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Natural Deep Eutectic Solvents – Solvents for the 21st Century

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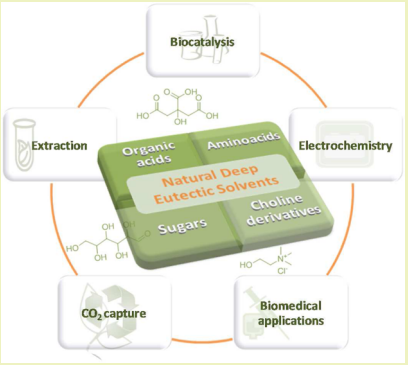
ABSTRACT: Green technology actively seeks new solvents to replace common organic solvents that present inherent toxicity and have high volatility, leading to evaporation of volatile organic compounds to the atmosphere. Over the past two decades, ionic liquids (ILs) have gained enormous attention from the scientific community, and the number of reported articles in the literature has grown exponentially. Nevertheless, IL "greenness" is often challenged, mainly due to their poor biodegradability, biocompatibility, and sustainability. An alternative to ILs are deep eutectic solvents (DES). Deep eutectic solvents are defined as a mixture of two or more components, which may be solid or liquid and that at a particular composition present a high melting point depression becoming liquids at room temperature. When the compounds that constitute the DES are primary metabolites, namely, aminoacids, organic acids, sugars, or choline derivatives, the DES are so called natural deep eutectic solvents (NADES). NADES fully represent green chemistry principles. Can natural deep eutectic solvents be foreseen as the next generation solvents and can a similar path to ionic liquids be outlined? The current state of the art concerning the advances made on these solvents in the past few years is reviewed in this paper, which is more than an overview on the different applications for which they have been suggested, particularly, biocatalysis, electrochemistry, and extraction of new data. Citotoxicity of different NADES was evaluated and compared to conventional imidazolium-based ionic liquids, and hints at the extraction of phenolic compounds from green coffee beans and on the foaming effect of NADES are revealed. Future perspectives on the major directions toward which the research on NADES is envisaged are here discussed, and these comprised undoubtedly a wide range of chemically related subjects.



KEYWORDS: *Natural deep eutectic solvents, Ionic liquids, Green chemistry, Choline chloride, Physical–chemistry properties*

INTRODUCTION

Green technology actively seeks new solvents to replace common organic solvents that present inherent toxicity and have high volatility, leading to evaporation of volatile organic compounds to the atmosphere. Over the past two decades, ionic liquids (ILs) have gained much attention from the scientific community, and the number of reported articles in the literature has grown exponentially. Ionic liquids are molten salts, liquid at room temperature, whose enormous potential arises from particular characteristics of these liquids, namely, their physicochemical properties (viscosity, density, hydrophilicity, and solubility), which can be tuned by the combination of different cations and anions.[1,2] ILs have found applications in very diverse areas and serve very different purposes. They can be used, for example, as solvents for biocatalytic processes,[3] as extractions solvents,[4,5] and for electrochemical applications.[6] Recently, it was found that ILs have the ability to dissolve and increase the processability of renewable natural biopolymers that can find applications in the biomedical area.[7] Nevertheless, IL "greenness" is often challenged, mainly due to their poor biodegradability, biocompatibility, and sustainability.

An alternative to ILs are deep eutectic solvents (DES), which may also have an ionic character but consist of a mixture of organic compounds having a melting point significantly lower than that of either individual component.[8] Figure 1 presents a schematic diagram of the solid–liquid boundaries of a mixture of two solids depending on the composition of the mixture.

The most common DES are based on choline chloride (ChCl), carboxylic acids, and other hydrogen-bond donors, e.g., urea, citric acid, succinic acid and glycerol. DES have similar characteristics to ILs but are cheaper to produce (lower cost of the raw materials), less toxic, and often biodegradable.[9] Recently, Dai and co-workers have reported a large number of stable natural deep eutectic solvents (NADES) based on natural compounds, particularly primary metabolites, such as organic acids, amino acids, and sugars.[10,11] Examples of

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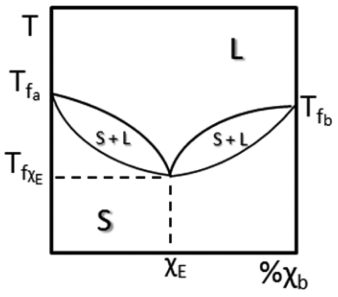

Figure 1. Schematic phase diagram of the melting temperatures of different mixtures.

different molecules that can be combined to produce natural deep eutectic solvents are presented in Figure 2. NADES from different mixtures of these compounds were prepared in this work.

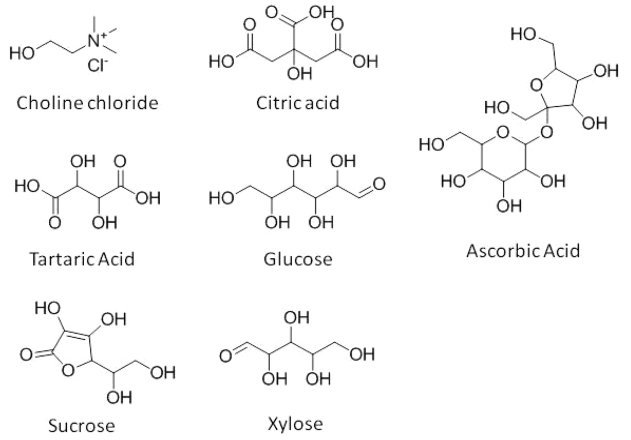

Figure 2. Chemical structure of different compounds with the ability to form natural deep eutectic solvents.

DES or NADES are obtained by the complexation between a hydrogen acceptor and a hydrogen-bond donor. The charge delocalization occurring is hereafter responsible for the decrease in melting point of the mixture relative to the melting points of the raw materials (Figure 1).[9] A major advantage of NADES over ILs is the facility to prepare these solvents. NADES can be prepared from the mixture of concentrated aqueous solutions containing each compound, from a melt of a first component in which the second is dissolved or from the solid mixture of the two components heated to a predetermined value.[12] Large combinations of NADES could be envisaged, and the possibilities that arise from the ability of tailor-made new solvents with the most adequate properties for a given application are enormous.

HISTORICAL PERSPECTIVE AND APPLICATIONS OF DES

Eutectic solvents were scarcely reported in the literature until the beginning of the 21st century. The few manuscripts reported in the 1990s are related with some specific applications of these liquid mixtures. A brief overview of the applications and major fundamental studies reported to date are schematically represented in Figure 3.

In 1994, Gill and co-workers[13,14] reported eutectic mixtures as substrates for enzymatic reactions. This pioneer work demonstrated that enzymes are able to retain their activity when dissolved in eutectic mixtures, providing a better reaction media than the conventional organic solvents. It would only be four years later that more scientific developments in this field would be reported. Erbeldinger et al. report in their work the use of heterogeneous eutectic mixtures for enzymatic synthesis that yield up to 80 wt % recovery. These findings have, hereafter, a notorious impact concerning large systems and novel industrial developments.[15] Biocatalytic processes represent a step further in comparison to enzymatic reactions in deep eutectic solvents. The work of Gutierrez published in 2010 described the possibility to maintain viability of whole organisms, such as bacteria in nonaqueous solvents.[16] Enzyme catalysis and whole-cell biocatalysis processes were reviewed by Clouthier.[8] The large amount of work reported on this topic is due to the need to improve biocatalyst immobilization, stabilization, and recycling to reduce process costs. In this sense, deep eutectic solvents are fostered as greener alternatives to ionic liquids, which have found numerous applications in biocatalysis.[17−21]

In 1995, a work published in *Nature* revealed the possibility to use eutectic mixtures as an alternative for emulsion crystallization providing a more cost-effective strategy for the separation and purification of molecular mixtures.[22]

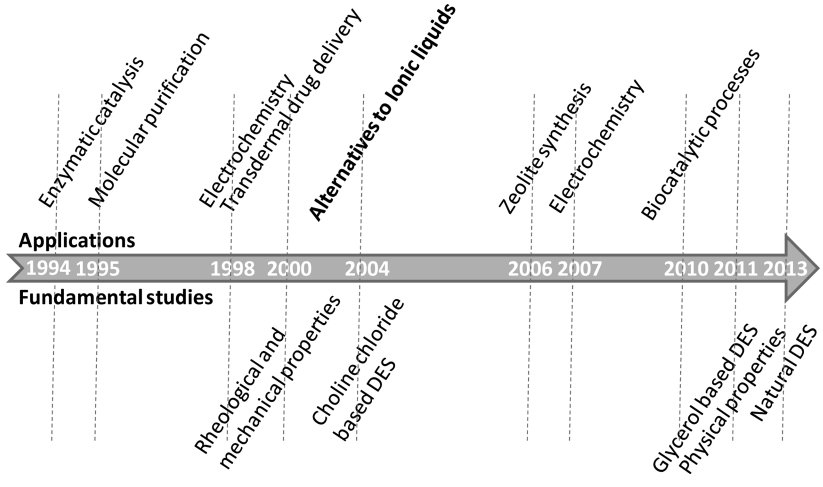

Figure 3. Timeline of reported developments, both on applications and fundamental studies on deep eutectic solvents.

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In 1998, Stott and co-workers demonstrate, for the first time, the possibility to develop drug delivery devices, particularly for transdermal drug delivery.[23] In their work, mixtures of a pharmaceutical active compound and different terpenes as enhancers of skin permeation are successfully described. The possibility to couple an active compound with a second component and prepare hereafter bioactive eutectic systems opens a broad spectrum, which concerns future developments on pharmaceutics and biomedical applications.

The first hint that DES could be used as versatile alternatives for ionic liquids was instigated in 2004.[24] From this year onward, a significant amount of literature on DES, particularly on the physicochemical properties and thermodynamics of these systems has been published. The knowledge of the fundamental properties can build the basis to push forward the broad applications of deep eutectic solvents.

Conventional production of zeolites requires the presence of an organic species to direct structure growth. Parnham and co-workers describe the possibility to use ionic liquids and/or deep eutectic solvents following an ionothermal synthesis.[25] These reactions are characterized by the fact that the organic species can act simultaneously as solvent and template. DES present the advantage that as the reaction progresses, one of the components that constitute the DES breaks down, providing the organic template needed for the reaction. The preparation of alumino, zirconium, or zinc phosphate zeolites following this reaction has been described recently in the literature.[26−28]

Cyclic voltammetry and chronoamperometry measurements, reported in 2007, for choline chloride-based DES have broadened the application spectra of these class of solvents, namely, for electrochemistry.[29] One particular application within this field of research is electrodeposition of metals, which may benefit from the high solubility of metal salts in nonaqueous solutions and the high conductivity of ionic liquids and deep eutectic solvents.[30]

DES have gained increasing scientific interest, and the attentions of the scientific community have now started to be directed toward the understanding of the characteristics of these fluids that make them extremely particular. Literature research in *ISI Web of Knowledge* indicates 380 references regarding deep eutectic solvents. A thorough exploitation of this number indicates an exponential increase in the publications in the last seven years, as depicted in Figure 4.

The major scientific fields that the research carried out in this subject address are chemistry, physics, materials science and electrochemistry and these manuscripts mostly refer to fundamental and basic research (Figure 5). This highlights

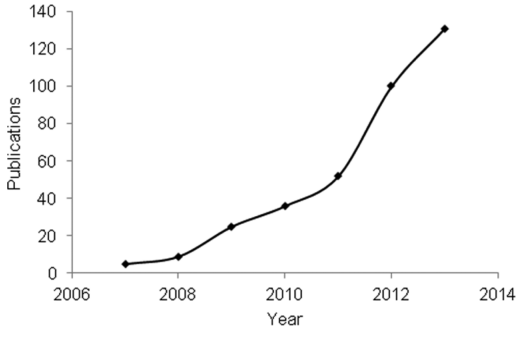

Figure 4. Number of publications in deep eutectic solvents in the past seven years (source ISI Web of Knowledge).

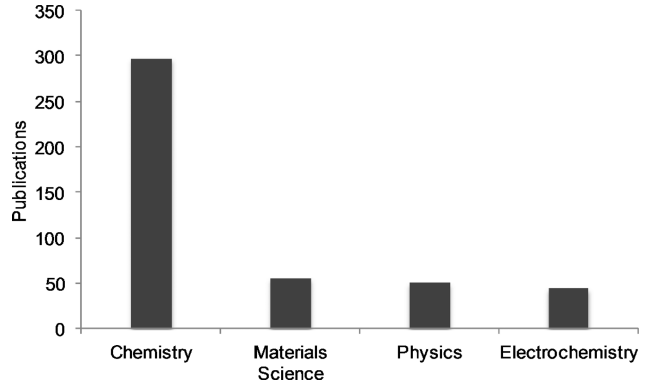

Figure 5. Distribution of the number of publications in deep eutectic solvents per scientific field in the past seven years (source *ISI Web of Knowledge*).

the lack of knowledge in this discipline broadening the spectra of new applications yet to be unraveled. Interesting to notice is the distribution worldwide of these publications (Figure 6). Europe is the most active region of research followed by China and other countries of Asia, while USA and Canada come only in fourth place.

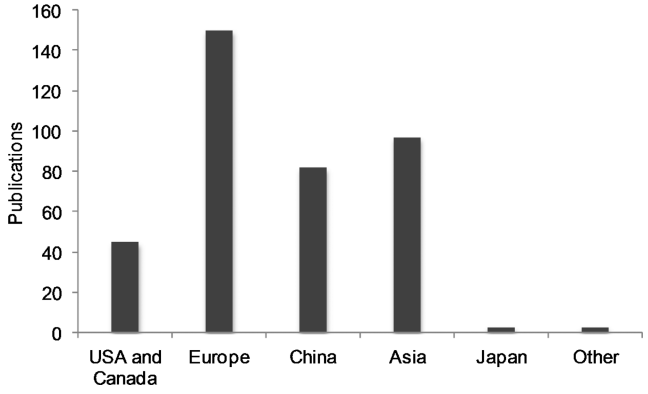

Figure 6. Worldwide distribution of the number of publications in deep eutectic solvents in the past seven years (source *ISI Web of Knowledge*).

FUTURE PERSPECTIVES

Future developments on deep eutectic solvents and natural deep eutectic solvents will rely on the characterization of the fundamental properties of these solvents, the understanding of phase behavior of the components, and the interactions established between the pairs that constitute the eutectic mixture. It is foreseen that major contributions will be published in the field of physical chemistry and thermodynamics of the systems, similarly to what was observed for the ionic liquids in early 1990s.

Biodegradability and Toxicity. One of the major advantages of DES compared to ILs is the fact that these solvents can be synthesized by natural primary metabolites. This fact provides indications that the toxicity of these systems should be significantly lower than ILs. Hayyan and co-workers have evaluated the citotoxicity and toxicity of three phosphonium-based DES using brine shrimp and two bacteria strains, gram positive and gram negative.[31,32] Regarding citotoxicity of DES toward brine shrimp, this study provides an indication that toxicity is dependent on composition,

viscosity, and concentration of DES, as would be expected. Furthermore, in this work, the authors conclude that in comparison with aqueous solutions of the singular molecules that constitute DES the mixture of the two components in the eutectic composition has a greater toxic effect. The toxicity of these DES against bacteria can provide indication that these substances could be used as antibacterial agents. This was explained by the disruption of the cell walls of the bacteria due to the presence of delocalized charges in these types of liquids.

Another study reported by Hou and co-workers[33] provides more insights on the toxicity and degradation of cholinium amino acid ionic liquids. In this study, the authors provide information on the toxicity of DES toward acetylcholinesterase (AChE), an essential enzyme present in the nervous system of nearly all higher organisms. The inhibitory effect of the DES studied was 1 order of magnitude lower when compared to one imidazolium-based IL [Bmim][BF_4]; however, the effect can be significantly different depending on the constituent amino acid of the DES. Additionally, authors also investigated the antimicrobial and antibacterial activity of the different DES prepared.

In our work, we have followed ISO guideline ISO/EN 10.993 to test the citotoxicity of 11 different NADES and two different ILs. In this test, the viability of cells after contact with solutions with a concentration of 25 mg/mL of both NADES and IL was determined. The different solutions were placed in contact with a model cell line of L929 fibroblast-like cells seeded on a polystyrene tissue culture plate at a concentration of 1.5×10^4 cells/mL and were tested after the MTS assay. This assay is based on the bioreduction of a tetrazolium compound, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphofenyl)-2H-tetrazolium (MTS) into a water-soluble brown formazan product, which can be quantified by UV-spectroscopy. Table 1 presents the different NADES tested.

Table 1. List of Natural Deep Eutectic Solvents Tested for Citotoxicity

	NADES		
number	component 1	component 2	mole ratio
1	choline chloride	D(+) Glucose	1:1
2	choline chloride	citric acid	1:1
3	choline chloride	citric acid	2:1
4	choline chloride	sucrose	4:1
5	choline chloride	sucrose	1:1
6	choline chloride	L(+) tartaric acid	2:1
7	choline chloride	D-xylose	2:1
8	choline chloride	D-xylose	3:1
9	citric acid	sucrose	1:1
10	citric acid	D(+) glucose	1:1
11	D(+) glucose	L(+) tartaric acid	1:1
12	1-butyl-3-methylimidazolium acetate		
13	1-butyl-3-methylimidazolium chloride		

Figure 7 presents the cell viability results obtained in comparison with two imidazolium-based ionic liquids.

The results suggest that the presence of tartaric acid has a detrimental effect on the metabolic activity of the cells. However, the analysis of the results obtained does not indicate a clear trend concerning the citotoxic effect and the constituents of the NADES. Frade et al. report the citotoxicity of different magnetic ionic liquids following the same test. In this work, the authors conclude that the viability depends on the concentration, but in general, the prepared choline-based solvents are noncitotoxic.[34]

Biodegradation and the environmental impact of new solvents is a critical issue in their design. On the basis of the biodegradability and environmental impact of the singular components of DES, it would be foreseen that these solvents are more biodegradable and present much lower environmental impact than the one of conventional ionic liquids. Biodegradation, particularly anaerobic degradation, was studied by Hou and co-workers[33] who have investigated the ability of different microorganisms to degrade DES. After 21 days, most of the compounds studied suffered a degradation of up to 80%, which was attributed mostly to the presence of the cholinium cation.

Biocatalysis. Although DES may be composed of known denaturating agents, such as citric acid and urea, many lipases are active in DES; for example, *Candida antartica* lipase B (CALB) shows high activity and stability in DES based on choline chloride.[18] Gorke et al. reported the used of these type of hydrolases in the transesterification of ethyl valerate with 1-butanol. Using DES, i.e., choline chloride/urea, as cosolvents, CALB showed an enhancement of 20−35 fold in enzymatic stability when compared to an aqueous solution of the isolated species.[35] Lindberg et al. have described the use of choline chloride-based DES as co-solvents in the hydrolysis of a chiral (1,2)-trans-2-methylstyrene oxide using epoxide hydrolases.[20] Lipases are widely used in pharmaceutical industry mainly due to their high enantio-, region-, and chemioselectivity; nevertheless, the downstream processing to obtain a pure component still poses some problems. The combination of ILs and supercritical fluids, such as supercritical carbon dioxide ($scCO_2$), was proposed as a viable solution by Reetz et al. In a two-phase separation system, the reaction would occur in the IL phase, while the product of interest would be extracted to the upper supercritical phase. Because ILs are practically insoluble in $scCO_2$, no solvent is lost to the supercritical phase. Moreover, the presence of the IL increases the selective of $scCO_2$ to the component of interest.[36−38] The drawback of this strategy is the high cost and biodegradability of the IL. As referred to before, NADES are cheap and natural-based solvents that have similar characteristics to ILs, more specifically the high solubility of $scCO_2$ in NADES, and due to their low vapor pressure, NADES will be almost insoluble in $scCO_2$.[39] This opens new possibilities for cheap and environmentally friendly biocatalytic process in biphasic systems.

5-Hydroxymethylfurfural (5-HMF) is a valuable building block that derives from biomass. The ester produced by the biocatalytic esterification of 5-HMF has industrial applications as surfactants, fungicides, monomers, etc. Krystof et al. have studied the lipase-catalyzed transesterification of HMF where DES are used as purification solvents in the 5-HMF ester extraction.[21]

NADES based on glycerol may be viable alternatives for the biocatalytic production of biodiesel. Lipases are widely used in the enzymatic production of biodiesel. Zhao et al. published in 2013 the use of DES in the enzymatic production of biodiesel using a choline chloride-based DES. In their work, the authors show that in reactions carried out in choline cloride/glycerol, CALB is able to maintain high biocatalytic activity and that these solvents can be used in the enzymatic transesterification of triglycerides with ethanol, resulting in high reaction yields.[17]

It is envisioned that NADES with their low cost, biodegradability, and biocompatibility will be widely pursued

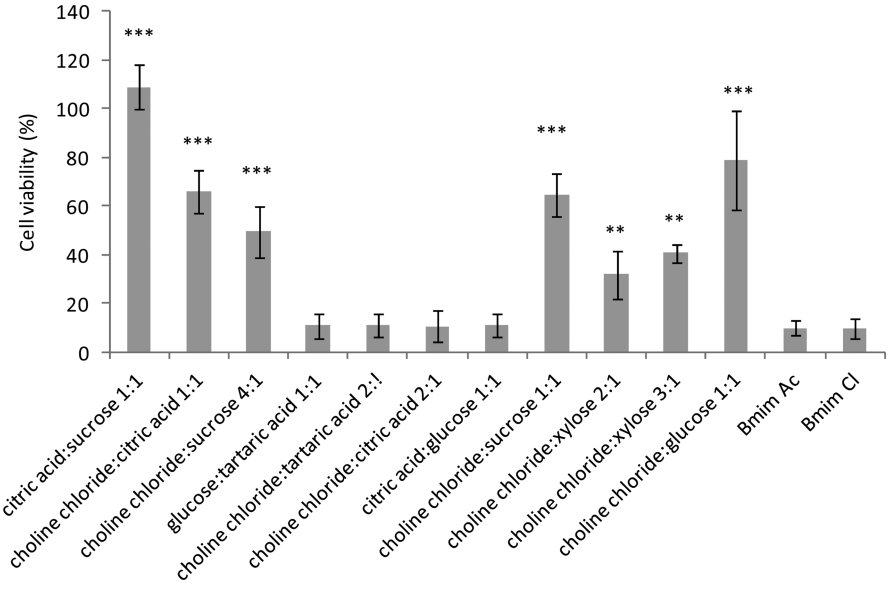

Figure 7. Citotoxicity studies of different NADES in comparison with two ionic liquids. ** denotes statistical significance at $p < 0.01$, and *** denotes $p < 0.001$ level.

as solvents for biocatalytic reactions, especially in pharmaceutical, nutraceutical, and cosmetic industries where product biocompatibility is a major issue. Moreover as shown in the work of Krystof et al., DES have also proven to be effective extraction agents.[21]

Extraction. The effectiveness of an extraction agent is dependent on its dissolution properties. DES have the ability of donating and accepting protons and electrons, which confers them the ability to form hydrogen bonds, increasing therefore their dissolution capability.[40] In fact, DES have been reported as extraction agents from chemical mixtures or for the extraction/dissolution of carbohydrates.[41]

Dai et al.[42] studied the extraction of phenolic compounds from safflower, using different NADES, lactic acid:glucose, glucose:choline choride, and fructose:glucose:sucrose. In that work, it was found that NADES have a high ability to extract phenolic compounds, which is related with the H-bond interactions that are established between the phenolic compounds and the NADES molecules. Physical properties of NADES also have a great influence in the extraction, such as polarity and viscosity. Optimizing all the parameters (viscosity, polarity, and temperature), these authors report higher phenolic compounds extraction yields using NADES compared with conventional solvents such as water and ethanol.

The solubility of poorly soluble molecules such as benzoic acid, griseo-fulvin, danazol, itraconazole, and AMG517 is reported to be 5 to 22,000 times higher in DES, i.e., ChCl/urea and ChCl/malonic acid, than in water.[43] This confirms the applicability of DES as extraction agents of bioactive molecules. Moreover, DES are also able to dissolve the more ionic transition metal oxides from minerals.[24]

Because NADES are greener and safer alternatives, it is not surprising that they have also been employed in extraction of natural products for pharmaceutical applications. NADES present good properties to be used as alternative extraction solvents, such as being liquid at room temperature (and sometimes even below 0 °C), having a viscosity that can be adjusted easily, and being sustainable and safe. Because NADES can dissolve both polar and nonpolar metabolites,[44] this envisages that they can serve as a solvent for the extraction of many types of natural compounds, depending on the physicochemical properties of each NADES.

In this work, we also present some preliminary results of the extraction of phenolic compounds from green coffee beans using different NADES solutions. The results are compared with results obtained from traditional extraction methods for phenols. The experimental procedure was based in the work of Dai et al.,[44] and the results are compared to extractions made using acetone and citric acid solution, following the determination of the total phenolic content as galic acid equivalents.[45] These preliminary results, shown in Table 2, clearly show the feasibility of using NADES as extraction agents of phenolic compounds from green coffee beans (GCBs) or other natural sources.

Table 2. Total Phenolic Content in Extract ($\mu g/g_{GCB}$)

ChCl:xylose (3:1)	ChCl:tartaric acid (1:1)	ChCl:citric acid (1:1)	acetone	citric acid
3.04	2.05	0.99	1.55	1.67

Carbon Dioxide Capture. The high solubility of CO_2 in ILs in addition to its ability to form carbamates in the presence of amine groups turned the attention of many researchers to this type of solvent for CO_2 capture.[46,47] One of the major issues in the chemical capture of CO_2 in aqueous amines by means of carbamate formation is water uptake in the gas stream. The loss of solvent may hinder the large scale application of the process. Due to their negligible vapor pressure, ILs are viewed as a viable alternative for CO_2 scavenging. Nevertheless, the high price of ILs still poses a major drawback in the industrial application of theses solvents. NADES can be viewed as a viable alternative. Like ILs, they have extremely low vapor pressure,[40,48] but NADES are much cheaper to produce. Moreover contrary to most ILs, NADES are biodegradable and biocompatible, which means that their disposal is straightforward and inexpensive. According to the Carbon Dioxide Information Analysis Center from the U.S. Department of Energy, CO_2 emissions are expected to reach 36

billion tonnes by the end of 2013. CO_2 capture, storage, and conversion are the main research topics for the 21st century. Amine-based NADES may be applied for the capture of CO_2, providing a cheaper alternative to the processes available today.

Biomedical Applications. For their versatility, nontoxicity, and biodegradability, DES have already found applications in the biomedical field. It is reported that DES can dissolve model drugs, increasing solubility, permeation, and absorption. Tuntarawongsa et al. have reported the preparation of a DES solution with therapeutical properties made from menthol and camphor and dissolved ibuprofen.[49] NADES dissolved considerable higher amounts of ibuprofen when compared to water. With the addition of a polymer, this resulted in a polymeric eutectic drug delivery system. Other authors have also reported on the DES ability for drug transdermal delivery using a DES composed of ibuprofen/terpene.[23]

The combination of NADES with bioactive molecules, such as ibuprofen, menthol, or mandelic acid, with biodegradable natural-based polymers and $scCO_2$, will be a viable alternative for the production of drug delivery systems, bone therapy scaffolds, and other biomedical applications. Doping of biopolymers with NADES creates a structure for the delivery of the therapeutic agent. By subjecting the doped biopolymer to $scCO_2$, a foaming process occurs where the porosity of the polymer increases, thus increasing the surface area. Moreover, by using a template, the polymer can be shaped to any desirable shape by this process. Our group has already tested the effectiveness of NADES in the $scCO_2$ foaming. Figure 8 shows

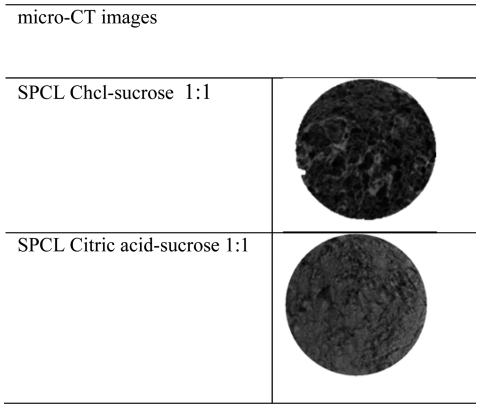

Figure 8. Representative micro-CT images of SPCL blended with 10% of NADES after $scCO_2$ foaming.

microcomputed tomography images of a natural-based polymer, namely, starch polycaprolactone 30:70 (SPCL), blended with different NADES after a supercritical foaming process (40 °C, 20.0 MPa, 2 h). These results reveal the morphological changes concerning the porosity of the samples obtained. In the presence of ChCl:sucrose (1:1), this effect is more evident than in the case of citric acid:sucrose (1:1).

Green Chemistry Metrics. NADES fully represent the green chemistry principles. The manufacturing process for NADES is simply a mixture of the two compounds. In some cases, either some heat or the solubilization of the compounds in water is necessary, meaning that water should be recovered by evaporation and condensation. This highlights the fact that no waste is generated during production and that no undesired byproducts are formed. Because NADES are actually a mixture of two compounds, the yield of the process may be considered 100%. There are several metrics to determine the sustainability of a process; however, the E-factor remains one of the most applied. The E-factor is defined by the total waste produced divided by the total amount of product. In the case of NADES, a theoretical E-factor of 0 can be achieved. The production of NADES does not involve any chemical reaction; therefore, the atom economy is also 100%.

Regarding carbon efficiency defined by the amount of carbon in the product divided by the amount of carbon in the reactants, a value of 100% can be assumed in the case where only the mixture of the two components is necessary. Nevertheless, if it is necessary to heat or to solubilize the initial components, more specific calculations should be made for the indirect carbon usage.

CONCLUSIONS

This paper provides an overview of the historical developments on deep eutectic solvents. The versatility of NADES is comparable to ionic liquids with their great advantages being their much lower cost and greenness based on the fact that NADES are composed by natural primary metabolites. Advances in different fields of research from biocatalysis, extraction, electrochemistry, and biomedical applications are expected to be boosted as more insights on fundamental and basic research on NADES is revealed. From an initial mere scientific curiosity, NADES are now foreseen as the next generation of solvents, and although a lot of research is yet to be pursued, this new class of solvents will definitely make great contributions to more sustainable industrial development.

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Notes

The authors declare no competing financial interest.

Biographies

Alexandre Paiva was born in Lisbon in 1978. He graduated in Chemical Engineering from the Faculdade de Ciências e Tecnologia of the Universidade Nova de Lisboa (FCT-UNL) in 2001. From 2001−2002, he worked as a researcher at FCT-UNL on phase equilibria of natural compounds and supercritical CO_2 and from 2002−2005 at the Instituto Superior Técnico, Lisbon, on particle generating from supercritical solutions. In 2008, he obtained is Ph.D. from the Technische Universität Hamburg-Harburg, Germany (TUHH) in biocatalic separation of isomers using supercritical technology. He currently holds a position as a post-doc researcher at REQUIMTE/FCT-UNL, where he is also an assistant professor in Biocalysis. His

main research interests are the valorization of agro-industrial residues using green technologies, supercritical extraction, hot compressed water technology, and the use of alternative green solvents for biopolymer processing.

Rita Craveiro is currently a Ph.D. student at the Faculdade de Ciências e Tecnologia of the Universidade Nova de Lisboa and and associated laboratory Requimte. She graduated in chemistry in 2008 from the Universidade de Coimbra and received her MSc in 2010 from the Universidade de Aveiro. Her main research interests include processing of biomaterials using alternative solvents such as ionic liquids, supercritical fluids, and deep eutectic solvents. Currently, she is the author and co-author of several scientific papers, mainly in the area of polymer processing and characterization.

Ivo Aroso was born in Porto, Portugal, in 1981. He holds a MSc degree in chemistry from the Faculdade de Ciências of the University of Porto (2003) and a post-graduation in materials processing from the School of Engineering of the University of Minho (2007). Since joining the 3B's Research Group in 2005, he has been actively involved in industrial-based research projects, namely, working with the largest cork company in Portugal, Corticeira Amorim. He has gathered strong expertise in the optimization of different extraction procedures using solvents, supercritical CO_2, and subcritical water methodologies and characterization of natural products, particularly, the identification of molecules through chromatographic techniques and characterization of the antioxidant potential. He is currently in the final stages of his work toward obtaining a Ph.D. degree in material science at the University of Minho.

Marta Martins was born in Porto, Portugal, in 1984. She obtained a BS degree in Chemistry Engineering (2008) and a MS degree in Chemistry Engineering—Energy Optimization in Chemical Industry (2010) from the School of Engineering of the Polytechnic of Porto (ISEP). She worked as a researcher in the Biomaterials and Nanotechnology Group of CIETI at ISEP from 2008 until 2011. At the 3B's Research Group, Marta is developing an innovative process to design novel biopolymers for biomedical devices and bone tissue engineering combining green technology, such as ionic liquids and supercritical fluid foaming.

Rui L. Reis, Ph.D., DSc, Hon. Causa MD, FBSE, is 46 years. He is the Vice-Rector for R&D of the University of Minho (UMinho), Portugal. He is the Director of the 3B's Research Group and of the ICVS/3B's PT Government Associate Laboratory of UMinho. He is also the CEO of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine (TERM) and the President and CSO of the company Stemmatters. He is the president-elect of Global TERMIS (Tissue Engineering & Regenerative Medicine International Society) and the editor-in-chief of the Journal of Tissue Engineering and Regenerative Medicine (Wiley-Blackwell).

Rui L. Reis's education background includes (i) a graduation in Metal. Eng., University of Porto, Portugal, 1990, (ii) a master degree in research on Mater. Sci. and Eng.—Biomaterials—obtained in a joint program of the six major technical Universities in Portugal, awarded by the University of Porto, Portugal, in 1994, (iii) a Ph.D. in Polymer Eng.—Biomaterials & Tissue Engineering from the University of Minho, Portugal, a degree that was prepared in co-operation with Brunel University, London, U.K., in 1999, and (iv) a Doctor of Science (D.Sc.) degree in Biomedical Engineering—Biomaterials & Tissue Engineering from the University of Minho, Portugal, in 2007. He is the Portuguese scientist with the most publications ever, being a co-author of 720 ISI listed publications (560 full papers in scientific journals),

around 200 book chapters, 30 patents, and 6 books. He is PI of projects totaling around 35 MEuros (around 20 MEuros for his UMinho group), including the very prestigious European Research Council (ERC) Advanced Grant.

He was awarded several major national and international scientific and innovation awards, including both the Jean Leray and George Winter Awards from the European Society for Biomaterials (ESB). He was also awarded a honoris causa degree by the University of Granada, Spain.

Rui L. Reis has been involved in biomaterials research since 1990. His main area of research is the development of biomaterials from natural origin polymers (starch, chitin, chitosan, casein, soy, algae-based materials, silk fibroin, gellan gum, carragenan, hyaluronic acid, xanthan, marine collagen, etc.) that in many cases his group originally proposed for a range of biomedical applications, including bone replacement and fixation, drug delivery carriers, partially degradable bone cements, and tissue engineering scaffolding. Lately, the research of his group has been increasingly focused on tissue engineering, regenerative medicine, stem cells, and drug delivery applications. His research group works with bone marrow, adipose-derived, umbilical cord (blood and matrix), amniotic origin (fluid and membrane), and embryonic stem cells

Ana Rita C. Duarte was born in Lisbon in 1978. Currently a research assistant at the 3B's Research Group at the Universidade do Minho, she graduated in Chemical Engineering from the Faculdade de Ciências e Tecnologia of the Universidade Nova de Lisboa in 2002 and completed her Ph.D. on exploring supercritical fluid technology for the preparation of controlled drug delivery systems in 2006 from the same university. In 2006−2007, she was a researcher at the Techniche Unversiteit Delft, The Netherlands. In 2007, she was a post-doc with the 3B's Research Group, where she is now currently working. The International Society for Advancement of Supercritical Fluids granted her thesis the Best Thesis Award in 2007. Presently, she has 48 papers listed in the Web of Knowledge with a total of 606 citations and an h-index of 17. Her main research interest is the use of green technologies for the development of biomaterials. The use of water, ionic liquids, and supercritical fluids, together with the exploration of natural deep eutectic solvents for natural-based polymer processing, are her main scientific interests.

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