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Green solvents for the extraction of bioactive compounds from natural products using ionic liquids and deep eutectic solvents

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Green technology is moving from an option to a must in modern industrial processing. Solvents are the core of the food, pharmaceutical, cosmetic, agrochemical, chemical, and biotechnological process technologies. In the past two decades, supercritical fluids, ionic liquids, and deep eutectic solvents became the most actively investigated as potential green solvents, especially in the field associated with food, flavors, fragrances, and medicinal plants processing. This review assesses recent information about the novel solvent technologies.

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Introduction

Daily life is to a great extent dependent on natural products: bulk products, such as food, beverages, clothes, fibers, building materials, and fuel; as well as fine chemicals, such as pharmaceuticals and cosmetics. These products require extensive processing, from very large scale such as cellulose and paper production, to small scale of fine chemicals, such as medicines, cosmetics, flavors, and fragrances. There are all kinds of regulations for these processes and the solvents used. For example, in food production, according to EU and national regulations, only a few solvents are allowed: water (with admixture of acid or base), volatile food materials, and some highly volatile solvents such as propane, butane, ethyl acetate, ethanol, CO_2, N_2O, and acetone [1,2]. For the allowed solvents, maximum residues are strictly defined. In industry, the value of the products is not only dependent on the production costs themselves but also on the way of production. Safety of the process, avoiding the use of chemicals that are potentially dangerous for human health and an environmentally friendly disposal of the waste products are nowadays important aspects that should also be taken into account in calculating real costs of production. In this context, green technology is becoming an essential part of processing. Green technology is defined as a way to improve production processes concerning long and short-term impact on an eco-system.

Production costs of any commodity can be lowered by developing novel products from the same production chain, for example, from the waste streams. With the rapidly growing industrial processing of natural products, the waste products are now getting more attention. The total annual waste from the food and beverages industry in the EU is estimated to be 37 million tons [3•]. For example, the biowaste in the potato industry is roughly 50% of the harvested amount of potatoes, consisting of peel and other waste [4]. In the case of oranges, approximately 90% of all the oranges produced in Florida (USA) are used for their juice, yielding every year 3.5 billion pounds waste (peel and pulp). In Europe, there is around 4.4 million tons tomato waste every year [4]. These huge amounts of biowaste drives the exploration for the production of novel products. Many bulk chemicals are now being produced by chemical modification or fermentation from the residual materials, for example, starch or D-glucose obtained from biowaste [5]. The isolation of high value bioactive substances such as polyphenols, proteins, pigments, and dietary fibers is now widely studied [3•].

This review assesses the use of novel solvents and their perspectives in bioprocesses.

Basic principles of supercritical fluids, ionic liquids and deep eutectic solvents

Through the years, supercritical fluids, ionic liquids, and deep eutectic solvents gained an important position in the field of green processes. Each solvent has its own specific physicochemical properties (see Table 1 for some typical examples) which consequently confine their applications. The first generation of green solvents were supercritical fluids. The supercritical phase occurs above a certain temperature (critical temperature, Tc) and pressure (critical pressure, Tp), it has properties somewhere in

Table 1

Comparison of the density and viscosity of ordinary gases, liquids, supercritical CO2[a], ionic liquids[b] and deep eutectic solvents[b]

		Density (g/cm^3)	Viscosity (g/cm s)
Gases		$(0.1–2) \times 10^{-3}$	$(1–3) \times 10^{-4}$
Organic solvents		0.6–1.6	$(0.2–3) \times 10^{-2}$
Supercritical CO_2	Tc[c], Pc[d]	0.47	3×10^{-4}
	Tc[c], 6Pc[d]	1.0	1×10^{-3}
Ionic liquids	C_4mimBF_4[e]	1.14	0.115
	$C_4mim(CF_3CO_2)_2N$	1.43	6.9×10^{-2}
Dee eutectic solvents	ChCl-ethylene glycol (1:2)	1.12	0.36
	ChCl-urea (1:2)	1.24	6.32

[a] Data taken from Ref. [6].
[b] Data taken from Ref. [19].
[c] Tc: critical temperature.
[d] Pc: critical pressure.
[e] C_4mim: butylmethylimidazolium.

between gas and liquid. With a typical density of a liquid (between 0.1 and 1.0 g/mL) and a characteristic dissolving power [6]. Since the end of 1970s, supercritical fluids are widely used for the extraction of chemicals from various sources including, for example, plants. One the most successful industrial applications is the decaffeination of coffee using $SC-CO_2$, examples are fractionation of butter oil from oil seeds and essential oils from spices [6,7••].

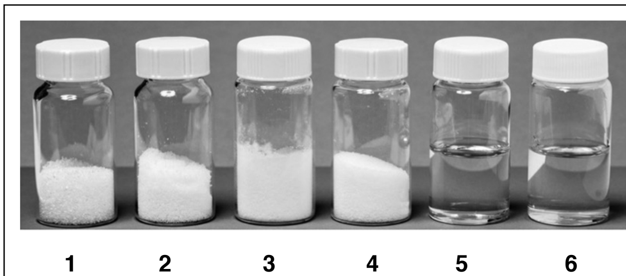
A rather new class of sustainable solvents are the synthetic ionic liquids. Applications and potential of ionic liquids are comprehensively reviewed by Plechkova and Seddon [8]. Ionic liquids are defined as liquid salt mixtures, in which individual ionic components bind with each other through ionic bonds and become liquid at room temperature in contrast to a single high-temperature molten salt [9]. They have characteristic physicochemical properties, which distinguish them from conventional organic solvents, such as an extremely low vapor pressure, high thermal stability and high conductivity, with a wide range of electrochemical, and polarity properties. Because of these features they replaced conventional organic solvents in many chemical processes, for example, extraction, enzyme reactions, chemical synthesis, and stabilization of labile compounds. This is extensively reviewed in many papers [8–10,11•,12,13]. Particularly, due to their negligible vapor pressure (<1 Pa), ILs have been proposed as green solvents after their discovery early 20th century [14]. The high expectations of ILs as green media were soon challenged [14,15••]. Among others their toxicity, costly synthesis and poor degradability hampered their application in industry [9,16,17]. To circumvent the problems of ILs, a new type of solvents was developed, the deep eutectic solvents (DES), also called deep eutectic ionic liquids (DEILs), low-melting mixtures (LMMs) or low transition temperature mixtures (LTTMs) [15••]. The first set of DES were obtained by mixing a quaternary ammonium salt with hydrogen bond donors (HBD) such as organic acids, urea or glycerol that form a complex with the halide anion of a quaternary ammonium salt (e.g. choline chloride) and various carboxylic acids [18]. The physicochemical properties of DES are similar to those of ILs, except that most DES are made of non-toxic, easily accessible, cheap sustainable compounds and include also non-ionogenic compounds [18,19]. Though further studies might be needed to prove that the DES are harmless for living organisms [20].

Recent applications of ionic liquids and deep eutectic solvents

The low polarity of $SC-CO_2$ hampers the extraction of most bioactive metabolites from natural sources. The development of ILs offered novel possibilities for these compounds. Therefore, ILs were studied for diverse applications making use of their above-mentioned favorable features. The drawbacks such as their high viscosity and polarity could be tailored by changing the cation–anion combinations [10]. ILs were first applied to extractions covering a wide range of bioactive secondary metabolites [21] and to media for chemical reactions (catalytic transformations) [22]. Large-scale processing of biopolymers such as cellulose and lignin in wood processing is a target, to open the world's largest untapped source of natural polymers [23–25]. The high costs and toxicity are major limitations for an industrial process.

Therefore, deep eutectic solvents (DES) have become an interesting alternative. Similar to ILs, DES are composed of two or more solid components in certain molar ratios which physically interact by, for example, hydrogen bonding, to form a eutectic mixture which is a liquid at a temperature far below the melting point of the individual compounds of the mixture and even far below room temperature [18]. Advantages of DES are biodegradability, recyclability, extremely low vapor pressure, low costs, and low toxicity of most commonly used compounds [10,15••,18,19]. Most DES are made from common bulk chemicals, often from natural origin [18,26,27]. To give a physical-chemically correct name many names have been proposed instead of DES, such as low-melting mixtures [28], and low transition temperature mixtures [29]. Even bio-ILs [30] has been used as a fashionable name to advertise their biodegradability and low toxicity. But nowadays DES and NADES (Natural DES) are the commonly used name to rather cover a concept than a strict physical–chemical phenomenon. The NADES are particularly related to the hypothesis of Choi *et al.* [31••] who proposed that the NADES act as a third liquid phase in all living organisms. They observed that a number of primary metabolites, such as sugars, organic acids and bases, and amino acids occur in high and similar molar concentrations. This raised the question about their meaning. After the first experiment with malic acid and choline chloride to see if this could give an ionic liquid was successful, many combinations of the abundant primary metabolites were shown to give liquids in certain molar ratios (Figure 1). The NADES can explain the multistep biosynthesis of non-water-soluble compounds, both for small molecules as well as for biopolymers. Also, storage of high concentrations of non-water-soluble compounds in, for example, flowers, cold and drought resistance, including resurrection plants, senescent dry seeds, seems to be related to the presence of typical NADES components [31••]. The NADES showed very high solubility of various non-water-soluble metabolites. They also dissolve enzymes, which upon dilution with water become active again [31••]. In general, NADES can be classified into five groups: ionic liquids, made from an acid and a base; neutral, made of sugars only, or sugars and other polyalcohols; neutral with acids, made of sugars/polyalcohols and organic acids; neutral with bases, made of sugars/polyalcohols and organic bases; and amino acids-containing NADES made of amino acids with organic acids/sugars. As they consist of common metabolites found in our daily food, toxicity is not a real issue. Like the ILs, NADES have a virtually zero vapor pressure and are viscous liquids. By increasing the temperature or adding small amounts of water (5–20%) the viscosity can be reduced to almost that of water. This is an important feature as it makes it possible to handle the NADES like water-based extracts in an industrial process. The disadvantage of the ILs of removing the solvent is the same for the NADES. However, with the difference that the NADES extracts itself can be applied as such as the NADES are non-toxic. So, in food and cosmetics the NADES extracts can be applied as such. The original expectations of NADES as universal solvents did not work out; in fact, they turned out to be highly selective solvents. That means for every application, the most suited NADES needs ought to be developed. The variables that one needs to consider are the class of NADES, the molar ratio of the constituents, and the water content. Interesting aspect is that highest solubility is not always correlating with highest extractability of a compound, yet there seems to be a matrix effect.

Figure 1



Typical natural eutectic solvents (NADES) 1: sucrose, 2: fructose, 3: glucose, 4: malic acid, 5: sucrose-fructose-glucose (1:1:1, mole/mole), 6: sucrose-malic acid (1:1, mole/mole). Remade with permission from Ref. [31] (Plant Physiol. 2011, 156, 1701-1705).

The strong points of NADES resulted in a large number of studies after the publication of the concept of NADES in 2011, that is, more than thousand scientific papers. This clearly shows the potential of NADES as green solvents. NADES, together with other DES, have been developed for applications in different chemical processes, particularly for the extraction of natural products. These applications have recently been reviewed [15••,32•,33•]. Also, the use in enzymatic or chemical reactions has been reviewed [34–36]. As mentioned above, the holy grail of the natural products is the hydrolysis of polysaccharides (cellulose) and lignin. As they must have been synthesized in a liquid form, there must be a NADES in plants in which cellulose and lignin have been dissolved during their synthesis. First partial results of dissolving these macromolecules have been reported [26,31••] and followed by many applications for polysaccharides, lignin and proteins [37,38]. Solubilization and stabilization of DNA and RNA has been reported [39–41]. Also, some different post-harvest treatments have been reported for agrochemical uses [42,43]. Some typical applications of NADES to the extraction of bioactive compounds, macromolecules and enzyme reactions are listed in Table 2.

Conclusion and perspectives

The classical extraction methods using organic solvents for biomaterials will remain, as they build upon many years of experience and have a strong scientific basis. However, the quest for more sustainable (bio)chemical processes will deliver novel solvents and process technologies. In the past decades, new solvents have already been discovered and developed for different applications, such as supercritical fluids, ILs and (NA)DES. Each of these solvents has its own advantages and drawbacks. Supercritical fluid technology with CO_2 is a proven technology in commercial large-scale production of extracts and pure compounds. However, the limitations such as a narrow range of polarity and high cost of high-pressure equipment hamper the extension of its applications to

Table 2

Typical applications of (natural) deep eutectic solvents [(NA)DES] to the production of natural products (referred to Ref. [25])

Category	(NA)DES ingredients[a] (mole ratio)	Sources and targets	Activities	References
Small molecules	Lactic acid, malic acid, proline, glucose, sorbitol and choline chloride	Carthamins from *Carthamus tinctorius* flowers	Extraction yields and stability improved	[44,45]
	Sugars, acids, amino acids and amines	Vanillin from vanilla pod	Solubility and extractability of vanillin tested	[46]
	Lactic acid–glycine with addition of water	Polyphenols from Greek medicinal plants	Ultrasound extraction assisted	[47]
	Choline chloride, 1,2-propanediol, glycerol, sugars and amino acids	Rutin from *Fagopyrum tataricum* hulls	Ultrasound extraction assisted, and biodegradability and biocompatibility of NADES tested	[48]
	1,2-Propanediol, lactic acid, malic acid, glucose, fructose, sucrose, proline and choline chloride	Anthocyanins from *Catharanthus rosues* flowers	Extractability and stability of anthocyanins tested	[49]
	Choline chloride, citric acid, malic acid, oxalic acid, glucose, fructose, xylose and glycerol	Anthocyanins from wine lees	Extraction parameters (time, ultrasound power and water content) optimized by DOE[b]	[50]
	Lactic acid–glucose–water (6:1:6)	Phenolics from olive oil	Extra virgin olive oil used for the extraction of phenolics to correlate UV spectrum of the oils	[51]
	Proline–glycerol (2:5)	Flavonoids from *Sophora immaturus* flowers	Extraction yields of flavonoids compared with methanol and SPE[c] applied for the recovery from DES	[52]
	Choline chloride with glycerol, oxalic acid, malic acid, sorbose, or proline	Phenolics from grape skin	Flavonoid glycosides and anthocyanins extracted by DES with ultrasound-assisted extraction	[53]
	Choline chloride, sugars, organic acids	Phenolics from *Cajanus cajan*	Extraction of the glycosides of flavone and isoflavane, phenylpropanoids, and coumarins analyzed by UPLC[d]	[54]
	Amino acids, sugars, urea, organic acids and choline chloride	Rutin	Solubility of rutin in diverse NADES measured. Proline–glutamic acid (2:1, mole ratio) used for pharmacokinetics of rutin	[55]
	Proline, xylitol, sorbitol, urea, organic acids, carnithine and acetylcarnithine	Berberine	Solubility of berberine and bioavailability of DES solution measured in mouse model	[56••]
	Betaine–glycerol–glucose (4:20:1, mole ratio)	Catechins from green tea extract	Extraction and storage of catechins as cosmetic ingredients measured	[57]
	1,2-Propanediol–choline chloride–water (1:1:1)	Resveratrol	Formulation of resveratrol in NADES and its bioactivity, the inhibition of matrix metallopretease-9 in mouse model measured	[58]
	Malic acid–choline chloride (1:1), malic acid–glucose (1:1), choline chloride–glucose (5:2), malic acid–proline (1:1), glucose–fructose–sucrose (1:1:1), glycerol–proline–sucrose (9:4:1).	Ginkgolides from *Ginkgo biloba* leaves and ginsenosides from ginseng leaves	Structure activity relationship of individual analogues in deep eutectic solvents and application HPTLC[e] to the recoveries of the compound	[59•]
	Glycerol–proline–sucrose (9:4:1)	Ginsenosides from ginseng roots	Improving extraction yields of saponins as well as their recovery by SPE[c] and bioavailability	[60]
Macro molecules	Citric acid, malic acid, maleic acid, fructose, sucrose, glycerol, trehalose and choline chloride,	5,10,15,20-tetrakis(4-hydroxyphenyl)-porphyrin (THPP)	Solubility and stability of THPP improved for antibacterial photodynamic therapy	[61]
	Fructose, glucose, sucrose, citric acid, tartaric acid, betaine and choline chloride	Gluten	Solubility of gluten and water effect on NADES solubility measured by immunoassay	[38]
	Malic acid–proline (1:1)	DNA of salmon testes	Solubility of DNA in NADES measured	[31••]
	Choline chloride–ethylene glycol (1:2)	DNA of salmon testes	Solubility and stability of DNA in DES measured	[39]
	Choline chloride–urea (1:2)	DNA and RNA	Stability of DNA and RNA measured	[41]
	PEG and quaternary ammonium salts	RNA	RNA extracted by aqueous biphasic systems using DES	[40]

Table 2 (*Continued*)

Category	(NA)DES ingredients[a] (mole ratio)	Sources and targets	Activities	References
Enzyme reactions	Lactic acid–choline chloride (5:1)	Cellulase in rice straw	Lignin isolation from lignocellulose by treatment of NADES	[37]
	Glycerol, betaine, malic acid and sorbitol	Laccase	Thermal stability and tertiary structure of the enzyme measured	[35]
	Malic acid–choline chloride (1:1)	Laccase	Feasibility test of the enzyme activity	[31••]
	Choline chloride, glycerol, ethylene glycol, urea, oxalic acid, malonic acid, ethylammonium chloride	Lipase B from *Candida antarctica*	Activity and stability of the enzyme measured	[62]
	Choline chloride, ethane diol, glycerol and urea	Epoxide hydrolase	Enzyme activity improved up to 20 times	[63]
	Choline chloride–glycerol (1:2), choline acetate–glycerol (2:3)	Lipase	A possibility for biodiesel production tested	[64]

[a] Several combinations from the ingredients in different mole ratio were used.
[b] DOE: design of experiment.
[c] SPE: solid phase extraction.
[d] UPLC: ultra-performance liquid chromatography.
[e] HPTLC: high performance thin layer chromatography.

more polar compounds. That is where the ILs and (NA) DES come into the picture. They cover a wider range of polarity covering both hydrophilic and hydrophobic features. In fact, they are really complementary to the supercritical solvents. Especially, the low toxicity of NADES is a major advantage for the application of NADES extracts as such in food, fodder, cosmetics, pharmaceuticals, and agrochemicals. For both enzymatic and chemical reactions, they provide novel media with among others a stabilizing effect on both enzymes and compounds. The use of the NADES as storage media for DNA, RNA and proteins is another interesting perspective. In fact, the first NADES extract has already been approved for cosmetics, and others will follow soon.

Conflict of interest statement

Nothing declared.

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