamethoxam, from aqueous environments was studied. Two different families of DES, that present two phase forming ability with aqueous solutions, were studied regarding their water stability. It was demonstrated that it is mandatory that both the HBA and the HBD are hydrophobic to attain the DES integrity, after being contacted with water. When one of the constituents of the DES, either HBD or HBA, is water soluble, partial disruption of the DEs occurs and variable amounts of the hydrophilic compound is found in the water phase, according to its water solubility. Using water stable DES based on DL-Menthol and organic acids, extraction efficiencies up to 80 % for the four studied neonicotinoids were obtained. These extraction efficiencies follow the hydrophobic character of the pesticides, although no direct link with the increase in DES hydrophobicity, through the increase of the chain length of the HBD, was observed. The appealing reusability of the DESs studied was also demonstrated, highlighting their relevant role as sustainable solvents in industrial applications.

5. Acknowledgements

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6. Supplementary Information

6.1. Characterization of Hydrophobic DESs studied in this work by ^{1}H and ^{13}C NMR spectra

A.1. DL-menthol: Octanoic acid (1:1)

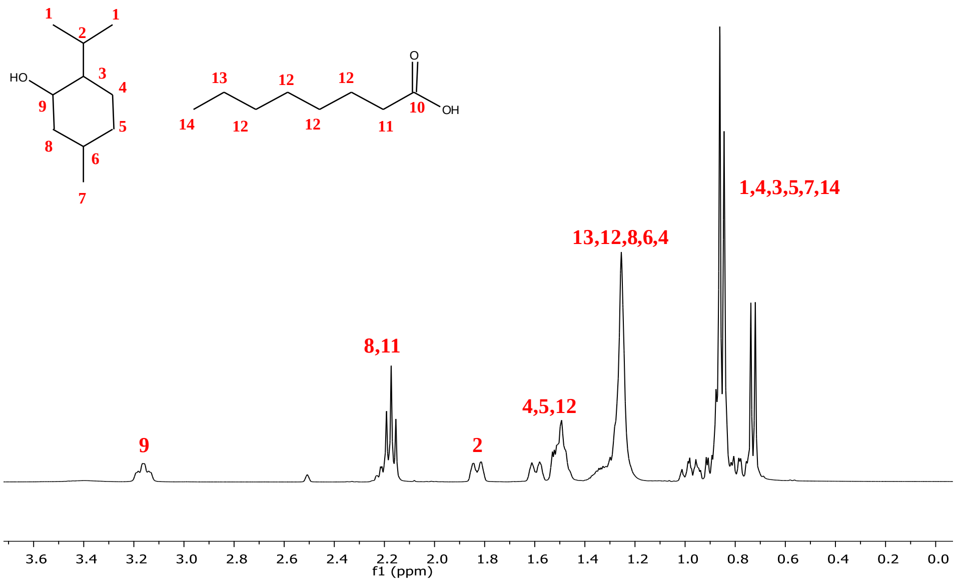


Figure S1. ^{1}H NMR spectra of DL-menthol 1:1 octanoic acid DES in DMSO. The structure and numbering of both compounds is also depicted.

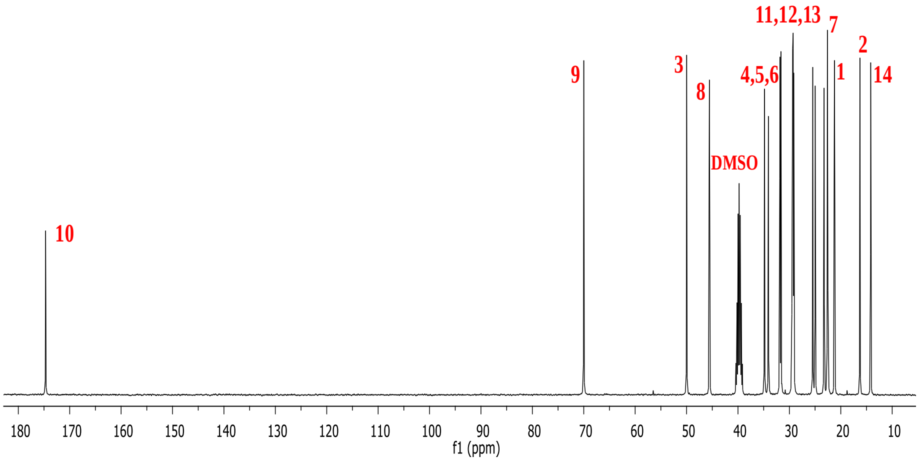


Figure S2. ^{13}C NMR spectra of DL-menthol 1:1 octanoic acid DES in DMSO. The structure and numbering of both compounds is depicted

A.2. DL-menthol: Decanoic acid (1:1)

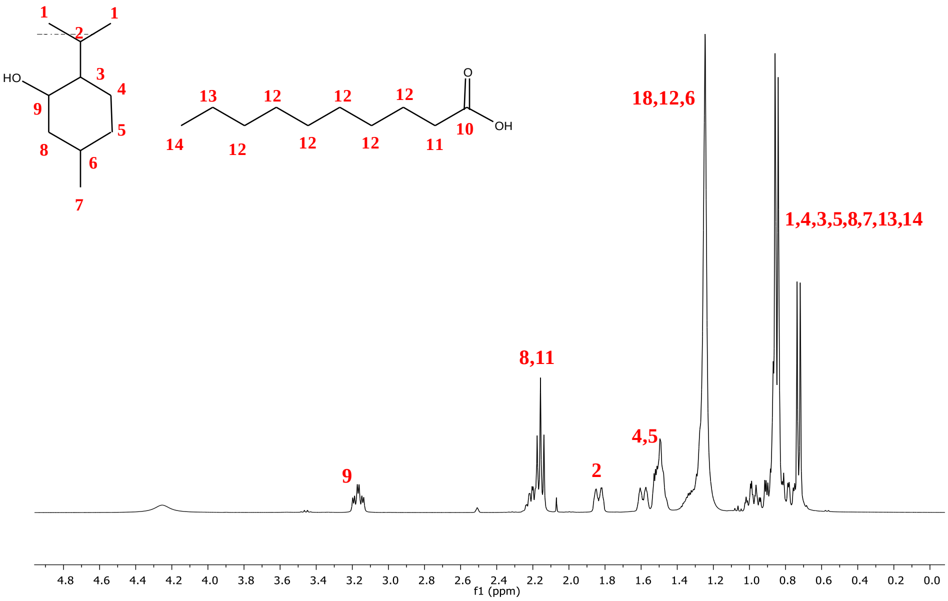


Figure S3. ^{1}H NMR spectra of DL-menthol 1:1 decanoic acid DES in DMSO. The structure and numbering of both compounds is also depicted.

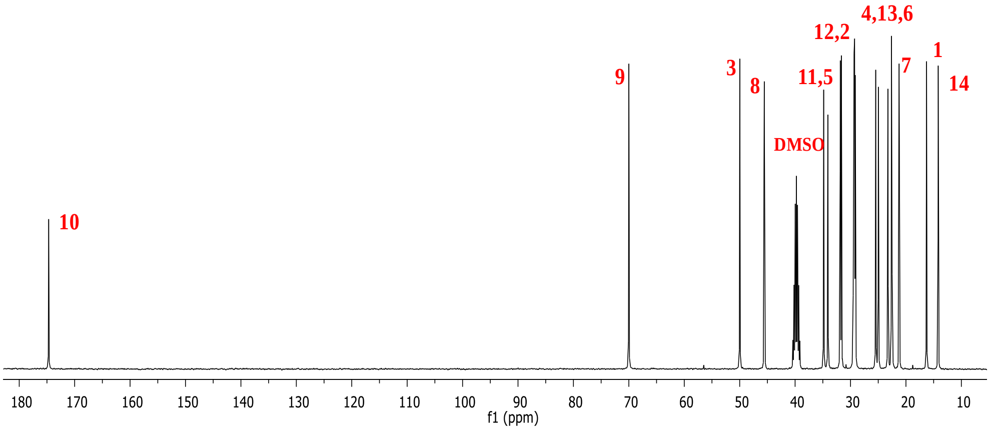


Figure S4. ^{13}C NMR spectra of DL-menthol 1:1 decanoic acid DES in DMSO. The structure and numbering of both compounds is depicted

A.3. DL-menthol: Dodecanoic acid (2:1)

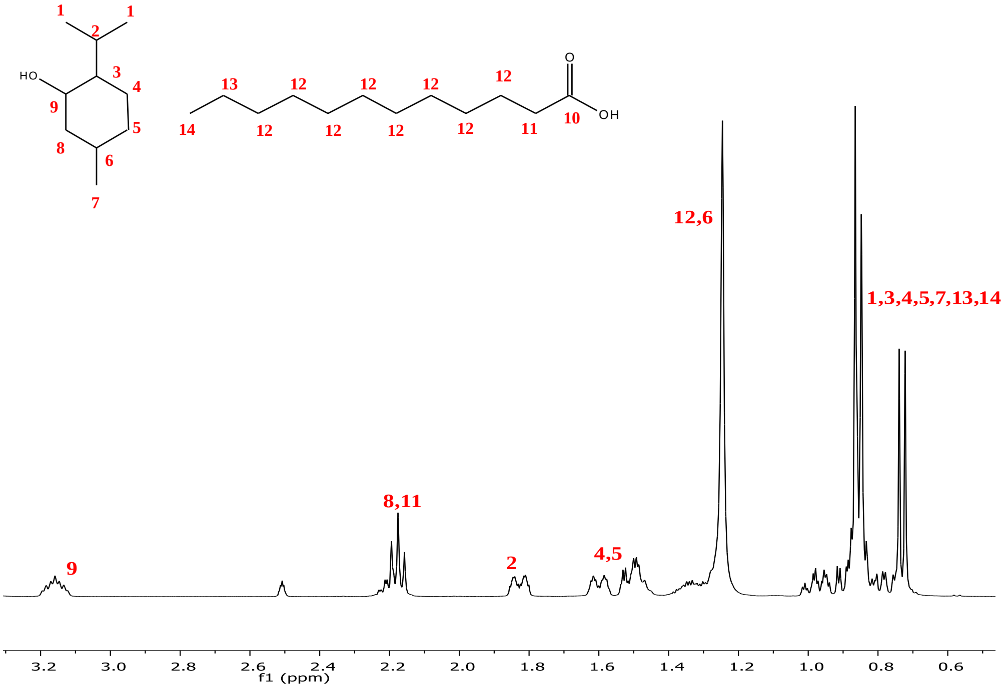


Figure S5. ^{1}H NMR spectra of DL-menthol 2:1 dodecanoic acid DES in DMSO. The structure and numbering of both compounds is also depicted.

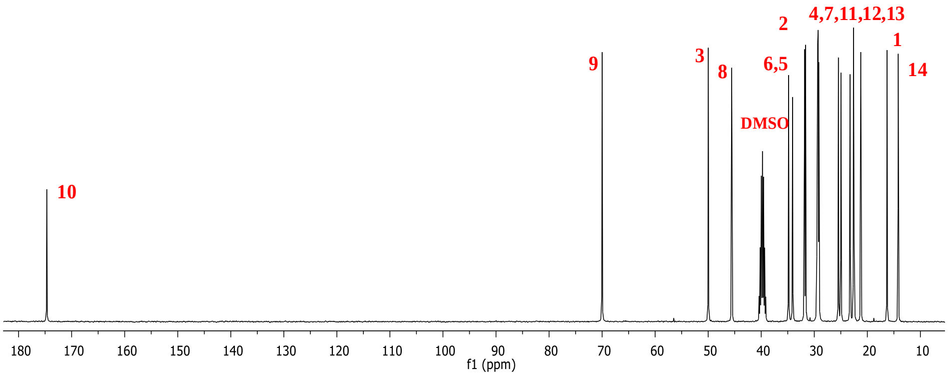
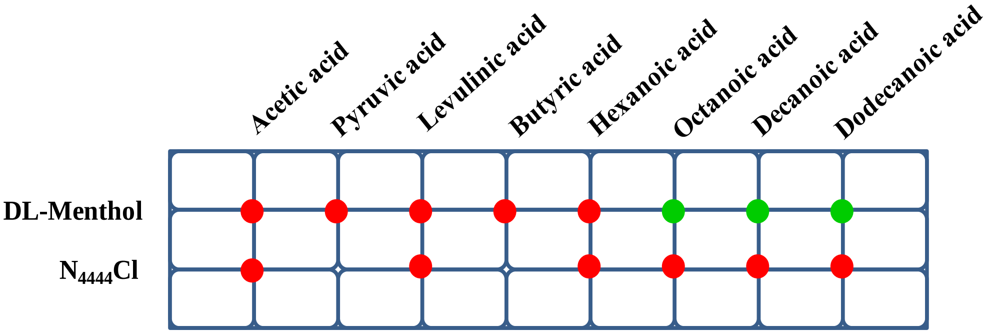


Figure S6. ^{13}C NMR spectra of DL-menthol 2:1 dodecanoic acid DES in DMSO. The structure and numbering of both compounds is depicted.

6.2. Examples of breakage of hydrogen bond in DESs with water by ^{1}H-NMR spectra



	Acetic acid	Pyruvic acid	Levulinic acid	Butyric acid	Hexanoic acid	Octanoic acid	Decanoic acid	Dodecanoic acid
DL-Menthol	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢
$N_{4444}Cl$	🔴		🔴		🔴	🔴	🔴	🔴

B.1. DL-menthol: Acetic acid (1:1)

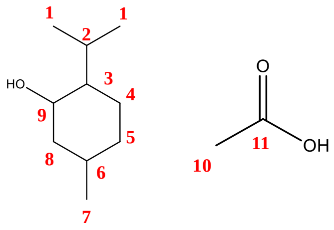


Table S1. Peak assignments and integrals of the ^{1}H-NMR spectrum of DES before and after mixing with water

Hydrophobic DESs		**Before mixing with water**			**After mixing with water**		
DL-menthol	**Acetic acid**	δ (ppm)	Shape	Integral	δ (ppm)	Shape	Integral
C (1,7,8,3) H_3,H_2,H		0.73-1.02	m	13.94	0.48-0.77	m	14.00
C (2) H		2.18	m	1.00	1.98	m	0.95
C (4) H_2		1.62;1.34	m	1.03	1.61;1.37	m	1.16
C (5) H_2		1.82;1.34	m	1.02	1.30	m	0.98
C (6) H		1.34	m	1.02	1.09	m	1.08
C (9) H		3.16	m	1.01	2.94	m	0.99
	C (10) H_3	1.91	s	3.00	1.74	s	0.33

B.2. DL-menthol: Levulinic acid (1:1)

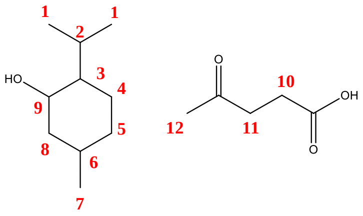


Table S2. Peak assignments and integrals of the ^{1}H-NMR spectrum of DES before and after mixing with water

Hydrophobic DESs		**Before mixing with water**			**After mixing with water**		
DL-menthol	**Levulinic acid**	δ (ppm)	Shape	Integral	δ (ppm)	Shape	Integral
C (1,7,8,3) H_3, H_2, H		0.75-1.01	m	14	0.72-0.99	m	14
C (2) H		2.19	m	1.02	2.19	m	1.04
C (4) H_2		1.58;1.34	m	1.03	1.60;1.34	m	1.02
C (5) H_2		1.81;1.34	m	1.02	1.83;1.34	m	1.02
C (6) H		1.34	m	1.02	1.33	m	1.05
C (9) H		3.16	m	1.03	3.17	m	1.00
	C (10) H_2	2.38	t	2.03	2.39	t	0.24
	C (11) H_2	2.65	t	2.03	2.65	t	0.23
	C (12) H_3	2.10	s	3.00	2.10	s	0.37

B.3. DL-menthol: Butyric acid (1:1)

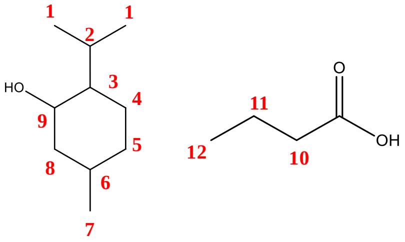


Table S3. Peak assignments and integrals of the ^{1}H-NMR spectrum of DES before and after mixing with water

Hydrophobic DESs		**Before mixing with water**			**After mixing with water**		
DL-menthol	**Butyric acid**	δ (ppm)	Shape	Integral	δ (ppm)	Shape	Integral
C (1,7,8,3,4) H_3,H_2,H	**C (12) H_3**	0.72-1.01	m	16.15	0.71-0.99	m	14.77
C (2) H	**C (10) H_2**	2.15-2.23	m	3.02	2.14-2.23	m	2.49
C (5) H		1.83	m	1.01	1.84	m	0.96
C (6) H		1.33	m	1.02	1.32	m	0.97
C (9) H		3.17	m	1.00	3.17	m	1.00
C (4, 5) H	**C (11) H_2**	1.49-1.61	m	4.02	1.49-1.54	m	3.40

B.4. DL-menthol: Octanoic acid (1:1)

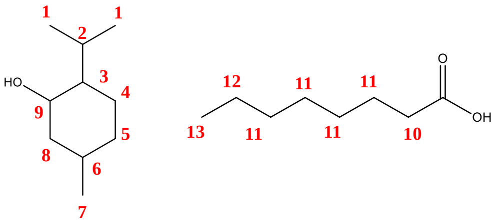


Table S4. Peak assignments and integrals of the ^{1}H-NMR spectrum of DES before and after mixing with water

Hydrophobic DESs		Before mixing with water			After mixing with water		
DL-menthol	**Octanoic acid**	δ (ppm)	Shape	Integral	δ (ppm)	Shape	Integral
C (1,7,8,4) H_3,H_2,H	**C (13) H_3**	0.72-1.02	m	16.05	0.47-0.63	m	16.18
C (2) H	**C (10) H_2**	2.15-2.23	m	2.98	1.90-1.96	m	3.02
C (4) H		1.59	m	1.01	1.33	m	1.03
C (5) H		1.83	m	0.99	1.60	m	1.01
C (6) H	**C (11) H_2**	1.26-1.33	m	9.00	1.00-1.11	m	9.00
C (9) H		3.16	m	0.98	2.92	m	1.03
C (3) H	**C (12) H_2**	1.48-1.62	m	2.99	1.19-1.26	m	3.01

B.5. DL-menthol: Dodecanoic acid (2:1)

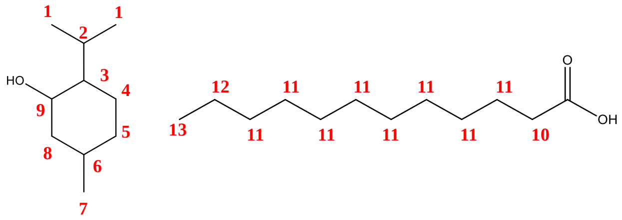
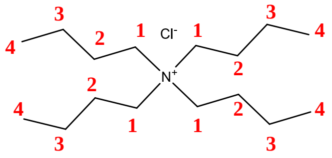


Table S5. Peak assignments and integrals of the ^{1}H-NMR spectrum of DES before and after mixing with water

Hydrophobic DESs		Before mixing with water			After mixing with water		
DL-menthol	**Dodecanoic acid**	δ (ppm)	Shape	Integral	δ (ppm)	Shape	Integral
C (1,7,8,4) H_3,H_2,H	**C (13) H_3**	0.72-0.98	m	29.05	0.72-0.99	m	29.00
C (2) H	**C (10) H_2**	2.16-2.23	m	4.05	2.15-2.23	m	4.01
C (4) H		1.60	m	2.04	1.60	m	2.01
C (5) H		1.83	m	2.03	1.83	m	2.05
C (6) H	**C (11) H_2**	1.25-1.38	m	18.12	1.24-1.34	m	18.02
C (9) H		3.16	m	2.00	3.16	m	2.00
C (3) H	**C (12) H_2**	1.49-1.53	m	4.01	1.47-1.54	m	4.02

B.6. $N_{4444}Cl$: Octanoic acid (1:2)



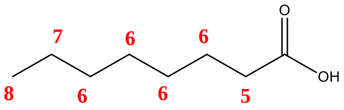


Table S6. Peak assignments and integrals of the ^{1}H-NMR spectrum of DES before and after mixing with water

Hydrophobic DESs		**Before mixing with water**			**After mixing with water**		
$N_{4444}Cl$	**Octanoic acid**	δ (ppm)	Shape	Integral	δ (ppm)	Shape	Integral
C (1) H_2		3.19	t	8.07	3.19	t	4.87
C (2,3) H_2	**C (6,7) H_2**	1.25-1.62	m	36.05	1.23-1.59	m	28.31
	C (5) H_2	2.19	t	4.02	217	t	4.00
C (4) H_3	**C (8) H_3**	0.84-0.96	m	18.05	0.82-0.94	m	15.03

B.7. $N_{4444}Cl$: Decanoic acid (1:2)

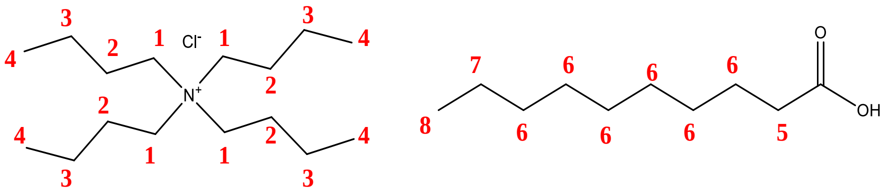


Table S7. Peak assignments and integrals of the ^1H-NMR spectrum of DES before and after mixing with water

Hydrophobic DESs		**Before mixing with water**			**After mixing with water**		
$N_{4444}Cl$	**Decanoic acid**	δ (ppm)	Shape	Integral	δ (ppm)	Shape	Integral
C (1) H_2		3.19	t	7.99	3.19	t	4.36
C (2,3) H_2	**C (6,7) H_2**	1.25-1.62	m	44.03	1.24-1.58	m	31.33
	C (5) H_2	2.1	t	4.01	2.17	t	4.00
C (4) H_3	**C (8) H_3**	0.84-0.96	m	18.01	0.82-0.94	m	13.74

6.3. Extraction efficiencies obtained using DES and ILs

Table S8. Extraction efficiencies (% EE) of pesticides using DESs and ILs

	Extraction Efficiencies (% EE)			
	Imidacloprid	**Thiamethoxam**	**Acetamiprid**	**Nitenpyram**
DL-Menthol: C_8	72.72 ± 0.12	43.66 ± 0.18	66.44 ± 0. 21	39.61 ± 0.09
DL-Menthol: C_{10}	53.06 ± 0.23	7.18 ± 0.11	45.00 ± 0.14	27.10 ± 0.16
DL-Menthol: C_{12}	66.64 ± 0.09	26.00 ± 0.08	77.45 ± 0.13	16.69 ± 0.07
$[C_2MIM][Ntf_2]$	90.96 ± 0.25	86.96 ± 0.19	82.08 ± 0.26	51.87 ± 0.11
$[C_4MIM][Ntf_2]$	95.09 ± 0.32	89.16 ± 0.26	94.54 ± 0.17	49.77 ± 0.20
$[C_6MIM][Ntf_2]$	92.66 ± 0.18	82.12 ± 0.35	87.83 ± 0.21	77.31 ± 0.24

Table S9. Distribution Ratio of pesticides between DES or IL phase and aqueous phase

	Distribution Ratio			
	Imidacloprid	**Thiamethoxam**	**Acetamiprid**	**Nitenpyram**
DL-Menthol: C_8	2.67	0.77	1.98	0.66
DL-Menthol: C_{10}	1.13	0.08	0.82	0.37
DL-Menthol: C_{12}	2.00	0.35	3.43	0.20
$[C_2MIM][Ntf_2]$	10.06	6.67	4.58	1.08
$[C_4MIM][Ntf_2]$	19.37	8.23	17.32	0.99
$[C_6MIM][Ntf_2]$	12.62	4.59	7.22	3.41

7. References

1. Abdel-Raouf, N.; Al-Homaidan, A. A.; Ibraheem, I. B. M., Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences* 2012, *19* (3), 257-275.

2. Fent, K.; Weston, A. A.; Caminada, D., Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* 2006, *76* (2), 122-159.

3. Luo, Y.; Guo, W.; Ngo, H. H.; Nghiem, L. D.; Hai, F. I.; Zhang, J.; Liang, S.; Wang, X. C., A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of The Total Environment* 2014, *473–474*, 619-641.

4. Sontheimer, H.; Brauch, H. J.; Kühn, W., Impact of different types of organic micropollutants present on sources of drinking water on the quality of drinking water. *Science of The Total Environment* 1985, *47*, 27-44.

5. Amin, M. T.; Alazba, A. A.; Manzoor, U., A Review of Removal of Pollutants from Water/Wastewater Using Different Types of Nanomaterials. *Advances in Materials Science and Engineering* 2014, *2014*, 24.

6. Gilden, R. C.; Huffling, K.; Sattler, B., Pesticides and Health Risks. *Journal of Obstetric, Gynecologic, & Neonatal Nursing* 2010, *39* (1), 103-110.

7. Stockholm Convention on Persistent Organic Pollutants. *Retrieved from file:///C:/Users/user/Downloads/UNEP-POPS-TREATY-NOTIF-CN524-2009.En.pdf* 2001.

8. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 Official Journal of the European Union 2013.

9. Hernández, A. F.; Parrón, T.; Tsatsakis, A. M.; Requena, M.; Alarcón, R.; López-Guarnido, O., Toxic effects of pesticide mixtures at a molecular level: Their relevance to human health. *Toxicology* 2013, *307*, 136-145.

10. Gupta, V. K.; Gupta, B.; Rastogi, A.; Agarwal, S.; Nayak, A., Pesticides removal from waste water by activated carbon prepared from waste rubber tire. *Water Research* 2011, *45* (13), 4047-4055.

11. Ormad, M. P.; Miguel, N.; Claver, A.; Matesanz, J. M.; Ovelleiro, J. L., Pesticides removal in the process of drinking water production. *Chemosphere* 2008, *71* (1), 97-106.

12. Bonmatin, J. M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D. P.; Krupke, C.; Liess, M.; Long, E.; Marzaro, M.; Mitchell, E. A. D.; Noome, D. A.; Simon-Delso, N.; Tapparo, A., Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research International* 2015, *22*, 35-67.

13. Jeschke, P.; Nauen, R.; Schindler, M.; Elbert, A., Overview of the Status and Global Strategy for Neonicotinoids. *Journal of Agricultural and Food Chemistry* 2011, *59* (7), 2897-2908.

14. Matsuda, K.; Buckingham, S. D.; Kleier, D.; Rauh, J. J.; Grauso, M.; Sattelle, D. B., Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences* 2001, *22* (11), 573-580.

15. Dlugolecka, M. D., Alf-Goran; Plaza, Elizabeta, Low concentrations of high priority' – Pharmaceutical and Personal Care Products (PPCPs); occurrence and removal at wastewater treatment plants. *Vatten* 2006, *62*, 139-148.

16. Rodriguez, C.; Van Buynder, P.; Lugg, R.; Blair, P.; Devine, B.; Cook, A.; Weinstein, P., Indirect Potable Reuse: A Sustainable Water Supply Alternative. *International Journal of Environmental Research and Public Health* 2009, *6* (3), 1174-1203.

17. Muga, H. E.; Mihelcic, J. R., Sustainability of wastewater treatment technologies. *Journal of Environmental Management* 2008, *88* (3), 437-447.

18. Hannah, S. A.; Austern, B. M.; Eralp, A. E.; Wise, R. H., Comparative Removal of Toxic Pollutants by Six Wastewater Treatment Processes. *Journal (Water Pollution Control Federation)* 1986, *58* (1), 27-34.

19. Grassi, M.; Kaykioglu, G.; Belgiorno, V.; Lofrano, G., Removal of Emerging Contaminants from Water and Wastewater by Adsorption Process. In *Emerging Compounds Removal from Wastewater: Natural and Solar Based Treatments*, Lofrano, G., Ed. Springer Netherlands: Dordrecht, 2012; pp 15-37.

20. Ali, I.; Gupta, V. K., Advances in water treatment by adsorption technology. *Nat. Protocols* 2007, *1* (6), 2661-2667.

21. Carballa M., O. F., Lema J. M., Removal of pharmaceuticals and personal care products (PPCPs) from municipal wastewaters by physico-chemical processes. . *Electronic Journal of Environmental, Agriculture and Food Chemistry* 2003, *2*, 309-313.

22. López-Montilla, J. C.; Pandey, S.; Shah, D. O.; Crisalle, O. D., Removal of non-ionic organic pollutants from water via liquid–liquid extraction. *Water Research* 2005, *39* (9), 1907-1913.

23. Moscoso, F.; Deive, F.; Esperança, J.; Rodríguez, A., Pesticide Removal from Aqueous Solutions by Adding Salting Out Agents. *International Journal of Molecular Sciences* 2013, *14* (10), 20954-20965.

24. Fortuny, A.; Coll, M. T.; Sastre, A. M., Ionic liquids as a carrier for chloride reduction from brackish water using hollow fiber renewal liquid membrane. *Desalination* 2014, *343* (0), 54-59.

25. Lozano, L. J.; Godínez, C.; de los Ríos, A. P.; Hernández-Fernández, F. J.; Sánchez-Segado, S.; Alguacil, F. J., Recent advances in supported ionic liquid membrane technology. *Journal of Membrane Science* 2011, *376* (1–2), 1-14.

26. Lemus, J.; Palomar, J.; Gilarranz, M.; Rodriguez, J., Characterization of Supported Ionic Liquid Phase (SILP) materials prepared from different supports. *Adsorption* 2011, *17* (3), 561-571.

27. Zheng, X.; He, L.; Duan, Y.; Jiang, X.; Xiang, G.; Zhao, W.; Zhang, S., Poly(ionic liquid) immobilized magnetic nanoparticles as new adsorbent for extraction and enrichment of organophosphorus pesticides from tea drinks. *Journal of Chromatography A* 2014, *1358* (0), 39-45.

28. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R. L.; Duarte, A. R. C., Natural Deep Eutectic Solvents – Solvents for the 21st Century. *ACS Sustainable Chemistry & Engineering* 2014, *2* (5), 1063-1071.

29. Francisco, M.; van den Bruinhorst, A.; Kroon, M. C., Low-Transition-Temperature Mixtures (LTTMs): A New Generation of Designer Solvents. *Angewandte Chemie International Edition* 2013, *52* (11), 3074-3085.

30. Silva, N. H. C. S.; Pinto, R. J. B.; Freire, C. S. R.; Marrucho, I. M., Production of lysozyme nanofibers using deep eutectic solvent aqueous solutions. *Colloids and Surfaces B: Biointerfaces* 2016, *147*, 36-44.

31. Passos, H.; Tavares, D. J. P.; Ferreira, A. M.; Freire, M. G.; Coutinho, J. A. P., Are Aqueous Biphasic Systems Composed of Deep Eutectic Solvents Ternary or Quaternary Systems? *ACS Sustainable Chemistry & Engineering* 2016, *4* (5), 2881-2886.

32. Ribeiro, B. D.; Florindo, C.; Iff, L. C.; Coelho, M. A. Z.; Marrucho, I. M., Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. *ACS Sustainable Chemistry & Engineering* 2015, *3* (10), 2469-2477.

33. van Osch, D. J. G. P.; Zubeir, L. F.; van den Bruinhorst, A.; Rocha, M. A. A.; Kroon, M. C., Hydrophobic deep eutectic solvents as water-immiscible extractants. *Green Chemistry* 2015, *17* (9), 4518-4521.

34. van Osch, D. J. G. P.; Parmentier, D.; Dietz, C. H. J. T.; van den Bruinhorst, A.; Tuinier, R.; Kroon, M. C., Removal of alkali and transition metal ions from water with hydrophobic deep eutectic solvents. *Chemical Communications* 2016, *52* (80), 11987-11990.

35. Mjalli, F. S.; Ahmad, O., Density of aqueous choline chloride-based ionic liquids analogues. *Thermochimica Acta* 2017, *647*, 8-14.

36. Pandey, A.; Pandey, S., Solvatochromic Probe Behavior within Choline Chloride-Based Deep Eutectic Solvents: Effect of Temperature and Water. *The Journal of Physical Chemistry B* 2014, *118* (50), 14652-14661.

37. Hayashi, Y.; Katsumoto, Y.; Omori, S.; Kishii, N.; Yasuda, A., Liquid Structure of the Urea−Water System Studied by Dielectric Spectroscopy. *The Journal of Physical Chemistry B* 2007, *111* (5), 1076-1080.

CHAPTER 3.2

Task specific hydrophobic deep eutectic solvents for purification of water contaminated with Bisphenol-A

Adapted from: **C. Florindo**, Nathalie V. Monteiro, Bernardo D. Ribeiro, L. C. Branco and I. M. Marrucho, Hydrophobic deep eutectic solvents for purification of water contaminated with bisphenol-A, Journal of Molecular Liquids, *in press*, 2019.

The author contributed to the execution of all characterization and properties experiments described in this chapter, as well as involved in the discussion and interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

The occurrence of micropollutants in aquatic ecosystems poses a serious public health and environmental problem. For that purpose, the main goal of this work is the removal of an endocrine disruptor micropollutant compound, Bisphenol-A (BPA), from aqueous environments using hydrophobic deep eutectic solvents (DES). In particular, DES composed of DL-Menthol or quaternary ammonium salts as hydrogen bond acceptors (HBA) and natural fatty acids as hydrogen bond donors (HBD) were prepared. Extraction efficiencies were maximized by optimizing the experimental parameters in the extraction BPA from water using a central composite design combined with a response surface methodology. Thus, the effect of several parameters, such as stirring speed, contact time, DES/water ratio and initial BPA concentration on the extraction efficiencies were evaluated.

1. Introduction

Currently, one of the major societal challenges is to provide good quality water to everyone, by dramatically reducing or completely eliminating the current hazardous micropollutants present in the water resources around the globe.[1, 2] In the coming decades, water scarcity, even in regions considered to be currently rich in water, puts on the 2030 agenda for the sustainable development the urgent development of innovative and sustainable water solutions so that clean water and sanitation can be provided to everyone.[3]

Micropollutants have been emerging as a new class of pollutants since, despite their very small concentration in water streams, their presence can be related to numerous harmful effects on the health of animals and humans, such as carcinogenic and endocrine disrupting effects, antibiotic resistance of microorganisms, etc.[4-6] Heavy metals, pharmaceuticals, personal care products, dyes, waterproofing treatments, plasticizers, pesticides, and others, are all classified as micropollutants.[7] Among these, Bisphenol-A (BPA) is an emerging micropollutant of special importance since it has been detected in increasing concentrations globally in water sources, as conventional water treatment plants are incapable of removing it completely. BPA has been largely used as a plasticizer from food and drink plastic packaging to plastic medical devices. However, BPA is a also well-known endocrine disruptor compound, that mimics the estrogen hormone, thus affecting the normal development and function of human and wildlife reproductive system.8 Although the European Union and Canada have recognized its harmful effects and banned its use in baby bottles since 2011,[9,6] there is a long way to go until its full legislative control and consequent removal from water sources.

The development of effective and sustainable innovative water cleaning technologies, typically to complement the existing ones, is a crucial step to achieve the targeted goal of sustainable development. Different extraction techniques, such as aqueous biphasic systems,[10, 11] liquid membranes,[12-14] adsorption[15, 16] and liquid-liquid extraction,[17-19] have been tested for removal of BPA from water, with good extraction efficiencies. However, most of these techniques are not sustainable, since they are based on high cost solvents or materials of difficult recycle and reuse, not to mention their hazardousness and consequent water contamination. As a result, benign and recyclable solvents have been searched and developed, so that a truly circular technology can be achieved. Deep eutectic solvents (DES) have been highlighted as sustainable solvents and showed to provide sustainable solutions in numerous scientific and technological fields.[20, 21] In recent years, DES have emerged as alternative solvents for numerous applications.[20] The ease of preparation, the possibility to use natural and biodegradable components makes DES versatile alternative green solvents.[22] DES can be defined as mixtures of two or more compounds composed by hydrogen bond donors (HBD) and acceptors (HBA), which can form liquids at room temperature.[23] DES emerged as green, sustainable and more advantageous solvents compared to ionic liquids and traditional organic solvents, especially considering their environmentally friendly character, biodegradability, low price and straightforward preparation. This class of solvents showed great potential for a diversity of applications, such as in electrochemistry,[24] nanotechnology,[25] stabilization of DNA and RNA,[26] extraction and separation,[27, 28] catalysis,[29] organic synthesis,[30] among others.

Since most DES reported in the literature are water soluble, limiting their application in several processes, the development of hydrophobic DES has been attracting the attention of researchers. The preparation and application of several hydrophobic DES in extraction processes, such as removal of metal ions from water, indium extraction from hydrochloric and oxalic acids, removal of pesticides and endocrine disruptor compounds from water, among others [4, 17, 31, 32] has already been reported.

The main goal of this work is to evaluate the removal of BPA, from aqueous environments using hydrophobic DES. For that purpose, two different families of hydrophobic DES, namely ionic and neutral, were developed and their ability to extract BPA was evaluated through liquid-liquid extraction. In particular, DES using natural components, as DL-Menthol which can be used as hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), and different quaternary ammonium salts with long alkyl chain, such as [N_{7777}]Br, [N_{8888}]Br and [N_{8881}]Br, as HBA combining several natural carboxylic acids as HBD, such as octanoic and decanoic acid, were prepared. Chemical structures of DESs and micropollutant used in this work, and their respective acronyms are depicted in Figure 1 as well as in Table S1 in Supplementary Information.

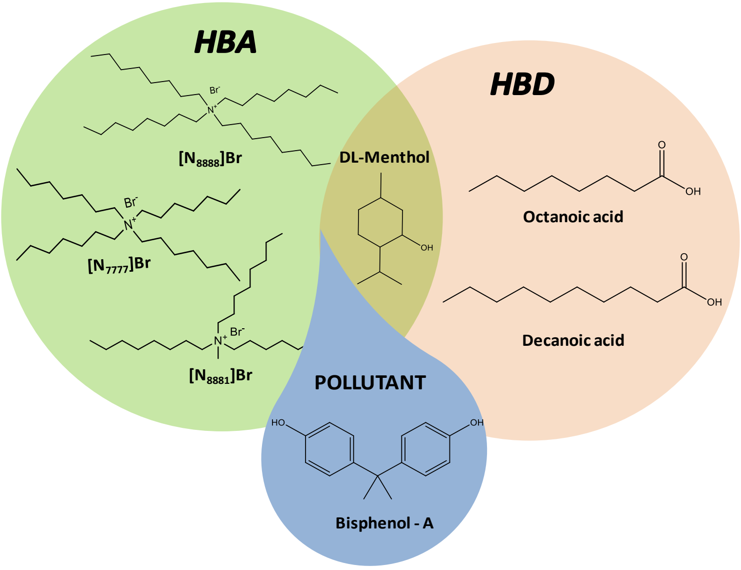


Figure 1. Chemical structures and respective acronyms of DESs and micropollutant studied in this work.

Moreover, the most influential experimental parameters on the extraction of BPA from water were identified and optimized using a central composite design (CCD). The optimization of extraction conditions is of utmost importance, since it is crucial to reduce the energy and operation associated cost, leading to environmentally friendly process.

2. Experimental Section

2.1. Materials

DL-Menthol (purity ≥ 95%), tetraheptylammonium bromide ([N_{7777}]Br) (purity ≥ 99%), tetraoctylammonium bromide ([N_{8888}]Br) (purity ≥ 98%), methyltrioctylammonium bromide ([N_{8881}]Br) (purity ≥ 97%), octanoic acid (C_8) (purity ≥ 98%) and decanoic acid (C_{10}) (purity ≥ 98%) were purchased from Sigma-Aldrich and used as received. The micropollutant Bisphenol-A (mass fraction purity ≥ 99 %) was purchased from Sigma Aldrich and used with no further purification. All aqueous solutions were prepared using high purity water (Milli-Q water) with a specific conductance <0.1 mS/cm.

2.2. Experimental Methodologies

2.2.1. Preparation of DESs

Hydrophobic DES were prepared according to the methodology proposed in our previous work.[33] All DES were prepared by mixing the two components in previously established proportions in a glass vial with a mechanical stirrer at 500 rpm and 333.15 K, until a homogeneous liquid was formed. In the preparation of DES, an analytical high-precision balance with an uncertainty of ± 10^{-5} g, was used to weigh the known masses of the individual compounds into bottles with caps. The HBAs (DL-menthol, tetraoctylammonium bromide ($[N_{8888}]Br$), tetraheptylammonium bromide ($[N_{7777}]Br$), methyltrioctylammonium bromide ($[N_{8881}]Br$) were first dried in a high vacuum pump at 40 °C for at least four days, while the carboxylic acids were used without any further purification, since they usually have very low amount of water. Their water contents were determined by Karl Fischer titration (< 500 ppm). All prepared hydrophobic DES were completely characterized by ^{1}H and ^{13}C NMR and FTIR spectroscopy, in order to confirm their expected structures and final purities.

2.2.2. Liquid-liquid extraction procedure

Liquid-liquid extractions were carried out at room temperature using the prepared hydrophobic DESs as extractants. BPA can be found in water environments at very low concentrations, usually ranging from ng/L to mg/L. In this work, in order to use a simple and fast screening technique, UV-vis spectroscopy, slightly greater concentrations were prepared using stock aqueous solutions of BPA of 0.1 g/L, 0.05 g/L and 0.025 g/L.

Mixtures composed by each one of the prepared hydrophobic DES and the aqueous solution of BPA were initially stirred vigorously during 10 min, which was the minimum time to reach equilibrium, and then left to settle for 12h, to ensure complete separation of phases. The extractions were carried out at (25.0±0.1) ^{o}C. Thereafter, the two phases were carefully separated using a needled syringe and the concentration of the remaining micropollutant in the water-rich phase was measured using UV–vis spectroscopy with a Shimadzu model UV-1800–Pharma-Spec spectrophotometer. Furthermore, to eliminate possible interferences of DES and their interaction with micropollutant in the quantification, only the concentration of BPA in the water phase was experimentally determined. A calibration curve was previously established for BPA in Milli-Q water (curves with $R^2 > 0.997$). All the experiments were carried out in triplicates so that standard deviations of the measurements could be calculated.

3. Results and Discussion

3.1. Performance of different DESs as extractants

In this work, hydrophobic DES were prepared using ionic hydrophobic compounds, such as quaternary ammonium salts with long alkyl chains, namely $[N_{7777}]$Br, $[N_{8888}]$Br and $[N_{8881}]$Br and carboxylic acids, while neutral hydrophobic DES were prepared combining a natural compound, DL-menthol, with carboxylic acids. These DES were prepared at a molar ratio of 1:2, which was determined to be liquid in previous works where phase diagrams of each system were measured. The prepared DESs are listed in Table S1 in Supporting Information.

Terms like deep eutectic solvents, eutectic solvents, eutectic mixtures or low transition temperature mixtures have been used in literature until now to designate this class of compounds, which means that the acronym DES has been used to designate eutectic mixtures that are liquid at room temperature and thus can be used as solvents. In this work we have used deep eutectics (those based on quaternary ammonium salts) and not deep eutectics (those based on DL-Menthol). Although we acknowledge here this difference, we have used the acronym DES for all the solvents used in this work, independently of the fact of the depression in the melting point being deep or not.

Nine hydrophobic DESs were initially screened for extraction of Bisphenol-A from water environments. The extraction efficiencies, EE %, were calculated from the Bisphenol-A (BPA) concentration in the water-rich phase before, $\mathrm{C}_{\mathrm{BPA},0}^{\mathrm{aq}}$, and after the extraction, $\mathrm{C}_{\mathrm{BPA},1}^{\mathrm{aq}}$:

$$\mathrm{EE}\ (\%) = \frac{\mathrm{C}_{\mathrm{BPA},0}^{\mathrm{aq}} - \mathrm{C}_{\mathrm{BPA},1}^{\mathrm{aq}}}{\mathrm{C}_{\mathrm{BPA},0}^{\mathrm{aq}}} \times 100 \qquad \text{(eq. 1)}$$

The experimental data relative to extraction efficiencies for the bisphenol-A using several DESs are displayed in Figure 2 and listed in Table S2 in Supplementary Information.

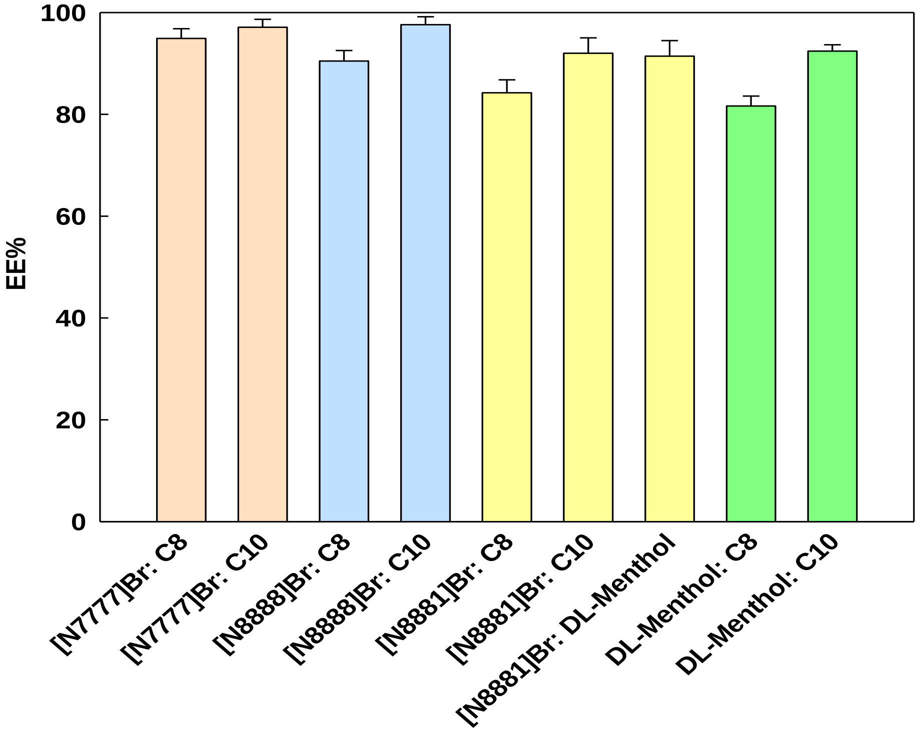


Figure 2. Extraction efficiencies (EE %) of Bisphenol-A obtained for all DESs used in this work ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, DES/water ratio = 1/1, temperature = 25 ºC, contact time = 10 min).

Good extraction efficiencies (> 85%) of BPA from contaminated water were achieved using all the selected hydrophobic DES. These large extraction efficiency values to remove BPA from water are essentially linked to the DES hydrophobicity, since BPA possess a very low water solubility (~120mg/L at 25 ºC) and a high octanol-water partition coefficient (K_{ow}) of 3.40,[34] leading to a large affinity for the organic hydrophobic DES phase. However, some difference in the extraction efficiencies between the studied DES can be observed. Analysing the data in Figure 2, it is clear that the extraction efficiencies depend on the choice of the components of DES, especially on the HBD. Moreover, the extraction efficiencies increased with the increase of the alkyl chain length in both the fatty acid and also in the quaternary ammonium salt, which corroborates our previous remarks on the fact that the more hydrophobic the DES are, the higher the extraction efficiencies.[35] Thus, the extraction efficiencies of BPA follow the HBA order: [N_{8888}]Br: HBD (~98% EE) > [N_{7777}]Br: HBD (~95% EE) > [N_{8881}]Br: HBD (~91% EE) ≥ DL-Menthol: HBD (~87% EE). Regarding the HBD of DES, the trend of the extraction efficiencies can be organized in the following order [N_{7777}]Br > [N_{8888}]Br > [N_{8881}]Br > DL-Menthol for C_8 acid, and [N_{7777}]Br ~ [N_{8888}]Br > [N_{8881}]Br > DL-Menthol for C_{10} acid, where the latter is the DES with the lowest EE%.

For all the HBAs studied there is a small difference in the BPA extraction between octanoic and decanoic acid, except for [N_{7777}]Br where similar results within the experimental error were obtained. Looking at [N_{8881}]Br as HBA, it can be seen that extraction efficiencies using DL-Menthol and decanoic acid as HBDs are also comparable, probably due their similar hydrophilicity. Consequently, it can be concluded that the extraction of BPA from water seems to be directly related to water solubility of components of the hydrophobic DES.

3.2. Screening of experimental parameters influencing the extraction efficiency

The optimization of all experimental parameters of a process is perhaps the most important aspect for its implementation at an industrial level, since it largely influences the cost/efficiency ratio. Therefore, in this section, the influence of experimental variables, such as mass ratio of HBA:HBD of DES, contact time, stirring speed of mixing, DES to water ratio and initial concentration of micropollutant, in the extraction efficiencies is evaluated and discussed in detail.

3.2.1. HBA: HBD molar ratio

As it has been recognized by several authors, the molar ratio between HBA and HBD is one of the crucial factors influencing the physicochemical properties of DESs.[36] In order to explore the impact of using different molar ratio of eutectic mixtures, where they are still liquid at working temperature, on the extraction efficiencies of BPA, four molar HBA: HBD ratios (1:2, 1:3, 1:4 and 1:5) were selected and used to prepare DES belonging to the three quaternary ammonium salts DES families ($[N_{7777}]Br$, $[N_{8888}]Br$ and $[N_{8881}]Br$) and DL-Menthol DES family. The reason to select these specific molar ratios of HBA: HBD is due to the fact that higher proportions of salt than acid would not lead to liquid phase at room temperature. The extraction results of BPA from aqueous solutions using different molar HBA: HBD proportions of different ionic and neutral DES are presented in Figure 3 and listed in Table S3 in Supplementary Information.

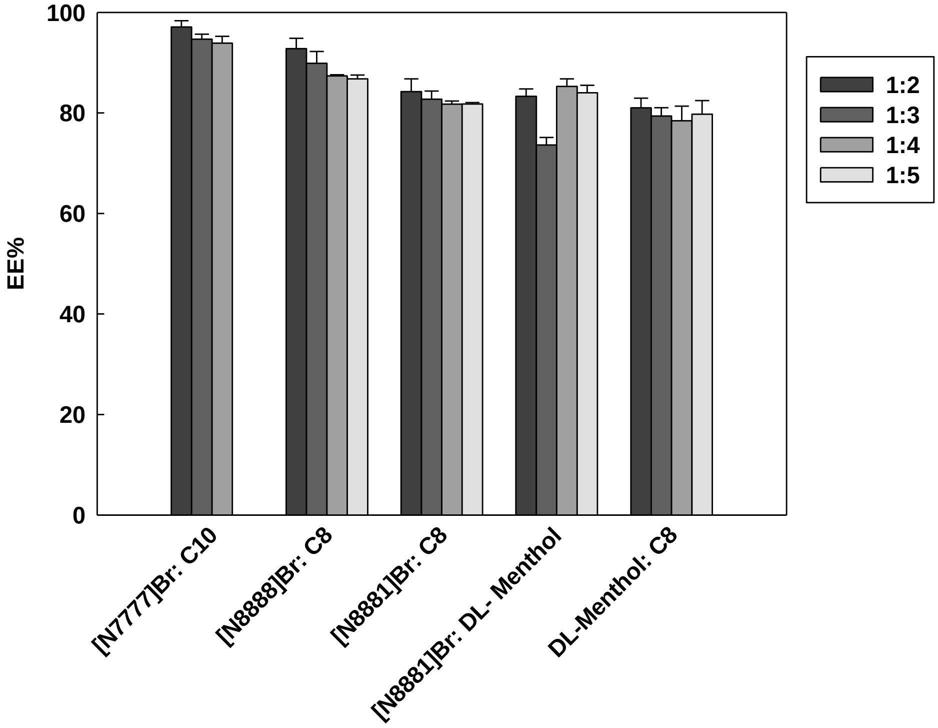


Figure 3. Influence of HBA: HBD ratio of five DESs families on the extraction efficiencies (EE %) of BPA ([C_0]BPA = 0.1 g/L, Stirring speed = 300 rpm, DES/water ratio = 1/1, Temperature = 25 ºC, Contact time = 10 min).

It can be seen that the molar ratios do not significantly influence the extraction efficiencies for the studied DES, and [N_{8888}]Br: C_8 is the one where larger differences in the BPA extraction efficiencies with the HBA:HBD molar ratios were observed. Thus, in general, it can be concluded that the incorporation of more acid (increasing from 1:2 to 1:5) does not favour the extraction efficiencies of BPA for both ionic and neutral DES. Since the best performance for all the studied systems was always achieved using the lowest HBA: HBD molar ratio (1:2), this ratio was chosen to carry out further studies.

3.2.2. Effect of initial concentration of BPA in water

In order to explore the effect of initial concentration of micropollutant on the extraction efficiency of BPA, the initial concentration was varied from 0.1 g/L to 0.025 g/L (100, 50 and 25 ppm) and the obtained results are presented in Figure 4 and listed in Table S4 in Supplementary Information.

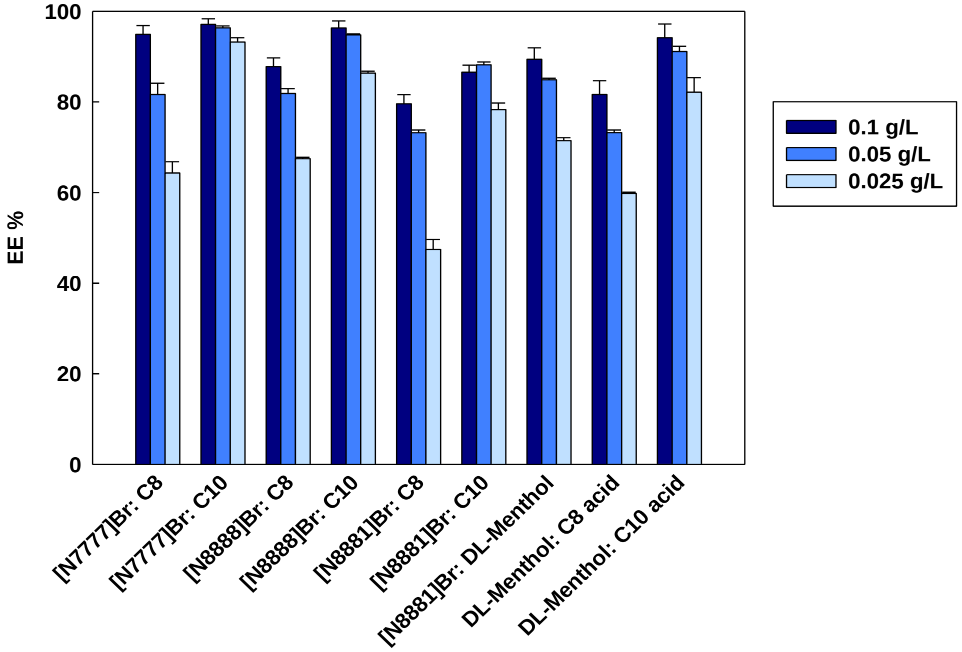


Figure 4. Influence of initial concentration of Bisphenol-A in water on the extraction efficiencies (EE %) for all DESs used in this work (stirring speed = 300 rpm, DES/water ratio = 1/1, temperature = 25 ºC, contact time = 10 min).

It can be seen that the initial concentration of the BPA greatly influences the EE% of BPA for the majority of the studied DES. Overall, extraction efficiencies decrease as the concentration of BPA decreases as follows: 0.1 g/L > 0.05 g/L > 0.025 g/L. However, for DES composed of decanoic acid as HBD, this decrease in %EE is much smaller than for DES composed of more hydrophilic HBD, such as octanoic acid or DL-Menthol. This fact is true for the all studied DES, both ionic and neutral. This behavior of the EE% with BPA initial concentration is linked to hydrophobic character of the HBD: the extraction efficiencies of BPA carried out with DES composed of decanoic acid are less affected by the initial concentration of micropollutant than those composed of octanoic acid or DL-menthol as HBD. This gives the opportunity to tune the DES chemical structure through the HBD in order to maintain the extraction efficiency. In some cases, such as $[N_{7777}]Br$: C_{10}, the differences achieved in the EE% at different initial concentrations of BPA are negligible. In conclusion, the best DES to efficiently remove BPA at low concentrations are the ionic most hydrophobic, $[N_{7777}]Br$: C_{10} and $[N_{8888}]Br$: C_{10}, and therefore the less effective are the most hydrophilic, $[N_{8881}]Br$: C_8 and DL-Menthol: C_8.

3.2.3. Effect of contact time

Contact time is a crucial parameter that reflects the kinetics of the separation process. A fast kinetics, as thus a short contact time, is usually desired. In order to find the optimal time to carry out the extraction of BPA from aqueous solutions, the best DES of each family, those with HBA: HBD ratio of (1:2), were selected to perform these experiments. Figure 5 shows the extraction efficiencies of BPA from water as a function of time at 25 ºC and also listed in Table S5 in Supplementary Information.

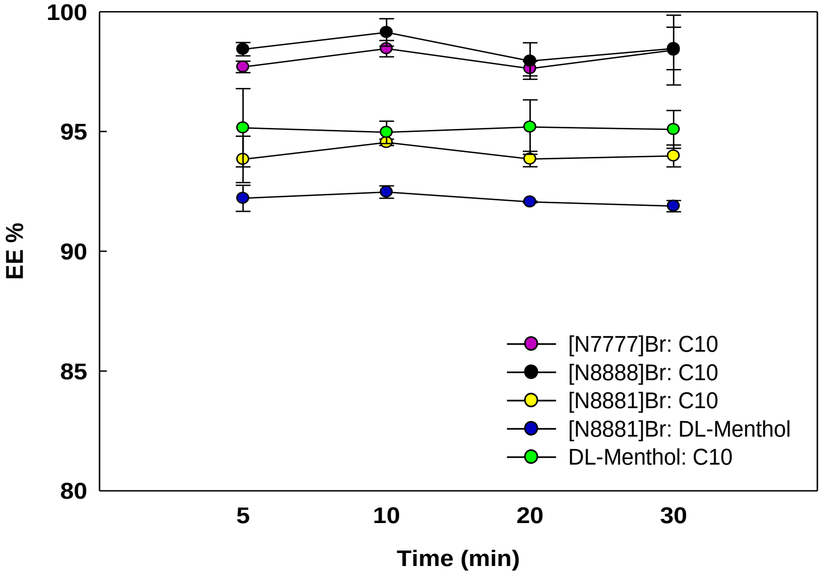


Figure 5. Influence of contact time on the extraction efficiencies (EE %) for the best deep eutectic solvents used in this work ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, temperature = 25 ºC, DES/water ratio = 1/1).

It can be concluded that the extraction process of BPA using the proposed DESs is quite fast, and the equilibrium can be reached in 5 to 10 min. Thus, the contact time was set to 10 min to ensure that the equilibrium is attained for all DESs.

3.2.4. Effect of stirring speed

Stirring speed is another parameter that might influence the extraction efficiency of BPA. The influence of stirring speed on the extraction efficiencies of BPA for the most performing DES used in this work, which are $[N_{7777}]Br$: C_{10}, $[N_{8888}]Br$: C_{10}, $[N_{8881}]Br$: C_{10}, $[N_{8881}]Br$: DL-Menthol and DL-Menthol: C_{10}, is shown in Figure 6 and listed in Table S6 in Supplementary Information.

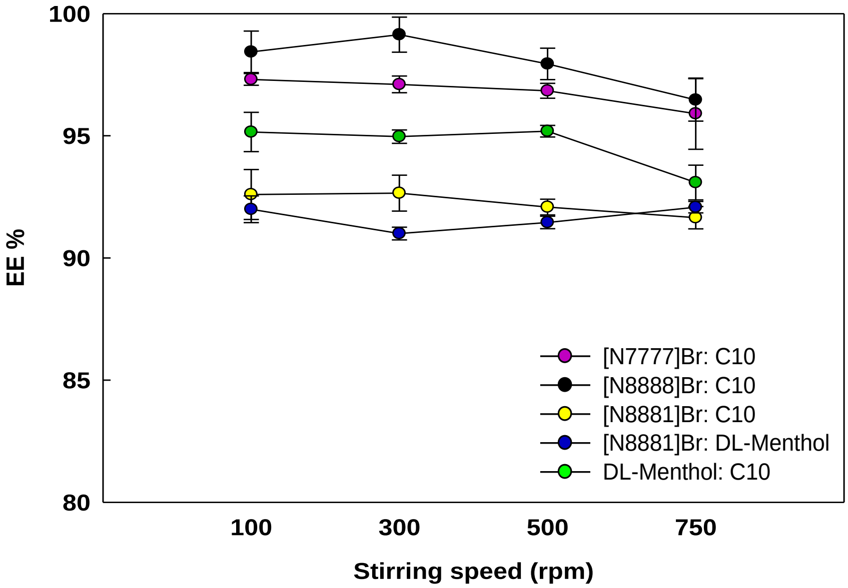


Figure 6. Influence of stirring speed on the extraction efficiencies (EE %) for the best DESs used in this work ($[C_0]_{BPA}$ = 0.1 g/L, DES/water ratio = 1/1, temperature = 25 ºC, contact time = 10 min).

These results show that the increase of the stirring speed leads to a minor decrease in the EE% for some DES, especially for the highest stirring speed, 750 rpm. This can probably be due to the different solubilities of water in DES[35] and the fact that the stirring speed might affect the dispersion of water in the studied DES. On the other hand, for $[N_{8881}]$Br: C_{10} this effect is almost negligible, taking into account the error bars. In conclusion, not taking into account the highest stirring speed, after 300 rpm, no significant increase in EE% is obtained. Thus, 300 rpm was chosen as the optimal extraction stirring speed, for energetic considerations.

3.2.5. Effect of DES/water mass ratio

In terms of cost of an industrial extraction process, it is desirable to obtain higher extraction efficiencies for a minimum amount of solvent. The effect of the mass ratio of the DES/water phases in the BPA extraction efficiencies was studied and the results are shown in Figure 7 and listed in Table S7 in Supplementary Information.

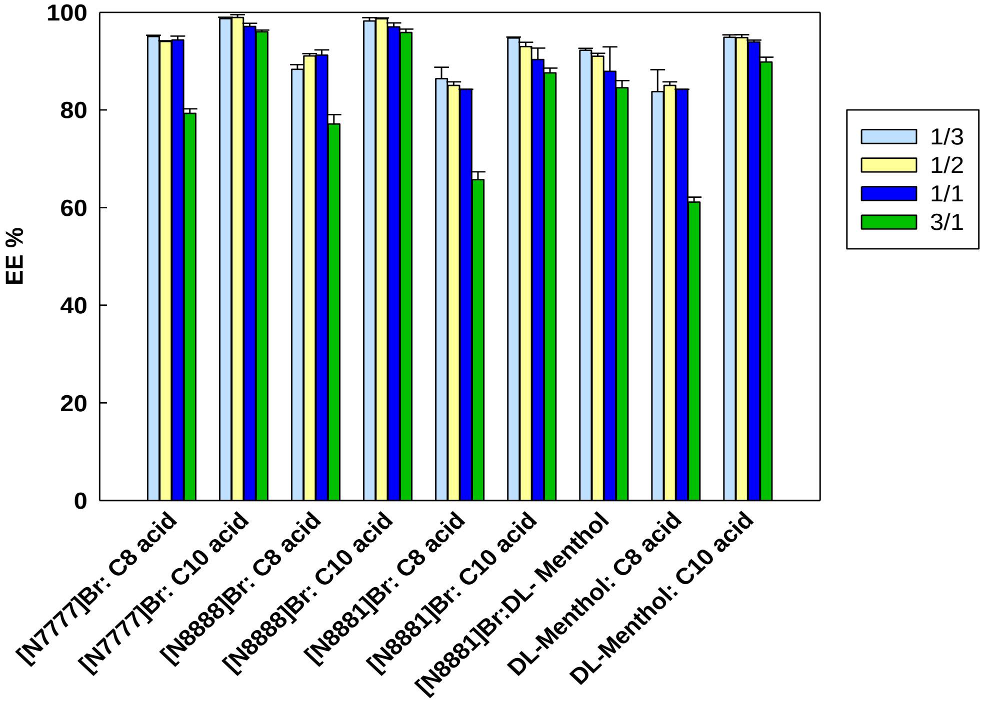


Figure 7. Effect of DES/water ratio on the extraction efficiencies (EE %) for all DESs used in this work ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, temperature = 25 ºC, contact time = 10 min).

It can be observed that the increase of DES/water mass ratio does not have a significant effect in the BPA extraction efficiencies. An exception is the case of 3/1 DES/water ratio, where a large decrease in the extraction efficiencies is observed for DES based on octanoic acid as HBD. These results are probably due to the lower hydrophobicity of these DES and higher solubility of water in them.[35]

In general, these results show that only a small mass of DES is needed to treat large volumes of contaminated water, which is probably due to high hydrophobicity of the DES and the very low solubility of BPA in water. These results are of particular importance when the DES starting materials are expensive, such as in the case of hydrophobic quaternary ammonium salts with long alkyl chains.

3.3. Optimization of the extraction efficiency using Experimental Design

The response surface methodology (RSM) is a useful and efficient statistical tool which allows optimizing the response of a system to the change of a set of independent variables. In this work, a Central Composite Design (CCD) was used to determine a set of experimental conditions leading to an optimal extraction efficiency of BPA. From the previously carried out studies, two experimental parameters, initial concentration of micropollutant and the DES/water mass ratio, could be identified as important for the extraction of Bisphenol-A from water environments. Temperature is also another experimental parameter that, generally, greatly influences the solubility and, consequently, the extraction processes. Since $[N_{8888}]Br$: C_{10} (1:2) DES presented the highest extraction efficiency values, it was selected to be used in the in CCD, where the optimal temperature, DES/water ratio and initial concentration of BPA parameter to achieve the highest extraction efficiencies. The levels of the studied variables are shown in Table 1 and the experimental plan using CCD is listed in Table S9 in Supplementary Information.

Table 1. Optimization of BPA extraction for [N_{8888}]Br: C_{10} (1:2) using a experimental central composite design.

Experimental Factors	- 1.68	- 1	0	+ 1	+ 1.68
Temperature	19	26	35	45	50
DES/water Ratio	0.24	0.96	2	3.05	3.76
Initial Concentration (C_0) of BPA	0.03	0.04	0.06	0.08	0.09

A fixed extraction time of 10 min and stirring speed of 300 rpm were used, since these parameters do not influence the extraction efficiencies. The experimental data obtained were analyzed by Pareto analysis of variance (ANOVA) and fitted to a second-order polynomial equation using multiple regression analysis. Detailed data are provided in Supplementary Information.

The statistical design software STATISTICA 6.0 (Statsoft Inc., Tulsa, U.S.A.) was used to analyze the data and graphically display the results. Central composite design was implemented for the experimental design analysis, using the response surface and the desirability approach, which converts each response variable in an individual desirability function ranging from 0 to 1. This design allows the interpretation of the association between the response of the percentage of extraction efficiencies and the independent variables that influence the BPA extraction from water. The influence of each parameter in BPA extraction efficiency using a CCD is illustrated in Figure 8 and listed in Table S10 in Supplementary Information.

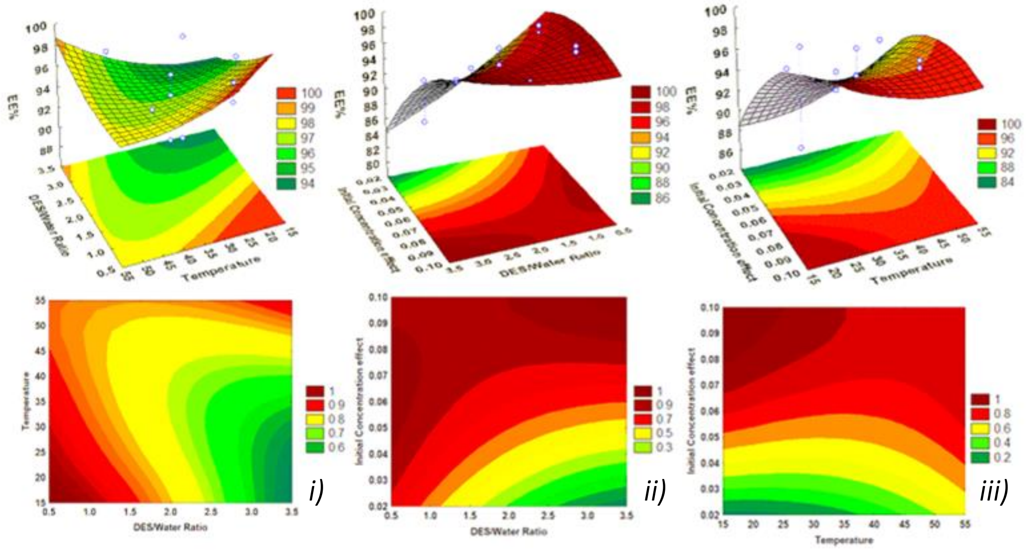


Figure 8. Response surface (top) and desirability plots (bottom) of the extraction efficiencies (EE %) of BPA from water using [N$_{8888}$]Br: C$_{10}$ DES with the combined effects of: i) Temperature and DES/water ratio; ii) initial concentration of BPA in water and DES/water ratio; and iii) initial concentration of BPA in water and Temperature.

An *F*-test and *p*-value were applied to the obtained model to check the significance of each coefficient. The low *p*-value (< 0.0001) and the high *F*-test indicate the suitability of the model. The repeatability of the model was assured through an average of three replicas of extraction efficiencies experiments with 95.77 ± 0.02 of EE%.

In Figure 8 ii) a steep decrease of the extraction efficiencies of BPA with initial concentration of BPA, independently of temperature and DES/water ratio, can be observed. The response curve temperature versus DES/water mass ratio are relatively flat, with a maximum at low temperatures and low DES/water mass ratio, revealing that after all the small effect of these two parameters. It is important to note that the most important factor is the initial concentration of BPA as well as the interaction between temperature and DES/water ratio. These results were consistent with ANOVA table and also showed that higher temperatures are not beneficial or required for this extraction process, as much lower temperatures or even room temperature. It means that in most cases, higher extraction efficiencies were achieved at 25 °C or lower temperatures.

As shown in Figure 8, and as previously demonstrated in the experimental work, by adjusting the different factors similar extraction efficiencies can be obtained. This result is very attractive for industrial application, since the process can be performed at low temperatures, lowering costs in terms of energy.

3.4. Reuse of DES

From an economic point of view, it is very important to have a solvent that can be used for multiple extractions, without losing its extraction capacity. In order to evaluate the possibility of reuse the DES, sequential extraction cycles were carried out, always using the same DES in contact with “fresh” contaminated water phase. Since $[N_{8888}]Br$: C_{10} allowed to achieve the highest extraction efficiencies, this DES was chosen. The results for five consecutive DES reuse cycles are shown in Figure 9 and listed in Table S8 in Supplementary Information.

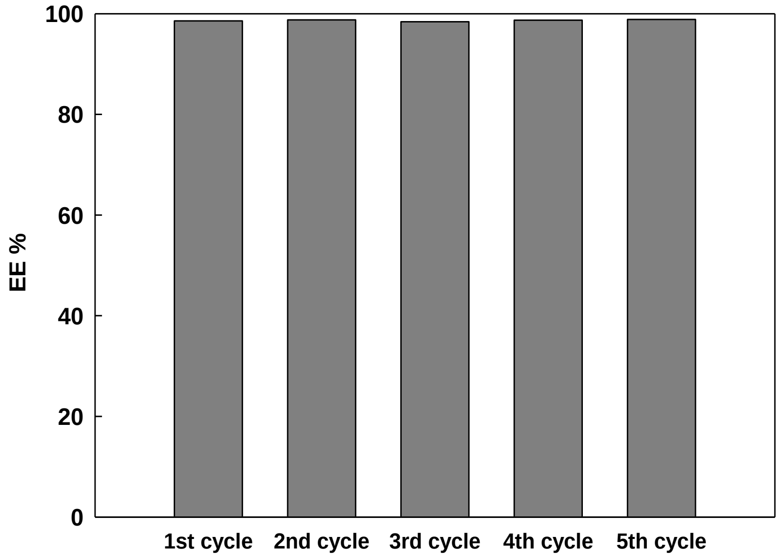


Figure 9. Extraction capability (EE %) of [N_{8888}]Br: C_{10} DES during several cycles ($[C_0]_{BPA}$ = 0.1 g/L, DES/water ratio = 1/1, stirring speed = 300 rpm, temperature = 25 ºC, contact time = 10 min).

From the analysis of Figure 9 it is obvious that [N_{8888}]Br: C_{10} DES could be reused up to five times without loss of its BPA extraction capability. This is directly related to the solubility of BPA in DES which was verified that is around five hundred times higher than in water. As mentioned before this is an important property from the economic and the sustainable point of view.

4. Conclusions

Hydrophobic DES have attracted considerable attention as task specific solvents for the extraction micropollutants, which typically are also hydrophobic, without contamination of water streams. In the present work, several hydrophobic DES, prepared using natural hydrophobic compounds such as DL-Menthol and fatty acids and several quaternary ammonium salts, were prepared and their efficiency in the extraction of an emerging micropollutant, Bisphenol-A, from water environments, evaluated. The results showed that $[N_{8888}]Br$: C_{10} can extract almost 100% of BPA from water environments under the optimal conditions. The recycling of DES is also possible due to its large capacity for this micropollutant.

5. Acknowledgements

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6. Supplementary Information

6.1. Deep Eutectic Solvents Preparation and characterization

Table S1. List of the hydrophobic DESs prepared in this work.

HBA	HBD	Abbreviation	MW (g/mol)	Molar Ratio
Tetraheptylammonium bromide, [N_{7777}]Br	Octanoic acid, C_8 acid	[N_{7777}]Br: C_8	259.70	1:2
	Decanoic acid, C_{10} acid	[N_{7777}]Br: C_{10}	278.40	1:2
Tetraoctylammonium bromide, [N_{8888}]Br	Octanoic acid, C_8 acid	[N_{8888}]Br: C_8	278.40	1:2
	Decanoic acid, C_{10} acid	[N_{8888}]Br: C_{10}	297.10	1:2
Methyltrioctylammonium bromide, [N_{8881}]Br	Octanoic acid, C_8 acid	[N_{8881}]Br: C_8	245.68	1:2
	Decanoic acid, C_{10} acid	[N_{8881}]Br: C_{10}	264.38	1:2
	DL-Menthol	[N_{8881}]Br: DL-Menthol	253.71	1:2
DL-Menthol	Octanoic acid, C_8 acid	DL-Menthol: C_8	148.23	1:2
	Decanoic acid, C_{10} acid	DL-Menthol: C_{10}	166.93	1:2

6.2. Characterization of the best DES studied, [N_{8888}]Br: C_{10}

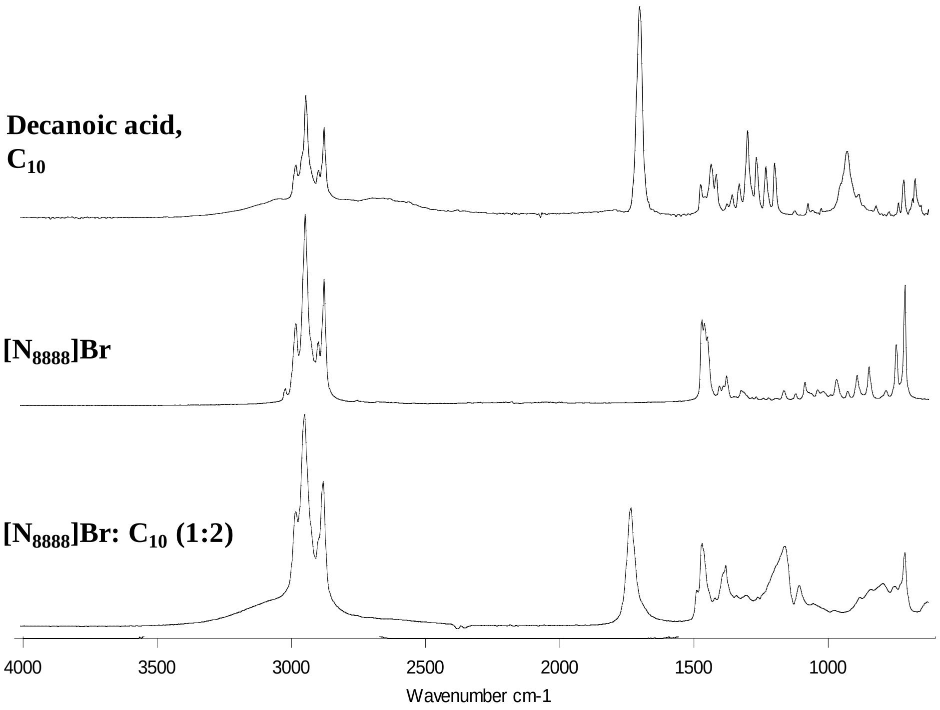

Figure S1. FTIR analysis of the hydrophobic DESs ([N_{8888}]Br: C_{10}) studied in this work.

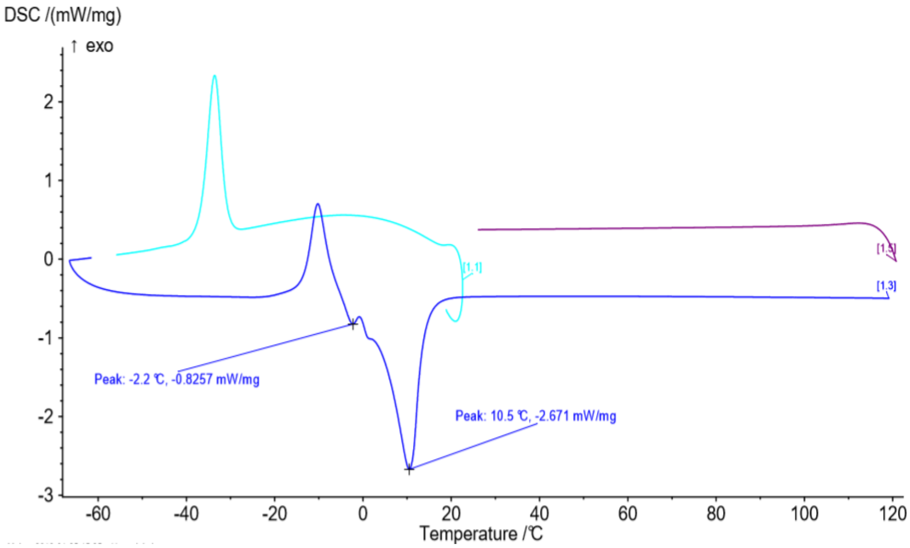

Figure S2. DSC analysis of the hydrophobic DESs ([N$_{8888}$]Br: C$_{10}$) studied in this work.

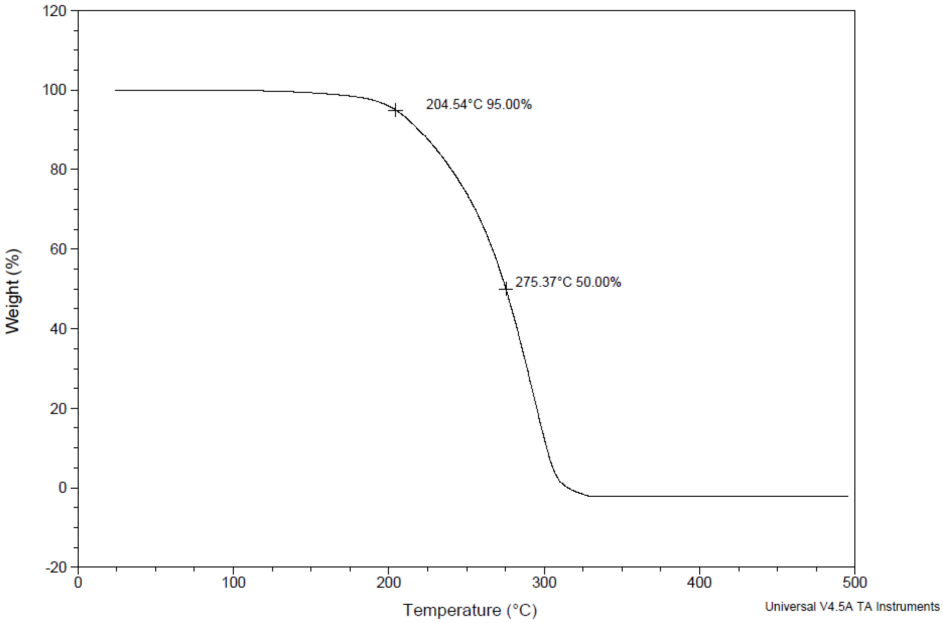

Figure S3. TGA analysis of the hydrophobic DESs ([N$_{8888}$]Br: C$_{10}$) studied in this work.

6.3. Liquid-liquid extraction technique

Table S2. Extraction efficiencies (EE %) of Bisphenol - A obtained for all DESs (1:2 molar ratio) used in this work ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, DES/water ratio = 1/1, temperature = 25 ºC, contact time = 10 min).

DESs	**Extraction Efficiencies (EE%)**
$[N_{7777}]$Br: C_8	94.91 ± 1.919
$[N_{7777}]$Br: C_{10}	97.10 ± 1.547
$[N_{8888}]$Br: C_8	90.48 ± 2.039
$[N_{8888}]$Br: C_{10}	97.61 ± 1.567
$[N_{8881}]$Br: C_8	84.24 ± 2.523
$[N_{8881}]$Br: C_{10}	91.99 ± 3.026
$[N_{8881}]$Br: DL-Menthol	91.45 ± 3.026
DL-Menthol: C_8	81.65 ± 1.938
DL-Menthol: C_{10}	92.43 ± 1.231

Table S3. Influence of ratio between HBA and HBD in some DESs on the extraction efficiencies (EE %) ($[C_0]_{BPA}$ = 0.1 g/L, Stirring speed = 300 rpm, DES/water ratio = 1/1, Temperature = 25 ºC, Contact time = 10 min).

DESs	**Extraction Efficiencies (EE %)**			
	1:2	**1:3**	**1:4**	**1:5**
$[N_{7777}]$Br: C_{10}	97.10 ± 1.234	94.69 ± 0.971	93.89 ± 1.342	
$[N_{8888}]$Br: C_8	92.80 ± 2.039	89.89 ± 2.322	87.36 ± 0.238	86.77 ± 0.786
$[N_{8881}]$Br: C_8	84.24 ± 2.523	82.75 ± 1.609	81.75 ± 0.613	81.78 ± 0.274
$[N_{8881}]$Br: DL-Menthol	83.30 ± 1.478	73.63 ± 1.500	85.29 ± 1.483	84.02 ± 1.582
DL-Menthol: C_8	81.01 ± 1.938	79.39 ± 1.655	78.45 ± 2.898	79.76 ± 2.696

Table S4. Influence of initial concentration of Bisphenol-A on the extraction efficiencies (EE %) for all DESs used in this work (Stirring speed = 300 rpm, DES/water ratio = 1/1, Temperature = 25 ºC, Contact time = 10 min).

DESs	**Extraction Efficiencies (EE %)**		
	100 ppm	**50 ppm**	**25 ppm**
[N_{7777}]Br: C_8	94.91 ± 1.938	81.63 ± 2.5034	64.31 ± 2.473
[N_{7777}]Br: C_{10}	97.10 ± 1.231	96.34 ± 0.442	93.20 ± 0.981
[N_{8888}]Br: C_8	87.79 ± 1.919	81.87 ± 1.058	67.47 ± 0.314
[N_{8888}]Br: C_{10}	97.31 ± 1.547	94.79 ± 0.216	86.35 ± 0.432
[N_{8881}]Br: C_8	79.57 ± 2.039	73.20 ± 0.583	47.46 ± 2.198
[N_{8881}]Br: C_{10}	86.54 ± 1.567	88.19 ± 0.615	78.30 ± 1.452
[N_{8881}]Br: DL-Menthol	89.41 ± 2.523	84.88 ± 0.356	71.44 ± 0.648
DL-Menthol: C_8	81.65 ± 3.026	73.22 ± 0.583	59.81 ± 0.255
DL-Menthol: C_{10}	94.17 ± 2.892	91.12 ± 1.155	82.15 ± 3.219

Table S5. Influence of contact time on the extraction efficiencies (EE %) for the best deep eutectic solvents used in this work ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, temperature = 25 ºC, DES/water ratio = 1/1).

DESs	**Extraction Efficiencies (EE %)**			
	5 min	**10 min**	**20 min**	**30 min**
[N_{7777}]Br: C_{10}	97.70 ± 0.240	98.46 ± 0.343	97.63 ± 0.306	98.40 ± 1.456
[N_{8888}]Br: C_{10}	98.44 ± 0.279	99.14 ± 0.573	97.94 ± 0.762	98.47 ± 0.887
[N_{8881}]Br: C_{10}	93.84 ± 0.970	94.55 ± 0.133	93.85 ± 0.322	93.98 ± 0.457
[N_{8881}]Br: DL-Menthol	92.21 ± 0.545	92.47 ± 0.259	92.06 ± 0.032	91.88 ± 0.234
DL-Menthol: C_{10}	95.16 ±1.633	94.97 ± 0.466	95.19 ± 1.139	95.09 ± 0.791

Table S6. Influence of stirring speed on the extraction efficiencies (EE %) for the best DESs used in this work ($[C_0]_{BPA}$ = 0.1 g/L, DES/water ratio = 1/1, temperature = 25 ºC, contact time = 10 min).

DESs	**Extraction Efficiencies (EE %)**			
	100 rpm	**300 rpm**	**500 rpm**	**750 rpm**
$[N_{7777}]$Br: C_{10}	97.31 ± 0.239	97.10 ± 0.343	96.84 ± 0.306	95.91 ± 1.456
$[N_{8888}]$Br: C_{10}	98.36 ± 0.852	99.14 ± 0.718	97.94 ± 0.643	96.47 ± 0.869
$[N_{8881}]$Br: C_{10}	92.60 ± 1.023	92.65 ± 0.733	92.08 ± 0.321	91.65 ± 0.467
$[N_{8881}]$Br: DL-Menthol	91.99 ± 0.545	91.00 ± 0.259	91.45 ± 0.256	92.08 ± 0.234
DL-Menthol: C_{10}	95.60 ± 0.805	94.53 ± 0.272	95.65 ± 0.238	93.09 ± 0.713

Table S7. Effect of DES/water ratio on the extraction efficiencies (EE %) for all DESs used in this work ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, temperature = 25 ºC, contact time = 10 min).

DESs	**Extraction Efficiencies (EE %)**			
	1/3	**1/2**	**1/1**	**3/1**
$[N_{7777}]$Br: C_8	95.06 ± 0.230	94.03 ± 0.128	94.35±0.793	79.31 ± 0.928
$[N_{7777}]$Br: C_{10}	98.70 ± 0.300	98.94 ± 0.600	97.10±0.650	96.00 ± 0.380
$[N_{8888}]$Br: C_8	88.33 ± 0.960	91.07 ± 0.485	91.24±1.071	77.12 ± 1.938
$[N_{8888}]$Br: C_{10}	98.23 ± 0.671	98.68 ± 0.190	97.03±0.815	95.86 ± 0.707
$[N_{8881}]$Br: C_8	86.40 ± 2.340	85.03 ± 0.730	84.22±0.023	65.72 ± 1.624
$[N_{8881}]$Br: C_{10}	94.74 ± 0.196	92.97 ± 0.888	90.33±2.350	87.60 ± 1.120
$[N_{8881}]$Br: DL-Menthol	92.21 ± 0.400	91.00 ± 0.600	87.89±5.022	84.55 ± 1.473
DL-Menthol: C_8	83.75 ± 4.494	85.03 ± 0.730	84.22±0.023	61.13 ± 0.967
DL-Menthol: C_{10}	94.88 ± 0.500	94.83 ± 0.600	93.91±0.400	89.82 ± 1.141

Table S8. Extraction capability (EE %) of [N_{8888}]Br: C_{10} DES during several cycles ([C_0]$_{BPA}$ = 0.1 g/L, DES/water ratio = 1/1, stirring speed = 300 rpm, temperature = 25 ºC, contact time = 10 min).

[N_{8888}]Br: C_{10}	**Extraction Efficiencies (EE%)**
1st cycle	98.5714
2nd cycle	98.7744
3rd cycle	98.3985
4th cycle	98.7068
5th cycle	98.8571

6.4. Experimental Design

Table S9. Experimental plan using CCD.

Experiment	Factor A	Factor B	Factor C
1	-1	-1	-1
2	-1	-1	1
3	-1	1	-1
4	-1	1	1
5	1	-1	-1
6	1	-1	1
7	1	1	-1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15 (C)	0	0	0
16 (C)	0	0	0
17 (C)	0	0	0

Table S10. Extraction efficiencies obtained and predicted for removal of Bisphenol-A from water environments using a central composite design (CCD).

Experiments	**DES/water Ratio**	**Temp. (ºC)**	**C_0 of BPA**	**Experimental EE (%)**	**Predicted EE (%)**
1	0.96	26.0	0.04	97.35	96.96
2	0.96	26.0	0.08	99.19	99.72
3	0.96	45.0	0.04	96.28	96.39
4	0.96	45.0	0.08	98.49	96.78
5	3.05	26.0	0.04	87.44	89.54
6	3.05	26.0	0.08	97.56	97.79
7	3.05	45.0	0.04	92.86	92.57
8	3.05	45.0	0.08	97.78	98.46
9	0.24	35.0	0.06	98.45	99.47
10	3.76	35.0	0.06	95.94	94.46
11	2.00	19.0	0.06	97.98	96.75
12	2.00	51.0	0.06	95.79	96.70
13	2.00	35.0	0.03	91.44	90.68
14	2.00	35.0	0.09	97.72	98.03
15 (C)	2.00	35.0	0.06	95.74	95.75
16 (C)	2.00	35.0	0.06	95.78	95.75
17 (C)	2.00	35.0	0.06	95.78	95.75

7. References

1. Rajasulochana, P.; Preethy, V., Comparison on efficiency of various techniques in treatment of waste and sewage water – A comprehensive review. *Resource-Efficient Technologies* 2016, 2, (4), 175-184.

2. Montgomery, M. A.; Elimelech, M., Water And Sanitation in Developing Countries: Including Health in the Equation. *Environmental Science & Technology* 2007, 41, (1), 17-24.

3. Shannon, M. A.; Bohn, P. W.; Elimelech, M.; Georgiadis, J. G.; Mariñas, B. J.; Mayes, A. M., Science and technology for water purification in the coming decades. *Nature* 2008, 452, 301.

4. Florindo, C.; Branco, L. C.; Marrucho, I. M., Development of hydrophobic deep eutectic solvents for extraction of pesticides from aqueous environments. *Fluid Phase Equilibria* 2017, 448, 135-142.

5. Margot, J.; Rossi, L.; Barry, D. A.; Holliger, C., A review of the fate of micropollutants in wastewater treatment plants. *Wiley Interdisciplinary Reviews: Water* 2015, 2, (5), 457-487.

6. Luo, Y.; Guo, W.; Ngo, H. H.; Nghiem, L. D.; Hai, F. I.; Zhang, J.; Liang, S.; Wang, X. C., A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of The Total Environment* 2014, 473-474, 619-641.

7. Kim, M.-K.; Zoh, K.-D., Occurrence and removals of micropollutants in water environment. *Environmental Engineering Research* 2016, 21, (4), 319-332.

8. Teng, C.; Goodwin, B.; Shockley, K.; Xia, M.; Huang, R.; Norris, J.; Merrick, B. A.; Jetten, A. M.; Austin, C. P.; Tice, R. R., Bisphenol A affects androgen receptor function via multiple mechanisms. *Chemico-Biological Interactions* 2013, 203, (3), 556-564.

9. Monneret, C., What is an endocrine disruptor? *Comptes Rendus Biologies* 2017, 340, (9), 403-405.

10. Almeida, H. F. D.; Marrucho, I. M.; Freire, M. G., Removal of Nonsteroidal Anti-Inflammatory Drugs from Aqueous Environments with Reusable Ionic-Liquid-Based Systems. *ACS Sustainable Chemistry & Engineering* 2017, 5, (3), 2428-2436.

11. Almeida, H. F. D.; Freire, M. G.; Marrucho, I. M., Improved extraction of fluoroquinolones with recyclable ionic-liquid-based aqueous biphasic systems. *Green Chemistry* 2016, 18, (9), 2717-2725.

12. Malik, M. A.; Hashim, M. A.; Nabi, F., Ionic liquids in supported liquid membrane technology. *Chemical Engineering Journal* 2011, 171, (1), 242-254.

13. Zhang, J.; Liu, H.; Jiang, L., Membrane-Based Strategy for Efficient Ionic Liquids/Water Separation Assisted by Superwettability. *Advanced Functional Materials* 2017, 27, (20), 1606544.

14. Dietz, C. H. J. T.; Kroon, M. C.; Di Stefano, M.; van Sint Annaland, M.; Gallucci, F., Selective separation of furfural and hydroxymethylfurfural from an aqueous solution using a supported hydrophobic deep eutectic solvent liquid membrane. *Faraday Discussions* 2018, 206, (0), 77-92.

15. Ali, I.; Gupta, V. K., Advances in water treatment by adsorption technology. *Nature Protocols* 2007, 1, 2661.

16. Sahiner, N.; Sagbas, S., Polymeric ionic liquid materials derived from natural source for adsorption purpose. *Separation and Purification Technology* 2018, 196, 208-216.

17. van Osch, D. J. G. P.; Zubeir, L. F.; van den Bruinhorst, A.; Rocha, M. A. A.; Kroon, M. C., Hydrophobic deep eutectic solvents as water-immiscible extractants. *Green Chemistry* 2015, 17, (9), 4518-4521.

18. Shi, C.; Jing, Y.; Xiao, J.; Wang, X.; Jia, Y., Liquid-liquid extraction of lithium using novel phosphonium ionic liquid as an extractant. *Hydrometallurgy* 2017, 169, 314-320.

19. Rocha, M. A. A.; Raeissi, S.; Hage, P.; Weggemans, W. M. A.; van Spronsen, J.; Peters, C. J.; Kroon, M. C., Recovery of volatile fatty acids from water using medium-chain fatty acids and a cosolvent. *Chemical Engineering Science* 2017, 165, 74-80.

20. Pena-Pereira, F.; Namieśnik, J., Ionic Liquids and Deep Eutectic Mixtures: Sustainable Solvents for Extraction Processes. *ChemSusChem* 2014, 7, (7), 1784-1800.

21. Zhao, H.; Xia, S.; Ma, P., Use of ionic liquids as ‘green’ solvents for extractions. *Journal of Chemical Technology & Biotechnology* 2005, 80, (10), 1089-1096.

22. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *Journal of the American Chemical Society* 2004, 126, (29), 9142-9147.

23. Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep Eutectic Solvents (DES) and Their Applications. *Chemical Reviews* 2014, 114, (21), 11060-11082.

24. Cruz, H.; Jordao, N.; Branco, L. C., Deep eutectic solvents (DES) as low-cost and green electrolytes for electrochromic devices. *Green Chemistry* 2017, 19, (7), 1653-1658.

25. Abo-Hamad, A.; Hayyan, M.; AlSaadi, M. A.; Hashim, M. A., Potential applications of deep eutectic solvents in nanotechnology. *Chemical Engineering Journal* 2015, 273, 551-567.

26. Zhao, H., DNA stability in ionic liquids and deep eutectic solvents. *Journal of Chemical Technology & Biotechnology* 2015, 90, (1), 19-25.

27. Tang, B.; Zhang, H.; Row, K. H., Application of deep eutectic solvents in the extraction and separation of target compounds from various samples. *Journal of Separation Science* 2015, 38, (6), 1053-1064.

28. García, G.; Aparicio, S.; Ullah, R.; Atilhan, M., Deep Eutectic Solvents: Physicochemical Properties and Gas Separation Applications. *Energy & Fuels* 2015, 29, (4), 2616-2644.

29. Williamson, S. T.; Shahbaz, K.; Mjalli, F. S.; AlNashef, I. M.; Farid, M. M., Application of deep eutectic solvents as catalysts for the esterification of oleic acid with glycerol. *Renewable Energy* 2017, 114, 480-488.

30. Alonso, D. A.; Baeza, A.; Chinchilla, R.; Guillena, G.; Pastor, I. M.; Ramón, D. J., Deep Eutectic Solvents: The Organic Reaction Medium of the Century. *European Journal of Organic Chemistry* 2016, 2016, (4), 612-632.

31. Tereshatov, E. E.; Boltoeva, M. Y.; Folden, C. M., First evidence of metal transfer into hydrophobic deep eutectic and low-transition-temperature mixtures: indium extraction from hydrochloric and oxalic acids. *Green Chemistry* 2016, 18, (17), 4616-4622.

32. Florindo, C.; Romero, L.; Rintoul, I.; Branco, L. C.; Marrucho, I. M., From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid–Based Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering* 2018, 6, (3), 3888-3895.

33. Ribeiro, B. D.; Florindo, C.; Iff, L. C.; Coelho, M. A. Z.; Marrucho, I. M., Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. *ACS Sustainable Chemistry & Engineering* 2015, 3, (10), 2469-2477.

34. Zhang, Y.; Causserand, C.; Aimar, P.; Cravedi, J. P., Removal of bisphenol A by a nanofiltration membrane in view of drinking water production. *Water Research* 2006, 40, (20), 3793-3799.

35. Florindo, C.; Lima, F.; Branco, L. C.; Marrucho, I. M., Hydrophobic Deep Eutectic Solvents: A Circular Approach to Purify Water Contaminated with Ciprofloxacin. *ACS Sustainable Chem. Eng*. 2019, 7, 17, 14739-14746.

36. Zhang, Q.; De Oliveira Vigier, K.; Royer, S.; Jerome, F., Deep eutectic solvents: syntheses, properties and applications. *Chemical Society Reviews* 2012, 41, (21), 7108-7146.

CHAPTER 3.3

Removal of ciprofloxacin from water environments using task-specific hydrophobic deep eutectic solvent

Adapted from: **C. Florindo**, Francine Lima, L. C. Branco and I. M. Marrucho, Hydrophobic Deep Eutectic Solvents: A Circular Approach to Purify Water Contaminated with Ciprofloxacin, ACS Sustainable Chem. Eng. 2019, 7, 14739-14746.

Francine Lima developed most of the laboratory work as part of her master's thesis based in the university of Stuttgart in collaboration with us, which was included in the doctoral project of the author. The author contributed to the execution of some final experiments described in this chapter to complete the work, as well as involved in the discussion, interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

Clean water became one of the major concerns of 21st century. The occurrence of micropollutants in aquatic ecosystems poses a serious public health and environmental problems, leading to true challenges in the development of sustainable and cost-effective alternative technologies for wastewater treatment processes. As a result, hydrophobic Deep Eutectic Solvents (DES) have been emerging as an easy-to-prepare, inexpensive and environmentally-benign media showing a high potential for water applications. The main goal of this work was the development of hydrophobic DES for the removal of ciprofloxacin, identified as one of the top ten priority micropollutants, from water environments. In particular, DES composed of natural neutral components, such as menthol and fatty acids, and also DES based on the combination of quaternary ammonium salts and natural fatty acids were evaluated as potential extractants. C_{12}: C_{10} displays the second highest solubility value of ciprofloxacin and the lowest water solubility which leads to the best extraction efficiency of ciprofloxacin. Moreover, extraction efficiencies were maximized by optimizing the experimental factors using a central composite design combined with a response surface methodology. Finally, aiming at full sustainability, a circular process was developed by recycling and reusing the hydrophobic DES through the use of activated carbon.

1. Introduction

The detection, identification and efficient removal of micropollutants in water still represent major challenges in environmental science.[1] Among the different micropollutants that can be found in water streams, active pharmaceutical ingredients (APIs) are probably those of greatest concern since they have been linked to serious public health problems.[2,5,6] According to OECD 2014 publication, the use of pharmaceuticals in Europe has almost tripled in the last 14 years, due to the increasing demand for drugs to treat age-related diseases, chronic issues, infections etc., typically caused by population growth.[3] Roughly 90% of the APIs prescribed end up in wastewater treatment plants (WWTPs), as they are not completely absorbed by the body, while the remaining portion is excreted.[4] This water contamination problem has been ignored for a long time, and only recently advances in analytical techniques allowed the knowledge and quantification of a vast majority of micropollutants. Despite their vanishing concentrations, APIs bio accumulate in living organisms, through the food chain, and are thus major concern. Consequently, the Global Water Research Coalition (GWRC) compiled a list of APIs that are known to be found in the water cycle and have a negative influence on human's health and ecosystems. Subsequently, Voogt et al. 2009[7], using well established criteria such as ecotoxicity, physical chemistry characteristics, toxicity, resistance to treatment etc, selected ten high priority emerging APIs micropollutants. The vanishing concentrations of these compounds together with their diverse chemical nature make the development of cost-effective and sustainable water treatment processes extremely difficult. Although adsorption8 is the technique of election in wastewater treatment plants, liquid-liquid extraction[9, 10] has been gaining advantage due to the recent boom in task specific alternative solvents field.

Deep Eutectic Solvents consist of a mixture of two or more compounds, usually both solids at room temperature, where one component act as hydrogen bond donor (HBD) and the other as hydrogen bond acceptor (HBA), and upon mixture, a liquid eutectic mixture is obtained.[11] Since the first publication in 2003,[12] a vast array of applications has been developed using these designer solvents.[11, 13, 14] Most reported DES in the literature are water soluble, but recently hydrophobic DES emerged and have been shown to effectively remove compounds present in aqueous phases, without its contamination.[14, 15] Lately, hydrophobic DES have been explored in the development of water cleaning applications, having already been successfully applied for removal of metal ions from water, indium extraction from hydrochloric and oxalic acids, removal of pesticides and endocrine disruptor compounds from water, among others.[15-18] To the best of our knowledge, the removal of APIs from water using this class of solvents is an almost non-explored topic. Only one recent study using hydrophobic DES as extractants of pharmaceutical contaminants from water phases has been published.[19] In that work, the authors used fatty acids/alcohols-based hydrophobic deep eutectic solvents to extract two antibiotics (levofloxacin and ciprofloxacin) present in water using liquid-liquid microextraction. This study showed that 1-octanol/ tricaprylylmethylammonium chloride-based DES allowed the best extraction efficiency with recoveries about 94.8%.

The main goal of this work is to evaluate the potential of hydrophobic DES as alternative extractant solvents for ciprofloxacin, one of the 10 high priority compounds. Ciprofloxacin is a common and widely used API, a broad-spectrum fluoroquinolone antibiotic, used to treat mild to moderate infections in the respiratory and urinary tract.[20] Usually, it can be found up to µg.L-1 levels in the aquatic environment. Two different families of hydrophobic DES were used as extractants: neutral DES, consisting of all neutral and natural compounds, such as DL-menthol and fatty acids as octanoic (C_8) and decanoic (C_{10}) acids; and charged DES, containing quaternary ammonium salts in their constitution, such as $[N_{7777}]Br$, $[N_{8888}]Br$ and $[N_{8881}]Br$. From the sustainability point of view, this last family of compounds are less appealing than the first one, since they are synthesized from petroleum-based compounds and are recalcitrant. Although neutral DES do not present a deep depression in the melting point of the eutectic mixtures, they are here designated as DES for sake of simplicity.

Since ciprofloxacin can exist in different forms, cationic, anionic or zwitterionic, depending on pH in aqueous solutions, it is important to test neutral and ionic hydrophobic DES to evaluate the effect of DES charges on the liquid-liquid extraction of ciprofloxacin. This work not only addresses the removal of ciprofloxacin as micropollutant in water streams but can also be of interest to the pharmaceutical industry, which need to treat their wastes before discharge. Thus, from the industrial application point of view, it is very important to optimize the extraction process, namely reducing associated energy and costs of the process, leading a complete environmentally friendly process. For that, the most influential experimental extraction parameters were optimized using a central composite design (CCD). Moreover, the reuse and recyclability of hydrophobic DES were also evaluated. Adsorption with activated carbon was chosen to remove ciprofloxacin from the hydrophobic DES since this technology is already implemented and available in water treatment plants and requires a very low mass to obtain high removal efficiency. On top of that, the use of activated carbon to clean DES and not to clean the water, avoids the contact of water with activated carbon and the recent health and environmental problems associated with the presence of microparticles.

2. Experimental Section

2.1. Materials

DL-menthol (purity ≥ 95%), tetraheptylammonium bromide ([N_{7777}]Br) (purity ≥ 99%), tetraoctylammonium bromide ([N_{8888}]Br) (purity ≥ 98%), methyltrioctylammonium bromide ([N_{8881}]Br) (purity ≥ 97%), octanoic acid (C_8) (purity ≥ 98%), decanoic acid (C_{10}) (purity ≥ 98%) and dodecanoic acid (C_{12}) (purity ≥ 98%) were purchased from Sigma-Aldrich and used as received. Ciprofloxacin micropollutant was generously provided by Bayer HealthCare AG with high purity (>99.8%) and were used as supplied. All aqueous solutions were prepared by weighting the required mass using an analytic laboratory balance Ohaus Adventurer® Analytical, through mixing high purity water (Milli-Q water). In order to obtain solutions with different pH’s, aqueous solutions of NaOH and HCl, for pH=11 and pH=4, respectively, were prepared.

2.2. Experimental Methodologies

2.2.1. Preparation of DESs

DES were prepared by mixing both components (HBD and HBA) in a glass vial at a certain temperature and stirring speed, until a liquid homogeneous mixture is attained. The water content of each pure hydrophobic DESs was determined by Karl Fischer titration (Metrohm 870 KF Titrino Plus) and the water content obtained was < 500 ppm. Based on previous studies,[16, 18, 21-23] the molar ratio corresponding to the eutectic point of each system was selected to carry out the extraction experiments. The ratios used to prepare the hydrophobic DES used in this work are shown in Table 1.

Table 1. Hydrophobic DESs molar ratios studied in this work.

Hydrophobic DES studied	Molar Ratio
C_{12}: C_8	1:3
C_{12}: C_{10}	1:2
DL-menthol: C_8	1:1
DL-menthol: C_{10}	1:1
DL-menthol: C_{12}	2:1
$[N_{7777}]$Br: C_{10}	1:2
$[N_{8881}]$Br: DL-menthol	1:2
$[N_{8881}]$Br:C_8	1:2
$[N_{8881}]$Br:C_{10}	1:2
$[N_{8888}]$Br:C_{10}	1:2

2.2.2. Solubility of ciprofloxacin in different hydrophobic DESs

To assess the solubility of ciprofloxacin in the studied hydrophobic DES, small amounts of pure pharmaceutical ingredient (around 10 mg per addition) were added to all prepared DES at room temperature. The flasks were stirred for at least 12 hours and observed afterwards. If all the solid added was dissolved (homogeneous mixture was observed) another small amount of solid was added. This procedure was repeated until the saturation point (heterogeneous mixture) was visually detected.

2.2.3. Miscibility of water and DESs

In order to determine the water content present in hydrophobic DES at room temperature, all prepared DES were vigorously mixed with water in a 1:1 (DES: water) molar proportion in a glass vial during the time required to reach equilibrium, which was previously determined. After an efficient and complete separation of both phases, the DES phase was sampled with a needled syringe and injected in the Karl Fischer Coulometer Metrohm® to measure the water content.

2.2.4. Liquid-liquid extraction procedure

The prepared hydrophobic DES were used as extractant solvents of ciprofloxacin present in water at room temperature. For that purpose, an aqueous stock solution of ciprofloxacin of known concentration in water was previously prepared and diluted to prepare standards so that a calibration curve of the micropollutant in Milli-Q water (curve with R2 > 0.993) was established. Each prepared hydrophobic DES and water containing ciprofloxacin were vigorously stirred for adequate time to ensure that equilibrium was reached, which was previously established trough an equilibrium curve. All extractions were performed at room temperature and then left to settle for a minimum of 12h to ensure complete phase separation.

Using a needled syringe, the two phases were carefully sampled and the concentration of the remaining ciprofloxacin in the water-rich phase was measured using UV–vis spectroscopy with a Shimadzu model UV-1800 – Pharma-Spec spectrophotometer. To eliminate possible interferences of DES in the ciprofloxacin quantification, the DES-rich phase was not quantified. For each sample and experiment, three replicates were performed.

2.2.5. Reuse and Recycling of DES

The potential reuse of DES for several cycles of ciprofloxacin extraction was also performed. After complete phase separation, the upper phase (DES-rich phase) was collected and then exposed again to fresh water contaminated with micropollutant, at the same mass ratio (1:1). This procedure was repeated five times, yielding five subsequent extraction cycles.

In order to recycle the DES containing ciprofloxacin from its re-use in several consecutive extractions, adsorption with activated carbon (AC) was used to remove the ciprofloxacin from DES. For that purpose, DES phase containing ciprofloxacin was mixed with an amount of AC in a glass vial at room temperature, and subsequently filtered using a hydrophobic polytetrafluorethylene (PTFE) syringe filter with a pore size of 0.22µm, to ensure the complete removal of the AC from DES phase. Finally, in order to qualitatively check the absence of ciprofloxacin from the regenerated DES phase, the extractant was dissolved in acetonitrile and analyzed using an UV-1800 Shimadzu® Spectrophotometer.

3. Results and discussion

3.1. Optimization of Experimental Extraction Conditions

To assess the efficiency of the extraction of ciprofloxacin from water, the concentrations of ciprofloxacin in the aqueous phase before (C_o) and after extraction (C) were used through the following expression:

$$EE\,\% = \left(\frac{Co-C}{Co}\right) \text{x}\ 100 \qquad \text{(eq.1)}$$

In order to maximize the extraction efficiency of ciprofloxacin, several experimental parameters, such as contact time, stirring speed, ratio between DES and water phases, initial concentration of micropollutant and the effect of pH in water, were optimized.

3.1.1. Influence of pH

Fluoroquinolones have two protonable groups, meaning that they exist in three distinct forms, depending on the pH, with different solubility in water.[24, 25] In Figure 1, the speciation profile of ciprofloxacin as a function of pH is presented. It can be seen that the cationic specie is dominant at pH between 3 to 5, the zwitterionic specie between 6 and 9 and the anionic specie at pH higher than 10.

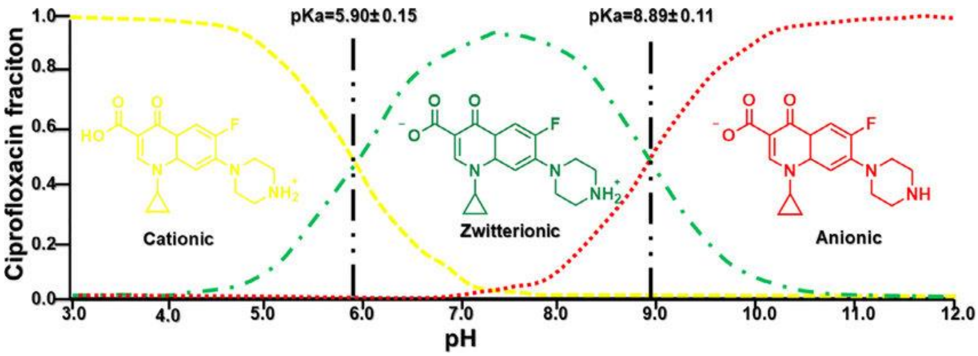

Figure 1. Distinct forms of ciprofloxacin at different pH values.[26]

In order to study the effect of pH in the extraction of ciprofloxacin from water, experiments were carried out at pH 3.3, 6.4 and 11.2 to attain the cationic, zwitterionic and anionic form, respectively. The extraction efficiencies at these pH values with the selected hydrophobic DES are presented in Figure 2.

Independently of the pH, it can be observed that neutral hydrophobic DES display much higher ciprofloxacin extraction efficiencies (from 40 to 90 %), than ionic DES based on ammonium salts (from 0 - 20%, typically around 0%).

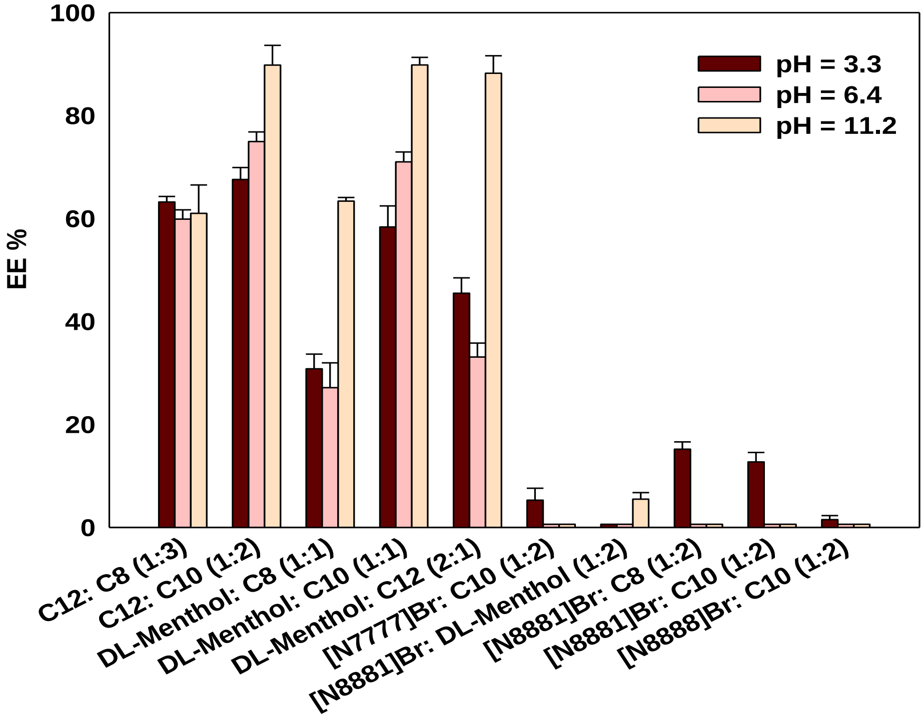

Figure 2. Extraction efficiencies (EE %) of ciprofloxacin at different pH values using different hydrophobic DES (contact time = 10 min, DES/water ratio = 1/1, stirring speed = 300rpm, temperature = 25 ºC).

Generally, literature shows that ionic hydrophobic DES are more efficient extractants of a wide range of compounds, from volatile fatty acids,[14] pesticides[15] to Bisphenol-A,[18] than neutral hydrophobic DES. Curiously, such behavior is not observed in this work, even when ciprofloxacin is in the ionic form. This fact rules out ion exchange extraction mechanism and evidences the complex interplay of intermolecular forces between DES and ciprofloxacin. Excellent extraction efficiencies were attained when cheap, neutral and all-natural DES are used, showing that it is possible to implement sustainable water cleaning processes based on these solvents.[23] Ionic hydrophobic DES, such as those based on quaternary ammonium salts, are more toxic and expensive than neutral all-natural DES.

As it can be seen from Figure 2, pH has a great influence on the extraction efficiency, especially for neutral hydrophobic DES, with higher extraction efficiencies obtained at higher pH values. If we consider that at pH higher than 4.9,[25-27] the pKa of both acids, the acids are deprotonated and ciprofloxacin is either in the zwiterionic (pH = 6.4) or anionic (pH = 11.2) form, electrostatic interactions seem to be responsible for the high extraction efficiencies of neutral hydrophobic DES. Note that the ionic DES here studied also contain acids but the probably the presence of ammonium cation introduces strong interactions among the DES components, including deprotonated acid, not leaving sufficient available charges for the interaction with ciprofloxacin. These results clearly show the importance of DES chemical design and the understanding of interactions between DES and different solutes at the molecular level.

Considering these results, C_{12}: C_{10} DES was chosen to proceed to the optimization of the other experimental parameters, since it provides a good combination of efficiency (one of the best extractants of ciprofloxacin from water) and sustainability, as it is based on all natural non-toxic compounds DES. Nevertheless, the solubility in water of some of these DES have already been reported before by us and some other authors,[15,16,18] using NMR, FTIR and UV spectroscopies and it was concluded that no traces of these DESs were detected in the aqueous phase up to the detection limit of experimental techniques used.

3.1.2. Influence of contact time

The influence of the contact time in the extraction of ciprofloxacin using C12:C10 hydrophobic DES at different pH values of the aqueous solution of ciprofloxacin is presented in Figure 3.

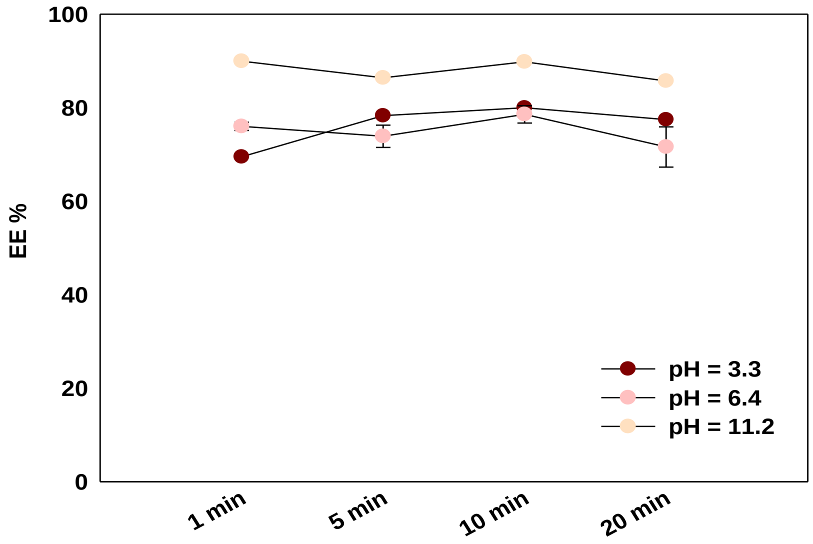

Figure 3. Effect of the contact time in the extraction efficiencies (EE %) of ciprofloxacin at different pHs using C_{12}: C_{10} hydrophobic DES (1:2) ($[C_{ip}]$ = 10ppm, stirring speed = 300 rpm, DES/water ratio = 1/1, temperature = 25 ^{o}C).

It can be observed that the process of the extraction of ciprofloxacin from water is rather fast, since the equilibration time for all extractions was found to be around 1 min for pH = 6.4 and 11.25 and 5 min for pH = 3.3. In order to ensure that all systems were in equilibrium during the extraction process, 10 minutes was the period of time chosen to carry out the following experiments.

3.1.3. Influence of stirring speed

The influence of stirring speed at different pH conditions was tested for four different stirring speeds, 100, 200, 300 and 500 rpm. The results are shown in Figure 4.

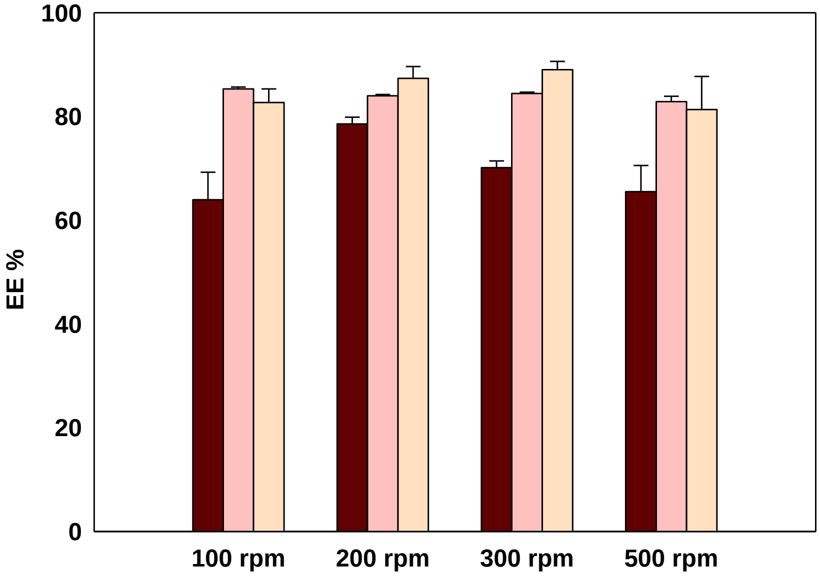

Figure 4. Effect of the stirring speed in the extraction efficiencies (EE %) of ciprofloxacin at different pHs (▬ pH=3.3, ▬ pH=6.4, ▬ pH=11.2) using C_{12}:C_{10} (1:2) hydrophobic DES ([Cip] = 10ppm, contact time = 10 min, DES/water ratio = 1/1, temperature = 25 ^{o}C).

As it can be seen, the stirring speed also does not have a significant influence in the extraction efficiencies. Only minor increase in extraction efficiency of ciprofloxacin was observed at acidic pH conditions (~3.3) for stirring speed of 200 rpm. Consequently, the stirring speed was fixed at 300 rpm.

3.1.4. Influence of DES/water ratio and initial concentration of ciprofloxacin

The initial concentration of micropollutant in water environments is a key factor to be considered, since it generally largely affects the extraction efficiency. In addition, the mass ratio of DES and water phases is also another very important factor to be considered, especially from an economical point of view, since, in the end, it might be determinant on the feasibility of the implementation of the extraction process.

For this purpose, aqueous solutions of three different concentrations of ciprofloxacin (10ppm, 5ppm and 3ppm) as well as different DES/water ratios (varied from 3/1 to 1/3) were tested using C_{12}: C_{10} hydrophobic DES as extractant. The obtained results are presented in Figure 5.

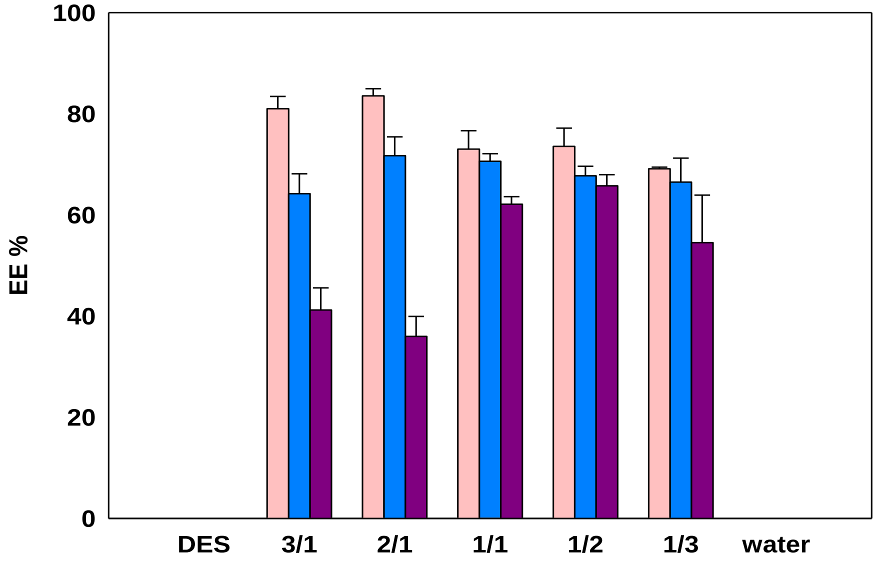

Figure 5. Effect of the initial concentration (— 10ppm — 5ppm — 3ppm) of ciprofloxacin in water on the extraction efficiencies (EE %) using C_{12}: C_{10} hydrophobic DES (contact time = 10 min, DES/water ratio = 1/1, pH = 6.4, stirring speed = 300rpm, temperature = 25 ºC).

As it can be seen from Figure 5, for large DES phase/water phase mass ratios, the lower the concentration of ciprofloxacin, the lower the extraction efficiency. For high concentration (10 ppm) of ciprofloxacin, the extraction efficiency does not change much with the phase mass ratio. In general, it can be observed that a change in the mass ratio of DES phase to the water does not have a significant effect in the extraction efficiencies. However, a maximum extraction efficiency is reached using a DES/water phase mass ratio of 2/1. However, taking into account the results obtained for the phase mass ratios 3/1, 2/1 and 1/1, it is possible to conclude that a high amount of DES leads to increase in the removal of the micropollutant from water. Nevertheless, this increase is not very significant, considering the associated error. The results obtained can probably be explained by the high hydrophobicity of the DES used and the very low solubility of ciprofloxacin in water.

At intermediate concentration (~5ppm) of ciprofloxacin no significant changes were observed for the different DES /water mass ratios. At the lowest ciprofloxacin concentration (3ppm), the decrease of DES /water mass ratio, increased the extraction efficiency, with a maximum at 1 to 2 of DES/water mass ratio. Therefore, it can be concluded that for lower concentrations of ciprofloxacin, which are the most technologically relevant, the DES/water mass ratio should be 1/2.

3.2. Experimental Design

To obtain refined statistical data an experimental design was performed using the software STATISTICA by Statsoft® to analyze the influence of some important parameters and to statistically validate and confirm the results obtained in the extraction experiments performed. Temperature, pH and DES/water mass ratio were the experimental parameters chosen to be evaluated, and the results of STATISTICA are plotted in Figure 6.

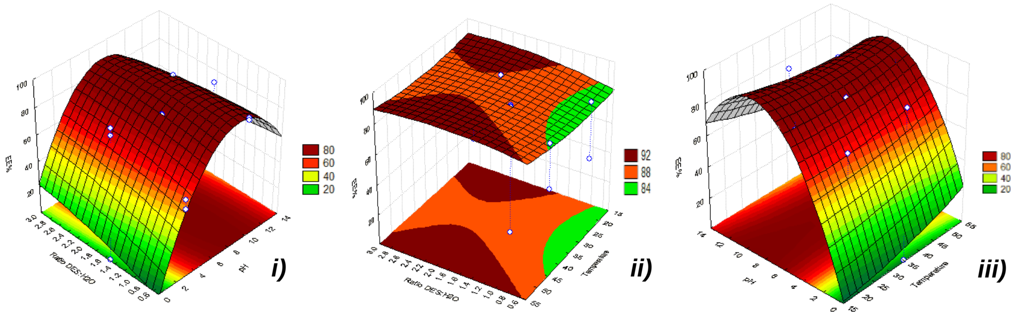

Figure 6. Response surfaces of the extraction efficiencies (EE %) of ciprofloxacin from water using C_{12}: C_{10} DES with the combined effects of: i) DES/water ratio and pH; ii) DES/water ratio and temperature and iii) pH and temperature (contact time = 10 min and stirring speed = 300 rpm).

In general, from Figure 6 it can be seen the great influence of pH on the extraction process of ciprofloxacin from water. More precisely, Figure 6 i) which analyses the influence of the DES/water ratio and pH shows the great influence of the pH in these experiments, with the surface plot reaching its highest extraction efficiencies at pH conditions between 8 and 12. Moreover, it is possible to observe that the lowest extraction efficiencies (around 0%) correspond to an extremely low pH (around 0.11). This agrees with the findings from the liquid-liquid extractions, where the extraction efficiencies improved with the increase of pH. Regarding the DES/water ratio, this experimental parameter does not seem to significantly influence extraction process for the ratios studied. The influence of DES/water ratio and temperature were also analyzed in Figure 6 ii) and both temperature and DES/water ratio did not have noteworthy influence on the extraction of ciprofloxacin from water. In fact, these results can be very advantageous for the extraction process since extractions can be carried out at low temperatures and with small mass of DES. Again, in Figure 6 iii), where the combined influence of pH and temperature is analyzed, the great influence of pH in the extraction efficiency is again observed. In this way, it can be concluded that the experimental design confirmed the crucial role of pH in the extraction efficiencies of ciprofloxacin, probably due to the speciation of this micropollutant and also of the acid in the DES.

4. Extraction Mechanism

4.1. Miscibility of DES and water

The use of hydrophobic DES is determinant in its use in the proposed application of water decontamination, since an hydrophobic DES not only will not leach,[15, 16] but also enables its re-use and recycling. The hydrophobicity of DES can be evaluated by its solubility in water and conversely by the solubility of water in the DES. Both these properties are directly related with the DES components properties, in particular their individual solubility in water and water in the DES components. For ciprofloxacin extraction, DES must not leak to the aqueous phase, which was prevented using DES components with very low water solubility, and also must not solubilize water. Since ciprofloxacin has a low water solubility, the incorporation of water in the DES will decrease ciprofloxacin solubility in DES phase. Solubility of water in the selected DES was measured using Karl-Fischer equipment, and the obtained results are presented in Figure 7.

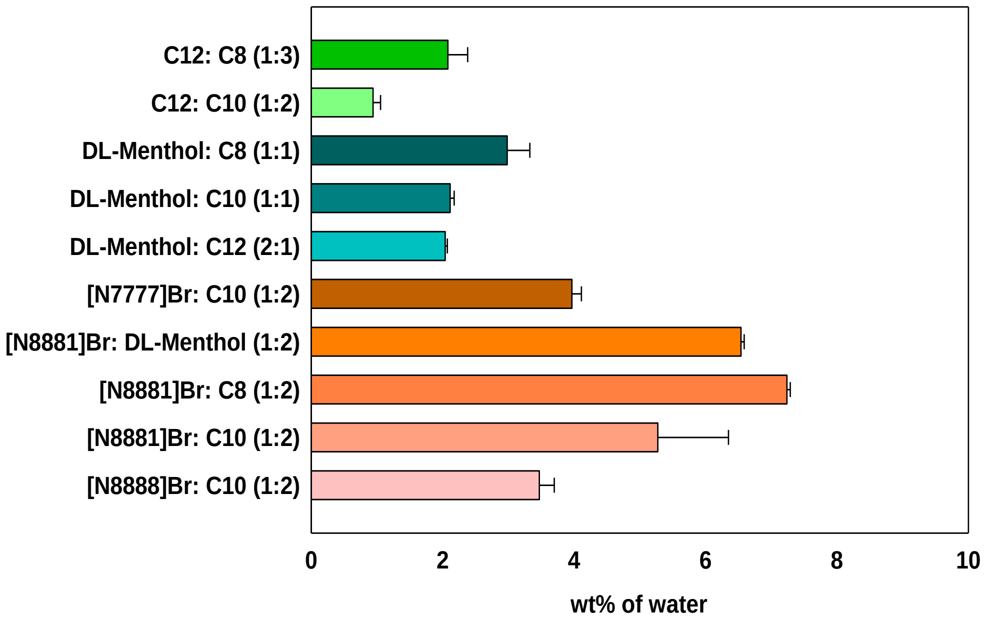

Figure 7. Water solubility in all hydrophobic DES studied in this work at neutral pH and 25 ºC.

From Figure 7, it is possible to distinguish two different behaviors: neutral hydrophobic DES, which typically have up to 3 wt% of water solubility, and ionic hydrophobic DES, whose water solubility vary between 3 and 8 wt%. The water solubility of the DES under study can be ranked by following order of its HBA components: fatty acids < DL-menthol < quaternary ammonium salts, where the latter has greatest water solubility. Comparing the HBD of DES, it can be seen that DES composed of acids with longer alkyl chains (C_{10}) are more hydrophobic than those composed acids with smaller alkyl chains (C_8), as expected. The values for the solubility of water in [N_{8888}]Br: C_{10} and DL-menthol: C_{10} DES here presented are in good agreement with those reported by Held et al.,[28] where average values of 2.78 wt% and 2.16 wt% were obtained by these authors.

Moreover, comparing the ciprofloxacin extraction results plotted in Figure 2 at neutral pH (~pH =6.4) with the DES water solubility displayed in Figure 7, it can be concluded that this parameter seems to consistently correlated with the extraction efficiencies of ciprofloxacin, since the DES with the lowest water solubility, C_{12}: C_{10}, is also that showing the highest extraction efficiency of ciprofloxacin. Conversely, DES with the poorest extraction efficiencies, $[N_{8881}]$Br: DL-Menthol and $[N_{8881}]$Br: C_{10}, are also those with the highest water solubility. Hence, besides the interaction between DES components and ciprofloxacin, DES with low water solubility, thus affording a more hydrophobic phase, seem to be an essential factor for the removal of ciprofloxacin from water phases.

4.2. Solubility of ciprofloxacin in DES

Another important parameter that needs to be evaluated is solubility of ciprofloxacin in the selected hydrophobic DES. If solubility rules the extraction mechanism of ciprofloxacin from water, a high solubility of this micropollutant in DES is desirable. The obtained results for this parameter are presented in Figure 8.

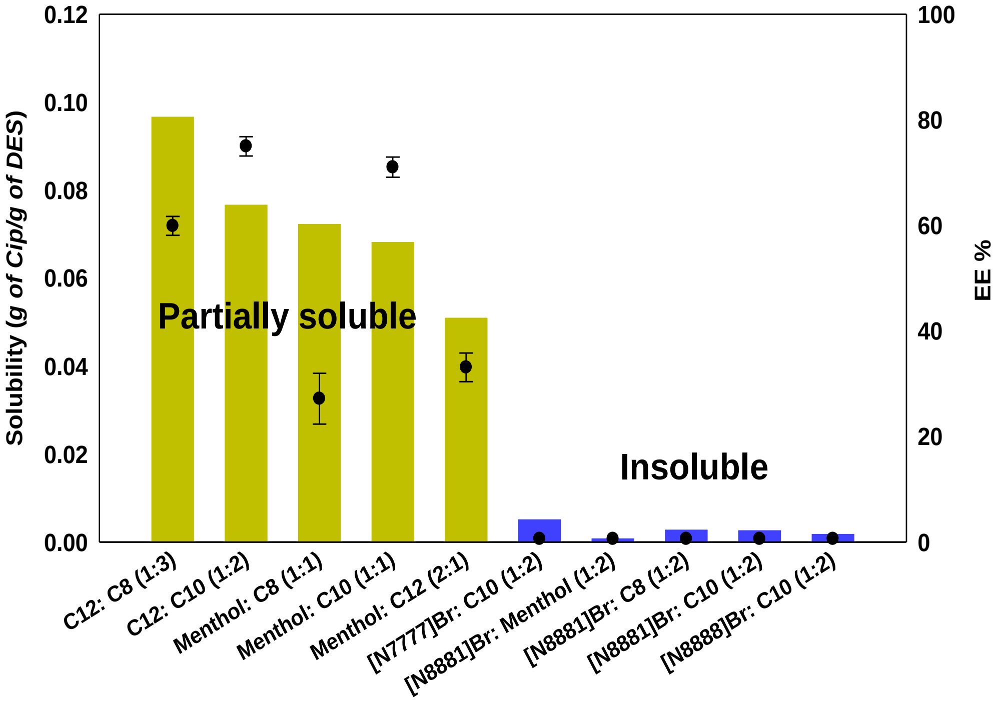

Figure 8. Comparison of solubility of ciprofloxacin (symbols) in the hydrophobic DES studied in this work and the respective extraction efficiencies in % (bars) at 25 ºC.

As can be seen from the Figure 8, solubilities of ciprofloxacin in the studied hydrophobic DES show the same pattern as extraction efficiencies of ciprofloxacin from water using the same hydrophobic DES. Nevertheless, although C_{12}: C_8 is the DES that can solubilize the highest amount of ciprofloxacin (0.097g/g DES), it does not present the highest extraction efficiencies. This is probably linked to the fact that it does not exhibit the smallest water solubility (2 wt%). C_{12}: C_{10} displays the second highest solubility value of ciprofloxacin and the lowest water solubility which leads to the best extraction efficiency of ciprofloxacin. Conversely, ionic hydrophobic DES, with high water solubilities in DES, present low solubilities of ciprofloxacin, and thus low extraction efficiencies of this micropollutant. To sum up, solubility of both water and ciprofloxacin in DES seem to be the ruling parameters for an efficient extraction of this micropollutant from water phases.

5. Reuse and recycle of DES

From an economic and sustainable point-of-view, the minimization of the amount of solvents used is very important in the implementation of the any separation process. In order to test the re-use of C_{12}: C_{10} hydrophobic DES, the DES-rich phase was collected after each extraction and reused in several consecutive extraction cycles. The obtained results are presented in Figure 9.

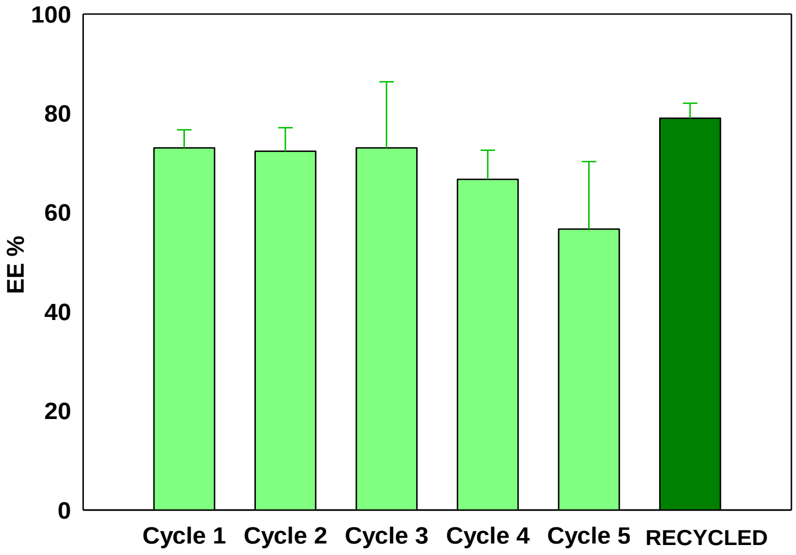

Figure 9. Re-use and recycle of C_{12}: C_{10} DES after several cycles of extraction of ciprofloxacin from water and after cleaning using activated carbon ([Cip] = 10 ppm, pH= 6.4, contact time = 10 min, DES/water ratio = 1/1, stirring speed = 300 rpm, temperature = 25 ºC).

It is possible to observe that C_{12}: C_{10} hydrophobic DES maintains its capacity to remove ciprofloxacin from the water environment, for at least 4 cycles. Only in the 5th cycle, the extraction capacity of the DES started to slowly decrease, which is an indication that hydrophobic DES is almost reaching the saturation point.

In order to achieve full sustainability, the implementation of a circular process, where DES can be cleaned and recycled, is a mandatory. For that purpose, adsorption using activated carbon (AC) was used to remove ciprofloxacin from the reused DES, where ciprofloxacin is concentrated. The example of the procedure used to recycle hydrophobic DES is illustrated in Figure 10.

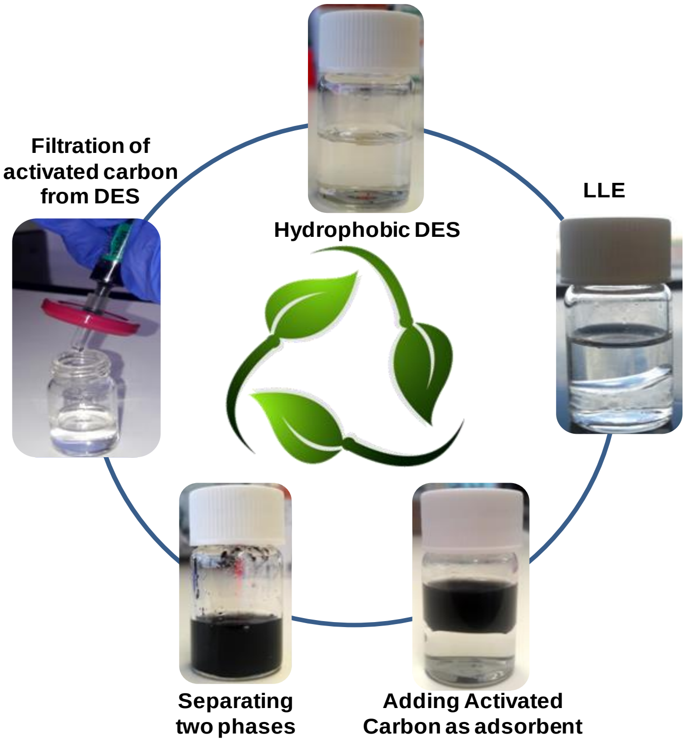

Figure 10. Scheme of circular process of recycling of hydrophobic DES contaminated with ciprofloxacin.

As it was mentioned before, the use of AC in contact with DES and not in contact with water presents the advantage of non-contamination of the water phase with carbon particulate matter. After cleaning C_{12}: C_{10} hydrophobic

DES using AC, the DES was used again in the liquid-liquid extraction of ciprofloxacin.

The results shown in Figure 9 reveal slightly higher removal extraction efficiencies of ciprofloxacin (82%) than those determined before using fresh DES (76%). Possible explanation for this could be the fact that some impurities present in the components of the DES were also removed during the contact with activated carbon. During the recycle experiments it is relevant to mention that DES phase was carefully analyzed in order to avoid it containing AC, which can be seen in Figure 10 where DES appears clean. The recycle of DES using activated carbon is thus viable and clean hydrophobic neutral DES can be used again to perform liquid-liquid extraction. The fact that DES can be cleaned up and recycles brings positive aspects for the point-of-view of application, since only a small amount of DES is needed.

6. Conclusions

Due to their advantageous properties, easy to prepare, typically composed of natural compounds with low toxicity and properties can be adjusted through the use of different components, DES have been increasingly attracting attention as truly green designer solvents that enable the development of sustainable processes. In the present work, neutral and ionic hydrophobic DES were prepared and their performance as extractants of ciprofloxacin, a high priority pharmaceutical micropollutant, from water tested. The effect of several experimental variables in the extraction efficiencies was studied and it was concluded that DES composed of natural and neutral compounds offered the best extraction efficiencies. In particular, C_{12}: C_{10} proved to be the best extractant, displaying the second highest solubility value of ciprofloxacin and the lowest water solubility which leads to the best extraction efficiency of ciprofloxacin.

Moreover, pH was found to have a dominant role in the extraction efficiencies since ciprofloxacin is better extracted in the anionic form, while temperature and DES/water ratio do not significant influence the extraction process. Ciprofloxacin extraction is clearly linked to the hydrophobicity of the DES, in particular to the water solubility in the DES. An economical and sustainable feasible circular process was developed using activated carbon to recycle the DES phase, showing that it is possible to work with solvents composed exclusively natural compounds achieving high extraction efficiencies.

7. Acknowledgements

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8. References

1. Geissen, V.; Mol, H.; Klumpp, E.; Umlauf, G.; Nadal, M.; van der Ploeg, M.; van de Zee, S. E. A. T. M.; Ritsema, C. J., Emerging pollutants in the environment: A challenge for water resource management. International Soil and Water Conservation Research 2015, 3, (1), 57-65.

2. Jiang, J.-Q.; Zhou, Z.; Sharma, V. K., Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in wastewater — A review from global views. Microchemical Journal 2013, 110, 292-300.

3. Kümmerer, K., Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. Springer: 2001.

4. H. Jones, O. A.; Voulvoulis, N.; Lester, J. N., Human Pharmaceuticals in Wastewater Treatment Processes. Critical Reviews in Environmental Science and Technology 2005, 35, (4), 401-427.

5. Brodin, T.; Piovano, S.; Fick, J.; Klaminder, J.; Heynen, M.; Jonsson, M., Ecological effects of pharmaceuticals in aquatic systems--impacts through behavioural alterations. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 2014, 369, (1656), 20130580.

6. Luo, Y.; Guo, W.; Ngo, H. H.; Nghiem, L. D.; Hai, F. I.; Zhang, J.; Liang, S.; Wang, X. C., A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of The Total Environment 2014, 473-474, 619-641.

7. de Voogt, P.; Janex-Habibi, M. L.; Sacher, F.; Puijker, L.; Mons, M., Development of a common priority list of pharmaceuticals relevant for the water cycle. Water Science and Technology 2009, 59, (1), 39-46.

8. Ali, I.; Gupta, V. K., Advances in water treatment by adsorption technology. Nature Protocols 2007, 1, 2661.

9. López-Montilla, J. C.; Pandey, S.; Shah, D. O.; Crisalle, O. D., Removal of non-ionic organic pollutants from water via liquid–liquid extraction. Water Research 2005, 39, (9), 1907-1913.

10. de los Ríos, A. P.; Hernández-Fernández, F. J.; Lozano, L. J.; Sánchez, S.; Moreno, J. I.; Godínez, C., Removal of Metal Ions from Aqueous Solutions by Extraction with Ionic Liquids. Journal of Chemical & Engineering Data 2010, 55, (2), 605-608.

11. Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep Eutectic Solvents (DES) and Their Applications. Chemical Reviews 2014, 114, (21), 11060-11082.

12. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. Journal of the American Chemical Society 2004, 126, (29), 9142-9147.

13. Mbous, Y. P.; Hayyan, M.; Hayyan, A.; Wong, W. F.; Hashim, M. A.; Looi, C. Y., Applications of deep eutectic solvents in biotechnology and bioengineering—Promises and challenges. Biotechnology Advances 2017, 35, (2), 105-134.

14. Shishov, A.; Bulatov, A.; Locatelli, M.; Carradori, S.; Andruch, V., Application of deep eutectic solvents in analytical chemistry. A review. Microchemical Journal 2017, 135, 33-38.

15. Florindo, C.; Branco, L. C.; Marrucho, I. M., Development of hydrophobic deep eutectic solvents for extraction of pesticides from aqueous environments. Fluid Phase Equilibria 2017, 448, 135-142.

16. van Osch, D. J. G. P.; Zubeir, L. F.; van den Bruinhorst, A.; Rocha, M. A. A.; Kroon, M. C., Hydrophobic deep eutectic solvents as water-immiscible extractants. Green Chemistry 2015, 17, (9), 4518-4521.

17. Tereshatov, E. E.; Boltoeva, M. Y.; Folden, C. M., First evidence of metal transfer into hydrophobic deep eutectic and low-transition-temperature mixtures: indium extraction from hydrochloric and oxalic acids. Green Chemistry 2016, 18, (17), 4616-4622.

18. Florindo, C.; Romero, L.; Rintoul, I.; Branco, L. C.; Marrucho, I. M., From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid–Based Deep Eutectic Solvents. ACS Sustainable Chemistry & Engineering 2018, 6, (3), 3888-3895.

19. Kyung, T. W. D. Y. H. R., Evaluation of fatty acid/alcohol-based hydrophobic deep eutectic solvents as media for extracting antibiotics from environmental water. Analytical and Bioanalytical Chemistry 2018, 410, 7325–7336.

20. Florindo, C.; Costa, A.; Matos, C.; Nunes, S. L.; Matias, A. N.; Duarte, C. M. M.; Rebelo, L. P. N.; Branco, L. C.; Marrucho, I. M., Novel organic salts based on fluoroquinolone drugs: Synthesis, bioavailability and toxicological profiles. International Journal of Pharmaceutics 2014, 469, (1), 179-189.

21. Ribeiro, B. D.; Florindo, C.; Iff, L. C.; Coelho, M. A. Z.; Marrucho, I. M., Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. ACS Sustainable Chemistry & Engineering 2015, 3, (10), 2469-2477.

22. Martins, M. A. R., et al., Tunable Hydrophobic Eutectic Solvents Based on Terpenes and Monocarboxylic Acids. ACS Sustainable Chemistry & Engineering 2018, 6, (7), 8836-8846.

23. Pontes, P. V. A., et al., Measurement and PC-SAFT modeling of solid-liquid equilibrium of deep eutectic solvents of quaternary ammonium chlorides and carboxylic acids. Fluid Phase Equilibria 2017, 448, 69-80.

24. Roca Jalil, M. E.; Baschini, M.; Sapag, K., Influence of pH and antibiotic solubility on the removal of ciprofloxacin from aqueous media using montmorillonite. Applied Clay Science 2015, 114, 69-76.

25. Jain, A.; Jain, S.; Pahwa, R.; Yar, M. S., Ciprofloxacin: review on developments in synthetic, analytical, and medicinal aspects AU - Sharma, Prabodh Chander. Journal of Enzyme Inhibition and Medicinal Chemistry 2010, 25, (4), 577-589.

26. Palacio, D. A.; Rivas, B. L.; Urbano, B. F., Ultrafiltration membranes with three water-soluble polyelectrolyte copolymers to remove ciprofloxacin from aqueous systems. Chemical Engineering Journal 2018, 351, 85-93.

27. Cherrington, C. A.; Hinton, M.; Mead, G. C.; Chopra, I., Organic Acids: Chemistry, Antibacterial Activity and Practical Applications. In Advances in Microbial Physiology, Rose, A. H.; Tempest, D. W., Eds. Academic Press: 1991; Vol. 32, pp 87-108.

28. Dietz, C. H.J.T., Erve, A., Kroon, M. C., Annaland, M. v. S., Gallucci, F., Held, C., Thermodynamic properties of hydrophobic deep eutectic solvents and solubility of water and HMF in them: Measurements and PC-SAFT modeling, Fluid Phase Equilibria 2019, 489, 75-82.

CHAPTER 3.4

Hydrophobic deep eutectic solvents as adsorbent materials for removal of micropollutants from water sources

Adapted from: **C. Florindo**, Leonardo Miakawa, L. C. Branco and I. M. Marrucho, Task Hydrophobic deep eutectic solvents as adsorbent materials for removal of micropollutants from water sources, 2019 *(pending patent).*

The author contributed to the execution of all experimental work described in this chapter, as well as involved on the discussion and interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

The easy preparation of hydrophobic deep eutectic solvents adsorbent materials through their support in porous membranes enables the development of highly efficient and cost-effective adsorption technology for the removal micropollutants from water. Real water samples collected from the largest river in Portugal were successfully tested using this novel technology.

1. Communication

In 2018, water crisis was ranked for the 7th consecutive year among the top 5 global societal risks in the World Economic Forum’s Global Risks Report 2018.[1] One of the most important aspects of water crisis is the pollution of fresh water sources. The recent development of advanced sophisticated analytical tools, which allow the detection compounds in very small concentrations, called the world´s attention to the presence of a large diversity of micropollutants in water streams, from effluents of wastewater treatment plants, to groundwater and even drinking water.[2] International agencies and programmes, such as Environmental Protection Agency, Global Water Research Coalition, European Water Framework, have established “watch lists” of priority compounds that need to be controlled, aiming at regulating the limiting concentration values of these micropollutants, since most of them are persistent, toxic and bioaccumulative leading to serious public health problems.[3, 4] In the case of European Union, anti-inflammatory pharmaceutical compounds, such as diclofenac, have been included in this list.[5] In general, cheap, over-the-counter drugs, with poor solubility profiles, represent a threat to environment and human health. Diclofenac is one of the most frequently reported active pharmaceutical ingredients in ambient monitoring studies worldwide, which is widely used and detected in high concentrations in drinking water.[6] Consequently, there is an urgent need to redesign the currently available technologies for waste water treatment plants that are still not able to solve this problem. Although several alternative strategies that recently have been proposed to purify water and remove micropollutants, such as liquid-liquid extraction,[7-9] aqueous biphasic systems,[10, 11] liquid membranes[12-14] and adsorption,[15-17] none of them has yet been implemented mainly due to high cost and lack of sustainability.

In this work, we propose to advance on adsorption technologies through the development of a highly efficient adsorption material, resulting from the impregnation of hydrophobic eutectic solvents on an inert porous material. Deep Eutectic Solvents (DESs) are defined as low melting mixtures of one salt and one or more compounds that are liquid at or around room temperature.[18] They are also called designer solvents since their properties can be easily tuned by the adequate combination of the salt cation and anions and also the other components. Soon eutectic solvents fully composed of neutral and natural compounds the so-called NADES, were proposed. The diversity of possible combinations of the starting materials provides a powerful tool to control the physical properties of DES. One of the great advantages of using these cheap, natural, designer solvents is their simple and easy synthetic procedure, without any solvent, or purification steps needed. DES have been playing key role in the development of liquid-liquid and solid-liquid extraction technologies in very diverse fields, including extraction of compounds from aqueous media. Despite the great advantages of liquid-liquid extraction in terms of extraction efficiencies and low cost of equipment, the long periods of phase separation, the phase contamination and the high amounts of solvent needed severely hinders its application in wastewater treatment plants. In this context, adsorption comes up as an attractive technique for contaminant removal due to its simplicity, low cost, and wide applicability.

The advantages of adsorption technologies in the removal of pharmaceutical pollutants from water is clearly highlighted in a recent work,[17] where highly sophisticated materials, fluorine-bearing covalent organic frameworks (COFs) crystalline porous materials[17] have been developed to obtain high efficiency in the removal of ibuprofen from water. Nevertheless, the complex and expensive synthesis of the COF and the use of organic solvents in the regeneration steps of the material are still major drawbacks. Therefore, sustainability and circular economy always need to be taken into account upon development of a promising industrial process.

Herein, we report the development of cost-effective adsorbent material for removal of diclofenac from aqueous environments. The adsorbent material was prepared through the impregnation a hydrophobic DES into an inert porous support and used in direct contact with the aqueous phase. Aiming at full sustainability, a circular process was also developed by re-using several times the supported DES material.

A scheme describing the preparation of DES-based adsorbent material is presented in Figure 1. Briefly, it consists on the impregnation, under vacuum, of a selected hydrophobic DES in a porous PVDF support (for more details, see the Experimental Section in supplementary Information). This support was uniformly cut in small circles with a defined size through the use of a mould.

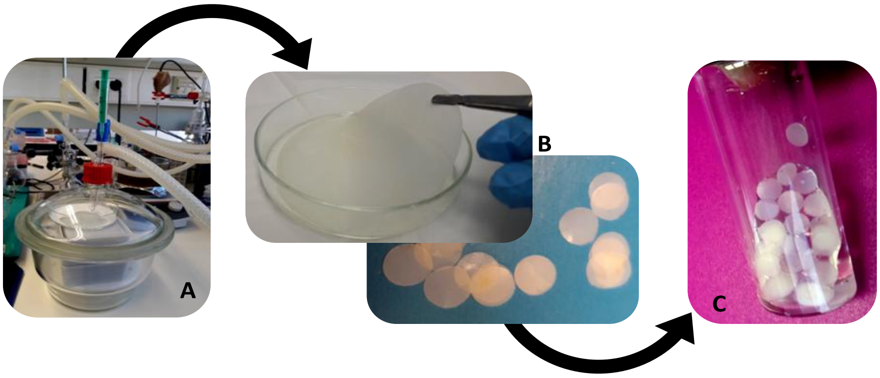

Figure 1. Scheme of the preparation of supported DESs: A) impregnation of DES in pores of PVDF support using a vacuum chamber B) removal of DES-based adsorbent material and cut into well-defined sizes and C) use of Des-

based adsorbent material in contact with water contaminated with the micropollutant.

A vast array of hydrophobic DESs and other eutectic mixtures was first tested in liquid-liquid extraction and the extraction parameters optimized. All the details concerning the screening of DESs, liquid-liquid extractions and optimization of parameters can be found in the Supplementary Information. These DESs were selected due to their negligible solubility in water, to avoid contamination of the water phase. From this initial screening, two DESs were selected to develop the adsorption material: one containing a salt, $[N_{8888}]Br: C_{10}$ (1:2), and the other containing two fatty acids, $C_{12}: C_{10}$ (1:2). These two eutectic mixtures were chosen not only due to their excellent performance, with extraction efficiencies over 85%, but also their fast kinetics, where equilibrium was achieved 5 min after contact.
The chemical structures and acronyms of these two eutectic solvents, as well as diclofenac, are depicted in Figure 2.

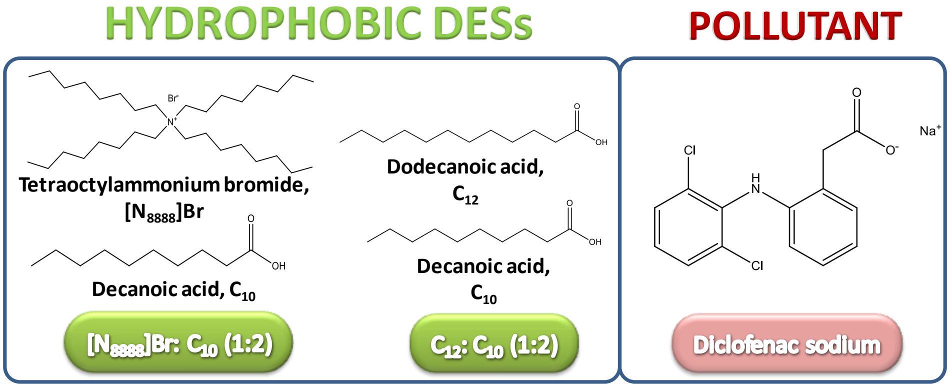

Figure 2. Chemical structures as well as abbreviations of the hydrophobic DESs and target micropollutant mainly studied in this work.

These hydrophobic DESs were impregnated in PVDF porous filters using vacuum and their performance as adsorbents to remove diclofenac from contaminated water was evaluated. The effect of several parameters, namely extraction time and mass of DES in the adsorption material, in the extraction of diclofenac was assessed. The extraction efficiencies (EE %) were calculated from the Diclofenac Salt (Dic) concentration in the water-rich phase before, $C_{DIC,0}^{aq}$, and after the extraction, $C_{DIC,1}^{aq}$:

$$EE\ (\%) = \frac{\mathrm{C_{DIC,0}^{aq}} - \mathrm{C_{DIC,1}^{aq}}}{\mathrm{C_{DIC,0}^{aq}}} \text{ x } 100 \qquad \text{(eq. 1)}$$

In order to evaluate the effect of the DES mass used in the extraction, small adsorption circles of two different sizes; 5 mm and 2 mm, corresponding to 1 mg and 0.1 mg of DES/ adsorption particle, respectively, were prepared and used to extract diclofenac from water. The main results are summarized in Figure 3.

One of the promising advantages of this novel adsorption technology compared to the liquid-liquid extraction is the quick and easy separation of the DES-based adsorbent particles from the aqueous phase by simple filtration, without any time-consuming separation. However, the most relevant aspect is undoubtedly the amount of DESs used, which is significantly smaller in DES adsorbent material than in liquid-liquid extraction, for the same extraction efficiency.

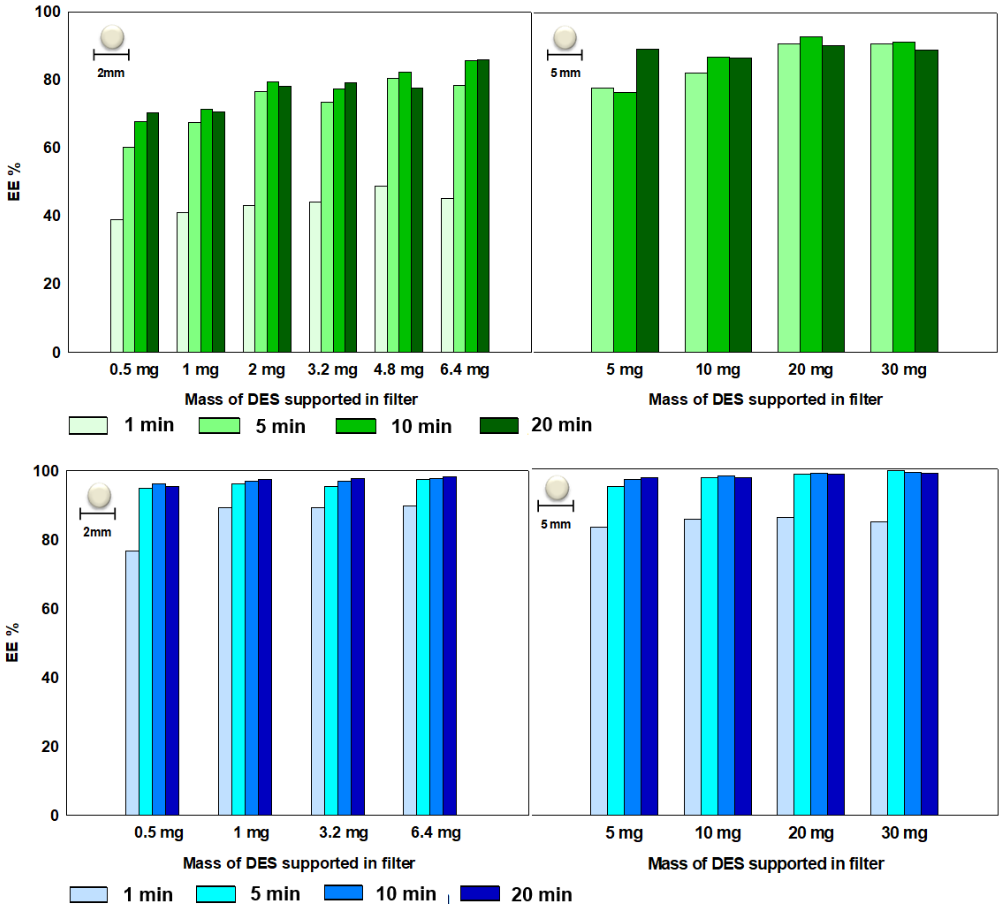

Figure 3. Effect of extraction time and the mass of supported DES in the extraction sodium diclofenac from water using C_{12}: C_{10} (1:2) (green) and $[N_{8888}]Br$: C_{10} (1:2) (blue). Parameters: size of adsorption material 2mm (~0.1mg of DES) and 5 mm of diameter (~1 mg of DES) (initial concentration = 0.03 g/L, stirring speed = 300rpm, temperature = 25 ºC).

From Figure 3, it can be seen that the maximum extraction efficiencies (around 85 and 98%, depending on the DES) were typically achieved between 5 and 10 minutes, for both masses of the two tested DES. However, it should be mentioned that the extraction time is directly influenced by the mass of DES adsorbent material used. In the case of C_{12}: C_{10} (1:2), only 6.4

mg of DES are needed to achieve 85% of extraction efficiency, which is a remarkable result. In the case of [N_{8888}]Br: C_{10} (1:2), the results are even less dependent of the mass and time of the DESs used: only 0.5 mg of DES and 5 min of extraction are required to reach a maximum extraction efficiency of 95%. These results clearly show that if high extraction kinetics can be attained, these DES-based adsorbent materials can be very promising not only from the point of view of their efficiency but also from the economical point of view.

Another important point when developing a sustainable process is the reusability of adsorbent material. For that purpose, several cycles of extraction were performed, where fresh water with the micropollutant was put in contact with the same DES adsorbent material in each one of the re-use cycles. In this way, the same mass of adsorbent material (30mg) for both families of DES, C_{12}: C_{10} (1:2) and [N_{8888}]Br: C_{10} (1:2), were used and were reused up to four times. From Figure 4, it can be seen that the extraction capacity of both DES adsorbent materials remains unchanged after 4 reuse cycles.

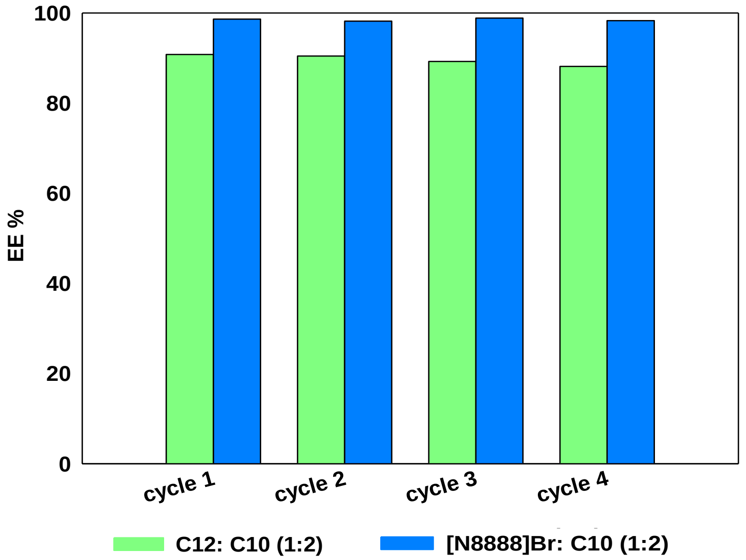

Figure 4. Extraction Efficiencies of the reuse of C_{12}: C_{10} (1:2) (green) and $[N_{8888}]Br$: C_{10} (1:2) (blue) DES based adsorbent material to extract diclofenac from water (mass of DES adsorbent = 30 mg, initial concentration = 0.03 g/L, contact time = 10min stirring speed = 300rpm, temperature = 25 ^{o}C).

In order to test a broader applicability of the DES adsorbent material to remove other micropollutants present in fresh water streams, ciprofloxacin and bisphenol-A were also tested. Diclofenac, ciprofloxacin and bisphenol-A have quite different chemical structures and thus physical chemical properties. For example, diclofenac, ciprofloxacin and bisphenol-A have 21.4 g/L,[19] 0.14 g/L,[20] and 0.38 g/L[21] of water solubility. In Figure 5, a brief comparison of the extraction efficiencies of liquid-liquid extraction and adsorption of these 3 micropollutants is presented.

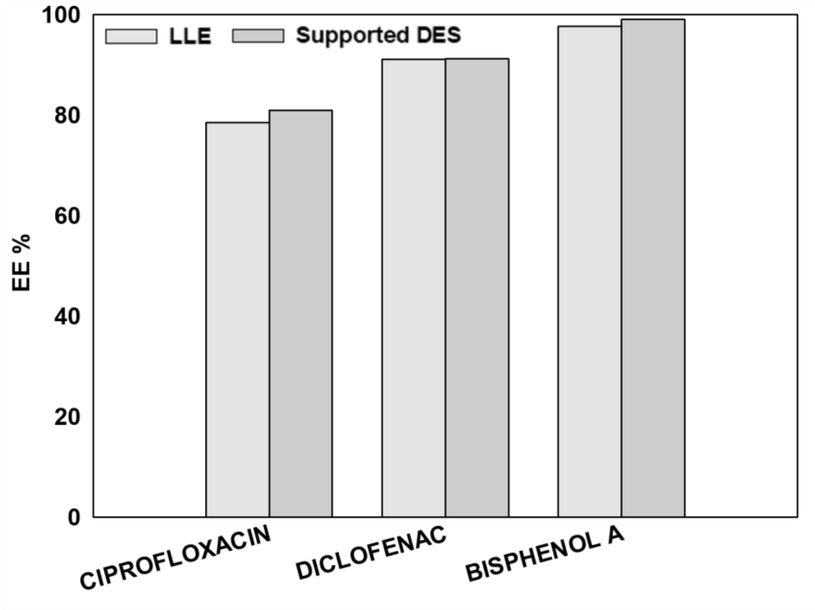

Figure 5. Brief comparison of extraction efficiencies obtained for LLE and adsorption both using DES for 3 different micropollutants from water: ciprofloxacin 0.001 g/L of ciprofloxacin, 0.03 g/L of diclofenac, and 0.1 g/L of Bisphenol-A (contact time = 10 min, stirring speed = 300 rpm, temperature = 25 ºC, DES/water ratio of LLE = 1/1 and DES/water ratio of adsorption = 15 mg/g of water).

It can be observed that both DES solvents and DES adsorbents yielded similar excellent %EE for different micropollutants under study.

Furthermore, to completely evaluate the proposed adsorbent technology using supported hydrophobic DESs taking a step forward to industrial application, some relevant parameters such as lower concentrations, mixture of micropollutants and the effect of real water samples were evaluated in the optimized conditions. The main results are summarized in Figure 6 and other results are presented in Supplementary Information. Unfortunately, with the adopted method in HPLC-UV, it was not possible to quantify ciprofloxacin because it elutes in the dead volume. However, from the preliminary and qualitative results, ciprofloxacin presented good results of extraction, being the proposed adsorption method also suitable for this micropollutant. Moreover, it should be noted that even though a quantification was not possible, the mixture samples present all micropollutants previously studied, ciprofloxacin, bisphenol-A (BPA) and diclofenac (DIC).

From Figure 6, it can be seen the performance of two different supported hydrophobic DES with real and simulated water samples.

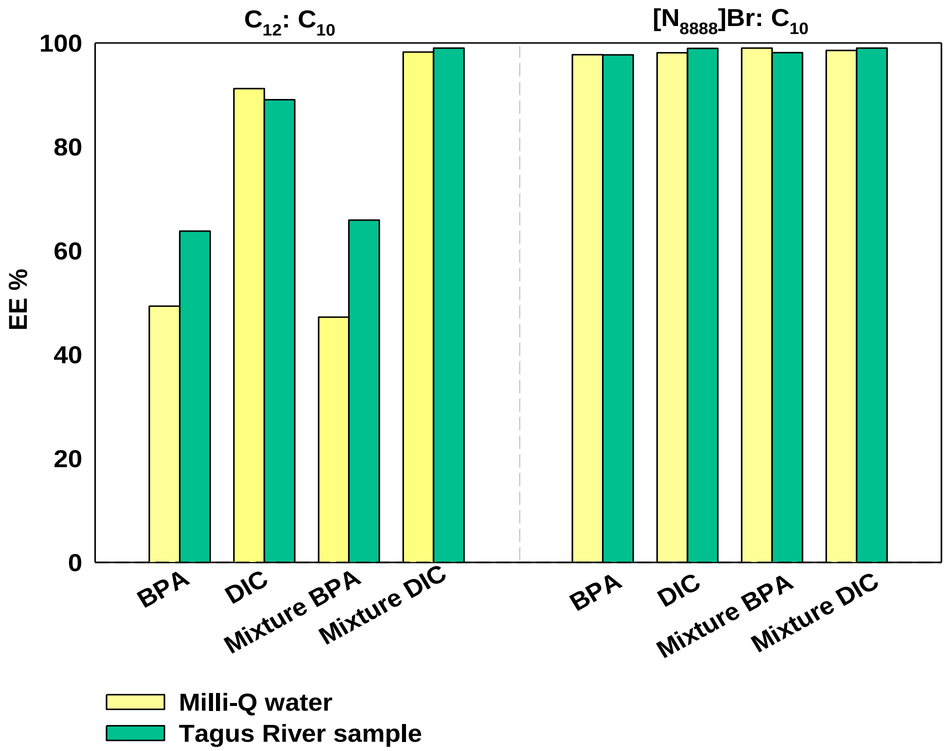

Figure 6. Comparison of extraction efficiencies obtained for adsorption method, detected by HPLC-UV, using two different DES for different micropollutants in Milli-Q water and Tagus river samples with 0.002 g/L of diclofenac, and 0.002 g/L of Bisphenol-A (contact time = 10 min, stirring speed = 300 rpm, temperature = 25 ºC, supported DES/water ratio of adsorption = 15 mg/2g of water).

From the results, it can be concluded that the novel technology proposed here works very well at lower concentrations (< 2ppm) as well as being able to extract from a complex mixture and also from real water samples with complex matrices. Moreover, it was once again confirmed that the structure of the DES and the micropollutant influence the performance of the adsorption technology used. It was noted that both hydrophobic DES have very good performances, however the best system to extract all micropollutants and with great extraction efficiencies (> 95%) was $[N_{8888}]Br: C_{10}$.

Comparing the extraction of the individual compounds and using mixtures of various micropollutants, these can be removed with the same success without any interference. Directly comparing the extraction of micropollutants in simulated water and taking a step forward to a real application using real water samples, the extraction efficiency remains appealing, presenting the same remarkable results.

Adsorption is an advantageous alternative approach to traditional technologies since it is simple, fast, low-cost and effective. As far as relevance for an implementation of a process following the tested conditions, it was highlighted that LLE experiments required the use of 100g of DES, whereas 250mg of supported DES adsorbent per liter of water sufficed to achieve the highest extraction efficiencies, for the same experimental conditions.

2. Conclusions

In conclusion, the development of adsorbent materials based on hydrophobic DES is a promising approach for water cleaning technologies. The proposed adsorption process offers several advantages compared to the liquid-liquid traditional process such as the fast and easy separation, the reusability without compromising capacity and the small amount of DES needed, which can be very important in the case of DESs composed of expensive compounds.

3. Acknowledgements

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4. Supplementary Information

4.1. Experimental details

Materials. DL-Menthol (purity ≥ 95%), tetraheptylammonium bromide ($[N_{7777}]Br$) (purity ≥ 99%), tetraoctylammonium bromide ($[N_{8888}]Br$) (purity ≥ 98%), methyltrioctylammonium bromide ($[N_{8881}]Br$) (purity ≥ 97%), octanoic acid (C_8) (purity ≥ 98%) and decanoic acid (C_{10}) (purity ≥ 98%) were purchased from Sigma-Aldrich and used as received. Durapore porous hydrophobic poly(vinylidene fluoride) (PVDF) filter provided by Millipore Corporation (USA) with a pore size of 0.22 μm and an average thickness of 125 μm, were used as the support to maintain the DES as a carrier in the porous filter. Diclofenac sodium micropollutant (mass fraction purity ≥ 99 %) was purchased from Sigma Aldrich and used with no further purification. All aqueous solutions were prepared using high purity water (Milli-Q water) with a specific conductance <0.1 mS/cm.

4.2. Experimental Methodologies

Preparation of DESs. All hydrophobic DESs were prepared by mixing the HBA and HBD components in a glass vial with a mechanical stirrer in a certain mole ratio and temperature until a homogeneous liquid appeared and DES stable with no apparent precipitate. Hydrophobic DESs used in this work and respective molar ratio are the following: DL- Menthol: C_8 (1:1), DL-Menthol: C_{10} (1:1), DL-Menthol: C_{12} (2:1), C_{12}: C_8 (1:3), C_{12}: C_{10} (1:2) [N_{7777}]Br: C_{10} (1:2), [N_{8888}]Br: C_{10} (1:2), [N_{8881}]Br: C_{10} (1:2) and [N_{8881}]Br: DL-Menthol (1:2). For the preparation of DESs, an analytical high-precision balance was used, with an uncertainty of ± 10-5 g and known masses of each component were added. DL-Menthol, tetraoctylammonium bromide ([N_{8888}]Br), tetraheptylammonium bromide ([N_{7777}]Br), methyltrioctylammonium bromide ([N_{8881}]Br) were first dried in a high vacuum pump at 40 °C for at least four days, while the carboxylic acids were used without any further purification. Their water content was determined by Karl Fischer titration (< 500 ppm). All hydrophobic DESs used in this work were completely characterized by ^{1}H and ^{13}C NMR and FTIR spectroscopy, in order to confirm their structures and final purities.

Solubility of micropollutant in DES. In order to evaluate the possible relation between the solubility of the diclofenac sodium in the DES, and the extraction efficiency obtained with them, a study of solubility was conducted. Thus, small quantities of micropollutant sodium diclofenac were added to known masses of the hydrophobic DES at 25 °C until the solutions became turbid, indicating the saturation point. The results obtained are summarized in Figure S1.

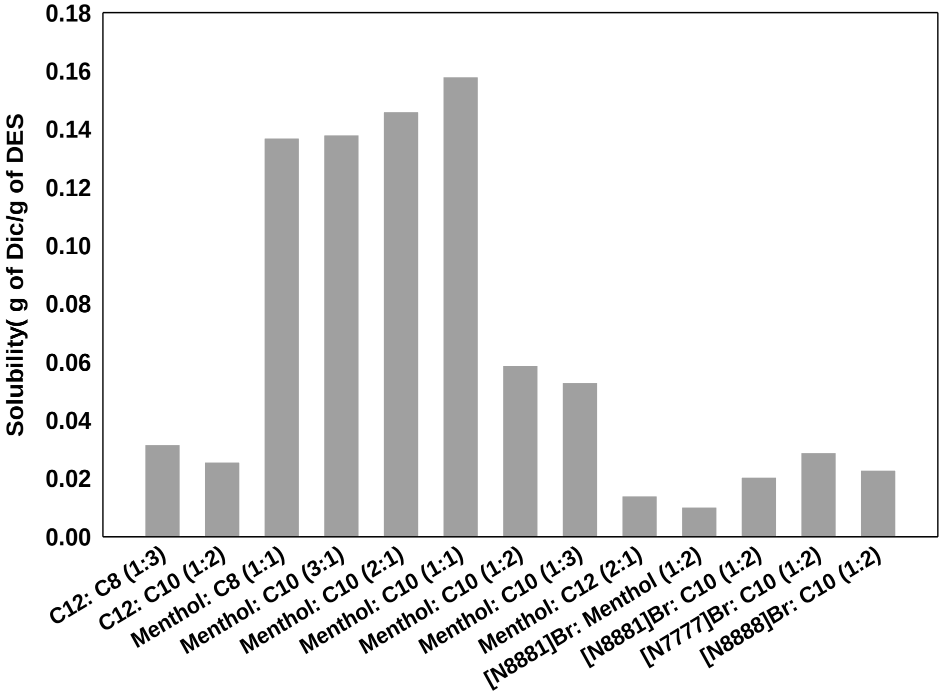

Figure S1. Solubility of diclofenac sodium salt in different hydrophobic DESs.

These results show that the solubility is not the only determinant factor in extraction efficiency, since it can be observed that the micropollutant compound is significantly less soluble in the family of salts than in the menthol's family, even if this first family had shown the best extraction efficiencies. Therefore, it is possible to conclude that other factors, as the hydrophobicity and the affinity between the DES and the micropollutant, are also decisive to increase and influence extraction efficiency. Furthermore, it is possible to observe that the capacity of the DES to absorb diclofenac sodium salt is much higher than the quantity that is introduced in one-step extraction, which leads to conclude that it is possible to use these hydrophobic DESs several times before losing its extraction ability.

Liquid-liquid extraction procedure. Liquid-liquid extractions were carried out at room temperature using hydrophobic DESs as extractants. A stock aqueous solution of sodium diclofenac in water was previously prepared. Mixtures composed by each one of the prepared hydrophobic DESs and the aqueous solution of micropollutant were initially stirred vigorously during a minimal time to ensure that equilibrium was reached, at room temperature and then left to settle for a minimum of 12h to ensure complete phase separation. Thereafter, the two phases were carefully separated using a needled syringe and the concentration of the remaining micropollutant in the water-rich phase was measured using UV–vis spectroscopy with a Shimadzu model UV-1800 – Pharma-Spec spectrophotometer. DES-rich phases were very concentrated and thus several dilutions were needed for their analysis through UV-vis. Furthermore, to eliminate possible interferences of DESs and their interaction with micropollutant in the quantification, the DES-rich phase was not quantified. A calibration curve was previously established in Milli-Q water (curve with $R^2 > 0.990$). A minimum of three replicates of each sample were performed for each experiment.

Supported Hydrophobic Deep Eutectic Solvents. The PVDF inert support was introduced in a vacuum chamber for a certain time to remove the air within the pores and facilitate the filter wetting, maximizing the amount of DES in the support. Then, drops of the DES were spread on the porous support using a syringe, while keeping the vacuum inside the chamber. As the liquid penetrated into the pores, the filter changed its appearance and became transparent. It was then left inside the chamber under vacuum for another hour. In Figure 1, a scheme of the preparation of supported DESs is presented. Finally, the supported DES was taken out of the chamber and its surface was cleaned with soft tissue paper to remove excess DES. In the end, the amount of the DESs immobilized was determined gravimetrically by weighing the filter before and after impregnation. Supported DES was then cut into small well-defined fragments using a metallic form of 5mm and 2mm and was used as adsorbent in contact with water contaminated with micropollutant.

Matrix effect in adsorption of micropollutants. After the screening for the extraction of different micropollutants and hydrophobic DESs, the system composed of C_{12}: C_{10} and $[N_{8888}]$Br: C_{10} were selected for further studies. Furthermore, in order to mimic representative scenarios of typical concentrations of micropollutants found in aqueous environments, aqueous solutions of individual micropollutants and mixtures of all micropollutants in water milli-Q and in real water samples were evaluated. All micropollutants as well as their mixtures were prepared at concentrations of circa 0.002 g/L.

For the studies involving lower concentrations of micropollutants, their mixtures as well as the effect of using real water samples, high-performance liquid chromatography (HPLC) Agilent Infinity 1100 was used to quantify several micropollutants, namely BPA and diclofenac, using calibration curves previously determined. The chromatographic separation was achieved using a Thermo Hypersil-Keystone Kromasil C18 analytical column with a size of 250 mm x 4.6 mm. The operating temperature of the column was set at 25 °C. The mobile phase used was a mixture of methanol and water with concentrated formic acid with a volume ratio of 70:30, respectively. The elution was performed at a flowrate of 0.8 $mL{\cdot}min^{-1}$ and the injection volume was 20 µL. The wavelength of the UV detector was set at 275 nm for the quantification of micropollutants. Extraction efficiencies were determined as the ratio between the concentrations of each micropollutant in the water-rich phase and those in the initial aqueous solution/sample. The calibration curves of micropollutants were determined with model systems (distilled water) and real water samples from the Tagus River in Lisbon. Since in the real sample, no detectable levels of the target micropollutants were found, the sample was spiked with 0.002 g/L of each micropollutant.

4.3. Liquid-liquid extraction details

4.3.1. Comparison of performance of different families of DESs as extractants

Previous work has been showing that hydrophobic DESs are excellent tailor-made water-immiscible solvents able to extract a range of compounds from aqueous environments. For this purpose, there are already reported hydrophobic DES based on different compounds, such as quaternary ammonium salts with long alkyl chains, namely [N$_{7777}$]Br, [N$_{8888}$]Br and [N$_{8881}$]Br, natural compound, DL-menthol and exclusively composed of fatty acids, octanoic, decanoic and dodecanoic acid.

Therefore, three different families of hydrophobic DESs were selected for the extraction of the diclofenac sodium salt from the water samples. All DESs were prepared as homogeneous liquids without any precipitation, with the following molar ratio. The hydrophobic DESs prepared in this work are organized by family of HBA and illustrated in Figure S2.

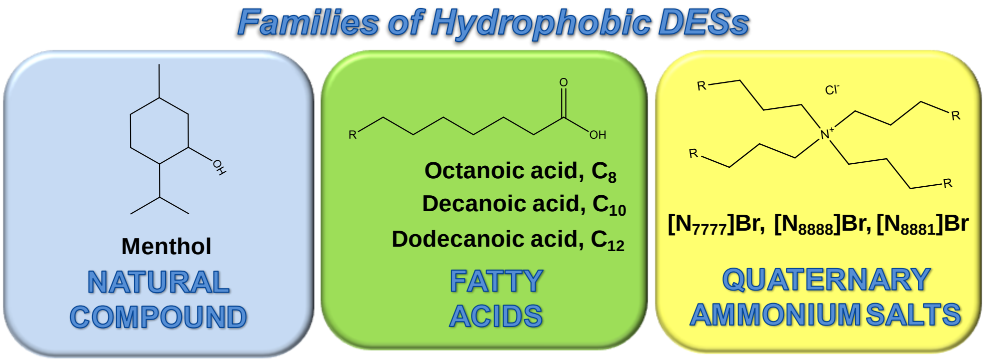

Figure S2. Chemical structure and acronym of different families of hydrophobic DESs studied in this work.

First of all, a screening of several hydrophobic DESs applied to the extraction of micropollutant diclofenac sodium salt from water environments, was evaluated. The extraction efficiencies (EE %) were calculated from the Diclofenac Sodium Salt (Dic) concentration in the water-rich phase before, $C_{DIC,0}^{aq}$, and after the extraction, $C_{DIC,1}^{aq}$:

$$EE\ (\%) = \frac{\mathrm{C_{DIC,0}^{aq}} - \mathrm{C_{DIC,1}^{aq}}}{\mathrm{C_{DIC,0}^{aq}}}\ \mathrm{x}\ 100 \qquad \text{(eq. S1)}$$

The obtained experimental results relative to extraction efficiencies for this micropollutant using several and different families of hydrophobic DESs are displayed in Figure S3.

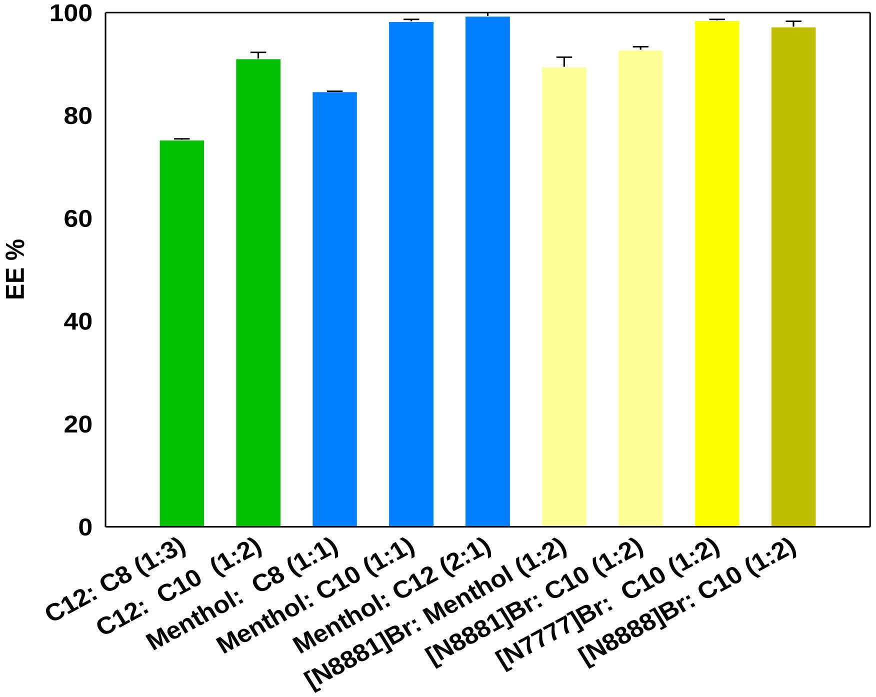

Figure S3. Extraction efficiencies (EE %) of diclofenac sodium salt using different families of hydrophobic DESs used in this work ($[C_0]_{Dic}$ = 0.03 g/L, stirring speed = 300 rpm, DES/water ratio = 1/1, temperature = 25 ºC, contact time = 10 min).

These results show that when the hydrophobicity of the DES increases, its extraction efficiency also increases. This could be seen in the menthol's and fatty acids families, where the increasing of the alkyl chain provides a visible optimization on the extraction of micropollutant. The same can be also observed in the quaternary ammonium salts family.

All the families of hydrophobic DESs presented a high extraction efficiency result, which means that all studied DESs have potential to extract diclofenac sodium from water environments. These results can be explained either by a high solubility of diclofenac sodium in the hydrophobic DESs or by the possible affinity between the DES and the micropollutant due to their structural similarities.

4.3.2. Evaluation of Different Parameters in the Extraction of Diclofenac Sodium

Effect of time on extraction. Different contact times were employed in the extraction process in order to verify if the system had already reached the equilibrium. The results obtained with the different contact times are presented in Figure S4.

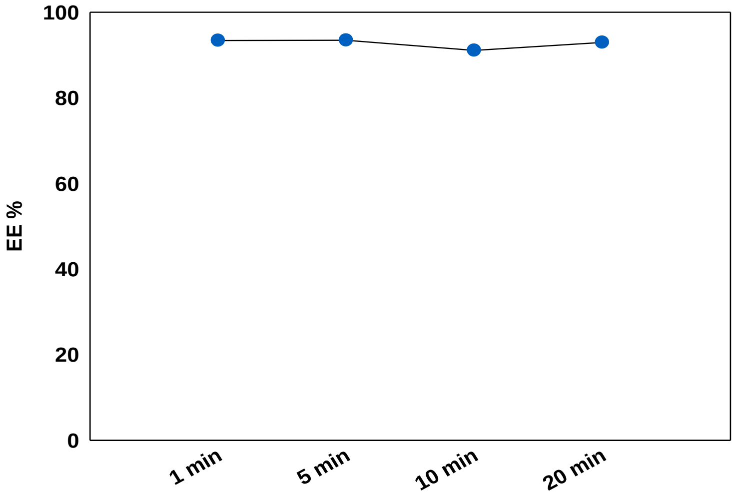

Figure S4. Effect of the contact time in the extraction efficiencies (EE %) of diclofenac sodium salt using the hydrophobic DESs C_{12}: C_{10} (1:2) ($[C_0]_{Dic}$ = 0.03 g/L, stirring speed = 300 rpm, DES/water ratio = 1/1, temperature = 25 ºC).

From Figure S4, it is possible to perceive that the contact time does not affect the extraction efficiency of the diclofenac sodium, since the system had already reached the equilibrium after 1 minute. Furthermore, it is possible to conclude that the development of the extraction presents a maximum value, and it is not always possible extract the totality of the polluting agent for certain experimental conditions.

Effect of stirring speed. Different stirring speeds were also tested in order to evaluate if stronger agitations would provide better contact between the DES and the aqueous phases, without breaking the DES hydrogen bonds. The stirring speeds were tested in a range from 100 to 700 rpm and obtained results are presented in Figure S5.

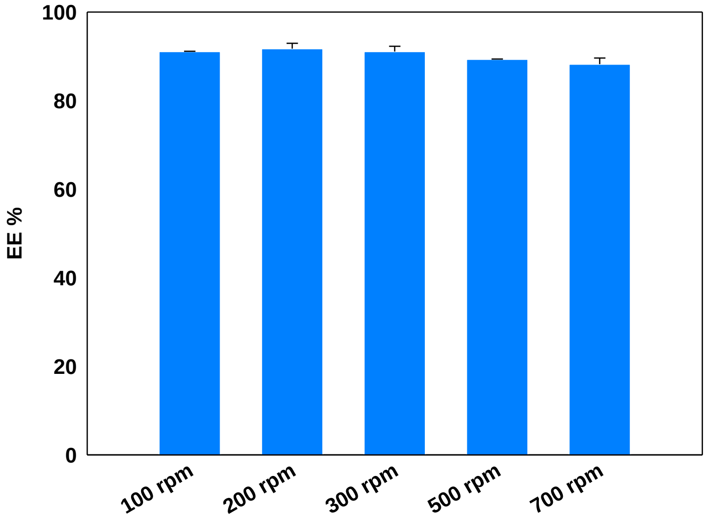

Figure S5. Effect of the stirring speed in the extraction efficiencies (EE %) of diclofenac sodium salt using the hydrophobic DESs C_{12}: C_{10} (1:2) ($[C_0]_{Dic}$ = 0.03 g/L, contact time = 10 min, DES/water ratio = 1/1, temperature = 25 ºC).

These results show that the stirring speed does not have a considerable influence on extraction efficiency, since a low stirring speed of 100 rpm is enough to extract more than 85% of the micropollutant. Nevertheless, it was observed that for higher speeds, as 500 and 700 rpm, the extraction efficiency may even slightly decrease, because the contact between the two phases becomes too fast. Although, it is important to observe that even if the stirring speed does not have a huge impact on the extraction efficiency, the quality of the stirring speed does have a visible impact on extractions. It is important to maintain always the same experimental conditions.

Effect of the initial concentration of the micropollutant. Other very important factor to consider in an extraction process is the initial concentration of micropollutant, in this case, diclofenac sodium, in aqueous environments since the extraction process should be applicable for any polluted water sample. For this purpose, three different aqueous solutions of diclofenac sodium (ranging from 0.01 to 0.03 g/L) were tested for one hydrophobic DES of each family, namely C_{12}: C_{10} (1:2), DL-Menthol: C_{10} (1:1) and [N_{8888}]Br: C_{10} (1:2). The obtained results are presented in Figure S6.

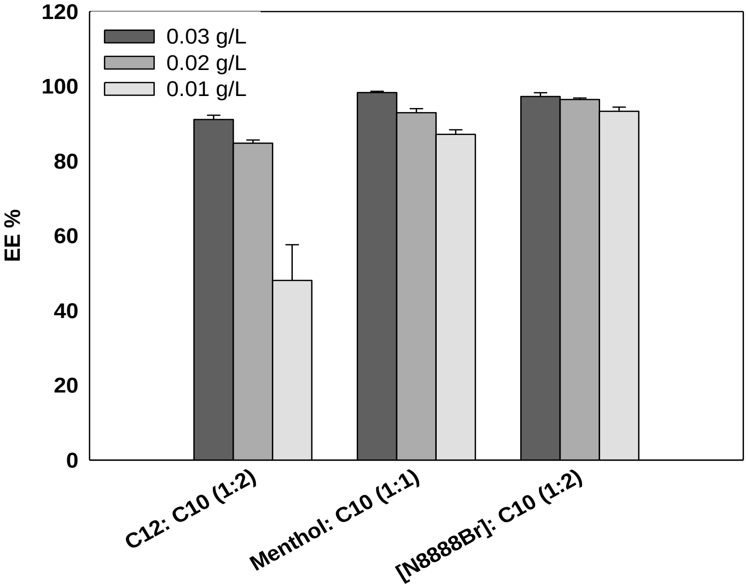

Figure S6. Effect of the initial concentration of diclofenac sodium salt in the extraction efficiencies (EE %) using different hydrophobic DESs (contact time = 10 min, DES/water ratio = 1/1, stirring speed = 300rpm, temperature = 25 ºC).

It can be observed that the extraction efficiency decreases for lower concentrations of diclofenac sodium, which means that this pollutant is more difficult to remove at lower concentrations in water. However, it essentially depends on the family or HBA of the hydrophobic DESs and can be ranked as follows: fatty acids family > menthol family > quaternary ammonium salt family, where the latter is the less pronounced. The quaternary ammonium salt family shows better and constant extraction efficiencies for lower concentrations than the other families. This can be explained by the chemical structural similarity between diclofenac sodium and [N_{8888}]Br: C_{10} (1:2), where both present ionic species. Therefore, it can be concluded that the hydrophobicity and the polarity of the hydrophobic DESs are crucial factors to the efficiency of the extraction process.

Effect of the ratio between DES and water phases. The ratio between the polluted water and the DES phase is also a key factor to be considered, since it will determine if the extraction process is feasible or not. Thus, different concentrations of aqueous solutions of diclofenac sodium (C = 0.03 g/L and C = 0.01 g/L) and two hydrophobic DESs ((C_{12}: C_{10} (1:2) and DL-Menthol: C_{10} (1:1)) were tested for using different DES/water ratios varied from 3/1 to 1/100. The obtained results are shown in Figures S7 and S8.

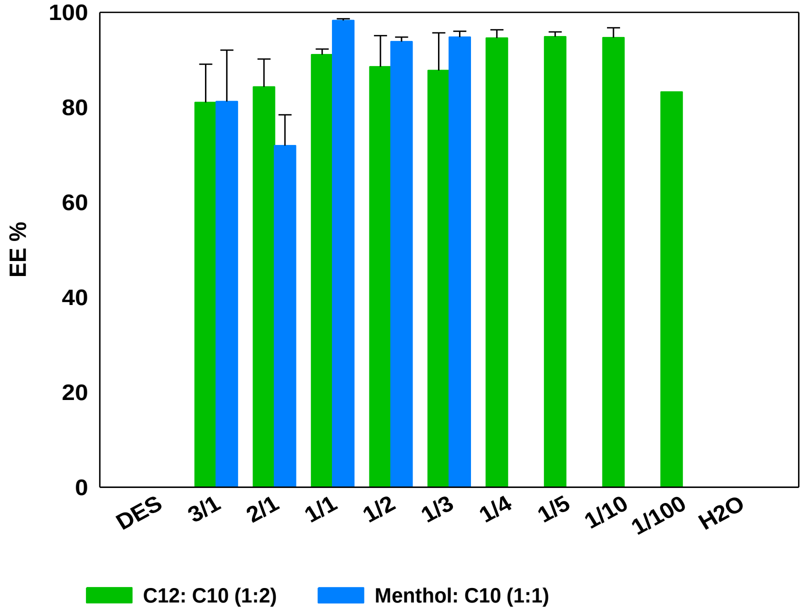

Figure S7. Effect of the DES/water ratio in the extraction efficiencies (EE %) using two hydrophobic DESs at a high concentration of micropollutant ($[C_0]_{Dic}$ = 0.03 g/L, contact time = 10 min, stirring speed = 300rpm, temperature = 25 ºC).

Regarding the results presented in Figure S7, it can be concluded that extraction efficiency increases for lower quantities of hydrophobic DES relative to the water phase. This can probably be explained by the high hydrophobicity of these solvents as well as the high solubility of micropollutant in DES. If DES is too abundant in the system, an optimized mass transfer can be challenging with the aqueous phase and the extraction can actually decrease, due to an unpromising mass to volume ratio. A small amount of DES introduced in the system ends up playing a relevant role regarding an appealing mass transfer as the DES can easily spread into the aqueous phase in the form of small droplets and the extraction can be improved.

Moreover, the effect of DES/water ratio can be exploited in order to compensate other important parameters, such as the improvement of the extraction in the less concentrated polluted water samples. Thus, a further study was conducted regarding the effect of the DES/water ratio for a diclofenac sodium aqueous solution with a lower concentration of 0.01 g/L, which had previously shown lower extractions efficiencies. The results are shown in Figure S8.

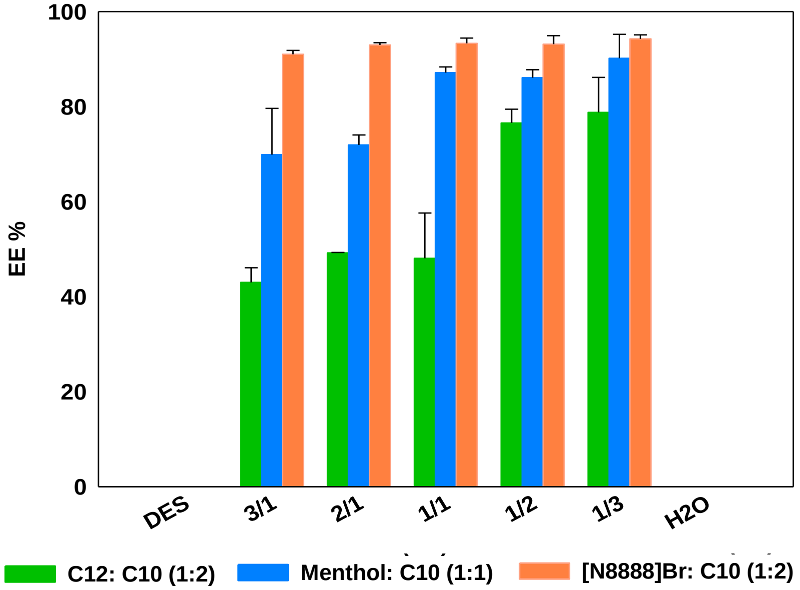

Figure S8. Effect of the DES/water ratio in the extraction efficiencies (EE %) using two hydrophobic DESs using a different initial concentration of micropollutant at low concentration of micropollutant ($[C_0]_{Dic}$ = 0.01 g/L, contact time = 10 min, stirring speed = 300rpm, temperature = 25 ^{o}C).

In general, it can be observed that the extraction efficiency was significantly enhanced for all the studied hydrophobic DESs. Once again, there are differences in the extraction efficiencies between the various families of hydrophobic DESs. It was found that for low concentrations, those that have the greatest effect on the ratio are menthol. It was verified that for low concentrations of diclofenac sodium, those that are more susceptible to be affected by DES/water ratio are from the fatty acid and menthol families.

This would be very interesting for a large-scale application, since it would provide high extraction efficiencies of micropollutant using lower quantities of DESs. It also proves that the liquid-liquid extraction will not be limited by the initial concentration of the micropollutant in water, since the effect of the ratio will be able to compensate the effect of the concentration.

Effect of the HBA: HBD ratio of DES. Another aspect to be explored in this field is the phase diagrams of hydrophobic DESs as well as the several liquid phase ratios at working conditions available in diagrams. The studied DES may also be found in the liquid form for different ratios HBA: HBD from those used previously and also different from the eutectic point. In this section, the effect of other compositions of C_{12}: C_{10} (1:2) and DL-Menthol: C_{10} (1:1) will be checked. The phase diagrams of the studied hydrophobic DESs are represented in Figure S9 and the obtained results are shown in Figure S10.

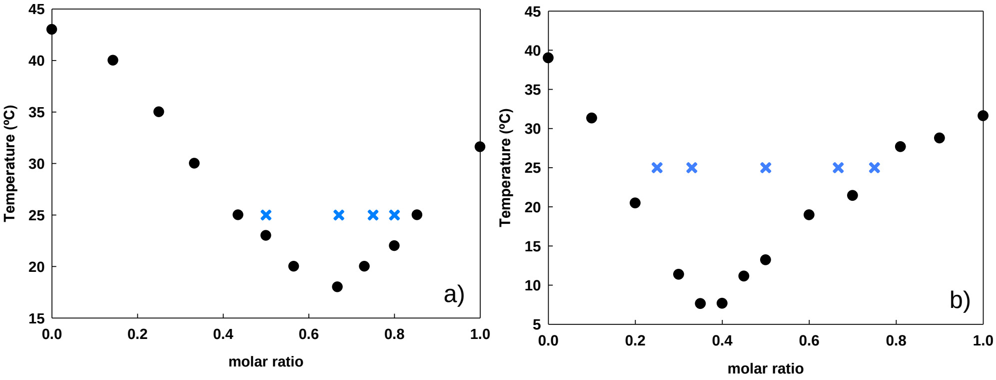

Figure S9. Phase diagrams of C_{12}: C_{10} (a) and DL-Menthol: C_{10} (b), respectively. The blue points represent the tested compositions of the DES to perform extractions.

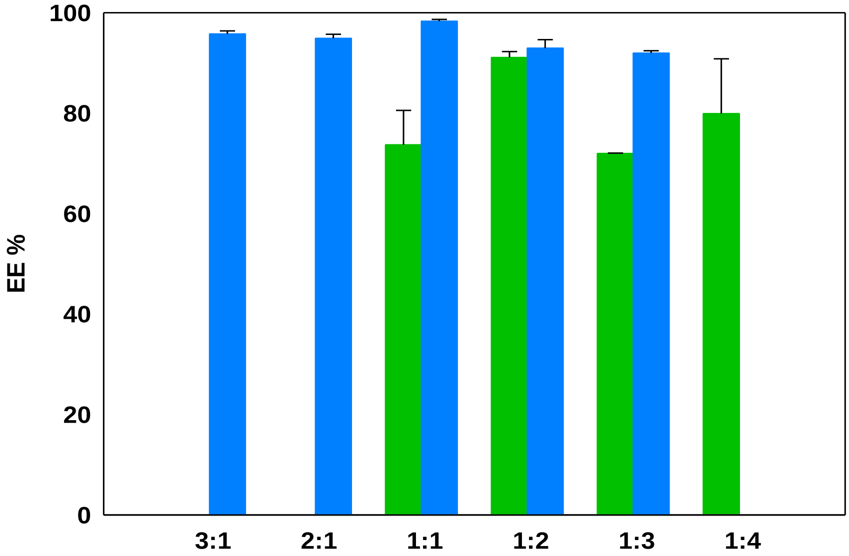

Figure S10. Liquid-liquid extractions of diclofenac sodium salt using C_{12}: C_{10} (green) and DL-Menthol: C_{10} (blue) in different ratios HBA: HBD as represented in the phase diagram above ($[C_0]_{Dic}$ = 0.03 g/L, contact time = 10 min, stirring speed = 300rpm, temperature = 25 ºC).

From the results, it can be concluded that the best results of extraction efficiencies obtained were found for C_{12}: C_{10} (1:2) and DL-Menthol: C_{10} (1:1) systems in the ratio which were used in the previous studies, as well as corresponding to the eutectic point indicated in the phase diagram.

However, some effect of HBA: HBD ratio is more visible for the case of DL-Menthol: C_{10}, which seems to be related to the family of DES used. As it can be seen, the HBA:HBD ratio had almost no effect on the extraction efficiencies, which means that other compositions from the phase diagram as well as different from eutectic point can be used, provided that DES remains liquid and stable at working conditions.

Reuse of the DES. From an economical point of view, it is vital for a novel solvent that it can be reused multiple times before losing its extraction capability. In this study, C_{12}: C_{10} (1:2) and [N_{8888}]Br: C_{10} (1:2) were selected to be reused in consecutive liquid-liquid extractions of different samples of a sodium diclofenac solution with a concentration of 0.03 g/L. After each extraction the DES phase was treated with “fresh” water in order to verify if it was possible to improve or maintain the extraction efficiency. The obtained results are summarized in Figure S11.

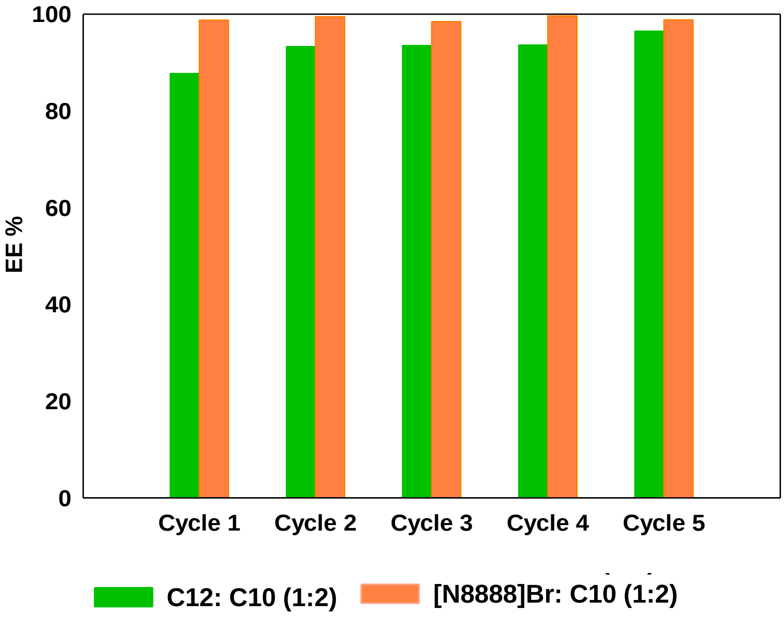

Figure S11. Several cycles of extraction of diclofenac sodium salt using C_{12}: C_{10} (green) and [N_{8888}]Br: C_{10} (orange) ($[C_0]_{Dic}$ = 0.03 g/L, contact time = 10 min, stirring speed = 300rpm, temperature = 25 ºC).

From the results, it can be concluded that in both DESs, C_{12}: C_{10} (1:2) and [N_{8888}]Br: C_{10} (1:2), after five cycles of its reutilization, it keeps its extraction capacity and it is even possible to observe that in some cases, the extraction efficiency can slightly increase in each cycle. This probably happens because the presence of the micropollutant in the used DESs, increases the affinity between the DES phase and the sodium diclofenac molecules to be extracted in the aqueous phase. Also, it is possible to see that the variations in the extraction efficiencies after several cycles are less visible in the case of $[N_{8888}]Br$: C_{10} (1:2), which presents constantly high extraction efficiencies. Therefore, it is possible to reuse the DES multiple times without losing its capacity to extract, becoming a more sustainable and attractive process.

***Impact of the saturation of DES on extraction efficiency*.** As the concentration of sodium diclofenac in the DES increases, it may reach a saturation limit in which the extraction efficiency of the process will decrease or to be incapable to extract more micropollutant. In order to evaluate that, samples of C_{12}: C_{10} (1:2) were saturated with different masses of sodium diclofenac in order to perform consecutive extractions until the system couldn't extract more pollutant. The results are presented in Figure S12.

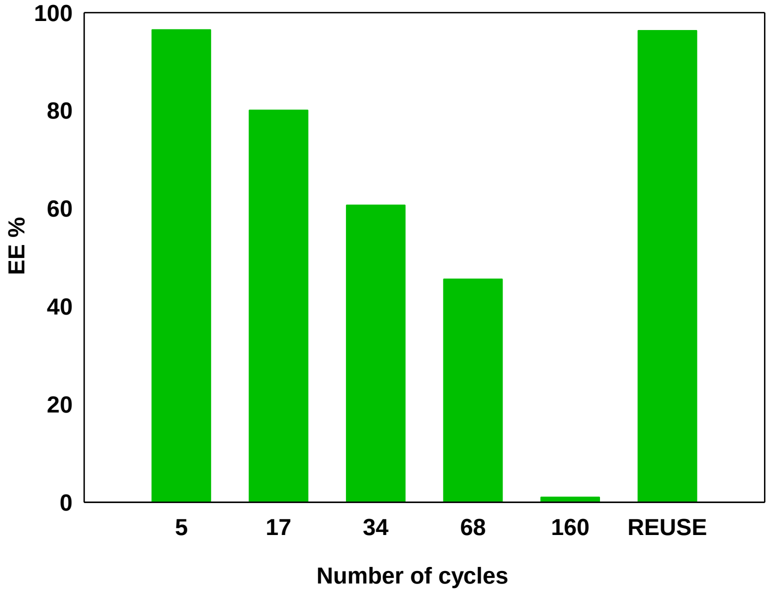

Figure S12. Consecutives extractions performed using the same hydrophobic DES (C_{12}: C_{10} (1:2)) until complete saturation as well as the recovering of initial DESs. ($[C_0]_{Dic}$ = 0.03 g/L, contact time = 10 min, stirring speed = 300rpm, temperature = 25 ºC).

These results display the significant extraction efficiency decreases upon the addition of sodium diclofenac in the DES. It can be observed that the extractions come to a stop before the solubility limit is reached (0.025 g DIC/g DES). Nevertheless, it is still possible to use the hydrophobic DESs for at least 17 reuse cycles obtaining extraction efficiencies higher than 80%. It is very interesting to see that this DES loses its total ability to extract the micropollutant after 160 cycles, which makes the process very sustainable in terms of reuse. After reaching the limit of extraction (around 68 cycles), it would be interesting to purify the DES, in order to reuse it.

For that, one of the most green and sustainable strategies used to purify water in wastewater treatment consists in using activated carbon which is a very porous material with a huge ability to extract pollutants. Therefore, activated carbon was used to purify diclofenac sodium from DES. Both purifications presented efficiencies of 100%, showing that the activated carbon has a great extraction capacity.

Moreover, activated carbon was used to purify polluted C_{12}: C_{10} (1:2) samples corresponding to 17 and 68 reuse cycles. In this study, the activated carbon and the polluted DES were mixed for 10 minutes, at 25 °C and with a stirring speed of 450 rpm. After the stirring, the DES was filtered with 0.2 µm PTFE filters. Finally, the DES samples were diluted in acetonitrile and analyzed by UV spectroscopy.

These results show that it is possible to purify the polluted DES efficiently using activated carbon. The necessary amount of activated carbon is significantly higher than the necessary amount for the purification of water samples. This may be due to the diclofenac being more stable in DES than in water. However, this purification step would not be a problem for an industrial application, since the water treatment stations already use activated carbon filters in their processes.

4.4. Adsorbent materials using hydrophobic DES details

4.4.1. Experiments of extraction of Bisphenol-A

In Figure S13, effect of extraction time, mass of DES used as adsorbent as well as reuse of supported DES in the extraction efficiency of BPA from contaminated water is depicted.

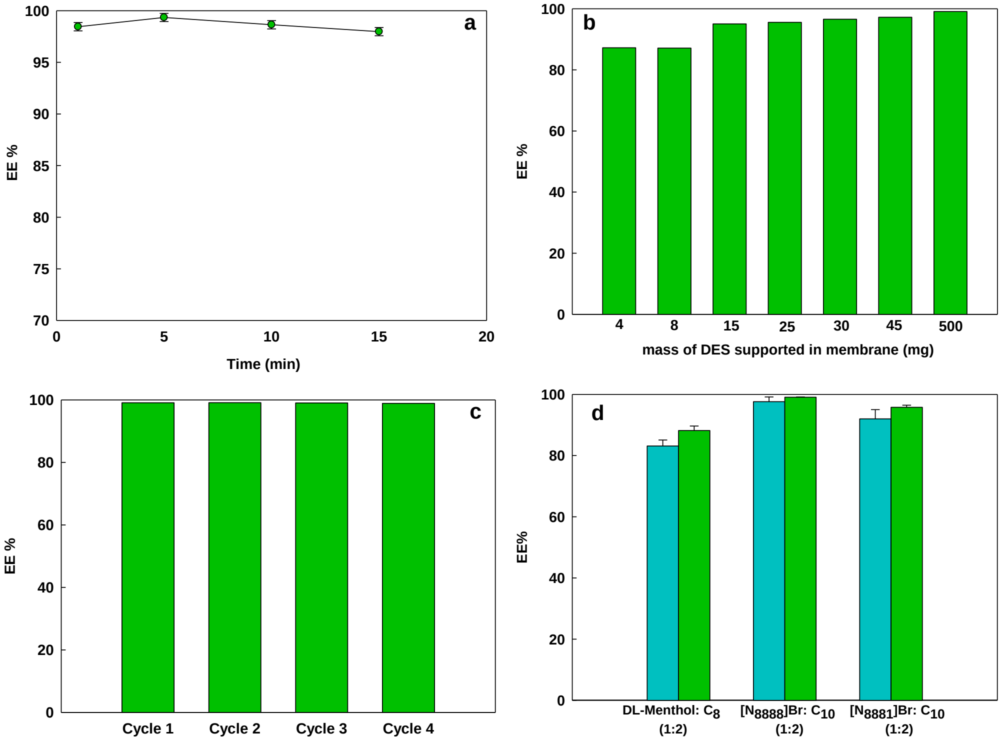

Figure S13. Extraction efficiencies (EE %) of BPA using supported hydrophobic DES ($[N_{8888}]Br$: C_{10}) as adsorbent material: a) effect of time ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, temperature = 25 ºC mass of DES = 500 mg), b) mass of DES supported used as adsorbent ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, contact time = 10 min, temperature = 25 ºC), c) reuse of supported DES ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, contact time = 10 min, temperature = 25 ºC, mass of DES = 500 mg), d) comparison of LLE (DES mass = 2 mL) and supported DES (DES mass = 500 mg) used as adsorbent for different hydrophobic DESs ($[C_0]_{BPA}$ = 0.1 g/L, stirring speed = 300 rpm, contact time = 10 min, temperature = 25 ºC).

From the obtained results it can be highlighted that supported DES shows a great performance in the extraction of BPA from water. The fast kinetics of the solubility/extraction process can clearly be seen in Figure S13 a), with a maximum of 5 min. In Figure S13 b) it can be observed that very small amounts of $[N_{8888}]Br: C_{10}$ allow high extraction efficiencies. In fact, with only 4 mg of DES it is possible to achieve 87 % of extraction efficiency, which is per se a remarkable result. When using 500 mg of DES, a maximum extraction efficiency of 99% could be attained, which is not very different from extraction efficiency (~95%) obtained when 15 mg of DES is used. Through direct comparison, in order to obtain two well defined and separated phases, a minimum of 2 mL of DES as solvent in LLE is required, while using DES as adsorptive material, 15 mg of DES is enough to obtain a similar extraction efficiency high extraction (~95%).

As in the case of LLE, it is also possible to reuse several times the DES as adsorbent material without losing its exceptional extractive capacity, as it can be seen in Figure S13 c), where 550 mg of DES adsorbent material is used.

It can also be observed in Figure S13 d) that both using DES as solvents and as adsorbents yielded similar excellent results. Moreover, it can be seen that the performance of %EE for different DES is the same. It means that the adsorption method can be reproduced for other pollutants as well as for DES with different families of HBA and HBD.

5. References

1. World Economic Forum, http://reports.weforum.org/global-risks-2018/, p. accessed: November 2018.

2. Zhang, Y., Geißen, S.-U. and Gal, C., Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. Chemosphere 2008, 73, 1151-1161.

3. Kümmerer, K., Antibiotics in the aquatic environment – A review – Part I. Chemosphere 2009, 75, 417-434.

4. Koopaei, N. N., and Abdollahi, M., Health risks associated with the pharmaceuticals in wastewater. DARU Journal of Pharmaceutical Sciences 2017, 25, 9.

5. Verlicchi, P., Hospital Wastewaters: Characteristics, Management, Treatment and Environmental Risks. Springer International Publishing, 2018.

6. Gros, M., Petrović, M., Ginebreda, A. and Barceló, D., Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environment International 2010, 36, 15-26.

7. Van Osch, D. J. G. P., Zubeir, L. F., Van Den Bruinhorst, A., Rocha, M. A. A., Kroon, M.C., Hydrophobic deep eutectic solvents as water-immiscible extractant. Green Chemistry, 2015, 17, 4518-4521.

8. Shi, C.; Jing, Y.; Xiao, J.; Wang, X. and Jia, Y., Liquid-liquid extraction of lithium using novel phosphonium ionic liquid as an extractant. Hydrometallurgy 2017, 169, 314-320.

9. Rocha, M. A. A. et. al., Recovery of volatile fatty acids from water using medium-chain fatty acids and a cosolvent. Chemical Engineering Science 2017, 165, 74-80.

10. Almeida, H. F. D., Marrucho, I. M. and Freire, M. G., Removal of Nonsteroidal Anti-Inflammatory Drugs from Aqueous Environments with Reusable Ionic-Liquid-Based Systems. ACS Sustainable Chemistry & Engineering 2017, 5, 2428-2436.

11. Almeida, H. F. D., Freire, M. G. and Marrucho, I. M., Improved extraction of fluoroquinolones with recyclable ionic-liquid-based aqueous biphasic systems. Green Chemistry 2016, 18, 2717-2725.

12. Malik, M. A., Hashim, M. A. and Nabi, F., Ionic liquids in supported liquid membrane technology. Chemical Engineering Journal 2011, 171, 242-254.

13. Zhang, J.; Liu, H. and Jiang, L., Membrane-Based Strategy for Efficient Ionic Liquids/Water Separation Assisted by Superwettability. Advanced Functional Materials 2017, 27, 1606544.

14. Dietz, C. H. J. T.; Kroon, M. C.; Stefano, M. D.; Annaland, M. V. S. and Gallucci, F., Selective separation of furfural and hydroxymethylfurfural from an aqueous solution using a supported hydrophobic deep eutectic solvent liquid membrane. Faraday Discussions 2018, 206, 77-92.

15. Ali, I. and Gupta, V. K., Advances in water treatment by adsorption technology. Nature Protocols 2007, 1, 2661-2667.

16. Sahiner, N. and Sagbas, S., Polymeric ionic liquid materials derived from natural source for adsorption purpose. Separation and Purification Technology, 2018, 196, 208-216.

17. Mellah, A. et al., Adsorption of Pharmaceutical Pollutants from Water Using Covalent Organic Frameworks. Chemistry - A European Journal 2018, 24 (42) , 10601-10605.

18. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L. and Rasheed, R. K., Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. Journal of the American Chemical Society, 2004, 126, 9142-9147.

19. Saei, A. A.; Jabbaribar, F.; Fakhree, M. A. A.; Acree, W. E. and Jouyban, A., Solubility of sodium diclofenac in binary water + alcohol solvent mixtures at 25°C. Journal of Drug Delivery Science and Technology 2008, 18, 149-151.

20. Florindo, C.; Costa, A.; Matos, C.; Nunes, S. L.; Matias, A. N.; Duarte, C. M. M.; Marrucho, I. M, Novel organic salts based on fluoroquinolone drugs: Synthesis, bioavailability and toxicological profiles. International Journal of Pharmaceutics 2014, 469 (1), 179-189.

21. Li, Jin-Hua; Zhou, Bao-Xue; and Cai, Wei-Min, The Solubility Behavior of Bisphenol A in the Presence of Surfactants. Journal of Chemical & Engineering Data 2007, 52 (6), 2511-2513.

CHAPTER 4

Hydrophilic vs. Hydrophobic Deep Eutectic Solvents

Miscible

Immiscible

From miscible to immiscible

CHAPTER 4.1

A closer look into Deep Eutectic Solvents: exploring intermolecular interactions using solvatochromic probes

Adapted from: **C. Florindo**, A. J. S. McIntosh, T. Welton, L. C. Branco, I. M. Marrucho, A closer look into Deep Eutectic Solvents: exploring intermolecular interactions using solvatochromic probes, Phys. Chem. Chem. Phys. (2018), 20, 206-213.

The author contributed to the execution of all characterization and properties experiments described in this chapter, as well as involved in the discussion and interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

Deep eutectic solvents (DESs) constitute a new class of ionic solvents that has been developing at a fast pace in recent years. Since these solvents are commonly suggested as green alternatives to organic solvents, it is important to understand their physical properties. In particular, polarity plays an important role in solvation phenomena. In this work, the polarity of different families of DESs was studied through solvatochromic responses of UV-vis absorption probes. Kamlet-Taft α, β, π^* and E_TN parameters were evaluated using different solvatochromic probes, as 2,6-Dichloro-4-(2,4,6-triphenyl-N-pyridino)-phenolate (Reichardt's betaine dye 33), 4-nitroaniline, and N,N-diethyl-4-nitroaniline for several families of DESs based on cholinium chloride, DL-Menthol and a quaternary ammonium salt ([N_{4444}]Cl). In addition, a study to understand the difference in polarity properties between DESs and the corresponding ILs, namely ILs based on cholinium cation and carboxylic acids as anions ([Ch][Lev], [Ch][Gly] and [Ch][Mal]), was carried out. The chemical structure of the hydrogen bond acceptor (HBA) in a DES clearly controls the dipolarity/polarizability afforded by the DES. Moreover, Kamlet-Taft parameters do not vary much within the family, but they differ among families based on different HBA, either for DESs containing salts ([Ch]Cl or [N_{4444}]Cl) or neutral compounds (DL-Menthol). A substitution of the HBD was also found to play an important role in solvatochromic probe behaviour for all the studied systems.

1. Introduction

Deep Eutectic Solvents (DESs) have been emerging since 2004 as a new generation of solvents with a great potential for a variety of applications.[1] DESs can be regarded as a new class of ionic solvents, typically composed of an organic salt and at least one hydrogen bond donor, which presents a lower melting point than any of its individual components.[2] The formation of a liquid compound at room temperature is due to the formation of hydrogen bonds between a hydrogen bond donor (HBD), and a hydrogen bond acceptor (HBA).[3] Bearing in mind that in a salt, the cation generally acts as an HBD and the anion as HBA, the DES formation is due to the addition of an extra HBD. DESs are currently attracting widespread scientific and technological interest as alternatives to ionic liquids (ILs).[4-5]

Although these solvents are generally compared to ILs, mainly due to their equivalently negligible vapour pressures at room temperature, they have important advantages such as their straightforward and green synthesis, that does not need any solvent and purification steps and their low toxicity and cost, since the compounds used are usually non-toxic, abundant and from renewable resources.[6-7] Just like ILs, one of the most attractive aspects of these alternative solvents is the ability to fine-tune their physical-chemical properties, including hydrogen bond donating/accepting ability and polarizability, through the easy manipulation of the chemical structures of the starting compounds. However, there is still a lack in the knowledge of some important solvent properties, such as polarity.

The exact meaning of “polarity” (the sum of all possible interactions between a solvent and any potential solute[8]) is complex as many different intra and inter aggregate/ion pair interactions, such as hydrogen bonding, *π*-interactions or van der Waals forces, are involved. The molecular dynamics of DESs systems have been recently are studied using ^{1}H pulsed field gradient NMR self-diffusion coefficients. This method is very useful to detail and understand the interactions between ions, host/guest molecules and complexes formation.[9-11] On the other hand, solvatochromic probes have also been used to investigate ILs and DES polarity. In particular, Kamlet–Taft parameters have been used to quantify the hydrogen-bond donating ability (α, acidity), the hydrogen bond accepting ability (β, basicity) and polarity/polarizability (π^*) of ILs and DESs.[5, 12-14] It should be remarked that polarity is not an absolute property of the pure liquid[15-16] and hence, there is no single correct value when comparing polarity scales.[15, 16] All polarity scales are relative and different scales give different polarities for the same solvent and even different relative polarities can arise.[17]

So far, only four studies[18-21] on the characterization of DESs polarities have been published. All studies are mainly focused on use of solvatochromic probes to characterize the polarity of the most popular DESs, namely cholinium chloride: urea (1:2), cholinium chloride: glycerol (1:2) and cholinium chloride: ethylene glycol (1:2). Highlighting the study developed by Pandey et al.,[14, 18] where they investigated the effect of temperature and the addition of water on the three DESs' polarity was also studied. They concluded that an increase in temperature results in reduced H-bond donor acidity of the DESs, while no temperature effect was observed on the dipolarity/polarizability and H-bond accepting basicity. It was also shown that addition of water to DESs resulted in increased dipolarity/polarizability and a decrease in H-bond accepting basicity. Very recently, Teles et al.[21] published a more detailed study of solvatochromic parameters of DES formed by ammonium-based salts and carboxylic acids. It was shown that the high acidity of the studied DESs was mainly due to the organic acid present in the mixture, and that an increase of the alkyl side chain of both the HBA and the HBD species leads to a lower ability of the solvent to donate protons. They concluded that DES composed of ammonium-based salts and carboxylic acids presented a higher capacity to donate and accept protons when compared to most of the ionic liquids or organic molecular solvents.

In the present work, the aim is to evaluate and discuss the polarity of DESs, which have different chemical structures, using the solvatochromic shift of different probes in the visible absorbance spectrum. Recently, we have compared the densities and viscosities of hydrophilic cholinium-based DESs and corresponding ILs.[4] We have also studied highly fluid hydrophobic DESs based on DL-Menthol[22] while other hydrophobic DESs based on quaternary ammonium salts have also been reported in literature.[23] Thus, it is now important to characterize all these different DESs from the point of view of polarity. In this way, a range of DESs, based on hydrophilic HBA (cholinium chloride) and a hydrophobic HBD (tetrabutylammonium chloride or DL-Menthol), combined with several different carboxylic acids, were studied in terms of normalised polarity (E_TN) and the Kamlet–Taft parameters (α, β and π^*). Chemical structures and respective acronyms of the ILs and DESs used in this work are presented in Figures 1 and 2, respectively. Additionally, a comparison of the solvatochromic parameters obtained for hydrophilic DESs and their corresponding cholinium-based ILs is also presented.

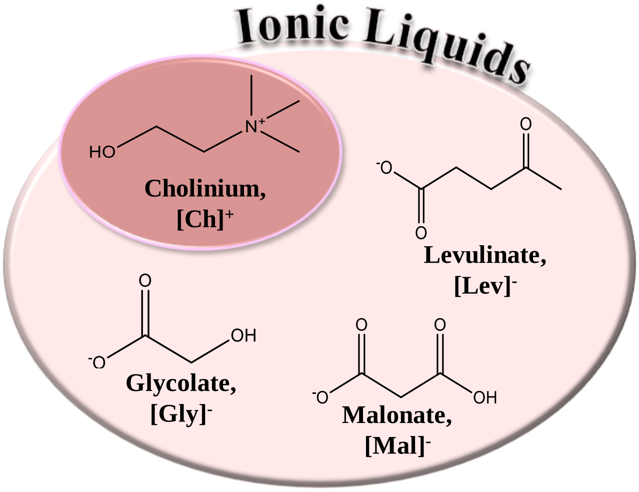

Figure 1. Chemical structures and respective acronyms of the cholinium-based ILs used in this work.

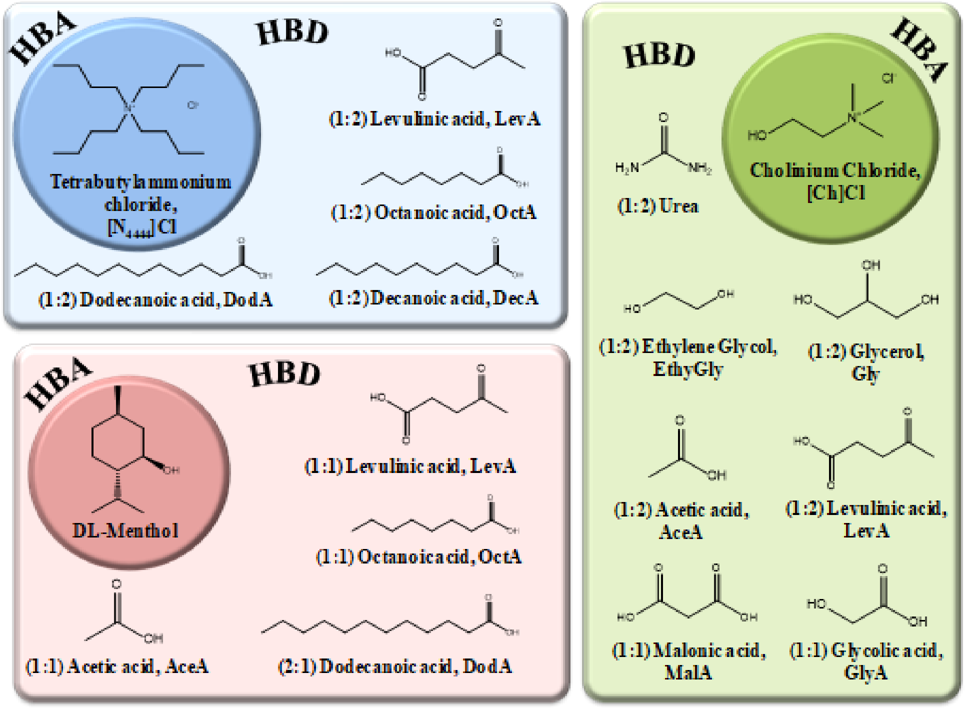

Figure 2. Chemical structures and respective acronyms of the DESs, using cholinium chloride ([Ch]Cl), DL-Menthol and tetrabutylammonium chloride ([N_{4444}]Cl) as hydrogen bond acceptors (HBA), studied in this work.

2. Experimental Section

2.1. Materials

2,6-Dichloro-4-(2,4,6-triphenyl-N-pyridino)-phenolate (Reichardt's betaine dye 33), 4-nitroaniline, and N,N-diethyl-4-nitroaniline were purchased from Fluka (≥ 97% mass fraction purity), Sigma Aldrich (≥ 99% mass fraction purity) and Frinton Laboratories, respectively, and were used as received. For the synthesis of the ILs the following reagents were used: Cholinium bicarbonate solution (80% mass fraction purity in H_2O), malonic acid (99% mass fraction purity), glycolic acid (99% mass fraction purity) were purchased from Sigma

Aldrich and Levulinic acid (98% mass fraction purity) was supplied by Acros Organics (> 98% mass fraction purity). Methanol and diethyl ether were purchased as AnalaR NORMAPUR (VWR chemicals) and dichloromethane was purchased as GPR Rectapur (VWR chemicals) and were used as received. For the synthesis of the deep eutectic solvents the following reagents were used: cholinium chloride ([Ch]Cl) (≥ 98% mass fraction purity) and tetrabutylammonium chloride ($[N_{4444}]Cl$) (> 97% mass fraction purity) were purchased from Sigma-Aldrich and were dried under vacuum prior to use. Urea, ethylene glycol, glycerol, levulinic acid, glycolic acid, malonic acid, acetic acid, octanoic acid, decanoic acid and dodecanoic acid (all ≥ 99% mass fraction purity) were purchased from Sigma-Aldrich and used as supplied.

2.2. Methods

2.2.1. Synthesis of ILs

The ILs used in this work, namely the cholinium levulinate ([Ch][Lev]), cholinium malonate ([Ch][Mal]) and cholinium glycolate ([Ch][Gly]), were prepared by dropwise addition of the corresponding acid (1:1) to aqueous cholinium bicarbonate, following an established procedure.[24] The mixtures were stirred at ambient temperature and pressure for 12 h. The resulting products were washed with diethyl ether to remove any unreacted acid. Excess water and traces of other volatile substances were removed first by rotary evaporation, and then by stirring and heating under vacuum. The chemical structures and the purities of the synthesized cholinium-based ILs were confirmed by ^{1}H and ^{13}C NMR. All the IL samples were dried prior to their use by stirring and heating under vacuum at moderate temperature (40 ºC, > 48 h, ca. 0.01 mbar). Their water contents were determined by Karl Fischer titration (831 KF Coulometer, Metrohm) and considered in all experiments.

2.2.2. Preparation of DESs

All DESs were prepared by mixing the HBA, cholinium chloride, DL-Menthol or tetrabutylammonium chloride, with the respective HBD, in a certain mole ratio. The resulting mixtures were then ground in a mortar with a pestle at room temperature until a homogeneous, colourless liquid had been formed. The water content was determined by Karl Fisher titration (model Metrohm 831 Karl Fisher coulometer).

2.2.3. Solvatochromic Probes

Stock solutions of all probes were prepared by dissolution in dichloromethane in pre-cleaned amber glass vials. The required amount of each probe was weighed using a Sartorius CPA Analytical Balance CPA224S with a precision of 0.1 mg. An appropriate amount of the probe solution was transferred from the stock to the 1 mm light path quartz cuvette. The dichloromethane was evaporated using a gentle stream of high purity nitrogen gas. A pre-calculated amount of DES was directly added to the cuvette and the solution thoroughly mixed. A Perkin-Elmer Lambda 25 double beam spectrophotometer was used for acquisition of the UV-Vis molecular absorbance data. All spectroscopic measurements were performed in triplicate starting from the sample preparation.

3. Results and Discussion

3.1. Betaine Dye scale

Due to the large shift in the lowest energy charge-transfer absorption band between polar and non-polar solvents, Reichardt’s dye or betaine dye 30 (2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl)phenolate) is one of the most widely used solvatochromic probes. However, its zwitterionic nature causes its solvatochromic behaviour to be strongly affected by the HBD character of the solvent: it is well known that hydrogen-bond donating solvents stabilize the ground state more than the excited state.[25] Since most of our DESs present strong acidic character (pH ≈ 1), they extensively interfere with this dye solvatochromic behaviour. To circumvent this problem, a derivative of Reichardt's dye named Reichardt's betaine dye 33 (2,6-dichloro-4-(2,4,6-triphenylpyridinium1-yl)phenolate) was used in this work. Molecular structures of the solvatochromic probes used are presented in Figure 3.

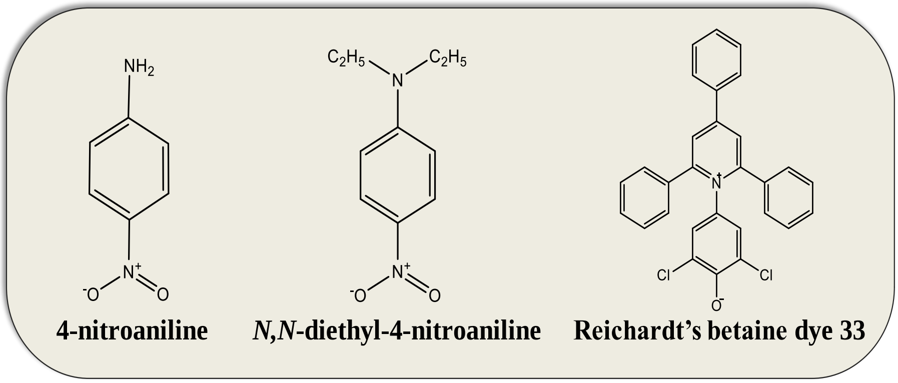

Figure 3. Chemical structures of the solvatochromic probes used in this work.

The solvatochromic parameter $E_T(33)$ was determined from the lowest energy of charge-transfer absorption band of dye in the solvent under study, and can be calculated using the following equation:

$$E_T(33) = \frac{28591}{\lambda\text{max}} \qquad \text{(eq. 1)}$$

where $E_T(33)$ is in kcal.mol^{-1} and λ_{max} (nm) is the wavelength corresponding to maximum of absorption of the probe in the solvent under study.[26]

$E_T(33)$ is used as a measure of the solvent's overall polarity, dipolarity/polarizability and/or HBD ability, arising from interactions between the solvent and the dye.[27] E_TN is a normalized polarity parameter and varies between 0 for TMS (extremely non-polar) and 1 for water (extremely polar). Thus, its use is recommended instead of $E_T(30)$.

Absorbance spectra of Reichardt's 33 were collected in several solvents (DESs and ILs) at room temperature, and the corresponding values of $E_T(33)$ converted into $E_T(30)$ values, through a linear regression analysis using the following equation (2)[14, 28]:

$$E_T(30) = 0.9953(\pm 0.0287) \times E_T(33) - 8.1132(\pm 1.6546) \qquad \text{(eq. 2)}$$

If Reichardt's dye is being used, the E_T^N polarity parameter is easily obtained by measuring the wavelength corresponding to the maximum of absorption of the dye in the solvent under study, according to the following equations:

$$E_T^N = \frac{[E_T(30)_{\text{solvent}} - E_T(30)_{\text{TMS}}]}{[E_T(30)_{\text{water}} - E_T(30)_{\text{TMS}}]} = \frac{E_T(30) - 30.7}{32.4} \qquad \text{(eq. 3)}$$

where $E_T(30)$ is given by equation (1).

In Figure 4, the absorbance spectra of Reichardt's 33 dissolved in several ILs and DESs are presented.

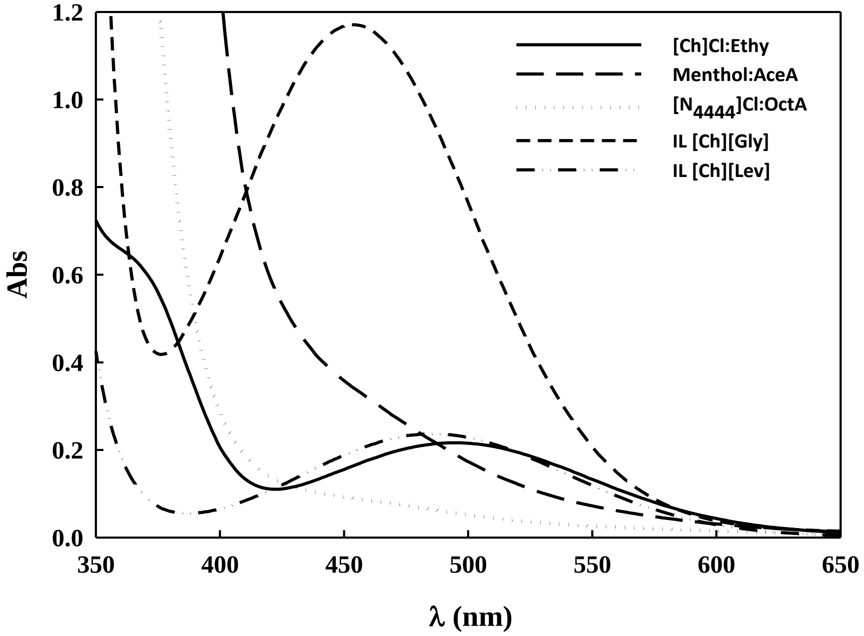

Figure 4. Example of the lowest energy intramolecular charge-transfer absorption band of Reichardt's betaine dye 33 for some ILs and DESs studied in this work.

It can be observed that the peaks for DL-Menthol: AceA and [N_{4444}]Cl: OctA in the region between 400 nm and 600 nm are difficult to detect. To overcome this issue, first derivatives of the spectra were used to determine the λ_{max}. The λ_{max} value, as well as $E_T(33)$ and E_TN calculated using equations (1) and (3), are listed in Table 1 for all the ILs and DESs studied in this work.

Table 1. UV-Vis absorbance maxima (λ_{max}) of Reichardt's 33, $E_T(33)$ and E_TN for all DESs and corresponding ILs.

		λ_{max} of Reichardt's 33 (nm)	**$E_T(33)$ (Kcal.mol^{-1})**	**E_TN**
Ionic Liquids	**[Ch][Lev]**	485.07	58.94	0.61
	[Ch][Mal]	428.73	66.69	0.85
	[Ch][Gly]	453.17	63.09	0.74
Deep Eutectic Solvents	**[Ch]Cl: LevA**	568.89	50.26	0.35
	[Ch]Cl: MalA	442.53	64.61	0.79
	[Ch]Cl: GlyA	562.44	50.83	0.36
	[Ch]Cl: Urea	434.84	65.75	0.81
	[Ch]Cl: Ethy	433.82	65.91	0.83
	[Ch]Cl: Gly	429.99	66.49	0.84
	DL-Menthol: AceA	457.80	62.45	0.72
	DL-Menthol: LevA	456.56	62.62	0.73
	DL-Menthol: OctA	453.12	63.10	0.74
	DL-Menthol: DodA	454.52	62.90	0.73
	[N_{4444}]Cl: OctA	464.71	61.52	0.69
	[N_{4444}]Cl: DecA	474.72	60.23	0.65
	[N_{4444}]Cl: DodA	464.36	61.57	0.69

In order to validate our experimental method, the $E_T(33)$ values for [Ch]Cl: Urea, [Ch]Cl: Ethy and [Ch]Cl: Gly were also measured in this work and compared with those published in literature 65.4[18], 65.7[18] and 66.4[20] Kcal mol^{-1}, respectively. There is a good agreement between our results listed in Table 1 and those reported in literature, thus validating the methodology here used. High E_TN values were obtained for the two families of hydrophobic DESs studied in this work, DL-Menthol-based and [N_{4444}]Cl-based, listed in Table 1, demonstrating that these solvents are hydrophobic and polar. For example, if one compares the values of DL-Menthol: OctA (0.74) or DL-Menthol: DodA (0.73) with those of the corresponding quaternary ammonium based DESs, [N_{4444}]Cl: OctA (0.69) and [N_{4444}]Cl: DodA (0.69), only small differences can be observed. Nevertheless, the E_TN values obtained for the DL-Menthol-based DESs are slightly higher than those obtained for $[N_{4444}]$Cl-based DESs, indicating the important role of HBA in defining the polarity of hydrophobic DESs. Another observation is that E_TN values are almost constant within the same HBA family. For example, the same E_TN value (0.73) was obtained for DL-Menthol: LevA and DL-Menthol: DodA, despite the obvious chemical differences between the HBD of the two DESs. In the same way, similar E_TN values were obtained for $[N_{4444}]$Cl-based DESs, despite the differences in the chain length of the HBDs used. These observations corroborate the conclusion that the HBA plays a more important role in overall polarity than the HBD in hydrophobic DESs.

Focusing now on the hydrophilic [Ch]Cl-based DESs, a wider range of E_TN can be observed: [Ch]Cl: MalA (0.79) has the highest E_TN value, while very similar values were obtained for the other two DESs, [Ch]Cl: LevA (0.35) and [Ch]Cl: GlyA (0.36). This means that the DES with HBD based on the diacid (MalA) as HBD is more polar than those based on monoacids with different chemical groups, such as a ketone (in LevA) or an alcohol group (in Gly). In other words, the interactions between the probe and the referred DESs decrease as the groups change from acid to alcohol to ketone, as expected.

Comparing the E_TN values obtained for hydrophilic and hydrophobic DESs for the same HBD, [Ch]Cl: LevA (0.35) and for DL-Menthol: LevA (0.73), it can be concluded that the latter is much more polar than the former. This is somehow surprising since [Ch]Cl: LevA contains an IL as HBA, while DL-Menthol: LevA contains a neutral molecule. Again, and as it was mentioned before when two hydrophobic DESs with the same HBD were compared, this can be attributed to the differences in the HBA: the chloride anion establishes a more stable hydrogen bond with the LevA than that between DL-Menthol and LevA, decreasing the HBD ability of the former and thus its interaction with the probe.

The values here obtained for the three studied ILs are very similar to those found in literature for other common and well-studied ILs. In general, the E_TN values for ILs vary significantly depending upon the nature of cation and anion, ranging from 0.5 to 0.7,[29] in agreement with the results here obtained: [Ch][Lev] (0.61), [Ch][Gly] (0.74), [Ch][Mal] (0.85). The explanation for this variation is essentially related to the length and the hydroxyl group functionalities of the alkyl chains of the anions and their ability to participate in hydrogen-bonding networks. Generally, for the same cation, the E_TN values of ILs decrease in the following order of anions: $[HCO_2]^-$ > $[NO_3]^-$ > $[BF_4]^-$ > $[NTf_2]^-$ > $[PF_6]^-$, which is the order of decreasing basicity of the anions.[30] Finally, comparing among the different ILs studied, where the anion is changed and the cation is kept constant, it can be observed that the E_TN scale is particularly sensitive to the HBD ability of the anion.

Comparing the DESs based on [Ch]Cl and acids with the corresponding ILs, ILs are generally much more polar than the corresponding DESs, since the E_TN for the ILs are generally higher than those of the corresponding DESs. This is in agreement with Pandey's observations, who studied DESs formed from choline chloride combined with 1,2-ethanediol, glycerol, and urea, in 1:2 molar ratios.[14, 18] Another important observation is that the polarity order observed in the DESs and ILs is maintained, that is, from the most polar to the less polar the following order is attained: MalA > GlyA > LevA.

3.2. Kamlet-Taft Scale

3.2.1. Hydrogen Bonding Acceptor Ability: β Parameter

The Kamlet-Taft HBA ability (β) is obtained by a solvatochromic comparison method, which compares solvent-induced shifts of the absorption bands of two probes. These probes are selected to be structurally very similar (i.e. homomorphic) except for their capacity as HBD, since one can act as HBD and the other cannot. Another characteristic of these probes is that they have a good correlation of both their spectra in non-HBA solvents, but with significant deviations in their spectra in hydrogen bond accepting solvents, so that the construction of a scale based upon these differences is possible.[16] Kamlet and Taft[31] proposed two pairs of probes, 4-nitroaniline/*N,N*-diethyl-4-nitroaniline or 4-nitrophenol/ 4-nitroanisole, to construct the β scale. In this work, the 4-nitroaniline/*N,N*-diethyl-4-nitroaniline probe set was used, since they are the most commonly used pair for ionic liquids.[32] The β values for the ILs and DESs under study are listed in Table 2 and were calculated using the following equations:

$$\beta = \frac{0.76\ (\Delta\bar{\upsilon}_{\mathrm{solvent}} - \Delta\bar{\upsilon}_{\mathrm{cycloexane}})}{\Delta\bar{\upsilon}_{\mathrm{DMSO}} - \Delta\bar{\upsilon}_{\mathrm{cyclohexane}}} \leftrightarrow \qquad \text{(eq. 4)}$$

$$\beta = \frac{1.035\ \bar{\upsilon}_{\mathrm{N,N-diethyl-4-nitroaniline}} + 2.64 - \bar{\upsilon}_{\mathrm{4-nitroaniline}}}{2.80} \qquad \text{(eq. 5)}$$

where $\Delta\bar{\upsilon} = \bar{\upsilon}_{\mathrm{N,N-diethyl-4-nitroaniline}} - \bar{\upsilon}_{\mathrm{4-nitroaniline}}$

Table 2. Kamlet–Taft parameters, using the dye set: Reichardt's 33, *N,N*-diethyl-4-nitroaniline and 4-nitroaniline.

		α	β	π^*
Ionic Liquids[a]	**[Ch][Lev]**	1.07	1.03	1.00
	[Ch][Mal]	1.55	0.62	1.04
	[Ch][Gly]	1.29	0.79	1.08
Deep Eutectic Solvents	**[Ch]Cl: AceA**	b	0.53	1.10
	[Ch]Cl: LevA	0.51	0.57	1.00
	[Ch]Cl: MalA	1.39	0.42	1.08
	[Ch]Cl: GlyA	0.49	0.50	1.08
	[Ch]Cl: Urea	1.42	0.50	1.14
	[Ch]Cl: Ethy	1.47	0.57	1.07
	[Ch]Cl: Gly	1.49	0.52	1.11
	DL-Menthol: AceA	1.64	0.60	0.53
	DL-Menthol: LevA	1.56	0.58	0.66
	DL-Menthol: OctA	1.77	0.50	0.41
	DL-Menthol: DodA	1.79	0.57	0.37
	[N_{4444}]Cl: LevA	b	0.82	1.06
	[N_{4444}]Cl: OctA	1.41	0.99	0.76
	[N_{4444}]Cl: DecA	1.36	0.97	0.73
	[N_{4444}]Cl: DodA	1.45	1.04	0.71

[a]Other ILs were synthesized in order to provide direct comparison between ILs and DESs, but solvatochromic probes measurements were not possible due their solid physical state. [b]No peak could be detected using the same probe for direct comparison.

In Figure 5, the obtained Kamlet-Taft β parameter for the studied DESs, organized by families of hydrogen bond acceptors, [Ch]Cl, [N_{4444}]Cl and DL-Menthol-based DESs, are presented.

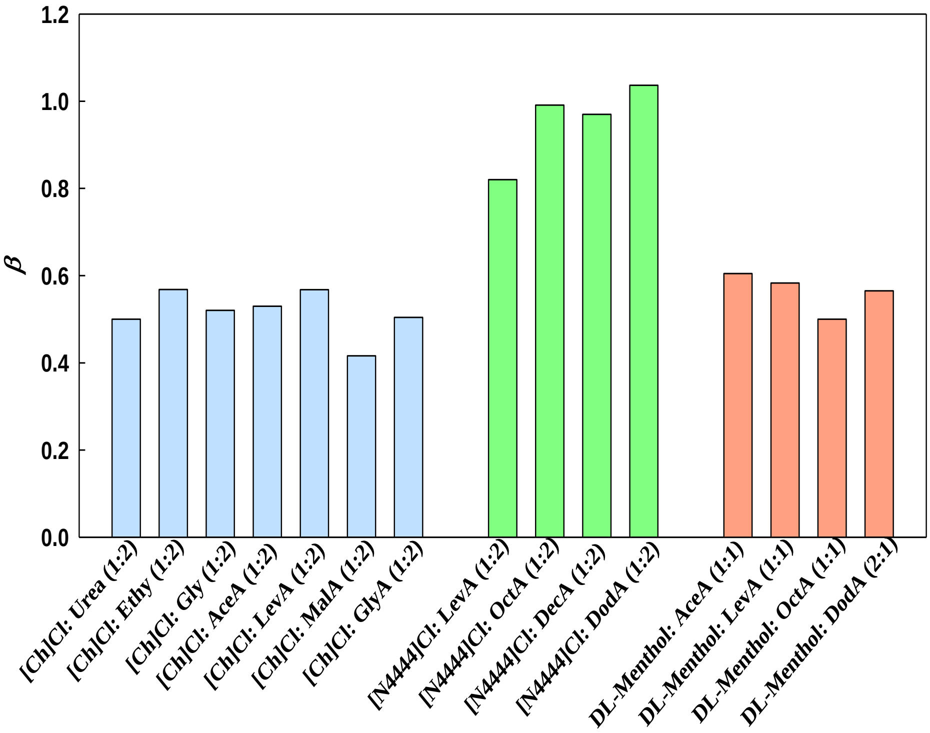

Figure 5. Kamlet-Taft β parameter obtained for all DESs studied in this work.

The *β* values obtained for [Ch]Cl: Urea, [Ch]Cl: Ethy and [Ch]Cl: Gly are in agreement with those reported in the literature by Pandey et al.,[18] where [Ch]Cl: Urea has the lowest *β* value, followed by [Ch]Cl: Gly and [Ch]Cl: Ethy. This can be attributed to the basicity of the HBD, since urea is by far the most basic compound, followed by glycerol and ethylene glycol, with pKa values of 0.10, 14.15 and 14.22, respectively. Regarding the *β* values obtained for [Ch]Cl: Urea, it is interesting to observe that the presence of the C=O group in urea does not increase the *β* value. In the case of [Ch]Cl: LevA, the effect of the C=O group in levulinic acid is still there, but the effect is smaller than for the IL analogue. Nevertheless, note that levulinic acid does have a carbonyl group which can act as a HBA.

It can be seen that the values for the [Ch]Cl-based and the DL-Menthol-based DESs are moderate and similar to each other, while those for [N$_{4444}$]Cl-based DESs are much higher. For example, the β value of [Ch]Cl: LevA (0.58) is similar to that of DL-Menthol: LevA (0.58) leading us to think that the influence of the HBA is negligible. However, comparing β values obtained for DL-Menthol-based DESs with those for [N_{4444}]Cl-based DESs for the same HBD, a large difference can be observed. For example, the β value for DL-Menthol: OctA (0.50) is almost half of that of [N_{4444}]Cl: OctA (0.97). This fact indicates that the nature of the HBA, or more precisely the interaction between the HBD and HBA which limits those with the probe, is of crucial importance in modulating the β results.

Regarding only the hydrophobic DESs studied in this work, it can be concluded that although very different values were obtained for the two families, DL-Menthol- and [N_{4444}]Cl-based DES, no large differences were obtained among the members of the same family, indicating the small effect of the HBDs on the acidity of the DESs. In particular, increasing alkyl chain of the HBD has very little effect on this descriptor in both families. Furthermore, the extra tunability of DES polarity by the easy introduction of different HBD/HBA ratios needs to be highlighted. In Figure 6, a comparison of the obtained Kamlet-Taft parameter for cholinium-based ILs and the corresponding DESs is presented.

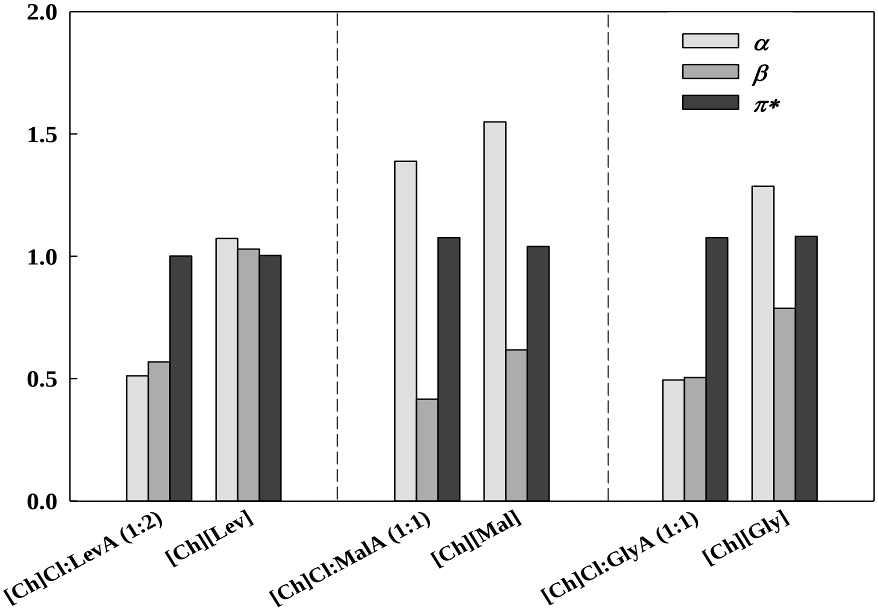

Figure 6. Comparison of Kamlet-Taft parameters obtained for cholinium-based ILs and corresponding DESs.

The *β* values for the studied cholinium-based ILs are consistently higher than those of the corresponding DESs. This is due to the fact that the IL anion is the deprotonated form of the acid while the DES contains the protonated form acid, and thus the deprotonated form should be less hydrogen bonding acceptor than the protonated form. To be highlighted the very high value of *β* for [Ch][Lev], with a value of 1.03, while the value found for [Ch]Cl: LevA is 0.58, similar to the other cholinium-based DESs. Moreover, [Ch]Cl: LevA is also composed of two moles of levulinic acid as opposed to the IL, where only 1 mole is present. As mentioned before the large *β* for [Ch][Lev] can be possibly explained due to the fact that levulinic acid anion can only act as HBA, while malonic and glycolic acid anions still have one acid group and one alcohol group, respectively and thus are able to act as HBA and HBD.

The higher values of β for the studied ILs, when compared to those of the corresponding DES, lead to the conclusion that the formation of DES, through establishment of hydrogen bonds, decreases the capacity of the DES to engage in further hydrogen bonds network as HBD. It has been demonstrated that for ILs the β value is dominated by the anion while the cation just plays a secondary effect.[33]

3.2.2. Hydrogen Bonding Donor Ability: α Parameter

The Kamlet-Taft parameter α provides a measure of a solvent's hydrogen-bond donating acidity (HBD) and is calculated using Reichardt's dye $E_T(33)$ parameter and the π^* parameter using the following equation:

$$\alpha = 0.0649\mathrm{E_T}(33) - 2.03 - 0.72\pi^* \qquad \text{(eq. 6)}$$

where $E_T(33)$ and π^* were calculated using equations (1) and (7), respectively. In Figure 7, the obtained Kamlet-Taft α parameter for the studied DESs, organized by families of hydrogen bond acceptors, [Ch]Cl-, [N_{4444}]Cl and DL-Menthol-based DESs, is presented.

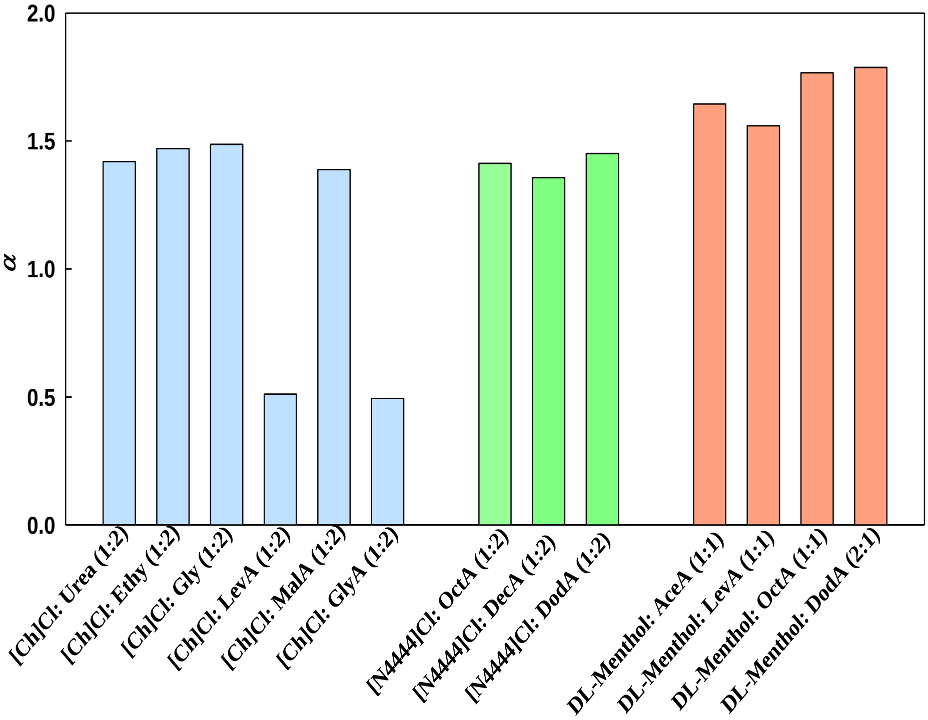

Figure 7. Kamlet-Taft *α* parameter obtained for all DESs studied in this work (Note: There is no value for [Ch]Cl: AceA and [N_{4444}]Cl: LevA because no peak could be detected using the same probe for direct comparison).

For the vast majority of the studied DESs and ILs, the *α* values obtained are higher than unity and thus higher than the values found for other common and well-studied ILs in the literature. However, minor effects can be highlighted. For example, in the family of ILs it can be seen that [Ch][Lev] has the lowest value (1.07), which is probably justified by the levulinic acid anion has no HBD functional group. In the [Ch][Mal] IL, the value of 1.55 can be related to the acid group in malonic acid anion that can act as a HBD. Finally, for [Ch][Gly], *α* value of 1.29 may be due to the OH group on glycolic acid anion that can act as a HBD, but not as strong as the acid group on malonic acid anion. Regarding the family of alcohol-based DESs, it is important to refer that there is also a small effect on *α* values (1.42 to 1.49) essentially due to the –OH group present on the ethyleneglycol and glycerol compounds. Concerning the family of cholinium-based DESs composed of carboxylic acids, the effect of the two acid groups in the malonic acid is translated in high value of *α* (1.39). However, in the case of glycolic acid the presence of the –OH group has little effect, leading to a lower *α* value (0.49).

Comparing the α values for the tetrabutylammonium-based ILs found in the literature[16] with the corresponding $[N_{4444}]Cl$-based DES obtained in this work, it can be concluded that α values for the ILs are much lower than those here obtained for the DES. For example, the *α* value for tetrabutylammonium 2-(cyclohexylamino)ethanesulfonate is 0.56, for tetrabutylammonium 2-[bis(2hydroxyethyl)amino]ethanesulfonate it is 0.31 and for tetrabutylammonium 2-hydroxy-4-morpholinepropanesulfate it is 0.28, which means the DES *α* values are 2 to 5 times higher than those observed in ILs. As already reported, and in line with the present study, there seems to be competition between the HBD and probe dye solute for the proton. The *α* values of the studied compounds are controlled by the ability of the compounds to act as a HBD moderated by its HBA ability.

3.2.3. Polarisablility/Dipolarity π* Parameter

The *π** parameter provides a measure of a solvent's dipolarity and polarizability. The variability in reported values for other systems, such as ILs, arises due to the number of specific dyes being used to determine *π**. As the solvent-dye interaction is unique, this resulted in the calculated value of *π** being unique to each dye.

The original *π** values were an average of the values for all of the solvatochromic dyes used, with a normalisation between 0 (cyclohexane) and 1 (dimethylsulfoxide). Since in this work, we used the three most commonly used Kamlet–Taft dyes, *N,N*-diethyl-4-nitroaniline, 4-nitroaniline and Reichardt's dye, the *π** parameter was calculated from spectroscopic data of the *N,N*-diethyl-4-nitroaniline probe in our solvents using the following equations:

$$\pi^* = \frac{\bar{\upsilon}_{\text{solvent}} - \bar{\upsilon}_{\text{cyclohexane}}}{\bar{\upsilon}_{\text{DMSO}} - \bar{\upsilon}_{\text{cyclohexane}}} \qquad \text{(eq. 7)}$$

$$\pi^* = 0.314(27.52 - \bar{\upsilon}_{\text{N,N-diethyl-4-nitroaniline}}) \qquad \text{(eq. 8)}$$

where $\bar{\upsilon}\ (cm^{-1}) = 10^7/\lambda_{max}(nm)$. In Figure 8, the obtained Kamlet-Taft π^* parameter for all the studied DESs, organized by families of hydrogen bond acceptors, [Ch]Cl-, [N$_{4444}$]Cl and DL-Menthol-based DESs, are presented.

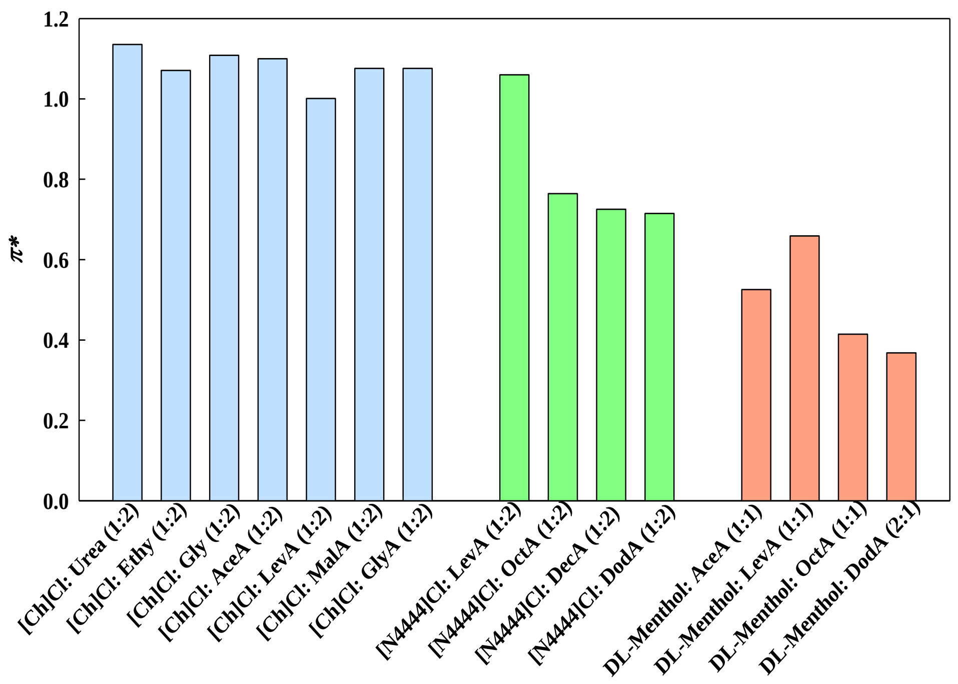

Figure 8. Kamlet-Taft π^* parameter obtained for all DESs studied in this work.

The π^* values obtained for the ILs and cholinium-based DESs are high in comparison with those of conventional organic solvents and ILs, which can be as high as 0.9.[34] π^* values can be affected by both the cation and anion,[35] and tend to be higher in ILs than most organic solvents due to the degree of delocalisation of the charge between the ions.

Major differences between the cholinium-based hydrophilic DESs and the hydrophobic DESs (either composed by DL-Menthol or [N_{4444}]Cl) were found. Both cholinium-based ILs and DESs studied in this work present similar π* values, around the unity, meaning that for these compounds the π* values are largely determined by the cholinium cation (HBA), and that chemical nature of the anion/does not greatly affect the dipolarity/polarizability. On the other hand, no significant differences were observed for the π* values of cholinium-based DESs containing acids and the corresponding ILs, meaning that no difference in terms of dipolarity/polarizability is found between ILs and the corresponding hydrophilic DESs. As for the hydrophobic DESs, it can be seen that both the DL-Menthol-based and the [N_{4444}]Cl-based families have lower π* values than the [Ch]Cl-based hydrophilic DESs, indicating that the former are less dipolar and/or polarisable than the latter. For example, for the π* value for [Ch]Cl: LevA (1.00) is much higher than that for DL-Menthol: LevA (0.66). Comparing the two families of hydrophobic DESs, DL-Menthol-based DESs have lower values of π* than the [N_{4444}]Cl-based DESs. This can be particularly appreciated when the HBD is maintained, such as in the case of DL-Menthol: OctA (0.41) and [N_{4444}]Cl: OctA (0.76). Thus, it can concluded that the HBA plays the dominant role for this descriptor since the studied compounds can be organized according to the following π* trend: Cholinium-based ILs ≈ Cholinium-based DESs > [N_{4444}]Cl-based DESs > DL-Menthol-based DESs, where the latter is the family with the lowest values of π*. This trend can be explained by the fact that the ILs and cholinium based DES are constituted by charged moieties with polar groups, thus are more polar than the [N_{4444}]Cl-based DESs, which are constituted by charged moieties with apolar alkyl chains, which are more polar than the DL-Menthol-based DESs, which are constituted by non-charged compounds. The HBD role in the π* parameter within each one of the families can also be clearly seen. The π*

value decreases with the increase of the alkyl chain of the HBD, as expected. The π^* values of DL-Menthol-based DESs decrease as follows: DL-Menthol: AceA > DL-Menthol: OctA > DL-Menthol: DodA, and the same trend is observed for the $[N_{4444}]$Cl-based DESs. Thus, it can be concluded that the increase of the non-polar part of the HBD decreases the overall polarizability of the DESs, as expected.

4. Conclusions

Owing to the very large gap in understanding solvent-solute interaction in DESs and considering that DESs and their corresponding ILs present a significant opportunity for a wide array of fields, the knowledge and understanding of their polarity in terms of their chemical structure is vital for their confident design for specific applications. To that end, solvatochromic data, polarity (E_T and E_TN) and Kamlet-Taft parameters (α, β, π^*) were obtained two different families of DES: those based on salts, such as cholinium chloride and tetrabutylammonium chloride, and those based on neutral compound, DL-Menthol. The polarity properties of the cholinium-based DESs were studied and compared for the first time with the corresponding ILs. A structural study on the influence of the HBD or the HBA, as well as increasing the alkyl chain length in a DES on polarity and on Kamlet-Taft parameters at room temperature was carried out. It was found that all the DES investigated display high values of hydrogen bonding acidity, probably due to the organic acids presented in all systems. On the other hand, the hydrogen bonding basicity in these compounds does not vary much within the same HBA family, but substantially differs from [Ch]Cl family to $[N_{4444}]$Cl family and DL-Menthol family.

It is also important to note that while one of the general characteristics of ILs is the dipolarity/polarizability parameter uniformly high irrespective of the chemical structures of the cations and anions, the same is not observed for

DESs. In a DES the molecular structure of the hydrogen bond acceptor (HBA) clearly controls the dipolarity/polarizability afforded by the DES. Moreover, the Kamlet-Taft and polarity parameters of several families of DESs based on different acceptors, namely salts (cholinium chloride and [N_{4444}]Cl) and a neutral compound (DL-Menthol) here reported demonstrate that DES displays a high capacity to donate and accept protons when compared to common solvents and also ILs. In summary, DESs polarity can be easily designed by the convenient choice of their components.

5. Acknowledgements

Catarina Florindo is grateful for the grant from COST - EXIL with reference COST Action CM1206 and for the financial support of FCT/MCTES for the PhD grant SFRH/BD/102313/2014. Isabel M. Marrucho and Luis C. Branco gratefully acknowledge for the contract under *Investigator FCT 2012* (IF/363/2012 and IF/0041/2013), respectively. This work was financed by CQE project (UID/QUI/00100/2013) and Research Units UID/QUI/00100/2013 (CQE) and UID/Multi/04551/2013 (GREEN-IT).

6. References

1. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Tambyrajah, V., Novel solvent properties of choline chloride/urea mixtures. *Chemical Communications* 2003, (1), 70-71.

2. Dai, Y.; van Spronsen, J.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y. H., Natural deep eutectic solvents as new potential media for green technology. *Analytica Chimica Acta* 2013, *766*, 61-68.

3. Hou, Y.; Gu, Y.; Zhang, S.; Yang, F.; Ding, H.; Shan, Y., Novel binary eutectic mixtures based on imidazole. *Journal of Molecular Liquids* 2008, *143* (2–3), 154-159.

4. Florindo, C.; Oliveira, F. S.; Rebelo, L. P. N.; Fernandes, A. M.; Marrucho, I. M., Insights into the Synthesis and Properties of Deep Eutectic Solvents

Based on Cholinium Chloride and Carboxylic Acids. *ACS Sustainable Chemistry & Engineering* 2014, *2* (10), 2416-2425.

5. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *Journal of the American Chemical Society* 2004, *126* (29), 9142-9147.

6. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R. L.; Duarte, A. R. C., Natural Deep Eutectic Solvents – Solvents for the 21st Century. *ACS Sustainable Chemistry & Engineering* 2014, *2* (5), 1063-1071.

7. Francisco, M.; van den Bruinhorst, A.; Kroon, M. C., Low-Transition-Temperature Mixtures (LTTMs): A New Generation of Designer Solvents. *Angewandte Chemie International Edition* 2013, *52* (11), 3074-3085.

8. Reichardt, C.; Welton, T., *Solvents and solvent effects in organic chemistry*. John Wiley & Sons: 2011.

9. D'Agostino, C.; Harris, R. C.; Abbott, A. P.; Gladden, L. F.; Mantle, M. D., Molecular motion and ion diffusion in choline chloride based deep eutectic solvents studied by 1H pulsed field gradient NMR spectroscopy. *Physical Chemistry Chemical Physics* 2011, *13* (48), 21383-21391.

10. Abbott, A. P.; D'Agostino, C.; Davis, S. J.; Gladden, L. F.; Mantle, M. D., Do group 1 metal salts form deep eutectic solvents? *Physical Chemistry Chemical Physics* 2016, *18* (36), 25528-25537.

11. D'Agostino, C.; Gladden, L. F.; Mantle, M. D.; Abbott, A. P.; Ahmed, E. I.; Al-Murshedi, A. Y. M.; Harris, R. C., Molecular and ionic diffusion in aqueous - deep eutectic solvent mixtures: probing inter-molecular interactions using PFG NMR. *Physical Chemistry Chemical Physics* 2015, *17* (23), 15297-15304.

12. Crowhurst, L.; Mawdsley, P. R.; Perez-Arlandis, J. M.; Salter, P. A.; Welton, T., Solvent-solute interactions in ionic liquids. *Physical Chemistry Chemical Physics* 2003, *5* (13), 2790-2794.

13. van Osch, D. J. G. P.; Kollau, L. J. B. M.; van den Bruinhorst, A.; Asikainen, S.; Rocha, M. A. A.; Kroon, M. C., Ionic liquids and deep eutectic solvents for lignocellulosic biomass fractionation. *Physical Chemistry Chemical Physics* 2017, *19* (4), 2636-2665.

14. Pandey, A.; Pandey, S., Solvatochromic Probe Behavior within Choline Chloride-Based Deep Eutectic Solvents: Effect of Temperature and Water. *The Journal of Physical Chemistry B* 2014, *118* (50), 14652-14661.

15. Spange, S.; Lungwitz, R.; Schade, A., Correlation of molecular structure and polarity of ionic liquids. *Journal of Molecular Liquids* 2014, *192*, 137-143.

16. Ab Rani, M. A.; Brant, A.; Crowhurst, L.; Dolan, A.; Lui, M.; Hassan, N. H.; Hallett, J. P.; Hunt, P. A.; Niedermeyer, H.; Perez-Arlandis, J. M.; Schrems, M.; Welton, T.; Wilding, R., Understanding the polarity of ionic liquids. *Physical Chemistry Chemical Physics* 2011, *13* (37), 16831-16840.

17. Katritzky, A. R.; Fara, D. C.; Yang, H.; Tämm, K.; Tamm, T.; Karelson, M., Quantitative Measures of Solvent Polarity. *Chemical Reviews* 2004, *104* (1), 175-198.

18. Pandey, A.; Rai, R.; Pal, M.; Pandey, S., How polar are choline chloride-based deep eutectic solvents? *Physical Chemistry Chemical Physics* 2014, *16* (4), 1559-1568.

19. Harifi-Mood, A. R.; Ghobadi, R.; Matić, S.; Minofar, B.; Řeha, D., Solvation analysis of some Solvatochromic probes in binary mixtures of reline, ethaline, and glyceline with DMSO. *Journal of Molecular Liquids* 2016, *222*, 845-853.

20. Abbott, A. P.; Harris, R. C.; Ryder, K. S.; D'Agostino, C.; Gladden, L. F.; Mantle, M. D., Glycerol eutectics as sustainable solvent systems. *Green Chemistry* 2011, *13* (1), 82-90.

21. Teles, A. R. R.; Capela, E. V.; Carmo, R. S.; Coutinho, J. A. P.; Silvestre, A. J. D.; Freire, M. G., Solvatochromic parameters of deep eutectic solvents formed by ammonium-based salts and carboxylic acids. *Fluid Phase Equilibria* 2017, *448*, 15-21.

22. Ribeiro, B. D.; Florindo, C.; Iff, L. C.; Coelho, M. A. Z.; Marrucho, I. M., Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents. *ACS Sustainable Chemistry & Engineering* 2015, *3* (10), 2469-2477.

23. van Osch, D. J. G. P.; Zubeir, L. F.; van den Bruinhorst, A.; Rocha, M. A. A.; Kroon, M. C., Hydrophobic deep eutectic solvents as water-immiscible extractants. *Green Chemistry* 2015, *17* (9), 4518-4521.

24. Mourão, T.; Tomé, L. C.; Florindo, C.; Rebelo, L. P. N.; Marrucho, I. M., Understanding the Role of Cholinium Carboxylate Ionic Liquids in PEG-

Based Aqueous Biphasic Systems. *ACS Sustainable Chemistry & Engineering* 2014, *2* (10), 2426-2434.

25. Reichardt, C.; Welton, T., Solute-Solvent Interactions. In *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA: 2010; pp 7-64.

26. Reichardt, C., Polarity of ionic liquids determined empirically by means of solvatochromic pyridinium N-phenolate betaine dyes. *Green Chemistry* 2005, *7* (5), 339-351.

27. Reichardt, C.; Welton, T., Empirical Parameters of Solvent Polarity. In *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA: 2010; pp 425-508.

28. Reichardt, C., Solvatochromic Dyes as Solvent Polarity Indicators. *Chemical Reviews* 1994, *94* (8), 2319-2358.

29. Jessop, P. G.; Jessop, D. A.; Fu, D.; Phan, L., Solvatochromic parameters for solvents of interest in green chemistry. *Green Chemistry* 2012, *14* (5), 1245-1259.

30. Reichardt, C.; Welton, T., Solvents and Green Chemistry. In *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA: 2010; pp 509-548.

31. Taft, R. W.; Kamlet, M. J., The solvatochromic comparison method. 2. The .alpha.-scale of solvent hydrogen-bond donor (HBD) acidities. *Journal of the American Chemical Society* 1976, *98* (10), 2886-2894.

32. Fox, D., *Molten Salts and Ionic Liquids 16*. Electrochemical Society: 2009.

33. Pitula, S.; Mudring, A.-V., Optical basicity of ionic liquids. *Physical Chemistry Chemical Physics* 2010, *12* (26), 7056-7063.

34. Kurnia, K. A.; Lima, F.; Cláudio, A. F. M.; Coutinho, J. A. P.; Freire, M. G., Hydrogen-bond acidity of ionic liquids: an extended scale. *Physical chemistry chemical physics : PCCP* 2015, *17* (29), 18980-18990.

35. Chiappe, C.; Pomelli, C. S.; Rajamani, S., Influence of Structural Variations in Cationic and Anionic Moieties on the Polarity of Ionic Liquids. *The Journal of Physical Chemistry B* 2011, *115* (31), 9653-9661.

CHAPTER 4.2

Supramolecular Hydrogel based on Sodium Deep Eutectic Solvent

Adapted from: **C. Florindo**, L. Celia-Silva, L. F. G. Martins, L. C. Branco and I. M. Marrucho, Supramolecular Hydrogel based on Sodium Deep Eutectic Solvent, Chem. Commun., 2018, 54, 7527-7530.

The author contributed to the execution of all characterization and properties experiments described in this chapter, as well as involved in the discussion and interpretation of results and the preparation of the manuscript.

CONTENT

Abstract

Supramolecular Hydrogel based on a metal containing Deep Eutectic Solvent (DES) is here presented for the first time. Phase diagram of DES-based hydrogel was drawn and its rheological properties determined.

1. Communication

Supramolecular hydrogels or low molecular-weight gelators (LMWG) are highly hydrated, porous materials, based on the formation of a fibrillar network by the self-assembly of molecular building blocks due to complementary non-covalent interactions, including hydrogen bonding, π-π interactions, hydrophobic interactions and metal ligand interaction.[1-2] They are typically formed through the use of ultrasounds, heating or pH change, which rearranges the aggregation of molecules by cleaving self-locked intramolecular hydrogen bonds or π-stacking to form crosslinked structures through intermolecular interactions, usually involving the participation of water molecules.[3] Supramolecular gels based on biocompatible compounds, as example ureas, amides, nucleobases, aminoacids, surfactants, sugars, fatty acids, among others, have been deeply investigated.[1] Recently, gelation induced by metal ions gained huge interest due to their fascinating properties and control over self-assembly by tuning metal-ligand coordination. Moreover, the incorporation of different metal ions in hydrogels allows a fine control of the gel mechanical properties, through the tuning of metal-ligand interaction.[4] Usually, supramolecular gels are composed by a long aliphatic chain connected with a polar head and the tendency of the head to interact in a three dimensional network enhances the probability of gel formation. Deep Eutectic Solvents (DES) have been arousing a lot of attention as new sustainable solvents not only due to their favourable properties such as easy preparation with no need of further purification, good biodegradability, low toxicity, low volatility and low prices,[5-6] but also to their application in a wide range of fields, such as catalysis, organic synthesis, electrochemical devices, solar technology, etc.[7] DES have found an unquestionable role in extraction and separation processes, for example in selective isolation and recovery of metals,[8] isolation and recovery of compounds from natural products,[9] desulfurization of fuels,[10] azeotrope breaking,[11] and water purification.[12-13]

DESs represent a new generation of unconventional solvents which are obtained just by the mixing two or more compounds, generally a salt and an aminoacid,[14] a carbohydrate,[15] an alcohol,[16] a carboxylic acid[17] etc, which act as hydrogen bond acceptor (HBA) and donor (HBD), respectively. This combination of a HBA and a HBD leads to the formation a final liquid compound, with a melting point much lower than those of individual starting components.[18-19] The control of the DES properties is essentially made through the chemistry of the chosen starting compounds, but other properties such as molar ratio and water uptake, also have crucial effect.[5] For example, most of the DESs reported are very hydrophilic and hygroscopic, due to the hydrophilic nature and the rapid establishment of a hydrogen bond network, at ambient conditions.[20-21] Lately, the development of hydrophobic DESs has also been explored and their stability when in contact with water studied.[12, 22] Long chain ammonium and phosphonium salts combined with hydrophobic acids and alcohols, or even two long chain fatty acids, or natural hydrophobic compounds combined with fatty acids have been used to prepare hydrophobic DES. In this work, we step forward and use a metal (sodium) salt derived from a long chain fatty acid (NaC_{12}) and a long chain fatty acid (C_{10}), as depicted in Figure 1, to prepare a DES. The use of a sodium-based salt with surfactant properties should provide DES with different properties from those based on ammonium and phosphonium salts with long hydrocarbon chains.

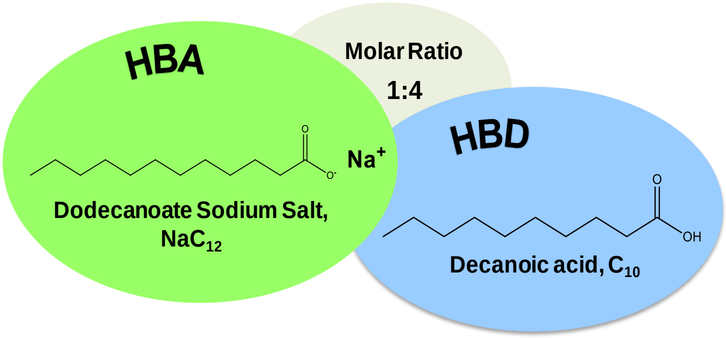

Figure 1. Chemical structure, respective acronym and molar ratio of the new sodium salt-based DES (NaC_{12}:C_{10} (1:4)) reported in this work.

The new sodium salt-based DESs may be of great interest and promise for applications, especially in medical and biotechnological fields, separations and extractions, purification of natural products and electrochemical applications.

Abbott[23] recently reported the preparation of a family of compounds using alkali metal salts, based on sodium, potassium, magnesium and calcium salts combined with glycerol. They concluded that although none of these mixtures showed eutectic behaviour, their physical properties are similar to hydrophilic DES.[23]

In order to ascertain about the eutectic behaviour of these new eutectic mixtures, the solid-liquid phase diagram was drawn, by combining several molar ratios of both components, NaC_{12} and C_{10}. The mixtures were put in a glass vial and heated at 80 ºC for DES preparation. The solid-liquid phase diagram was mapped using a visual method, where mixtures of known concentrations are heated up until only a liquid phase is observed and is shown in Figure S1 of Supplementary Information. Contrary to what was observed by Abbott's group, in the present case an eutectic point at 0.80 mole fraction of C_{10} was obtained, meaning NaC_{12}:C_{10} DES eutectic is formed at the molar ratio of (1:4), with a melting point of 22 ºC. Also, the eutectic mixture has a lower melting point than their corresponding starting materials, yielding a window of compositions in the liquid phase at room temperature. This was found by visual and confirmed by DSC analysis, as presented in thermograms in Supplementary Information. All details of preparation, characterization and thermophysical properties measurements of the newly prepared sodium salt-based DES can be found in ESI. It can be concluded that the thermophysical properties of NaC_{12}:C_{10} DES, namely density and viscosity, are similar to those reported for hydrophobic DES, with lower densities and viscosities than hydrophilic DES.

An important characteristic feature of a hydrogel is its water holding capacity.[24] When NaC_{12}:C_{10} DES is put in contact with water, the formation of a hydrogel can be observed. This water holding capacity and its consequent restructuring of the hydrogen bond network is an important characteristic feature of any hydrogel.[24]

The presence of the charged sodium atoms seems to have a decisive role in the formation of the hydrogel, since in our previous work reporting DES formed using two carboxylic acids with long alkyl chain, complete DES immiscibility with water was observed and, of course, no gel formation. In the present case, instead of two mutually immiscible liquid phases, a hydrogel was obtained. The more water was added, the more viscous the gel became. In Figure 2 is presented an illustration of the formation of the gel system for one selected composition (50 wt% of DES + 50 wt% of water).

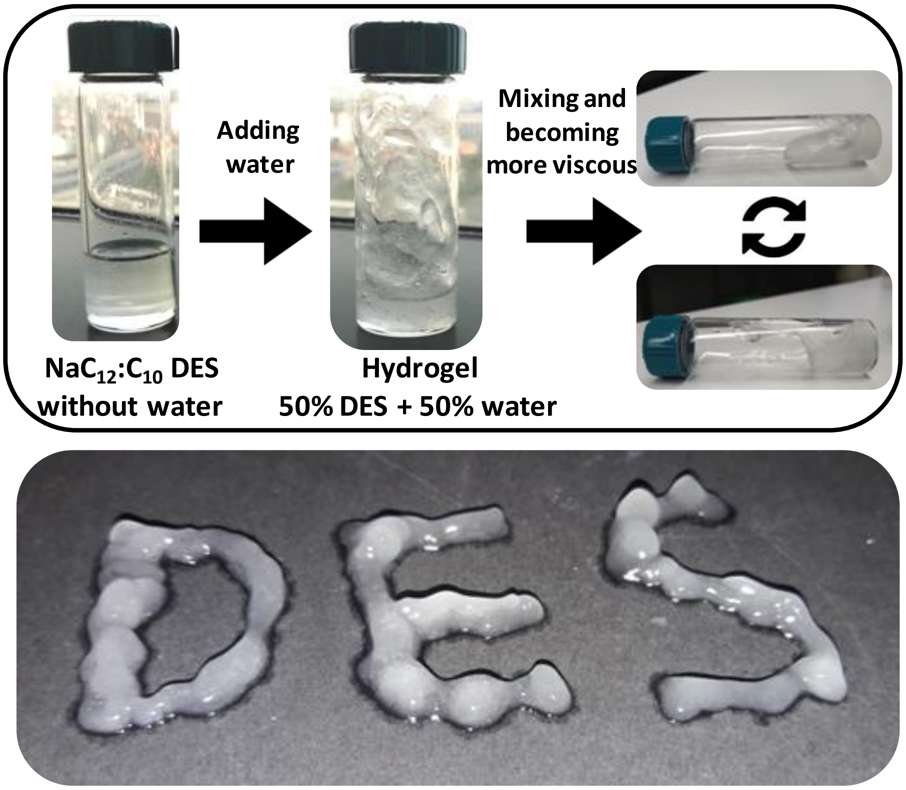

Figure 2. Illustration of the pure sodium salt-based DES, NaC_{12}: C_{10} (1:4 molar ratio) without water and after addition of 50wt% of water, becoming more viscous and more turbid, forming the gel system. Below, a photograph of the hydrogel composed of 50 wt% of DES and 50 wt% of water at 25 °C.

In order to better evaluate and understand the behaviour of NaC_{12}:C_{10} DES with water, the (*T, x*) phase diagram was measured using a visual method and it is shown in Figure 3.

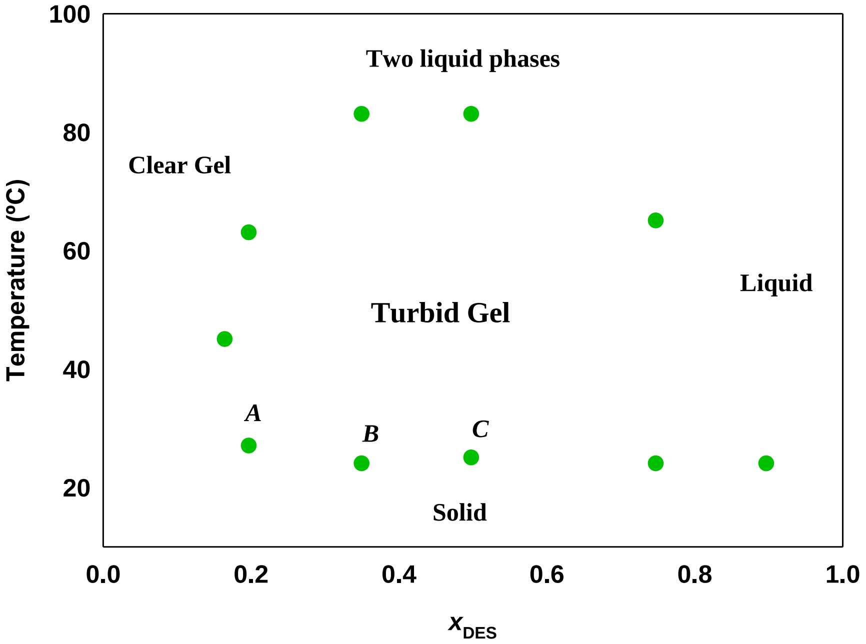

Figure 3. Temperature-composition sol-gel phase diagram of the sodium salt-based DES aqueous solutions. Green circles (●) indicate sol–gel transitions of NaC_{12}:C_{10} (1:4) for different compositions with temperature. A, B and C correspond to pure DES mole fractions of 0.197, 0.350 and 0.498 and 0.803, 0.650 and 0.502 of water. These A, B and C mixtures were selected for further studies.

It can be observed that mixtures of NaC_{12}:C_{10} DES and water exhibit stimuli sensitive and responsive phase transition to temperature and concentration of water, probably due to changes in the network structure.

According to Figure 3, at temperatures slightly above room temperature, NaC_{12}:C_{10} DES + water mixtures might yield either a clear gel, or a turbid gel or a liquid phase, depending on DES concentration. The turbid gel, obtained for molar ratio of DES between 0.30 and 0.70, yield phase separation at higher temperatures, and two liquid immiscible phases were obtained. Just like conventional supramolecular hydrogels, supramolecular hydrogels-based DES will certainly attract noticeable interest for their possible use in interesting applications, such as hygienic products, drug delivery systems, biomedical applications, sensors and pharmaceuticals, removal of dyes and metal ions and sewage water treatment, purification of water, among others.[24-27]

The rheological properties of the hydrogels were also obtained by investigating the viscometry in rotational mode, viscoelastic properties in oscillation mode as well as thixotropy and creep/recovery tests. A rotational rheometer from Malvern (Kinexus pro) was used with cone-and-plate geometry. The measurements were carried out for three binary mixtures of different compositions A, B and C, which correspond to a DES molar fraction of 0.197, 0.350 and 0.498 DES and 0.803, 0.650 and 0.502 of water, respectively, as marked in Figure 3, and two different temperatures within the gel region of the phase diagram. These hydrogels are markedly non-newtonian materials, presenting shear thinning behaviour, as can be seen in Figure 4, whose flow curves (Figure S8 from Supplementary Information) can be correlated by Herschel-Bulkley with flow consistence index (K), flow behaviour index (n) and yield stress (τ_0) quite dependent on composition (Table S2 in Supplementary Information). The application of high strains on the systems seems to easily break their gel structure leading to sol phase formation. Mixture C was particularly fragile exhibiting the lowest consistency index, almost negligible yield stress and the most marked shear thinning behaviour, with the lowest viscosity values for a given shear rate. Mixtures A and B showed similar behaviour.

The gel behaviour of the systems could be clearly seen in oscillation tests, where the elastic module (G') is consistently higher than the viscous module (G''), presented in Figure 5, with no cross-over detected in the frequency range imposed (0.01-100 s^{-1}) for low strains and phase angles always less than 15 º. However, these gels should be classified as weak, which can be seen by the shear thinning behaviour in flow tests (high strains) and the relatively narrow linear viscoelastic regimes (strains between 0.2 and 1 %).

Another indication of the viscoelastic behaviour of these hydrogels is the results of creep/recovery tests at a constant shear stress (sufficiently low not to break up the gel structure) which are shown in Figure S11 from Supplementary Information.

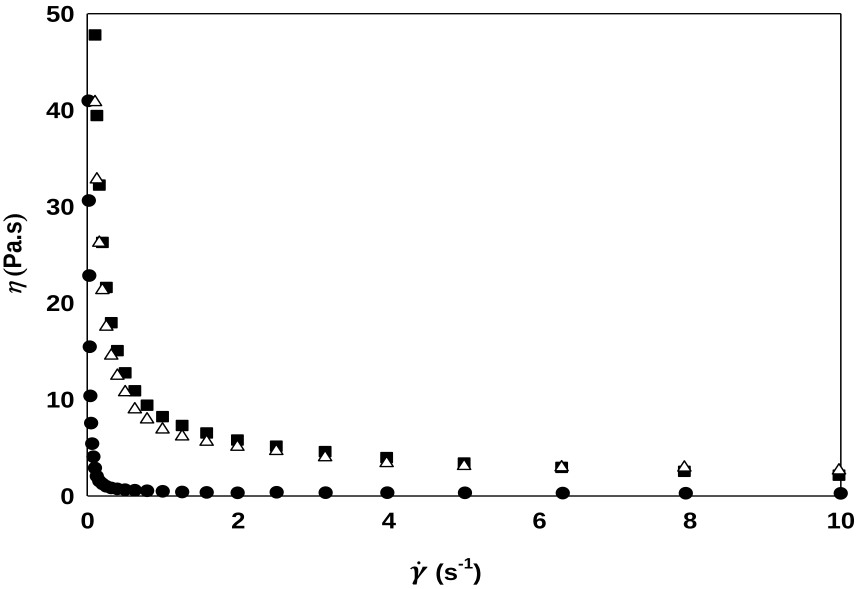

Figure 4. Viscosity (*η*) as a function of shear stress for three gel compositions with angular speed (*ω)* for composition A (Δ), B (■) and C (●) at 40 ºC.

Comparing the shear moduli obtained for the three studied compositions, it seems that these mixtures don't present a monotonic behaviour as a function of composition. This fact is also visible from the viscosity measurements as a function of shear rate. A more detailed study on composition dependence of rheological properties of these gels will be carried out to elucidate this point.

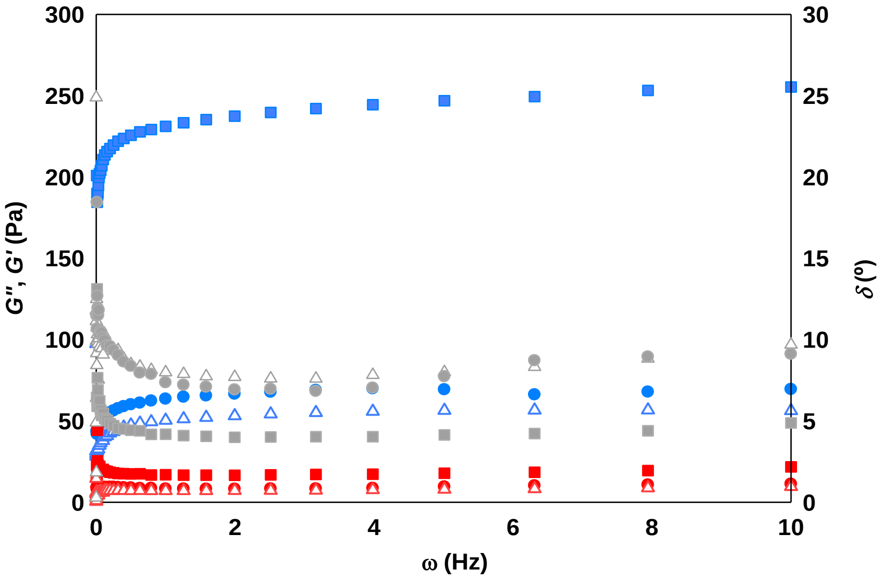

Figure 5. Oscillation experiments on frequency sweep mode for gel mixtures with angular speed (*ω) for composition A* (Δ), *B* (■) and *C* (●) at 40 ºC (▬ storage module (*G'*), ▬ loss module (*G''*) and ▬ phase angle (*δ*)).

After the steady state was attained, removing the stress allowed the gels to spring back, with ultimate recoil values of 0.0302 Pa^{-1} for a maximum creep compliance of 0.0595 Pa^{-1} [ω (DES) for composition A] and 0.342 Pa^{-1} for a maximum creep compliance of 1.469 Pa^{-1} [ω (DES) for composition C]. The fact that both values of ultimate recoil are non-zero, representing a fraction of 0.51 (A) and 0.23 (C) of the maximum compliance proves the significant spring back of the samples when stress is removed due to their elastic character (typical of gels).

The oscillation experiments in temperature sweep mode for fixed strain and oscillation frequency, allowed us to detect the low temperature limit of the gel phase corresponding to the point where a steep increase in storage module occurs, as can be seen in Figure 6 for mixture with composition A for which the phase transition temperature was found to be 16 ºC.

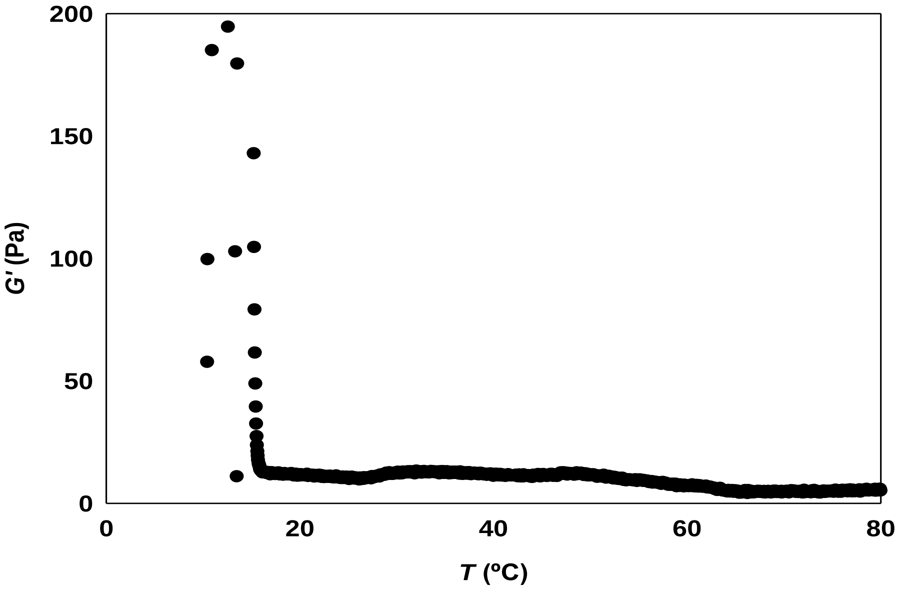

Figure 6. Oscillation experiments on temperature sweep mode for gel mixture with angular speed *(ω) of* DES mixture with composition A for an oscillation frequency of 1 Hz and a strain of 0.1%. Storage module as a function of temperature.

Finally, these hydrogels proved to be thixotropic as can be seen in the results of creep/recovery tests showed in Figure S12 from Supplementary Information. At constant low shear rates, apparent viscosity decreases with time. Upon the application of higher shear rates, the viscosity deeply decreases (breaking of the gel structure). However, once the high shear rate is removed, these gels present low rebuild times, which is an indication of the reversibility of the gel breaking process. Both the decrease of viscosity with time at constant shear rate and the rebuild of original structure in a finite time show the thixotropic nature of these gels.

2. Conclusions

For the first time a new metal-based DES, combining dodecanoate sodium salt as HBA and a carboxylic acid as HBD, that can behave as hydrogel in a presence of water was reported. This new hydrogel exhibits a temperature phase transition, and also a thermoreversible viscosity depending essentially on the water content.

3. Acknowledgements

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4. Supplementary Information

4.1. Experimental Details

Materials**.** Dodecanoate sodium salt (NaC_{12}) (purity 99-100%) and decanoic acid (C_{10}) (purity ≥ 98%) were purchased from Sigma-Aldrich. All materials were used without further purification. The water used was ultrapure; double distilled, passed through a reverse osmosis system and further treated with Milli-Q plus water purification apparatus.

Preparation Methodology of DES. Mixtures of sodium salt and carboxylic acid with long alkyl chain, in a proportion of 1 to 4 of mole ratio, respectively, were prepared by adding the acid to the sodium salt in a sealed glass vial until a homogeneous clear solution was formed. The water content of DES after its preparation and after drying was found to be about 3000 ppm and 280 ppm, respectively. These results were obtained by Karl Fischer titration (Metrohm 870 KF Titrino Plus). The influence of sodium ion on the DES behavior towards water was not evident during the synthesis of DESs since both dried DES (NaC_{12}: C_{10} and C_{12}: C_{10}) had approximately the same amount of water (150 to 200ppm). However, a clear difference can be observed in the study of DES hydrophobicity: one (C_{12}: C_{10}) gave rise to a two phase system, displaying a very hydrophobic behavior with a maximum water content of 5000 ppm, while the other (NaC_{12}: C_{10}) formed a more viscous phase giving rise to the gel system.

Preparation of the Hydrogel. After the synthesis of a mixture composed of a dodecanoate sodium salt and decanoic acid (1:4 mole ratio), the hydrogel was achieved by homogeneously mixing different amounts of DES and water, from 10 to 80 wt%, at 25 °C.

Solid-liquid phase diagram determination. The phase diagram was measured in a glass flask using the visual method at atmospheric pressure, under constant stirring and at different temperatures. Briefly, to perform this experiment it was started with pure compounds HBA and HBD and then made mixtures in varying molar proportions into several glass flasks. Each one of these mixtures, plus the pure HBA and pure HBD represent different compositions, as can be seen in Figure S1. In this case, we only evaluated the temperature at which the solid-liquid transition occurs for different DES compositions, holding pressure constant at 1atm. The eutectic systems compositions were determined by weight quantification of all components within ± 10^{-4} g.

The eutectic composition was obtained at only one temperature, the lowest melt temperature when compared to the starting materials. The above procedure was repeated to obtain sufficient data to construct the phase diagram of the studied DES, shown in Figure S1.

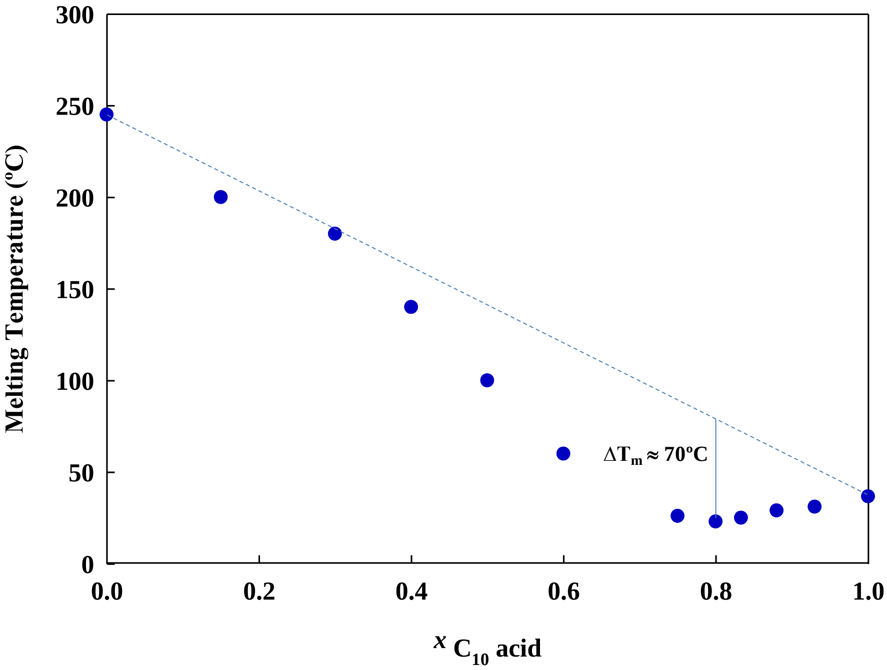

Figure S1. Experimental (T,x) phase diagram obtained for NaC_{12}:C_{10} DES.

Sol-Gel phase diagram determination. The sol–gel phase transition temperature for the hydrogel was determined using a vial containing different mass proportions of DES and water, as can be seen in Figure 3, in an oil bath with constant stirring speed with a temperature increment of 5 °C. The appearance of the gel state was determined by visual observation of the change in the fluidity when temperature changes. The temperature was measured with a precision of ± 1 °C.

4.2. Analytical Methodologies

Karl-Fischer measurements. In order to measure the quantity of water of DES after the preparation and drying, a Karl Fischer titration analysis (Metrohm 870 KF Titrino Plus) was used.

Thermophysical Properties. Measurements of viscosity and density of the DES were carried out in the temperature range from 20 up to 80 ºC and at atmospheric pressure, using an Anton Paar (model SVM 3000) automated rotational Stabinger viscometer-densimeter. The temperature uncertainty is ± 0.01 ºC. The relative uncertainty of the dynamic viscosity is ± 0.25 %, and the absolute uncertainty of the density is ± 0.0005 $g \cdot cm^{-3}$. A DSC (differential scanning calorimeter TA Instrument Model DSC Q200) was used to measure the thermal properties of the prepared DES. In the DSC, the samples were continuously purged with 50 mL min−1 of nitrogen. About 10 mg of the compound was crimped in an aluminium standard sample pan and analyzed under a nitrogen atmosphere by heating (10 $K\ min^{-1}$) - cooling (5 $K\ min^{-1}$) cycles between 193.15 and 373.15 K.

FTIR analysis. FTIR measurements were carried out using a Brüker IFS66/S FTIR spectrometer (Brüker Daltonics, MA, USA) with a single reflection ATR cell (DuraDisk, equipped with a diamond crystal). The data relative to spectral region were recorded between 4000 cm^{-1} and 600 cm^{-1} at room temperature. For each sample, 290 scans were recorded at a spectral resolution of 4 cm^{-1} and five replica spectra were collected in order to evaluate reproducibility (OPUS v5.0).

Rheological Properties. Rheological studies comprised viscometry measurements in rotational shear rate controlled mode (in general for shear rates between 0.001 and 100 s^{-1}), viscoelastic tests in oscillation mode using strain (for linear viscoelastic region – LVER), frequency (in general, frequency between 0.01 and 100 s^{-1}) and temperature (single frequency) sweep, creep/recovery and thixotropy tests. All the measurements were made in a Kinexus pro rotational rheometer from Malvern with cone and plate geometry (cone with 40 mm of diameter, an angle of 4º and a gap of 0.15 mm). Temperature was controlled by a Peltier element at the bottom of the fixed plate and measured with a precision of 0.01 ºC. The systems studied were gels with 0.803, 0.650 and 0.502 weight fractions of water at 40 and 60 ºC.

4.3. FTIR Analysis

As already proven, different intermolecular interactions are responsible for the formation of a eutectic mixture, depending on the compounds. In conventional DES based on a salt and a carboxylic acid, which acts as hydrogen bond acceptor and donor, respectively, the establishment of hydrogen bonds between the two compounds is responsible for the eutectic solvent formation. Thus, it is important to observe how dodecanoate sodium salt interacts with decanoic acid, in the DES prepared in this work. For that purpose, FTIR experiments were performed for the pure starting materials, dodecanoate sodium salt and decanoic acid, and the DES. The FTIR spectra obtained are presented in Figure S2.

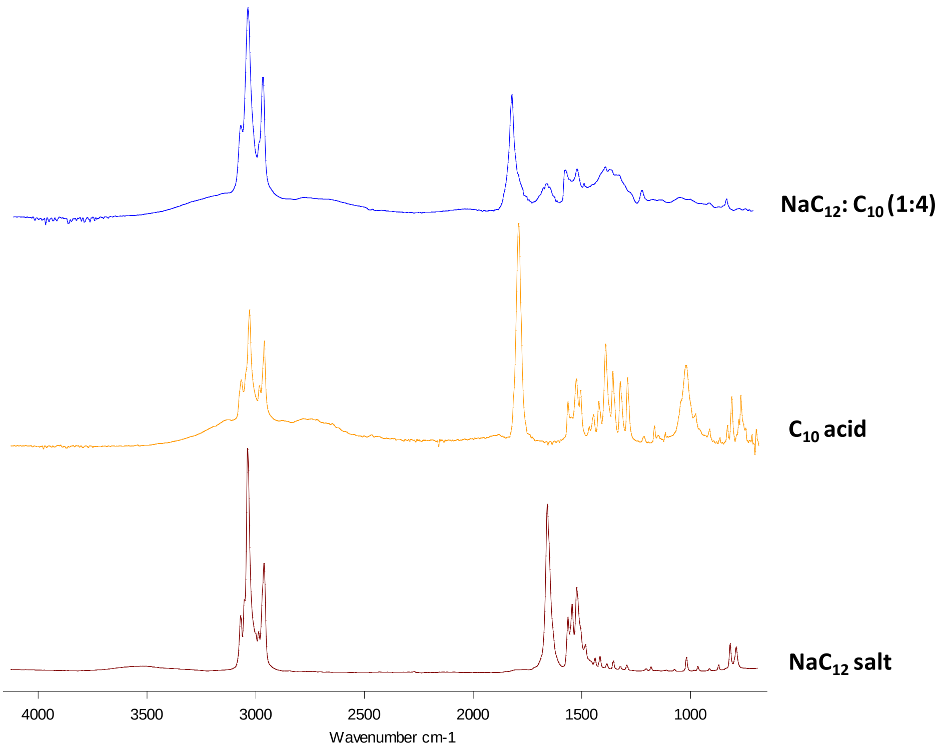

Figure S2. FTIR spectra of the pure dodecanoate sodium salt (NaC_{12} salt), decanoic acid (C_{10} acid) and NaC_{12}: C_{10} (1:4 mole ratio) DES.

FTIR analysis is also used to confirm the formation of the new compound, through the establishment of hydrogen bonds, between the salt and the hydrogen bond donor. As can be seen from the Figure S3, an intermolecular hydrogen-bond interaction between the hydrogen bond donor and the salt, mostly in the carboxyl group region, is formed. It can be observed that the carbonyl band from the HBD was originally located at lower wavenumber values (~1700 cm-1) and it broadens and shifts to higher values (~1750 cm-1) in the eutectic mixture. This is a strong indication of a new hydrogen bond formation, thus proving that a new compound was formed. This evidence can be further confirmed through the physical state of the compounds: initially, the physical state of both these compounds was solid and after DES formation an homogeneous liquid is obtained at room temperature.

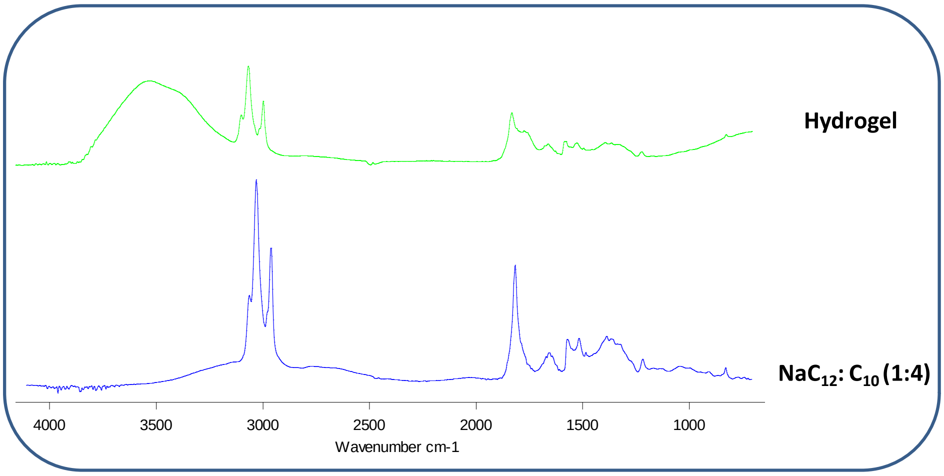

Figure S3. Comparison of FTIR spectra of the sodium salt-based DES (NaC_{12} salt) without water and the corresponding hydrogel (50 wt% DES of NaC_{12}: C_{10} (1:4 mole ratio) and 50 wt% of water).

4.4. DSC analysis of DES and Hydrogel

The new NaC_{12}:C_{10} DES and the corresponding hydrogel (50% of DES and 50% of water) were also characterized using differential scanning calorimetry (DSC). In order to prove the eutectic behaviour of this new system, in Figure S4, a comparison of DSC thermograms between the pure sodium salt-based (NaC_{12}:C_{10}) and the pure decanoic acid (C_{10} acid) starting material is presented.

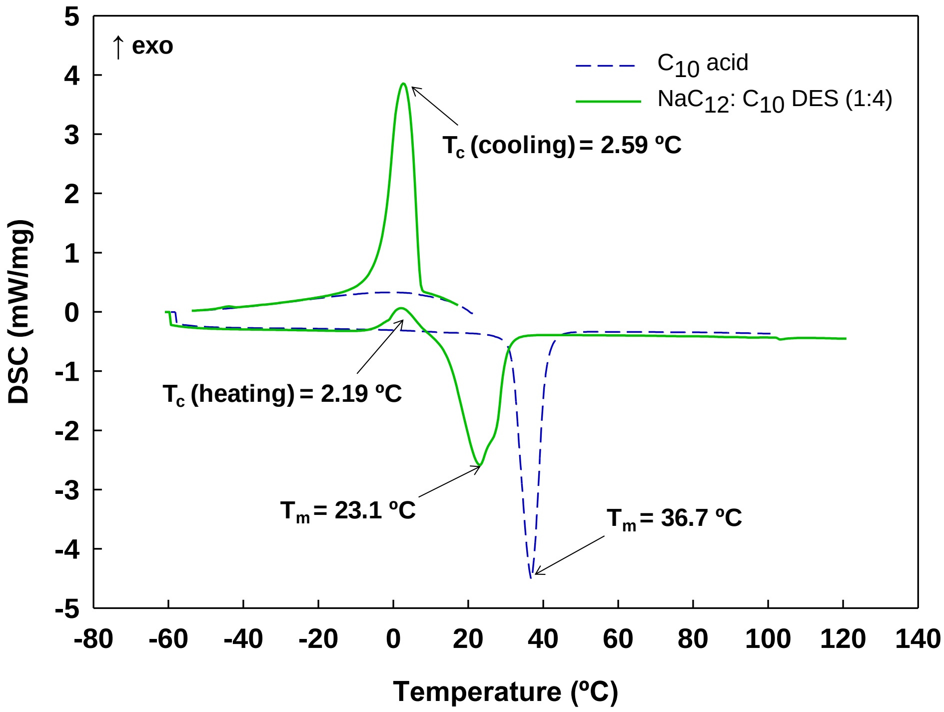

Figure S4. Comparison of DSC thermogram between the pure sodium salt-based (NaC_{12}:C_{10}) and the pure decanoic acid (C_{10} acid) starting material. (Note: Due to the limitations of the equipment, it was not possible to measure the melting point of the initial material (dodecanoate sodium salt), although this is well defined in the literature and in the safety data sheet, being at 245 ºC.)

As can be seen in Figure S4, there is a difference in melting temperatures of both pure compounds and the DES: sodium dodecanoate salt and decanoic acid have a melting point of 245 ºC and 36.7 ºC, respectively, and DES in a mole ratio of 1:4 presents a melting point of 23.1 ºC. DES (NaC_{12}:C_{10} (1:4)) also presents a cold crystallization at 2.59 ºC and a heat crystallization at 2.19 ºC, which does not occur for the starting material.

These results are in agreement with those obtained by visual inspection of the melting points and that are reported in the (T, x) phase diagram in Figure

S1: a lowering of ≈70 °C in the melting temperature of the DES compared that which should be obtained for an ideal mixtures is observed.

In Figure S5, DSC thermograms of hydrogel composed of 50 wt% of DES and water and the pure corresponding DES are presented and compared.

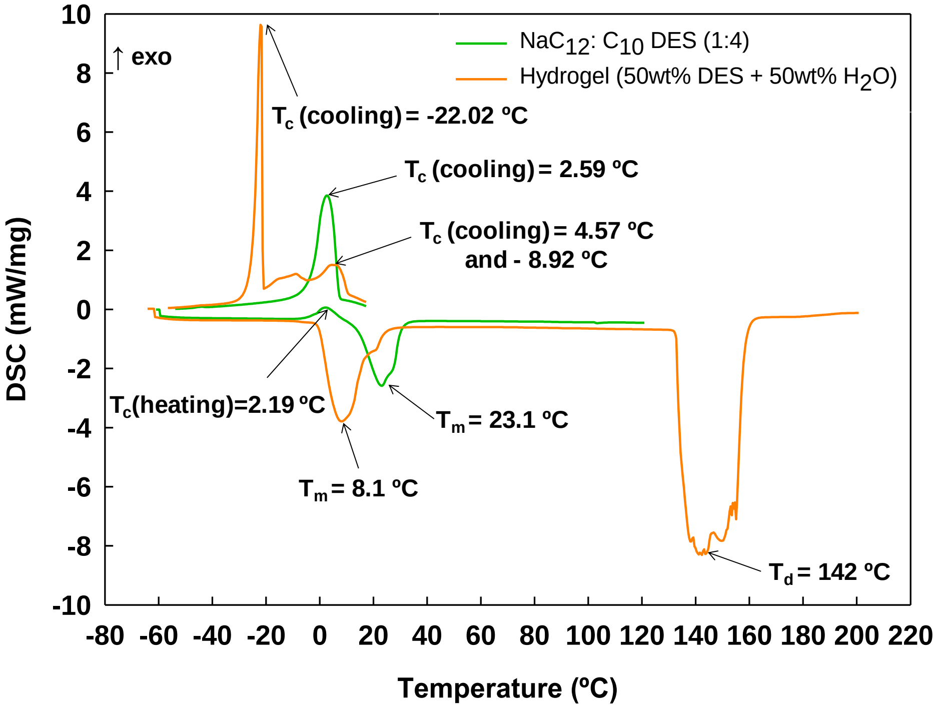

Figure S5. Comparison of DSC thermogram of the pure sodium salt-based DES (NaC_{12} salt) and the corresponding hydrogel (50wt% DES of NaC_{12}: C_{10} (1:4 mole ratio) and 50wt% of water).

Regarding results, it can be seen that hydrogel showed a completely different pattern on thermogram from DES. From DSC thermogram of hydrogel it is possible to see the different transition phases as well as showed the degradation of the polymeric network at 142 ºC and possible evaporation of water, after the melting temperature of the hydrogel. DES and hydrogel have a melting temperature of 23.1 and 8.1 ºC, respectively, as a difference of 15 ºC between them. However, this is expected since water has a melting point lowering effect, and the hydrogel present an amount of 50wt% of water in their composition. Also, in the DES profile can be seen the existence of heat crystallization at 2.19 ºC, which disappears when it becomes a hydrogel and stores water in its polymer network. Finally, regarding cold crystallization, it can be seen that both systems present them, but again with very different profiles: DES presents one big and isolated cold crystallization at 2.59 ºC and hydrogel presents three different cold crystallizations, a large and thin one at -22.02 ºC and two smaller and wider at 4.57 and -8.92 ºC. From all data present in these DSC thermograms, it can be concluded that the chemical structure of hydrogel is much more complex than that presented by DES.

4.5. Thermophysical properties, densities and viscosities

Density and viscosity are important properties of any solvent since they influence the mass transport phenomena, there by affecting their suitability for particular applications. The density decreases linearly with temperature for all dried samples, in the whole temperature range studied. A linear equation was used to express the correlation with the temperature:

$$\rho = a + bT \qquad \text{(eq. 1)}$$

where ρ corresponds to density in g·cm^{-3}, T is the temperature in ºC and a and b are the fitting parameters.

The experimental viscosity values were fitted as a function of temperature, using the Vogel-Fulcher-Tammann (VFT) model using the following equation:

$$\ln \eta = A_\eta + \frac{B_\eta}{(T - C_\eta)} \qquad \text{(eq. 2)}$$

where η is the viscosity in mPa·s, T is the temperature in ºC, and A_η, B_η, and C_η are adjustable parameters. The experimental density and viscosity results for the dried DES as a function of temperature are presented in Table S1 and in Figure S6.

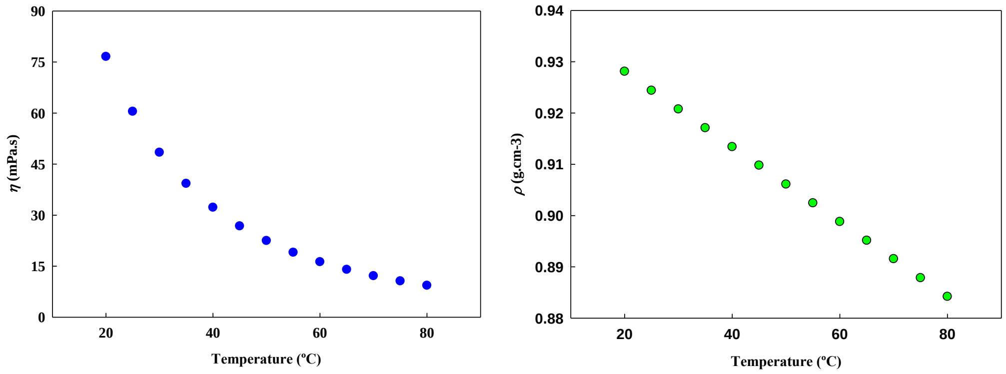

Figure S6. Viscosity (η (mPa.s)) and density (ρ (g·cm-3) data obtained for the NaC_{12}: C_{10} acid (1:4) DES.

Table S1. Experimental density, ρ (g·cm^{-3}), and viscosity, η (mPa·s), of the dried NaC_{12}:C_{10} DES as function of temperature (T/ºC).

***T* (ºC)**	***ρ* (g·cm^{-3})**	***η* (mPa·s)**
20	0.928	76.59
25	0.924	60.48
30	0.921	48.43
35	0.917	39.29
40	0.913	32.27
45	0.910	26.79
50	0.906	22.48
55	0.902	19.04
60	0.899	16.27
65	0.895	14.02
70	0.892	12.16
75	0.888	10.63
80	0.884	9.346

4.6. Rheological properties

Flow curves (shear rate between 0.005 and 10 s^{-1}) for three gel samples at 40 ºC are presented in Figure S7. For shear rates above 10 s^{-1} some mixtures become unstable.

The flow curve was fitted to Herschel-Bulkley model:

$$\tau = \tau_0 + K\dot{\gamma}^n \qquad \text{(eq. 3)}$$

with K being the consistence index, n the flow behaviour index and T_0 the yield stress, and the obtained parameters are presents in table S2.

Table S2. Flow consistence index (K), flow behaviour index (n) and yield stress (τ_0) for three gel mixtures.

Mixture	**K (Pa.s^n)**	**n**	**τ_0 (Pa)**
ω (DES) for composition A	3.56	0.81	3.38
ω (DES) for composition B	5.53	0.56	3.34
ω (DES) for composition C	0.30	0.89	0.17

In Figure S8, viscosity as a function of shear rate for gel mixture with ω (DES) for composition A at 40 ºC and 60 ºC is shown.

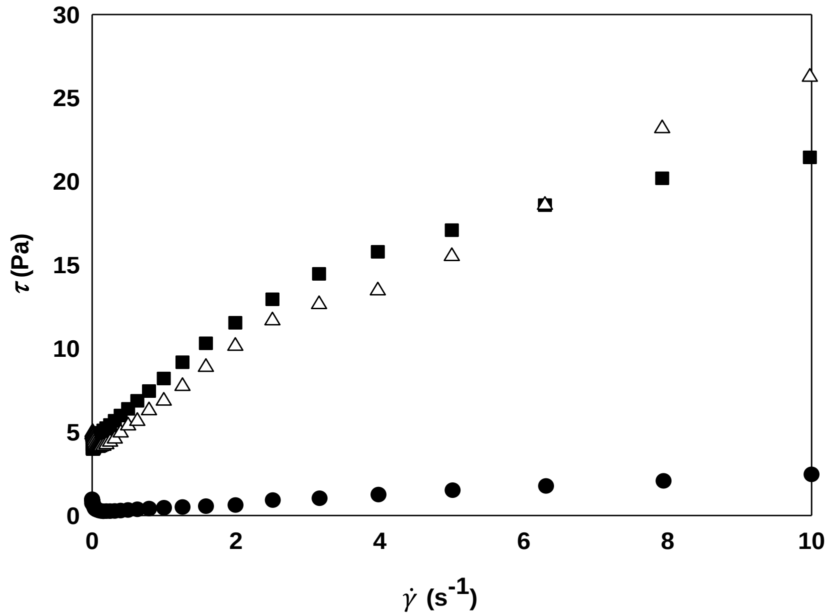

Figure S7. Flow curves (shear stress as a function of shear rate) for three gel mixtures with *ω* (DES) for composition A (Δ), B (■) and C (●) at 40 ºC.

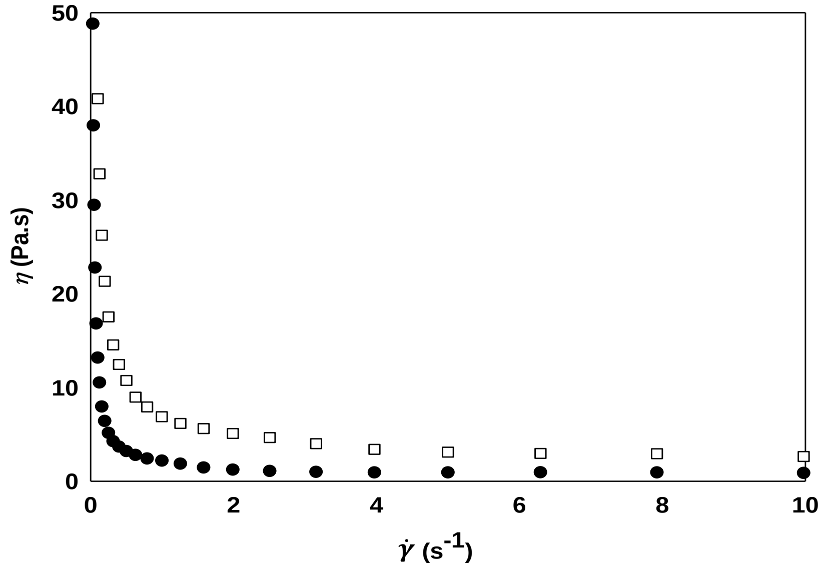

Figure S8. Viscosity as a function of shear rate for the gel mixture with ω(DES) composition A, at 40 ºC (☐) and 60 ºC (●).

The viscoelastic properties of gels were studied, first of all, by oscillation experiments with strain sweep, where linear viscoelastic regime (LVER) was obtained (Figure S9). From these results, strain values between 0.08 and 1 % were chosen for the remaining oscillation experiments, in order to ensure that the samples are kept in linear viscoelastic regime.

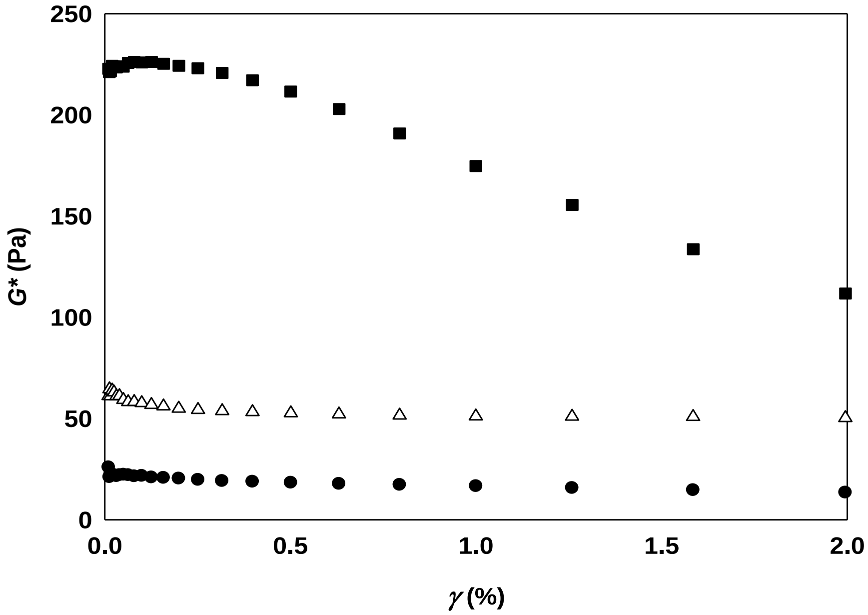

Figure S9. Oscillation experiments on strain sweep mode (complex module as a function of strain) for gel mixtures with ω(DES) for composition A (Δ), B (■) and C (●) at 40 ºC.

The creep/recovery tests were carried out at low shear stresses (0.4 to 0.5 Pa) and are shown in Figure S10 for two gel mixtures. In Figure S11, the thixotropy test for one gel mixture is shown for which shear rates of 0.01 s^{-1} (lower) and 25 s^{-1} (higher) were used. A rebuild time of 486 s was obtained.

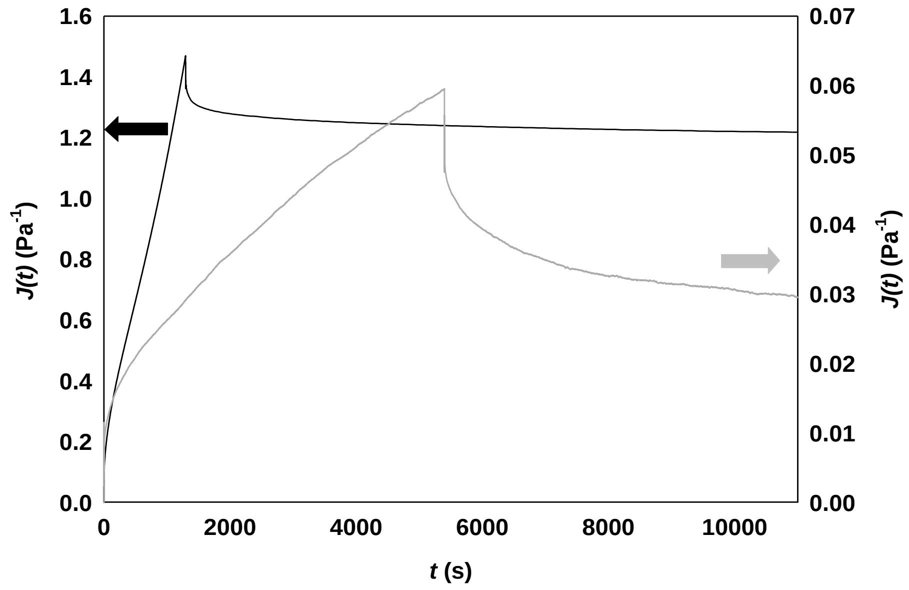

Figure S10. Creep/recovery tests. Shear compliance as a function of time for gel mixtures with ω(DES) equal to 0.197 for composition A (grey), and 0.498 C (black) at 40 °C.

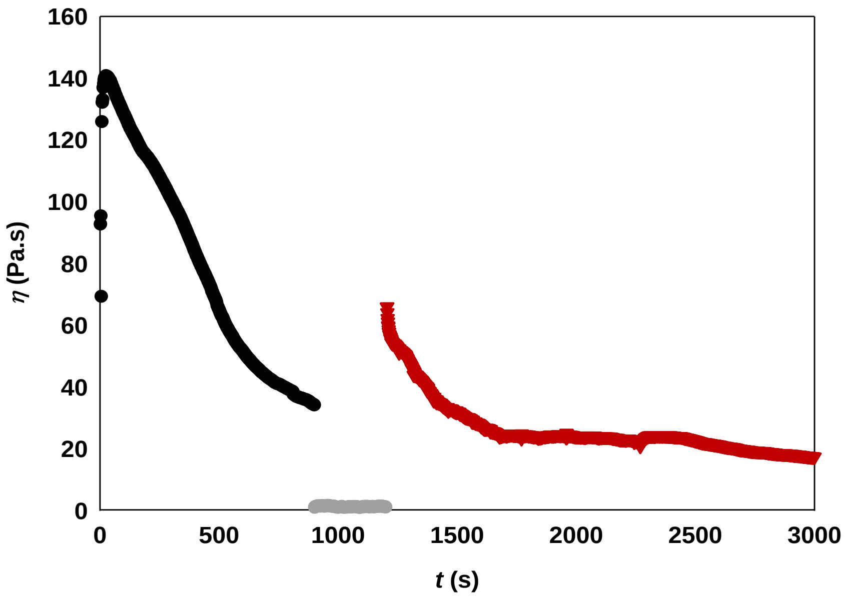

Figure S11. Thixotropy test for gel mixture with ω (DES) for composition A at 40 °C; Viscosity as a function of time for shear rates equal to 0.01 s^{-1} (▬ and ▬) and 25 s^{-1} (▬). Structure rebuild: ▬.

5. References

1. Steed, J. W., Supramolecular gel chemistry: developments over the last decade. *Chemical Communications* 2011, *47* (5), 1379-1383.

2. Bhattacharjee, S.; Bhattacharya, S., Role of synergistic [small pi]-[small pi] stacking and X-H[three dots, centered]Cl (X = C, N, O) H-bonding interactions in gelation and gel phase crystallization. *Chemical Communications* 2015, *51* (32), 7019-7022.

3. Basak, S.; Nanda, J.; Banerjee, A., Multi-stimuli responsive self-healing metallo-hydrogels: tuning of the gel recovery property. *Chemical Communications* 2014, *50* (18), 2356-2359.

4. Basak, S.; Singh, I.; Banerjee, A.; Kraatz, H.-B., Amino acid-based amphiphilic hydrogels: metal ion induced tuning of mechanical and thermal stability. *RSC Advances* 2017, *7* (24), 14461-14465.

5. Zhang, Q.; De Oliveira Vigier, K.; Royer, S.; Jerome, F., Deep eutectic solvents: syntheses, properties and applications. *Chemical Society Reviews* 2012, *41* (21), 7108-7146.

6. Dai, Y.; van Spronsen, J.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y. H., Natural deep eutectic solvents as new potential media for green technology. *Analytica Chimica Acta* 2013, *766*, 61-68.

7. Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep Eutectic Solvents (DESs) and Their Applications. *Chemical Reviews* 2014, *114* (21), 11060-11082.

8. van Osch, D. J. G. P.; Parmentier, D.; Dietz, C. H. J. T.; van den Bruinhorst, A.; Tuinier, R.; Kroon, M. C., Removal of alkali and transition metal ions from water with hydrophobic deep eutectic solvents. *Chemical Communications* 2016, *52* (80), 11987-11990.

9. Nam, M. W.; Zhao, J.; Lee, M. S.; Jeong, J. H.; Lee, J., Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: application to flavonoid extraction from Flos sophorae. *Green Chemistry* 2015, *17* (3), 1718-1727.

10. Niawanti, H.; Zullaikah, S.; Rachimoellah, M., Purification of biodiesel by choline chloride based deep eutectic solvent. *AIP Conference Proceedings* 2017, *1840* (1), 050006.

11. Gouveia, A. S. L.; Oliveira, F. S.; Kurnia, K. A.; Marrucho, I. M., Deep Eutectic Solvents as Azeotrope Breakers: Liquid–Liquid Extraction and

COSMO-RS Prediction. *ACS Sustainable Chemistry & Engineering* 2016, *4* (10), 5640-5650.

12. Florindo, C.; Branco, L. C.; Marrucho, I. M., Development of hydrophobic deep eutectic solvents for extraction of pesticides from aqueous environments. *Fluid Phase Equilibria* 2017, *448*, 135-142.

13. Florindo, C.; Romero, L.; Rintoul, I.; Branco, L. C.; Marrucho, I. M., From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid–Based Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering* 2018.

14. Mjalli, F. S.; Al-Hajri, R.; Al-Muhtaseb, A. a.; Ahmed, O.; Nagaraju, M., Novel amino acid-based ionic liquid analogues: neutral hydroxylic and sulfur-containing amino acids. *Asia-Pacific Journal of Chemical Engineering* 2016, *11* (5), 683-694.

15. Florindo, C.; Oliveira, M. M.; Branco, L. C.; Marrucho, I. M., Carbohydrates-based deep eutectic solvents: Thermophysical properties and rice straw dissolution. *Journal of Molecular Liquids* 2017, *247*, 441-447.

16. Krisanti, E.; Terahadi, F.; Fauzia, F.; Putri, S., Alcohol-based natural deep eutectic solvents (NADES) as green solvents for extraction of mangostins from Garcinia mangostana pericarp. *Planta Med* 2015, *81* (16), PW_163.

17. Florindo, C.; Oliveira, F. S.; Rebelo, L. P. N.; Fernandes, A. M.; Marrucho, I. M., Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids. *ACS Sustainable Chemistry & Engineering* 2014, *2* (10), 2416-2425.

18. Francisco, M.; van den Bruinhorst, A.; Kroon, M. C., Low-Transition-Temperature Mixtures (LTTMs): A New Generation of Designer Solvents. *Angewandte Chemie International Edition* 2013, *52* (11), 3074-3085.

19. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *Journal of the American Chemical Society* 2004, *126* (29), 9142-9147.

20. Hammond, O. S.; Bowron, D. T.; Edler, K. J., The Effect of Water upon Deep Eutectic Solvent Nanostructure: An Unusual Transition from Ionic Mixture to Aqueous Solution. *Angewandte Chemie (International Ed. in English)* 2017, *56* (33), 9782-9785.

21. Shah, D.; Mjalli, F. S., Effect of water on the thermo-physical properties of Reline: An experimental and molecular simulation based approach. *Physical Chemistry Chemical Physics* 2014, *16* (43), 23900-23907.

22. van Osch, D. J. G. P.; Zubeir, L. F.; van den Bruinhorst, A.; Rocha, M. A. A.; Kroon, M. C., Hydrophobic deep eutectic solvents as water-immiscible extractants. *Green Chemistry* 2015, *17* (9), 4518-4521.

23. Abbott, A. P.; D'Agostino, C.; Davis, S. J.; Gladden, L. F.; Mantle, M. D., Do group 1 metal salts form deep eutectic solvents? *Physical Chemistry Chemical Physics* 2016, *18* (36), 25528-25537.

24. Ahmed, E. M., Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research* 2015, *6* (2), 105-121.

25. Men, D.; Zhou, F.; Hang, L.; Li, X.; Duan, G.; Cai, W.; Li, Y., A functional hydrogel film attached with a 2D Au nanosphere array and its ultrahigh optical diffraction intensity as a visualized sensor. *Journal of Materials Chemistry C* 2016, *4* (11), 2117-2122.

26. Coukouma, A. E.; Asher, S. A., Increased volume responsiveness of macroporous hydrogels. *Sensors and Actuators B: Chemical* 2018, *255*, 2900-2903.

27. Sakthivel, M.; Franklin, D. S.; Guhanathan, S., pH-sensitive Itaconic acid based polymeric hydrogels for dye removal applications. *Ecotoxicology and Environmental Safety* **2016,** *134*, 427-432.

CHAPTER 5

FINAL REMARKS

Seeking new doors and opportunities in the field

CHAPTER 5.1

Concluding Remarks and Outlook

> *“Your work is going to fill a large part of your life, and the only way to be truly satisfied is to do what you believe is great work. And the only way to do great work is to love what you do. If you haven't found it yet, keep looking. Don't settle. As with all matters of the heart, you'll know when you find it."*
>
> Steve Jobs

CONTENT

One of the current concerns of XXI century is related to the continuous contamination and consequently increase of pollution in water streams by several micropollutants from industrial, animal and human activities. The presence of a wide diversity of micropollutants with different chemical functionalities, and their consequent bioaccumulation in the food chain, poses to a true challenge in the development of efficient water treatment technologies. Thus, it is of maximum priority to develop new efficient and cost-effective techniques to remove micropollutants from aqueous environments. Hence, the main objective of this PhD thesis is two folded: i) to explore the design and development of sustainable and hydrophobic deep eutectic solvents (DESs) and ii) their use in the implementation of purification processes for water contaminated with several micropollutants. As a result, in this chapter, the most important results achieved during this PhD project are highlighted.

Moreover, the main conclusions, challenges and an outlook for future research work about the development and application of novel sustainable hydrophobic deep eutectic solvents in water purification processes are presented.

1. Development of novel sustainable solvents

Deep Eutectic Solvents (DESs) are a recent class of sustainable solvents which are already extensively considered for applications in several fields. Their success lays on the many possibilities of combinations between components to tailor their properties, which makes them exceptional candidates for a wide range of applications.

DESs emerged as sustainable alternative to ILs overcoming some of their disadvantages, specifically the solid phase at low temperatures, the high cost due not only to the cost of the starting materials but also to the laborious purification steps that are needed, and also in terms of toxicity. With DESs the same degree of properties tunability can be achieved, using typically natural compounds, that can be easily biodegradable, with a very simple preparation method that allows for a substantial cost reduction in comparison with the ILs. Research on DES field has been mainly focused essentially on hydrophilic DESs. This thesis takes advantage of this opportunity and focuses mainly on the design and application of hydrophobic DESs, as a new class of sustainable solvents ready to replace conventional organic solvents.

The first publication concerning DESs came up in 2003, and so far, thousands of publications of the possible new combinations of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) to form new DESs are already known. However, the fundamental knowledge governing their formation and about their properties is still lacking. Later, more specifically in 2015, the first publications concerning hydrophobic deep eutectic solvents were reported. This is a promising class of solvents which have raised great interest, not only due to their hydrophobicity but also to their low viscosity, due to the establishment of fewer hydrogen bonds among the hydrophobic starting compounds, which typically are natural and low cost. Thus, these solvents can be contacted with water, and due to their vanishing water solubility, they remain chemically stable, enabling their reuse and recycle.

In this work, we proposed two novel, neutral, low viscous and sustainable class of hydrophobic DESs: i) the family of eutectic solvents composed of neutral natural occurring compounds, namely that based on DL-menthol combined with several carboxylic acids, and ii) another family based exclusively on fatty acids with long alkyl chains. These novel DES were very attractive in the field of solvents, since besides the fact that they are composed by cheap and natural compounds, they also have a very low quantity of water and possessing low viscosity values (< 200 mPa.s). Both these families have densities comparable to other reported hydrophobic DESs (889 to 942 kg.m^{-3}), but lower than the densities found for most hydrophilic DESs (around 1150 kg.m^{-3}). For other side, it is observed lower viscosity values comparing to already reported hydrophobic DESs in the literature, such as those based on DL-menthol and others containing several quaternary ammonium salts, which typically vary from 11 to 50 mPa.s and 173 to 783 mPa.s, respectively, and much lower than hydrophilic DESs. Another very important aspect is related to the leaching of DES components to water phase. The novel hydrophobic DES here used were designed to be chemically stable when in contact with water environments, which was proved using NMR spectroscopy. It was demonstrated that when one of the constituents of the DES, either HBD or HBA, is water soluble, partial disruption of the DES occurs and variable amounts of the hydrophilic compound is found in the water phase, according to its water solubility.

After the development of the novel eutectic solvents based natural compounds, this thesis steps forward and a metal (sodium) salt of a long chain fatty acid combined with a long chain fatty acid were used to prepare novel hydrophobic DES. However, when in contact with water, a peculiar supramolecular hydrogel behavior was observed due to the localized charge of the metal salt.

This hydrogel behavior was here attained for the first time. Moreover, it was also observed that this new hydrogel exhibits a temperature phase transition, and also thermoreversible viscosity depending essentially on the water content. The new sodium salt-based DES as well as hydrogels based on natural and cheap materials may be of great interest and promising for different applications, especially in medical and biotechnological fields as well as for separation and extraction processes and also in electrochemical applications.

2. Hydrophobic DES as a sustainable approach to water purification

As already stated, DES are a novel emerging class of solvents which have been gaining attention both from academic and industrial fields. Recently, hydrophobic DES have been proposed as sustainable alternatives to organic volatile solvents. Making justice to the exceptional properties of DESs, such as quick and straightforward preparation and the easy tailoring of the chemical and biological properties, which makes them attractive for specific application through to the judicious choice of their individual components, their water immiscibility also makes them very attractive and excellent candidates for water applications.

Thus, this thesis aimed to demonstrate one of the most promising applications of hydrophobic DES, as well as to open new paths of application of these solvents. It is well known that water treatment plants do not properly address the removal of micropollutants, and thus these compounds contaminate water sources of public drinking water systems. In this context, two different types of separation technologies were tested based on developed hydrophobic DESs, namely a liquid-liquid and solid-liquid extractions, with the goal of developing improved strategies to efficiently detect, remove and recover micropollutants from water streams.

In particular, liquid-liquid extraction (LLE) and supported hydrophobic DES as adsorbent materials were investigated for the extraction of three important classes of micropollutants found in the aquatic environment: pesticides (imidacloprid, thiamethoxam, nitenpyram and acetamiprid), a plastic additive (Bisphenol-A) and pharmaceutical compounds (ciprofloxacin and diclofenac).

For that purpose, two different families of hydrophobic DESs, one based on natural neutral ingredients (DL-Menthol and fatty acids) and the other based on quaternary ammonium salts were designed. Both these hydrogen bond acceptors (HBAs) were combined with acids with long alkyl chains, acting as hydrogen bond donors (HBDs), in several proportions, so that a liquid solvent at room temperature was obtained. Only chemically stable hydrophobic DESs were selected to be used as solvents in the extraction of the micropollutants so that no contamination of the water cycle is attained, while reuse and recycle of the DES was possible.

In what concerns the first approach, LLE, extraction efficiencies follow the hydrophobic character of the micropollutants, although no direct link with the increase in DES hydrophobicity, through the increase of the chain length of the HBD, was observed. From all micropollutants studied in this thesis (pesticides, Bisphenol-A, ciprofloxacin and diclofenac), pesticides were the most difficult family of micropollutant to remove from water (extraction efficiencies up to 80% for the four studied pesticides), whereas BPA and diclofenac was efficiently extracted with extraction efficiencies reaching 100% for most of the hydrophobic DES tested. The most curious result obtained using LLE approach was that regarding the removal of ciprofloxacin. Generally, literature reports that ionic hydrophobic DES are more efficient extractants. In this work, it was found that neutral hydrophobic DES display much higher ciprofloxacin extraction efficiencies (from 40 to 90 %) than ionic DES based on ammonium salts (from 0 - 20%, typically around 0%), even when ciprofloxacin is in the charged form.

Moreover, the appealing reusability and recyclability of the hydrophobic DESs used was also demonstrated, highlighting their possible relevant role as sustainable solvents in industrial applications. It was found that these hydrophobic DES had the ability to be used for numerous cycles of extraction without losing the ability to extract the micropollutants as well as being able to be cleaned up and recycled and reach extraction efficiencies up to almost 90%.

In what concerns the second approach used in this thesis to purify water samples, the supported hydrophobic DES, the adsorbent material was prepared through the impregnation of a hydrophobic DES into an inert porous support and used in direct contact with the aqueous phase in order to remove the same micropollutants. It was found that it is possible to obtain similar results as those observed in the LLE approach. The maximum extraction efficiencies (around 85 and 98%, depending essentially on the DES) were typically achieved between 5 and 10 minutes, with a much smaller mass than that required by the LLE approach. However, it should be noted that in this adsorption approach, the extraction time is directly influenced by the selected DES adsorbent material mass.

Finally, both separation technologies explored in this thesis were directly compared, and it was observed that both DES solvents and DES adsorbents yielded similar appealing results for different micropollutants under study.

However, adsorption is an advantageous alternative approach to traditional technologies including LLE, since it is simple, fast, cost-effective and efficient. As far as relevance for an implementation of a process following the tested conditions, it was highlighted that LLE experiments required the use of 100g of DES per liter of water, whereas 250mg of supported DES adsorbent is enough to achieve the highest extraction efficiencies, for the same experimental conditions. It is possible to conclude that the development of adsorbent materials based on hydrophobic DES is a promising approach for water cleaning technologies. The proposed adsorption process offers several advantages compared to the liquid-liquid traditional process such as the fast and easy separation, the reusability without compromising capacity and the small amount of DES needed, which can be very important in the case of DESs composed of expensive compounds.

To sum up, low cost and highly efficient methodologies, either based on liquid-liquid extraction or solid-liquid extraction, were developed using hydrophobic DES in this PhD thesis and it has been demonstrated that they can be efficiently introduced in a final stage of water purification process for the complete removal of micropollutants.

3. Outlook

In this PhD thesis, promising and innovative results for the development of novel hydrophobic DES and their use as efficient extractants in water purification technologies were achieved. The design of hydrophobic DESs chemical structures specifically for the removal of hydrophobic pollutants significantly improves the efficiency of separation processes. Additionally, the results presented herein attest the remarkable potential of DESs in general as designer solvents. As this work is only the tip of the iceberg in terms of application of DES with different properties, further applications concerning hydrophobic DESs should be explored. For example, following up the results here presented and taking advantage of the dramatic change in the mass of solvent from LLE to the adsorption process, while attaining the same performance, a flow system should be implemented using a packed column of hydrophobic DES so that a continuous removal process could be designed. Considering that the goal of the work is the application of hydrophobic DES as suitable materials to novel separation technologies to apply in a final stage of a water purification process, additional investigation on the technologies scale-up also are required. Taking into account the industrial implementation of the proposed methodologies using novel sustainable solvents, an overall sustainability study, involving economic and environmental perspectives, should be performed in order to meet the most appealing solution.

The separation strategies developed in this thesis can be very useful not only in purification of drinking water but also in the efficient, easy and cheap recover and recycle active pharmaceutical ingredients (APIs) present in waste streams of pharmaceutical industry to before their discharge into public waters. In the same vein, these solvents can be of great value in the purification steps of APIs, since they might provide alternative solubility solutions and polarity ranges to non-toxic volatile organic solvents.

Since the field of DES is still in its infancy, the identification of other starting compounds, especially nature derived, non-toxic and biodegradable compounds, can be used to prepare new eutectic mixtures, either deep or not so deep, providing different properties than those currently under study, is crucial for the foreseen boom of these sustainable solvents and their applications.

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