Deep Eutectic Solvents in Pharmaceutical Industry

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Neoteric solvents emerged as an alternative to organic solvents and are commonly called green solvents, since they have low toxicity, are biodegradable, are made with accessible and low-cost materials, and are quite easy to produce. Deep eutectic solvents (DESs) are defined as a mixture of two or more pure compounds, which, when combined in an appropriate ratio, give rise to a eutectic mixture that deviates from the ideal thermodynamic behaviour. This deviation is due to strong interactions between the initial components that act as hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs). The HBDs and HBAs interact in the DESs to form a dense network of molecules that give them remarkably interesting physical and chemical properties.

Keywords: green chemistry ; sustainability ; neoteric solvents ; deep eutectic solvents ; bioactive compounds

1. Deep Eutectic Solvents Classification

DESs can be classified as hydrophobic or hydrophilic according to their solubility in water [1]. The majority of the DESs are hydrophilic due to the extensive network of hydrogen bonds [2][3]. Hydrophobic DESs are defined as insoluble or very poorly soluble substances in water, composed of two or more compounds insoluble in water [2][4]. Hydrophobic DESs have been successfully applied in several areas, namely for water purification [5], in the preparation of new materials such as magnetic gels, in nanoparticles consisting of carbon nanotubes and graphene for the removal of organic micropollutants and metallic ions from water [6][7], in the capture of carbon dioxide [8], in electrolyte medium for solar cells [9], and for the extraction of bioactive compounds [10][11].

DESs are usually classified according to the type of compounds used in their preparation and are subdivided into four subclasses: natural deep eutectic solvents (NADESs), therapeutic deep eutectic solvents (THEDESs), polymeric deep eutectic solvents (PDESs) and poly-quasi eutectic solvents (PQDESs) [2].

NADESs were discovered in 2011 when trying to elucidate the solubility of intracellular compounds, which were insoluble in water and lipids [2]. NADESs contain in their composition cellular metabolites such as amino acids, alcohols, sugars, and organic acids [12][13][14]. In addition, water can also be part of its composition, forming a ternary system [15]. In nature, this type DESs in different cells and organisms. For example, nectar is nothing more than a mixture of sugars that are solid at room temperature when separated, but liquid when combined. Another example is honey, with such interesting and unique properties that are not yet fully understood, but with tested medical applications [13][16][17][18]. NADESs play a key role in cellular metabolism and in many biological processes such as resistance to drought, germination, and dehydration. In addition, all living organisms have a process called organ cryopreservation, which is a defence mechanism to withstand extreme conditions, such as temperature variations between winter and summer. NADESs act as cryoprotective agents for the simple fact that membranes, enzymes, and metabolites remain stable with the addition of this type of eutectic mixtures [2][12]. In terms of applications, NADESs have been used in biocatalysts processes [19][20], in the extraction of compounds [21][22], in the pre-treatment of biomass [23][24], in electrochemistry for the detection of bioactive materials [25], for drug solubilization [26], for permeation enhancement [27], and as extraction solvents [28][29].

THEDESs emerged as one of the strategies to promote the increased solubility, permeability, and, consequently, bioavailability of drugs [30][31]. THEDESs are a class of DESs that use at least one active pharmaceutical ingredient (API) as one of its components [32][33]. These solvents have raised a lot of interest and THEDESs are currently being studied, namely for increasing the solubility of drugs in aqueous solutions or increasing their permeability in different biological barriers such as the skin or intestinal wall, among others [27][34][35][36].

Another class of DESs are polymeric, so named because a portion of DES is polymerizable [2][37][38]. The polymer, when completely converted, can be used in various applications, namely in nanotechnology [39], electrochromatography [40], and gas capture [37]. In 2017, a new class of DESs was proposed: the quasi-polymeric deep eutectic solvents [37].

2. Deep Eutectic Solvents Synthesis

DESs can be synthesized in various ways depending on the equipment available (**Figure 1**). Independently of the type of equipment that is used, the synthesis involves the mixture of two (or more) components normally without the need of any solvent, and then energy is provided to the system for a certain amount of time in the form of a temperature increase (heating and stirring), irradiation (microwave and ultrasound), mechanical forces (grinding), or a combination between temperature and mechanical forces (twin screw extrusion). There are also methods in which the initial components are dissolved in a solvent (normally water) and then heated in a vacuum to evaporate the solvent (vacuum evaporation), or frozen and lyophilized (lyophilization) [2][41]. Another method that seems interesting in terms of sustainability is the use of concentrated solar radiation [42].

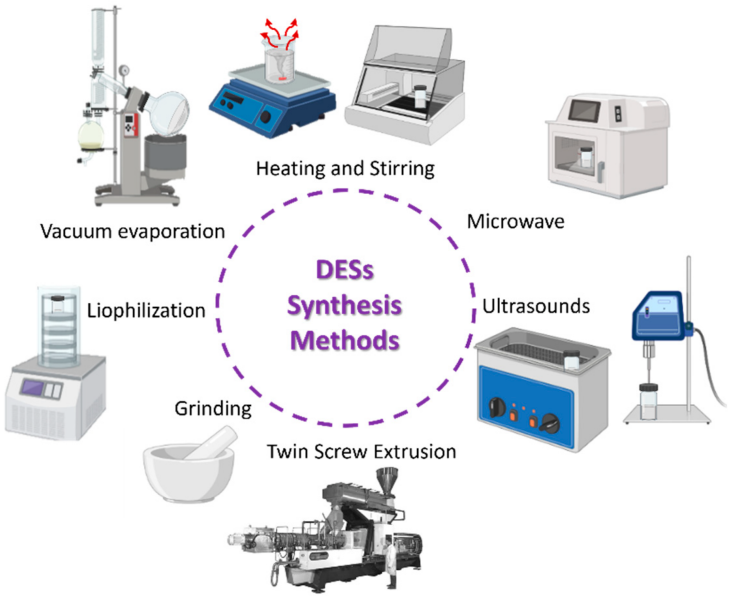

Figure 1. Deep eutectic solvent synthesis methods.

The temperature is important, and it should be carefully chosen due to the possibility of degradation of the initial compounds [2][15][43].

The time needed for the DESs to be synthesized may vary from minutes to hours depending on the method of preparation and on the initial components and their ratio.

3. Deep Eutectic Solvents Properties

Deep eutectic solvents have a set of properties that make them quite useful as extraction solvents. One of the features of DESs is the possibility of being used as extraction solvents for a wide range of solutes [44]. The main characteristic that makes them good extraction solvents is their solvation capacity, that is, the fact that they can accept and transfer protons and electrons, establishing hydrogen bonds with the compounds and retrieving them from their matrix [45]. DESs are known for their enormous capacity to dissolve very poorly soluble metabolites in water. They are also able to dissolve natural products such as rutin, paclitaxel, gingilido b and quercetin, starch, deoxyribonucleic acid (DNA), and high-molecular-weight proteins [29]. Dai et al. [46] verified that small molecules, such as rutin, paclitaxel, gingilido b, increased the solubility values in DESs when compared with water. It was found that, for example, rutin is 50–100 times more soluble in DESs than in water. DESs are also capable of stabilizing natural products. Natural pigments such as carthamine are more stable to light, elevated temperatures, and storage time in various DESs with sugars than in water or a 40% ethanol solution [47]. The same stabilizing effect was later observed in anthocyanins [48]. Recently, the effects of DESs in the stability of phlorotannins extracted from F. vesiculosus was studied for 360 days and compared with their stability and ethanol. It was found that the DESs enabled greater stability than ethanol [49].

Polarity expresses the strength of a solvent; that is, it determines its solvation power and is an important characteristic for a solvent [12][15][50]. The polarity of a DES can be adjusted by changing its constituents, making it more polar or apolar accordingly to necessity, improving the selectivity of the solvent towards a particular bioactive component or class of components. A relative polarity scale could be established, but there are few publications about the polarity of DESs. Among the most used scales is the Dimroth and Reichardt scale; however, these scales are not universal and depend on probes. This means that the polarity parameters obtained by different probes cannot be compared [2][15][51]. Variations in the polarity of DESs depend on the compositions of their individual constituents and are believed to be related to the

molecular structure of the HBD [50][51]. As a rule, polarity increases with increasing intermolecular attractions. Omar et al. [51] found that for the same DES choline chloride/glycerol in different molar ratios of 1:1, 1:2, and 1:3, the polarity values were of 58.49 kcal/mol, 58.00 kcal/mol, and 57.96 kcal/mol, respectively.

The thermal stability of DESs is an important property because it limits the maximum operating temperature at which DESs can be useful. Between the temperature of glass transition (Tg) and the decomposition temperature, the DES maintains its liquid state and the properties that arise from that condition [52]. Delgado-Mellado et al. [52] studied the thermal stability of eight different choline chloride-based DESs and found out that the volatility of the HBDs was the main contributor to the decomposition of the DESs. The authors also emphasized the importance of establishing the real range of operational temperatures for DESs to be able to use them at the industrial level. All DESs are normally glass formers with a Tg below 0 °C; however, this property can be modified with the inclusion of water in the DESs structure due to the plasticizing effect of water. Craveiro et al. showed that an increase of 5 wt% of water in a chlorine chloride/xylitol (2:1) DES decreased the T_g by 4 °C [53]. The presence of water (added or absorbed during preparation) can also influence the thermal stability of the DESs if the water is lost upon heating. This can be observed in thermogravimetric analysis with mass lost around 100 °C due to water evaporation [54].

Water plays a significant role in the physicochemical properties of DESs, and the incorporation of water into DESs either by adding it intentionally or by its absorption from the ambient air is inevitable; therefore, several authors have studied the role of water in DESs. Water can be an HBD or HBA and, in this way, can be a part of the structure of the DES, or it can play the role of the solvent used to decrease the density and viscosity of the DESs. Edler and al. [55] were the among the first to study the effect of water in DESs by studying a series of choline chloride/urea/water DESs by neutron total scattering and empirical potential structure refinement. They found out that until 42 wt% of water the DESs nanostructure is maintained due to the solvophobic sequestration of water into nanostructures domains around cholinium cations. At 51 wt% of water, this segregation is disrupted, and DES–water interactions are dominant, and above this level of water, the mixture was described as an aqueous solution of DESs components. The role of water as an additional HBD was shown by López-Salas et al. [56]. They studied the role of water in a ternary DESs system of resorcinol, urea, and choline chloride by ^{1}H NMR and Brillouin spectroscopy. They realized that the tetrahedral structure of water was distorted as a consequence of its incorporation as an additional HBD or HBA. This fact was confirmed by DSC showing the formation of a new eutectic solvent with a lower melting point when water was incorporated.

Water can also be incorporated into hydrophobic DESs, altering their properties. Kivelä et al. [57] studied the low water absorption by a 1:2 molar ratio of tetrabutyl ammonium chloride and decanoic acid and found out that even extremely low water content causes nanoscale phase segregation, reducing viscosity and fragility, increasing self-diffusion coefficients and conductivity, and enhancing local dynamics. Water interferes with the hydrogen bonding network by solvating the carboxylic acid group.

The existing studies show that physicochemical properties of DESs can be tailored by adding water in a controlled way [58]. The incorporation of water in the DESs can decrease viscosity by enhancing mass transfer from solid samples to the solution and increasing the extraction efficiency, but it also increases the polarity of the DESs making it more suitable for extracting more polar components [59]. However, if too much water is incorporated into the DES, the hydrogen bond network between the DES components can be disrupted ending up with an aqueous solution of the DES components [58].

The toxicity of any compound depends on its ability to cross or interact with biological membranes and is affected if its structure is disrupted [60]. DESs are considered green solvents, presenting a low toxicity, which comes from the use of initial constituents of natural or little toxic origin. However, studies of the toxicity, cytotoxicity and ecotoxicity of DESs and respective aqueous mixtures are still too rare for them to be classified as safe [12][61][62]. Initial studies showed that DESs were biodegradable and non-toxic [63]. However, some DESs proved to be more toxic than their initial constituents [64].

Toxicity and cytoxicity studies made with DESs composed by different HBAs and HBDs in Gram-positive and Gram-negative bacteria and shrimp larva gave diverse results depending on the DESs. Some DESs were shown to be toxic for some of the bacteria used, and their toxicity was associated with the pH and with the charge delocalization between the HBA and HBD. The DESs also showed higher cytoxicity when compared to the initial components. The authors concluded that the lack of oxygen and their high viscosity may be the reason for this behaviour [64]. Fish cells and a human cell line were used to study the toxicity of three choline chloride DESs. One of the DESs showed a moderate toxicity due to the formation of calcium ions in addition with a pH decrease when the DES was added to the culture medium [63]. Lapena et al. [60] studied and evaluated the ecotoxicity of six DESs on algae, bacteria, and crustaceans. The authors concluded that the inclusion of water in the DESs can change the DES toxicity because water can be a part of the DESs or can disrupt the intermolecular forces between the DESs' components. Sanches et al. [65] performed an ecotoxicological screening of

15 DESs using an extensive set of marine and freshwater bioassays. The main conclusion was that none of the DESs presented toxicity; however, both algal assays showed a certain degree of biostimulation, up to over a 100% growth increase in respect to controls, with 8 out of 15 compounds tested with *Raphidocelis subcapitata*. Therefore, their release into aquatic systems may represent a risk leading to ecosystem functioning impairments.

Juneidi et al. [66] evaluated the toxicological profile of ten DESs on fungi and establish that the toxicity of the acidic DESs was higher since it is known that acid compounds can cause cell membrane and protein damage. Nevertheless, the DESs showed a lower toxicity than the respective acids when used isolated. The authors consider that this decrease can be explained by a pH change during the formation of the DESs or by a synergetic effect between the two initial compounds. The acidity of DESs was also considered a problem in a study made by Passos et al. [67] which evaluated the toxicological profile of nine DESs on an enzyme. All the DESs were constituted by sugars, organic acids, and water. The variation in the sugars was found to have no relation with the toxicity of the DESs; however, the acidity of the organic acids was linked to have a direct relation with the increase in the DESs toxicity. Zhao et al. [68] studied twenty DESs that contained amines, alcohols, sugars, and organic acids as HBDs. Toxicity was evaluated for Gram-positive and Gram-negative bacteria. All DESs containing amines, alcohols, and sugars as HBDs showed no inhibition of any of the bacteria. Only the DESs constituted by the organic acids as HBDs significantly inhibited all bacteria (seven DESs out of twenty). Higher inhibition was found for Gram-negative bacteria. The authors concluded that the characteristic acidity of these compounds must be responsible for the damage caused to their outer membrane. This is extremely important, since Gram-negative bacteria have their own external membrane, which acts as a protective barrier, making them more resistant to external aggressions; therefore, these DESs are a hypothesis to combat this type of very resistant bacteria. Li et al. [69] proposed a rating scale for DES toxicity: Type 1, Type 2, and Type 3. Type 1 is the DES that has a higher toxicity then the individual constituents due to new interactions created during the formation of DES. Type 2 is the DES that has lower toxicity then the initial constituents. In this case, the properties that make the initial components toxic are modified in the DESs. Finally, Type 3 is the DESs whose toxicity is the combination of the toxicity of its constituents. The authors studied DESs with amino acids in their constitution and observed, for the first time, that DESs containing amino acids can also present toxicity.

Polar DESs are capable of co-extracting trace elements of metals during the extraction process; however, this has rarely been investigated. Shikov et al. [70] studied the ability of acid-based DESs to co-extract metallic elements from the roots of *Glycyrrhiza glabra* L. and its associated health risks. The authors found that several metals were co-extracted; however, the amount of metallic elements did not pose any health risks. According to the study, the HBA played a decisive role in the extraction of these elements. This type of toxicity should be further investigated since different types of DESs can co-extract elements that can be toxic for human health.

There are few studies that evaluate the biodegradability of DESs. All DESs biodegradability studies follow the Standard OECD No.301 D, which allows the classification of a compound as easily biodegradable or not in an aqueous aerobic medium. According to this standard, to consider a material easily biodegradable, it is necessary that the level of biodegradation is 60% on the 10th day out of 28 for respirometry methods [71]. Lapena et al. [60] studied the biodegradability of six DESs and concluded that the addition of water to the DESs affects their biodegradability, increasing or decreasing it depending on the DES. The number of hydroxyl groups was also a factor in the percentage of biodegradability of the DESs. The same conclusion was retrieved by Radošević et al. [63], who evaluated the aerobic biodegradability of three DESs with components containing different number of hydroxyl groups. The authors concluded that the higher the amount of hydroxyl groups, the higher the percentage of biodegradability.

The density of the DESs is an extremely important property due to the implication it has on their use and handling. Very high densities can cause the DESs not to flow, which can impair their processing. The density values of DESs are higher than those of their pure constituents, and as a rule, DESs have values higher than those of water, except for hydrophobic DESs [15][50][51][72][73]. DESs are usually highly viscous solvents, which can impede mass transfer and decrease the extraction efficiency [45]. Viscosity translates resistance to deformation at a given shear rate of a given fluid [1][43][74][75]. A liquid with low viscosities flows very easily, while more viscous ones flow more slowly. This is particularly important, as it will influence and determine its commercial applicability and the cost of the process [12][73][74]. The high density and viscosity of DESs can be circumvent by adding water to the DESs and/or handling them at temperatures higher than the ambient temperature.

One of the characteristics of the DESs is their low vapor pressure. This intrinsic characteristic of these compounds can be an advantage or a disadvantage depending on the application. In an extraction process, it is preferable that the DESs have a lower vapor pressure, considering that the extraction temperature is reached without the loss of extraction solvents by evaporation. However, if we intend to separate the DES from the extract a posteriori, a low vapor pressure is a

disadvantage because it hinders its evaporation, unless the DESs can be incorporated in the extracts or other methods are used to separate the DESs from the extracts [76][77][78].

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