

Review

Applications of Deep Eutectic Solvents Related to Health, Synthesis, and Extraction of Natural Based Chemicals

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Abstract: Deep eutectic solvents are liquid mixtures of solid components at room temperature, which present exceptional properties: high solvent capacity, high biodegradation, low volatile organic compound character, and relatively low toxicity. Furthermore, there are an important number of DES described, formed by different components and ratios, and thus, the studied applications are also numerous. In this review, we focused on the applications related to health. One of the most promising applications of DES is the development of oral liquid formulations of poorly soluble active pharmaceutical ingredients, although it currently remains at an early stage. We have analyzed the potential and limitations of DES with this regard. Furthermore, DES have been used as synthesis media. In this work, we revised the use of DES to obtain bioactive natural products via synthesis or extraction process. Finally, the usefulness of DES in other interesting applications for promoting health has been also examined: this is the case of genomics studies, nano-carriers for the encapsulation of anticancer drugs or stabilization of samples for medical purposes.



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1. Introduction

The scientific and technological progress carried out over time has resulted in many improvements in the quality of life, but also in visible damage and overexploitation of natural sources. The concept of green chemistry, established by Anastas and Warner, appeared as a sustainable development tool [1]. According to the authors, green chemistry is defined as the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances [1] and is based on 12 principles that give guidelines about the chemical processes, synthesis, use of raw materials and chemicals, energy efficiency, security, and health issues with the aim of moving toward sustainability. In the last decades, the development and use of new solvents, which follow the assumption of the green chemistry principles, have increased considerably with the objective of substituting polluting traditional solvents. Moreover, it is necessary that these new solvents show similar physicochemical properties to traditional solvents and are efficient for the applications desired. In addition, a low toxicity to the environment and health and a high biodegradability is necessary to promote their use. These kinds of solvents can be named as green solvents [2–4].

In the last few years, a new class of substances have emerged as promising green solvents, the so-called deep eutectic solvents (DESs). These new DESs have emerged as a realistic and tighter alternative than previous attempts [5,6], providing a new type of substance with better environmental and health properties, low production costs [7], and better biodegradability profiles [8].

DESs can be defined as a mixture of two or more chemicals (hydrogen bond donors and acceptors) that are solid at room temperature but when combined at a particular molar ratio, present a melting point depression and become liquid [9]. Generally, the components of the mixtures are safe and cheap [10]; more typical are quaternary salts such as choline

chloride or betaine, but others such as thymol, menthol, decanoic, or lauric acid have been used [11], acting as hydrogen bond acceptors. As hydrogen bond donors, it is common to use urea, amino acids, sugars, alcohols, amides, amines, or carboxylic acids [12,13].

In fact, the components of DESs are directly associated with their properties and thus can be designed by properly combining the components [10]. In general, DESs show several properties such as low preparation costs, environmentally-friendly, wide polarity range, low vapor pressure and volatility, non-inflammability, thermal and chemical stability, biodegradability, low toxicity, etc. Additionally, they present many possible combinations to obtain different kinds of solvents depending on their uses [14,15]. Components of DESs can interact through intermolecular forces, not only via covalent or ionic bonds and thus are considered as good candidates to replace ionic liquids or traditional solvents [16].

A few years ago, a new type of DES arose when Choi et al. [17] noticed that mixtures of natural products such as choline, sugars, and amino acids had a similar behavior to that of a DES when combined properly. These authors named these products as natural deep eutectic solvents (NADESs) [12]. At least 174 DESs have been described in the literature [16].

Due to the nature and versatility of DESs, these mixtures are attracting attention from the academic and industry sectors. It has been demonstrated that DESs can be used for many applications such as synthesis [18–20], separation processes [21,22], extraction [23–28], biocatalysis [29], nanomaterials, biotechnology [12,13], electrochemistry [30,31], food [32], cosmetics, pharmaceuticals [33–38], or as a biofuel [12,14,16,25,39]. DESs are also useful in the pharmaceutical industry as excipients for the delivery of hydrophobic drugs or as a vehicle [40]. In fact, the pharmaceutical industry requires the use of organic solvents as media for poor soluble drugs and DESs seem to be promising alternatives in this area [33]. Considering some of these properties, in this work, a revision focused on the health field applications was carried out. Figure 1 shows the most important DESs, properties, and main applications of this type of mixture.

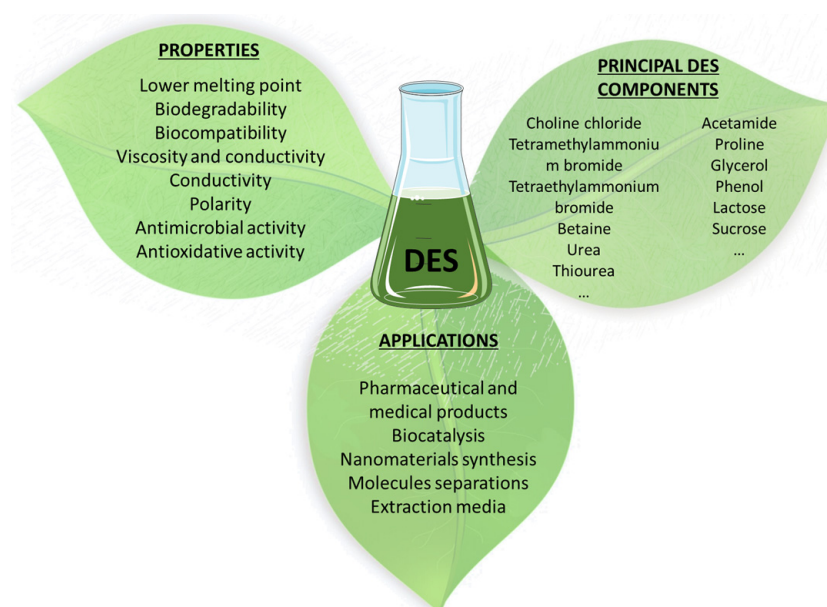


Figure 1. Properties and applications of the most important DESs.

2. Applications Related to Peculiar Solubility Properties of DESs

Considering the growing interest in the use of solvents which allow solubility to be improved in health application, DESs seem to be a good alternative [41]. Therefore, a solution for drugs with poor water solubility is necessary. Currently, to solve this problem, some mechanisms have been used such as new dosage forms, use of other chemical

structures (salts, esters), prodrugs, active metabolites, different routes of administration, etc. [42].

In fact, the use of adequate media for dealing with active pharmaceutical ingredients (APIs) with a poor water solubility is one of the most important challenges for the pharmaceutical industry. It is well known that solubility has a direct relationship on a therapeutic effect of a drug [43]. In addition, in the last few years, several studies have focused on the use of eutectic mixtures in the pharmaceutical area with the aim of improving the solubility and bioavailability of drugs [40,44]. In Figure 2, a scheme of the evolution of these new solvents is shown.

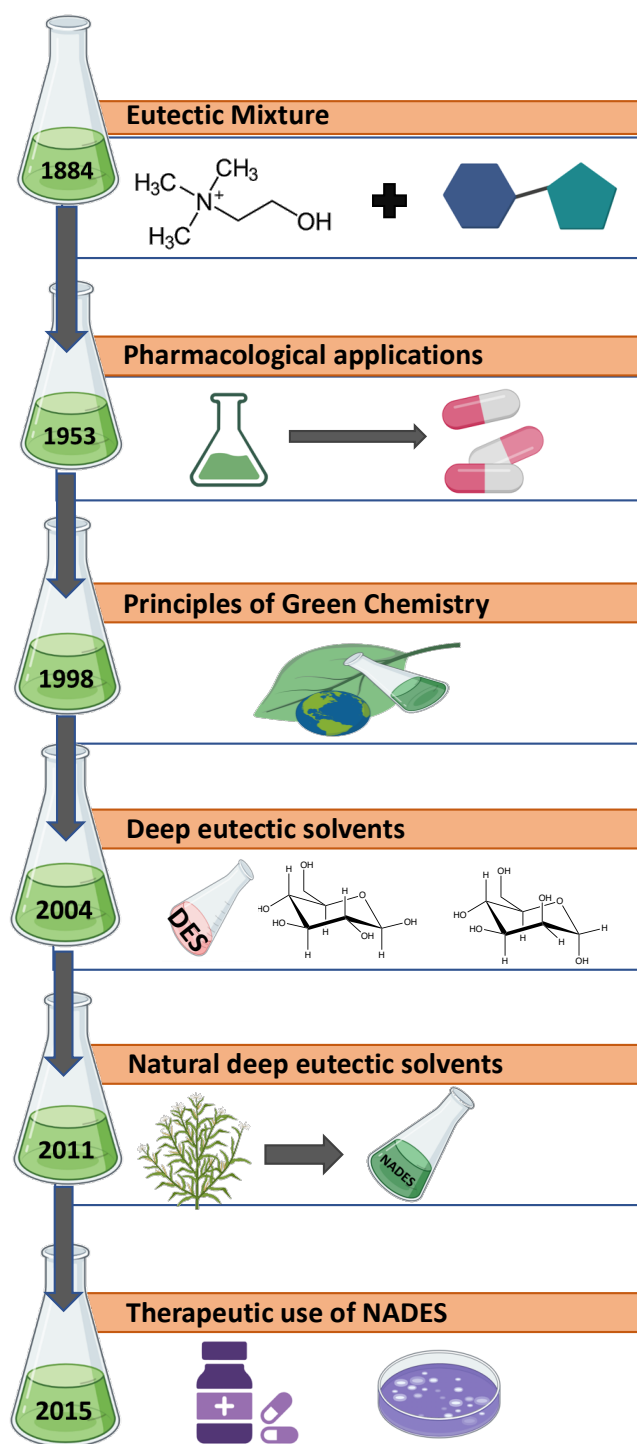


Figure 2. Evolution of DESs over time and their relationship with pharmaceutical applications.

Choline-based DESs are one of the most common eutectic mixtures found. The choline cation can be combined with different chemicals (alcohols, fatty acids, carboxylic acids, . . .) due to their ability as hydrogen bond acceptors. These kinds of mixtures allow one to increase the solubility of diverse drugs [33]. As a matter of fact, a mixture of a choline chloride-based DES with urea (ChCl:U (1:2)) increased the solubility of benzoic acid and AMG517 by 80 and 100 times, respectively, and the mixture of the same cation with malonic acid (ChCl:MaLA (1:1)) reached a solubilization of 320 and 20,000 times higher than that for the solubilization of danazol and AMG517 with respect to pure water [40]. Similarly, several reports have indicated that binary and ternary eutectic mixtures have been used to dissolve itraconazole, piroxicam, lidocaine, and posaconazole and the solubilization ability was augmented in all situations in comparison to water [45,46].

Other substances such as menthol, borneol, and camphor have been used as hydrogen bond acceptors to prepare eutectic mixtures and as vehicles for transdermal drug delivery, analgesic, antimicrobial, anti-inflammatory, and antitussive compounds [47] of testosterone, ibuprofen, lidocaine, ubiquinone, captopril, or fluconazole have been used. For instance, Yong et al. studied the *in vitro* dissolution of poorly water-soluble ibuprofen using polaxamer and menthol in an aqueous solution. It was observed that a relation 4 ibuprofen:6 menthol increased its solubility by 2.5, however, when the polaxamer was included, a 6-fold increase in the aqueous solubility of the drug was found [48]. Kamal and Haghtalab observed that the solubility of cefixime was increased when a mixture of cefixime and DES was prepared. In this case, DES was formed by choline chloride and glycolic acid in a 1:2 molar ratio [49]. The solubility of other active pharmaceutical ingredients with DES has also been analyzed. Another report has studied the solubility of nonsteroidal anti-inflammatory drugs (aspirin, acetaminophen, ibuprofen, ketoprofen, and naproxen) in the presence of deep eutectic solvents, observing that their solubilities increased by 100- to 5400-fold over water solution [50,51].

In Table 1, a summary of the drug solubility comparison between water and DES is presented.

Table 1. List of drug solubility in DESs.

Eutectic Mixture	Drug	Increased Solubility with Respect to Water Solution (T = 298.15 K)	Reference
Choline chloride and urea (1:2)	Benzoic acid	80 times	[40]
	AMG517	100 times	[40]
Choline chloride and malonic acid (1:1)	Danzol	320 times	[40]
	AMG517	20,000 times	[40]
Choline chloride and glycolic acid (1:2)	Itraconazole	6700 times	[45]
	Piroxicam	430 times	[46]
	Lidocaine	2. times	[46]
	Posaconazole	6400 times	[46]
Choline chloride, glycolic acid, and oxalic acid (1:1.7:0.3)	Itraconazol	50,000 times	[45]
	Piroxicam	135 times	[46]
	Lidocain	81 times	[46]
	Posaconazole	7400 times	[46]
Menthol	Ibuprofen	2.5 times	[48]
Menthol:Poloxamer		6 times	[48]
Choline chloride and glycolic acid (1:2)	Cefixime trihydrate	2418 times	[49]

In addition, other studies have focused on the application of DESs in terms of improving solubility, and more specifically for pharmaceutical applications. Thus, Araya-Sibaja et al. improved the solubility of the drug lovastatin in three binary eutectic mixtures with three different carboxylic acids (benzoic, salicylic, and cinnamic carboxylic acids), being higher for the system formed with salicylic acid. Moreover, the systems showed stability since there was no dissociation and the results achieved indicated that binary eutectic mixtures can be excellent for the preparation of pharmaceutical formulations [44]. Accordingly, another report observed that the use of a DES, composed of betaine and urea, stabilized two therapeutic B-lactam molecules, clavulanic acid and imipenem, with respect to the use of water and with no effect on their antimicrobial activity [52]. On the other hand, NADES can play an important role in small-molecule formulations such as hydrogels or as a vehicle of lipophilic bioactive ingredients [16]. Moreover, NADES also have an analogous structure to cyclodextrin, and thus can be used as an excipient in hydrophobic drug delivery systems [53].

Thereby, DESs could be interesting alternatives in pharmaceutical formulations, however, more knowledge is necessary at preclinical and clinical phases of implementation as well as their toxic effects in organisms.

Pharmaceutical Applications: Therapeutic Deep Eutectic Solvent-THEDES

The development of bioactive eutectic systems containing active principles increases the potential of these systems and opens a wide spectrum for future developments in pharmaceutical and biomedical applications.

One of these techniques, which can be used to increase the solubility of drugs, is related to the use of THEDES. A THEDES (DES + active pharmaceutical ingredient (API)) and their transdermal drug delivery was first described in 1998 by Stott et al. [54] who showed some mixtures of an API with different enhancers of skin permeation (terpenes). Additionally, two manuscripts reported an increase in the solubility of benzoic acid, danazol, and griseofulvin, an itraconazole using urea–choline chloride and malonic acid–choline chloride DES [9,40].

At the topical level, it was in the 1980s when the first eutectic mixture formulation (EMLA) was marketed, containing an 80% eutectic mixture of lidocaine and prilocaine [33,55]. In another study carried out by Gohel and Nagori, the release of fluconazole in a system made up of this type of substance and prepared as a transdermal spray was carried out to evaluate the antifungal activity in vivo in patients with ringworm was also evaluated. The results were also positive, demonstrating that this type of system improves drug release [56]. Finally, it has also been proven that some NADES combined with collagen allowed for the chemotactic properties and structure of collagen in topical formulations to be retained [57].

In a study carried out by Wang et al., the lidocaine:ibuprofen THEDES was developed and the permeability of API through a porous membrane was reported. Additionally, it was demonstrated that some differences between the dissolution and permeation of the API because of the formulation [9,58].

Some other THEDES based on choline chloride and menthol with acetylsalicylic acid, benzoic acid, and phenylacetic acid has been also described. The dissolution rate was studied, and it was found that the antibacterial activity of the API was retained for all THEDES [9].

Recently, a paper has been published using a choline and geranic acid deep eutectic solvent. In this case, this compound has been used for the treatment of rosacea; the study describes how to apply this THEDES from the synthesis to the medical application and shows the scale-up, characterization, stability analysis, mechanism of action, dose analysis, GLP toxicity, and human clinical study [59].

One of the key points that limit the use of THEDES is the lack of evidence related with safety, toxicity, and some other pharmaceutical issues such as pharmacokinetic behavior. However, there have been some studies that point out the safe use of most DESs.

Regarding the toxicity of DES, it has been tested both in vitro and in vivo. Focusing on in vitro tests, several analyses in bacteria (*Allivibrio fischeri*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Aspergillus niger*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, and propionibacterium) [60–62], virus (Herpes simplex), fish cells (CCO) [63], human cells (MCF-7, PC3, A375, OKF6, HepG2, HT29, and H413) [64], fungi (*Aspergillus niger*, *Candida albicans*, *Candida cylindracea*, *Lentinus tigrinus*, *Panerochaete chrysosporium*) [47] and plants (*Triticum aestivum* or *Allium sativum*) have been measured [65] while in vivo, some studies in animals (mice) [66] and marine organisms (*Hydra sinensis* and *Cyprinus carpio*) have been carried out [67,68].

Silva et al. studied the therapeutic role of DESs based on menthol and saturated fatty acids. They prepared and characterized some DESs using menthol and lauric acid, stearic acid, and myristic acid. After the preparation, they studied their cytotoxicity in the HaCaT cell line. They also carried out an antibacterial assay using *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. No significant halo has been observed for pure compounds, however, all THEDES presented antimicrobial activity being more effective at higher concentrations. These results can be explained because of the molar ratio (1 menthol:8 saturated fatty acid). Menthol amount was lower in THEDES than the pure compound, nevertheless, a synergistic interaction between menthol and saturated fatty acid has been observed [14]. Additionally, Silva et al., in another paper, prepared a THEDES, formed by perillyl alcohol and ibuprofen, and studied their antimicrobial activity and the cytotoxic profile in colorectal cancer (CRC). They observed that this system presented a possible alternative to conventional therapies because of its properties against microorganisms and toward CRC cells [69].

Roda et al. developed a THEDES delivery system for the treatment of tuberculosis. The system was prepared by encapsulating L-arginine based THEDES with a lipidic matrix and using supercritical technology. They analyzed the cell viability and fibroblast with THEDES encapsulated and concluded that the system was non-cytotoxic [70].

Pereira et al. evaluated the THEDES system based on limonene and saturated fatty acids, menthol, or ibuprofen for cancer treatment. The THEDES system presented anti-proliferative properties, however, they observed that THEDES formed by ibuprofen and limonene (1:4) was able to inhibit HT29 proliferation without compromising cell viability. In addition, they observed that this THEDES (ibuprofen and limonene) increased the anti-inflammatory activity, an important key to cancer treatment [71].

THEDES seems to be another promising solvent in order to improve the solubilities of the drugs and is important in parameters such as absorption and permeability. Their characteristics allow them to have good applications in the biomedical and pharmaceutical sectors. In this sense, several terpenic compounds, mainly menthol and camphor, have been demonstrated to improve solubility and dermal adsorption of different drugs, allowing for different formulations such as in the field of permeation enhancers [33].

Table 2 summarizes the successful development of several THEDES systems (API-DES) and the different kinds of studies carried out.

Table 2. Examples of API-DES reported by the literature, year, experimental study, achievement, and administration route.

THEDES System	Year	Experimental Study	Achievement	Administration Route	Reference
Ibuprofen:L-menthol Ibuprofen:thymol Ibuprofen:D-limonene Ibuprofene:cymene Ibuprofene:L-menthone	1998	Human epidermal membranes	Solubility enhancement	Transdermal	[54]
Methyl nicotinate:ibuprofen	2000	physicochemical studies	Enhance transdermal delivery	Transdermal	[72]
Menthol:coenzyme Q10	2002	Physicochemical studies	Formulation of self-nanoemulsified drug delivery system (SNEDDS) to improve the solution of coenzyme Q10	Oral *	[73]
Cannabidiol:phosphotidylcholine	2003	Mice	Enhance transdermal delivery and better accumulation (muscle and skin)	Transdermal	[74]
Fluconazole:camphor:menthol	2009	Rat skin (antifungicide activity, effective in vivo activity)	Improved drug transport	Transdermal	[56]
ritonavir:gelucire	2010	Albino Wistar rats	Increase of rate of absorption	Oral	[75]
Itraconazole:phenol	2012	Franz diffusion cells fitted with excised hairless mouse skins	Permeability enhancement	Topical	[76]
Ibuprofen:lauric acid Ibuprofen:palmitic acid	2013	Physicochemical studies	Information drug-exciptient and its compatibility	Topical *	[77]
Nimesulide:PEG Nimesulide:urea	2014	rats	Increase of the analgesic effect	Oral	[78]
Coenzyme Q10:lauric acid	2015	Physicochemical studies	Solubility enhancement	Topical	[79]
Lidocaine:tertracaine Lidocaine:camphor	2015	physicochemical studies	Liquid formulation	Topical *	[80]
acetylsalicylic acid:ChCl acetylsalicylic acid:menthol	2016	Microbiology studies in <i>E. coli</i> , <i>S. aureus</i> , <i>B. subtilis</i> .	Enhanced transporters and delivery vehicles for bioactive molecules	Oral *	[9]
Ibuprofen:lidocaine	2017	Transdermal Bioavailability in rats	Enhancers of skin permeation	Transdermal	[81]
Resorcinol:ChCl acetylsalicylic acid:ChCl salicylic acid:ChCl Paracetamol:ChCl ranitidine:glycerol diphenine:glycerol phenformin:glycerol ticlopidine:glycerol tetracycline:glycerol ranitidine:urea. adiphenine:urea adiphenine:acetylsalicylic acid	2017	Physicochemical studies	Solubility enhancement	Oral *	[82]

Table 2. Cont.

THEDES System	Year	Experimental Study	Achievement	Administration Route	Reference
Paeonol:menthol	2017	In vitro permeation and deposition study on mouse skin	Transdermal delivery	Transdermal	[83]
Ibuprofen:menthol	2017	Physicochemical studies	Solubility and permeability increases	Oral *	[84]
Felodipine:nicotinamide Felodipine:malonic acid	2017	Animal model	Improvement of AUC compared to free drug solution	Oral	[85]
Hydrochlorothiazide:atenolol	2017	Female Wistar rats	Decrease in systolic blood pressure was more pronounced when it was compared to physical mixtures	Oral	[86]
alpha-eprosartan:p-hydroxy benzoic acid	2017	Female Sprague-Dawley rats	Oral bioavailability and AUC increases	Oral	[87]
Ibersartan:nicotinic acid Ibersartan:ascorbic acid	2018	pharmacokinetic/pharmacodynamic studies and oxidative stress analysis in rats	Improvement of AUC	Oral	[88]
Caffeine:meloxicam Caffeine:aceclofenac Caffeine:flurbiprofen	2019	Male Wistar rats	Increase in solubility and effect	Oral	[89]
Ibuprofen:Limonene	2019	HT29 cell line	Enhancement of anti-inflammatory activity of ibuprofen	Oral *	[71]
Ibuprofen:1-tetradecanol Ibuprofen:1-octadecanol Ibuprofen:1-docosanol	2020	Physicochemical studies	In vitro release	Topical *	[90]
Diacerein:fumaric acid	2020	Pharmacokinetic study in Sprague-Dawley rats	Improvement of C _{max} and AUC	Oral	[91]
Diacerin:2,4-dihydroxybenzoic acid	2020	Pharmacokinetic study in Sprague-Dawley rats	Higher bioavailability and lower t _{max} compared to pure drug and	Oral	[92]
Citric acid:L-arginine:H ₂ O	2020	L929 fibroblasts and system characterization	Design and development of drug formulation	Inhalatory *	[70]
Lidocaine:ketoprofen Lidocaine:flurbiprofen Lidocaine:aceclofenac Lidocaine:meloxicam Lidocaine:tenoxicam	2021	Albino male rabbit ear model. Skin permeation studies using vertical glass Franz diffusion cells	Use of lidocaine as eutectic co-former for enhanced skin delivery of NSAIDs.	Transdermal	[93]
Ezetimibe:simvastatin:fenofibrate	2021	Physicochemical studies	New way of selecting therapeutic concentrations	Oral *	[94]
Curcumin:choline geranate deep eutectic solvent	2021	Domestic pig ears	Increase the transdermal permeation of curcumin, insulin, and bacteriophage particles. It will be use as non-invasive delivery	Transdermal	[95]

* This information is not described in the original manuscript, however, it was considered according to the original API application.

3. DES as Synthesis Media of Natural Based Chemicals

DESs have been widely used as green synthesis media since early 2000, timidly at first (with just a dozen articles a year), and with much greater intensity a few years later (Figure 3, Web of Science).

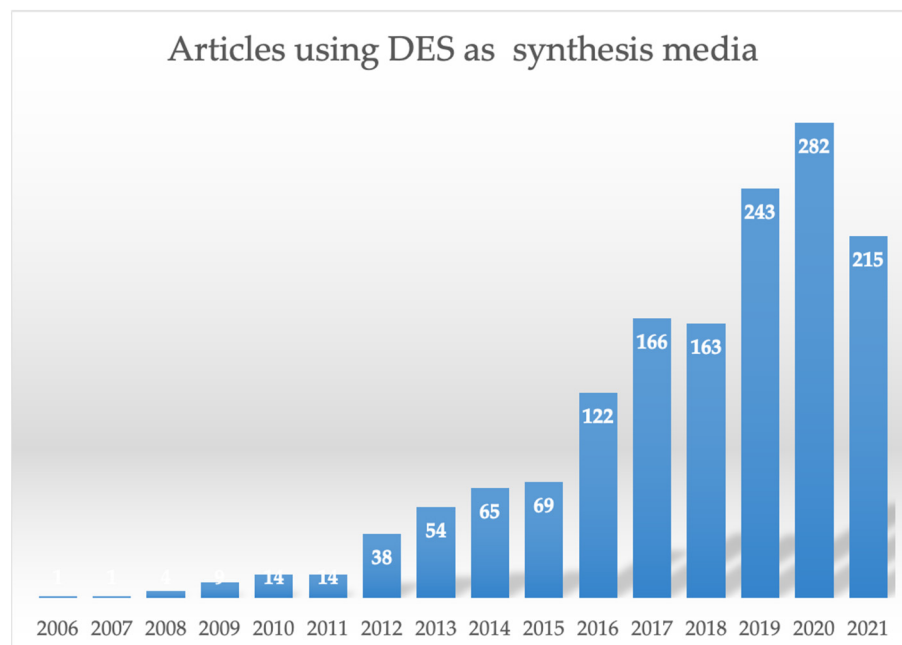


Figure 3. Manuscripts in which DESs have been used as synthesis media from 2006 to 20 October 2021. Source: Web of Science.

During 2000, DES have been used both as the solvent of the reaction and the source of the template (structure-directing agents); the so called-ionothermal synthesis. This strategy provided opportunities to develop new synthetic routes for the preparation of several organometallic chemicals [96–101].

At the early stage of the research, the green character of these compounds is already visible (biodegradability, reuse, low volatility) while unexpected catalytic properties have been found [102,103]. Quickly, DESs began to be studied as a mean of synthesis in the preparation of many types of chemical substances of a very different nature; inorganic materials such as silicas, metal charlcogenides, metal structures, organosilicas, metal oxides or metal salts [104], polymers [105], micro/nano structures such as microtubes [106], microporous materials [107], nanowires [108], or carbon absorbent [109] among many others [110].

Focusing on preparation of chemicals with natural bases, DES has been used in the synthesis of biodiesel. Habitually, biodiesel is prepared from animal fats or vegetable oils or biomass such as yeasts, molds, algae, soybean, or pine trees [111]. The traditional processes for preparing biodiesel present several limitations such as emulsification problems, corrosion and energy, waste production, and the saponification of fatty acids, among others. Investigations on the preparation of biodiesel using ionic liquids or DESs by means of enzymatic routes have revealed that less waste is produced and not such severe conditions are needed [111]. Nevertheless, these new processes are still challenging, and some difficulties must still be overcome (the compatibility with non-aqueous solvents, for instance).

On the other hand, DESs have been widely used for biotransformations such as (trans)esterifications, polymerization of lactone, aminolysis, or epoxidation involving lipase-catalyzed processes such as in [112] or epoxide hydrolase reactions [113].

Regarding the synthesis of natural products with biological and pharmaceutical applications, DESs have been useful in the preparation of quinazolinones (marketed as drugs due to their anticonvulsant, hypotensive, sedative, antidepressant, anti-inflammatory,

and anti-allergy properties) mediated cyclization strategy. Results indicated that the new process was cheaper, since the starting material, anthranilamide, is low-priced with respect to the initial raw material and greener, in good to excellent yields [114]. The same strategy was used to synthesize another type of alkaloid, a penipanoid type, with successful results [115].

Another important feature in the use of DESs for the synthesis of natural products is the facility of reuse and the decrease in the waste produced. For example, Annes et al. synthesized 2H-chromene (structural motif in natural products with important biological activity) via DESs in moderate and good yields, with the advantage of being able to recycle the used DES at least five times [116].

Finally, another popular strategy is the use of DES as biocompatible building blocks as a reactant, medium, and/or catalyst in the design of green novel synthetic protocols. The reaction of 4-chlorobenzaldehyde, malononitrile, and hydrazine hydrate was studied under two different reaction protocols (one-pot stepwise pattern and adding reactants simultaneously) in the presence of various deep eutectic solvents, without using any extra catalyst. Once again, the DES was reused and recycled four times with little loss of reactivity [117].

4. DES in Extraction Processes of Bioactive Products

DES, but more specifically, NADES based on natural compounds, have been selected as optimal solvents for extraction processes because of their properties [10,16,118]. NADES have superior solubilizing ability for natural products and are usually nonvolatile [16]. For example, Dai et al. (2014) found that NADESs have been able to enhance the stability of the extracted phenolic compounds [119]. The biocompatibility of NADES with biomaterials has even generated interest in biotechnological industries since they are also suitable solvents for the extraction of natural bioactive compounds from different organisms with higher efficacy compared to the conventional ones, as shown in different previous reviews [12,67]. Moreover, DES have been successfully used for the extraction of proteins very efficiently [120,121]. For example, NADES are more useful as dissolving media in biological assays in substitution of DMSO [16].

In relation to health, we are going to focus our attention on some studies on the extraction of bioactive compounds from medicinal plants, although DESs have been widely used for the extraction of other kind of compounds, with different ranges of hydrophilicity. This is because DES and NADES can include different combinations of compounds, and molar ratios of each component can be modified, allowing for a wide range of tailored designs, apart from the development of innovative extraction techniques [16,122,123]. The most common solvents include choline chloride as a hydrogen bond acceptor [123]. It has been demonstrated that a higher efficiency can be obtained using them instead of conventional solvents. Most bioactive compounds extracted with them include flavonoids, phenolic acids, and anthocyanins [123]. These kinds of extractions involve plants such as *Chamaecyparis obtusa* [124], *Larrea cuneifolia* [125], *Cinnamomum camphora* [126], or *Scutellaria baicalensis* [127], among others.

Nahkle et al. published a review of the microextraction techniques such as liquid-phase microextraction; hollow-fiber liquid-phase microextraction; dispersive liquid-liquid microextraction methods; deep eutectic solvent based on an aqueous two-phase system; ultrasound; microwave extraction; vortex extraction; heating and stirring or solid-phase extraction. They presented the advantages and disadvantages of each extraction method and showed how several properties (melting point, density, viscosity, surface tension, pH, solubility, or polarity) could directly affect the extraction efficiency [24].

Socas-Rodríguez et al. presented an important summary where they showed some studies that have used DESs as the extraction solvent. They collected information regarding the used sample, extracted bioactive compounds from plants, fruits or vegetables, DES (molar ratio and water content), extraction method, and, finally, analytical technique [128].

The food and pharmaceutical industry have also explored the application opportunities of DES due to the potential use of extracts as food additives, mainly, or even as candidate pharmaceutical agents because of their antimicrobial and antioxidant activity [129,130]. Therefore, extractions include the use of DESs with by-products of fruits such as grape skin or grape pomace or from onion seed and olive oil, tomato, or pear industries, among others [129,131,132]. This can be a way to revalorize agro-food industry waste or by-products. There is an additional application of NADES to increase food safety as reviewed by Misan et al. (2019) or Chen (2019) in an attempt to remove harmful toxins from food ingredients [130,133].

A recent review carried out by Redha showed a very good summary of several studies where some phenolic compounds were extracted from natural sources such as *Morus alba*, *Olea europaea*, *Junglas regia*, *Lycium barbarum*, *Citrus aurantium*, *Citrus paradise*, *Lonicera japonica Thunb*, *Camellia sinensis*, or *Citrus sinensis*, among others. In this manuscript, they showed the DES or NADES used (providing information about HBA and HBD and their molar ratios), the extraction method (detailing the temperature, times, frequencies, etc.), and the obtained yield in each method [134].

Moreover, bioextracts could even be used for dermal formulations as an antifungal agent in a pharmaceutical application [135].

Very recently, DESs have also been tested as a capillary coating for in-tube solid-phase microextraction for the bioanalysis of different kinds of biological samples [136].

The biological activity of DES/extract systems on cells have also been demonstrated. The individual components of NADES such as malic acid, citric acid, proline, and betaine possess antioxidative activity [137–139].

Grillo et al. studied the residues of blueberry processing used as a source of anthocyanins, which are valuable metabolites that possess a wide range of antiproliferative activity. In this study, choline chloride:lactic acid (ChCl:LA) was selected as the most suitable NADES based on extraction efficiency, cost, viscosity, and toxicity. This biological activity was tested in the HeLa cell line and compared to human skin cells (HaCaT). This antiproliferative effect observed with these extraction technologies using green solvents could be interesting for the food industry [140].

Moreover, Guo et al. showed that the essential oil obtained by deep eutectic solvent-homogenate based microwave-assisted hydrodistillation (DES-HMAHD) presented higher antioxidant activity, but lower antifungal activity, and these properties were related to its chemical composition [141].

5. Other Interesting Applications

It is also remarkable that DES can be used in many other applications of a very different nature, which shows the great versatility of this type of mixture. For instance, some molecular analytical methods are based on the use of biological samples that have been previously preserved. DESs have been used successfully for the stabilization of different kinds of samples prior to their analysis without interfering with the methods [142]. NADES can also improve the analytical method for the analysis of different drugs (aspirin, atorvastatin, metformin, metoprolol), as shown recently by Ramezani et al. (2020) [143].

Additionally, some authors have demonstrated the usefulness of DESs as antibacterial activity for the improvement of different candidate materials for medical purposes [144]. Gronlien et al. (2020) described the potential use of NADES with collagen in a practical application combining wound healing by collagen peptides and the antibacterial properties of the NADES [57].

More recently, even NADESs have been prepared to be used as nano-carriers for the encapsulation of anticancer drugs to prevent and treat breast cancer [145]. In addition, DESs can act as therapeutic agents by themselves. Hayyan et al. (2015) showed that different DESs have been toxic to some cancer cell lines such as human prostate cancer cell line (PC3), human malignant melanoma cell line (A375), human liver hepatocellular cell line (HepG2) and human colon adenocarcinoma cell line (HT29), human breast cancer

cell line (MCF-7), human oral keratinocyte cell line (OKF6) cells, and carcinoma-derived human oral keratinocyte cells (H413). Besides, toxicity in normal cells was lower than the cancerous ones [146]. Although certain types of DESs have been used as effective drug deliverers or even have medical or pharmaceutical activities, it is recognized that further investigation is needed prior to clinical use [41].

Metals and metal salts present high solubilities and electrical conductivities in DESs, so they are good candidates for the extraction/recycling of metals in solution. In addition, DESs can be used in industrial separation processes [147]. The study carried out by Oliveira et al. showed that the ChCl:levulinic acid DES had the highest distribution coefficient for separating ethanol from heptane [148]. The application of DES as media for the extractive desulfurization of fuels (e.g., SO₂ sequestration) is also growing in importance. In this area, DESs have also demonstrated some interesting properties in improvements in carbon dioxide (CO₂) capture [138].

On the other hand, DESs have been used in genomics, incorporating them in the study of genome and the nucleic acids (DNA/RNA) of organisms. DNA in DES dissolution showed novel behaviors of this nucleic acid [149].

6. Conclusions

DESs are tremendously versatile, being used for an important number of applications of very different natures. The main reason that explains this usefulness is the great solvation capacity that these mixtures show against different types of substances. This may be due to the supramolecular structure formed, where solutes can be dissolved inside the holes of this matrix (hole or liquid crystal theory) [17] and that the hydrophilic–hydrophilic interactions between solutes and DES components that make the solute form part of the DES matrix (binding theory) [150].

This solubility boosting makes DESs a potential answer for the well-known challenge of developing a formulation of poorly soluble API, which pharmaceutical science has been addressing for a long time. Furthermore, the possibility of including the API into the DES structure (THEDES) is another direction of research. With this regard, multidisciplinary studies (chemical and pharmaceutical) are needed since a lack of rigorous studies during the formulation processes (in vivo toxicity, pharmacokinetics studies, etc.) has been detected.

When DESs are used in the extraction and synthesis processes of natural compounds, the solubility and biocompatibility of building blocks are not unique reasons that make these mixtures so special: their reuse, recyclability, and benign environmental properties are also points in favor of eutectic mixtures. Therefore, it is expected that research into this field related to the obtention of high added value biological substances via DESs will continue to increase.

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