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Possibility of replacing conventional organic solvents with green Deep Eutectic Solvents in drug analysis

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Abstract

In recent years, Deep Eutectic Solvents (DESs) have attracted increasing attention in various scientific disciplines. Their unique properties, such as low toxicity, biodegradability, low cost and versatility, have made them an attractive alternative to conventional organic solvents. In pharmaceutical analysis, where the demand for environmentally friendly methods is growing, questions regarding whether DESs can replace organic solvents as "green solvents" have arisen. This paper explores the potential applications of DESs in drug analysis, and highlights the benefits and challenges they bring. Examples of the use of DESs in chromatographic techniques and in the extraction of drugs from various samples are presented. Additionally, research on the stability and toxicity of DESs in analytical applications is discussed. Based on the available data, it could be concluded that DESs have the potential to become an important component of analytical methods in pharmaceutical analysis and provide environmentally sustainable and efficient alternatives to conventional organic solvents. However, further research is needed to better understand their properties and optimize their application in drug analysis.

Key words: Deep Eutectic Solvents, green solvents, liquid chromatography, drug analysis

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Introduction

Due to increasing awareness of the Earth's pollution, the United Nations, under the panel "Transforming our world: The 2030 Agenda for Sustainable Development," has emphasized the importance of replacing conventional organic solvents with alternative "green solvents" (1). Deep eutectic solvents (DESs) were first discovered nearly two decades ago (2), and since then they have been continuously at the forefront of research. The first preparation of eutectic solvents, along with their definition, was provided by Abbott et al. (3). Eutectic solvents are a mixture of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), resulting in a mixture with a melting point lower than that of the individual components. However, in addition to the given definition, other definitions describing eutectic mixtures can be found in the literature, such as mixtures of Lewis and Brønsted acids and bases, which may contain various cationic and anionic species (4). Figure 1 depicts the DESs formation diagram.

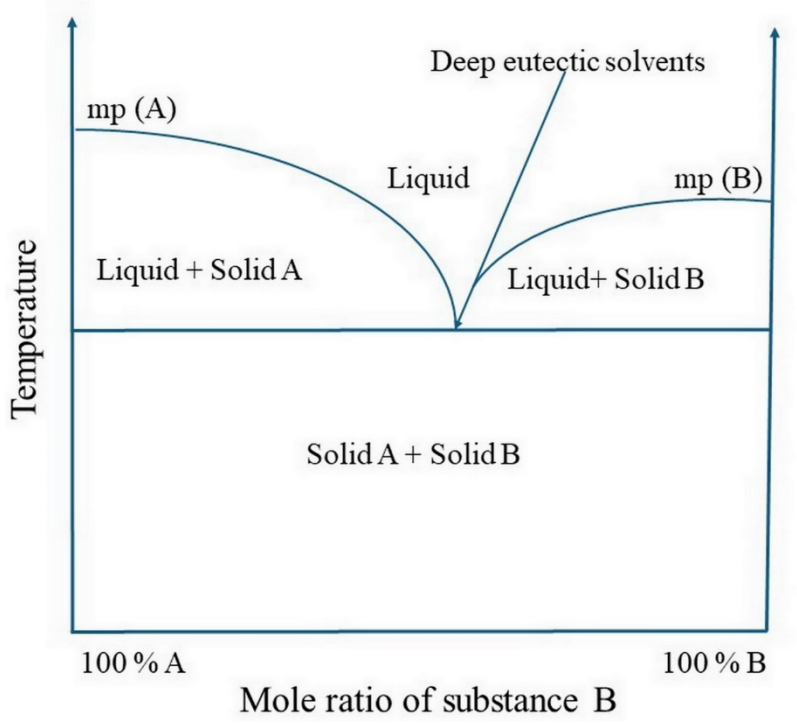

Figure 1. Deep Eutectic Solvent formation diagram
Slika 1. Dijagram formiranja eutektikih smeša

In the literature, a distinction is made between two types of solvents: eutectic solvents and ionic liquids (Ils). Ils were first described in the literature in 1914 and shortly afterwards became an important topic in chemical research. Originally, Ils were considered to be "green" solvents, but later research has shown that this is not the case. DESs were introduced as a "green" alternative to Ils and incorrectly categorized by some authors as their subclass. The fundamental difference between Ils and DESs lies in the interactions they form. In Ils, the predominant type of interaction is ionic, whereas in

DESs hydrogen bonding prevails. From the perspective of “green” chemistry, DESs are superior to Ils due to their biodegradability and low toxicity. In addition, DESs can be obtained by mixing naturally occurring components, which makes their return to nature harmless. Furthermore, the preparation of DESs is more economical, utilizes less resources and energy and is safer for the researcher. Although DESs have many advantages over Ils, viscosity as a physicochemical property is a limiting factor for DESs, which is overcome by synthesizing new DESs (5).

The most common and simplest method of obtaining eutectic solvents is to mix individual components and heat them in a flask at a temperature range from 50°C to 100°C until a DES is formed. However, it is known that mixtures of choline chloride (ChCl) and carboxylic acids can decompose at elevated temperatures due to esterification processes (6). As a possibility for obtaining eutectic mixtures, freeze-drying (7) and the grinding method (8) have also been mentioned in the literature. The preparation of eutectic solvents by heating is the most frequently described in the literature. Pandey and Pandey (9) provided a detailed method for the preparation of a eutectic mixture based on ChCl in a molar ratio of 1:1. In addition, the preparation of DESs from ChCl with urea, ethylene glycol and propylene glycol was described in detail. The characterization of DESs was carried out using FTIR and Raman spectroscopy. The influence of temperature and the addition of water on the formation of DESs was demonstrated (9).

Certain interactions that influence the properties of eutectic solvents are important for the formation of eutectic systems. Abassi et al. investigated the variation in solvation interactions of ChCl-based DESs formed using different HBDs (10). ChCl-based DESs composed of HBDs with shorter alkyl chain substituents and strong organic acids, which possessed lower hydrogen bond basicity and dispersive interactions and higher hydrogen bond acidity compared to those composed of different isomers of butanediol and hexanediol as HBDs. Choline acetate DESs possessed higher hydrogen bond basicity and dispersive interactions, as well as dipolar interactions, compared to those composed of ChCl as a HBA. The interactions between ChCl and alcohols (1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 2,3-butanediol, 1,3-propanediol, glycerol, 1,5-pentanediol, 1,2,5-pentanetriol and xylitol) were studied to evaluate the strength of hydrogen bonding. DESs were prepared at different molar ratios, and consequently infrared spectroscopy (IR) and nuclear magnetic resonance (NMR) spectra were recorded to investigate the mechanism of hydrogen bonding. It was concluded that hydrogen bonding occurs between the chlorine atoms of the ChCl and the hydrogen atoms of the alcohols. The strength of the bond decreases with the increase of the alkyl chain length in the alcohol (11).

The characterization of eutectic solvents is well-described in the literature. Fourier transform infrared spectroscopy (FT-IR) is widely accepted as a method for characterizing eutectic solvents. In addition, Raman spectroscopy is another method for structural analysis to confirm the formation of eutectic mixtures, as Raman spectra change with the formation of hydrogen bonds. NMR spectroscopy is invaluable for evaluating the formation of eutectic solvents. Thermogravimetric analysis is important for monitoring the rate of degradation of eutectic solvents (12, 13).

Classification of eutectic solvents

Eutectic solvents can be represented by the general formula Cat+X-zY, where Cat+ represents any ammonium, sulfonium, or phosphonium ion, X is a Lewis base, and Y is Lewis or Brønsted acid (z defines the number of Y molecules). The complex is formed between X and Y. The classification of DESs is carried out according to the nature of the reagent used to form the mixture (Table I) (14, 15).

Table I Classification of the Deep Eutetic Solvents (DESs) (14)

Tabela I Podela eutektičkih rastvarača (14)

Type	**General formula**	**Terms**	**Example (15)**
type I	$Cat^+X^-zMCl_x$	M = Zn, Sn, Fe, Al, Ga, In	$ZnCl_2$+ChCl
type II	$Cat^+X^-zMCl_x{\cdot}yH_2O$	M = Cr, Co, Cu, Ni, Fe	$CoCl_2$*$6H_2O$ + ChCl
type III	Cat^+X^-zRZ	Z = $CONH_2$, COOH, OH	urea + ChCl
type IV	$MCl_x + RZ = MCl_{x-1}^+{\cdot}RZ + MCl_{x+1}^-$	M = Al, Zn Z = $CONH_2$, OH	$ZnCl_2$ + urea

Cat+ stands for ammonium, sulfonium, or phosphonium ion; X - Lewis base; Y - Lewis or Brønsted acid; z - number of Y molecules; R – alkyl group, ChCl - choline chloride

Type I eutectic solvents are formed by the combination of a metal chloride and a quaternary ammonium salt, while type II DES eutectic solvents are a combination of a hydrate of a metal chloride and a quaternary ammonium salt. Type III eutectic solvents represent a mix of a carboxylic acid, an amide or a polyol with a quaternary ammonium salt. Type IV eutectic solvents are mixture of a metal chloride hydrate and an HBD. For a long time, the main division was into these four groups (type I-IV), but researchers have recently reported the existence of type V eutectic solvents, consisting of non-ionic molecular HBAs and HBDs (16).

In addition to the mentioned classification of DESs, a new one has been proposed (Figure 2). Since DESs are hydrophilic by nature, the search for hydrophobic DESs was a major challenge. Their discovery enabled the extraction of non-polar compounds and the formation of two layers with water in a separating funnel. Hydrophobic DESs can be divided into ionic and non-ionic ones, depending on their composition. Non-ionic DESs have an advantage over ionic DESs due to their cost-effectiveness and reusability (16). Another group of DESs includes magnetic DESs, which are obtained by adding a metal chloride to a previously prepared DES (i.e. [*tert* butyl acetate (TBAC)/ethylene glycol][FeCl4]). They react strongly to an external magnetic field. However, due to their nature, these solvents cannot be considered “green” and cannot be injected into liquid or gas chromatography systems (17). The switchable DESs have been poorly studied in the literature. These solvents are fascinating due to their nature; they change their polarity by the addition of a deprotonating agent or under the influence of temperature (18). The

preparation of switchable DESs from thymol as a HBA and a fatty acid (hexanoic acid) as a HBD has been described (19). This mixture has shown high selectivity in the extraction of triazole pesticides from orange peel, watermelon, and grapes.

Furthermore, in addition to binary DESs systems, ternary eutectic solvents were also introduced, i.e. guanidine hydrochloride (GH): ethiylen glycol : p-toluenesulfonic acid (PTSA) (20). Compared to binary DESs, ternary systems have a lower viscosity and lower melting points (21).

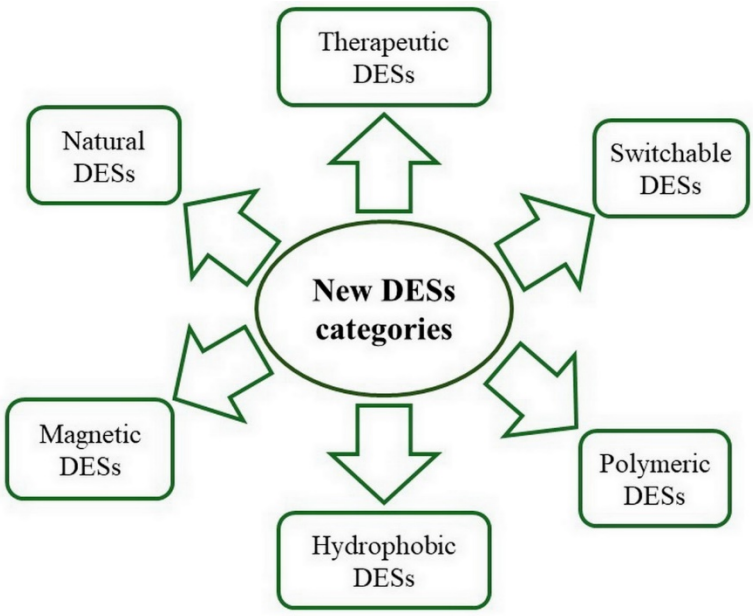

Figure 2. New classification of DESs
Slika 2. Nova klasifikacija eutektičkih smeša

Physico-chemical properties and toxicology of eutectic solvents

The properties of eutectic solvents are important for researchers. Eutectic solvents are polar and the most important physical properties of DESs include melting point, viscosity, polarity, pH and surface tension (22).

Eutectic solvent mixtures with a melting point below 50°C are of main interest for research. The melting point is influenced by the molar ratio of the components and the length of their alkyl chain.

Viscosity is also recognized as a critical property of eutectic solvents. Eutectic solvents are viscous liquids at room temperature, which is due to the hydrogen bond density in a small volume of solvent and the presence of Van der Waals forces. The viscosity is influenced by the type of constituents, their molar ratio, and the temperature. Low viscosity eutectic solvents are of great interest to researchers, and thus the overcoming of high viscosity is very important and challenging. The addition of water is one possibility, but there are also other methods (23). The high viscosity of DESs is

attributed to a dense network of hydrogen bonds. Large molecules and limited mobility within the dense hydrogen bond network contribute to high viscosity. Viscosity is largely dependent on the type of molecules they are made of. Therefore, to prepare DESs with low viscosity, one should pay attention to the type of ammonium salts, hydrogen bond donors and acceptors. As mentioned above, the dense network of hydrogen bonds and the limited space for molecular mobility can be overcome by using small cations or fluorinated hydrogen bond donors, which can contribute to a reduction in viscosity. In addition, temperature, together with the addition of water, leads to a decrease in viscosity.

Additionally, pH is one of the most important properties of DESs, and it depends on the HBA and HBD. The pH value has a major influence on DESs application in various areas. The pH could be altered by the addition of certain anions, which influences the shift to acidic or basic properties of these solvents. Moreover, temperature could have an influence on pH, as a linear increase in temperature leads to a decrease in pH (24).

Eutectic solvents are considered green solvents. A group of scientists investigated the toxicity and cytotoxicity of eutectic solvents based on ChCl. Glycerol, ethylene glycol, triethylene glycol and urea, as HBDs, were added to ChCl. After the study, the scientists reported that these mixtures showed neither toxicity to the bacteria tested nor cytotoxic effects. However, as this was the first study on this topic, the researchers themselves stated that further research and findings on this topic are needed and the term “non-toxic” should be used with caution (25).

In contrast to the first study, in which no toxic effects of eutectic solvents were found, a second study evaluating the toxic effects on *E. coli* showed contradictory results. It was found that ChCl-based eutectics have an antibacterial effect on *E. coli*. The toxicity depended on the molar ratio and concentration of the ingredients used to treat the microorganisms (26).

A recent study on the toxicity of eutectic solvents has recognized the limitations of studying the toxicity of these solvents (27). The study suggested that the previous experimental approaches were not appropriate due to the nature of eutectic solvents, and highlighted their generalized nature. An attempt was made to extrapolate the toxicity research to humans. The study also highlighted the possibility of using machine learning tools to obtain a clearer picture of the toxicity of these solvents. Finally, the researchers point out the low toxicity of studied DESs (27).

In addition to toxicity, the biodegradability of eutectic solvents is discussed in the literature. For a solvent to be described as “green”, it means that it is easily biodegraded. Reviewing the biodegradability of the DESs, it was found that all research on biodegradability uses a single test, the closed bottle test. All eutectic mixtures contained chloride in their composition (different DESs based on ChCl as HBA, while the HBDs were glycerol, ethylene glycol, urea, oxalic acid, acetamide, malonic acid, citric acid, glucose, and fructose), and were found to be readily biodegradable in all cases. It was also suggested to consider the use of *in silico* method, i.e. to create a unique database, to reduce the number of experiments performed and to start faster application of DESs in different industries (28).

Application of Deep Eutectic Solvents

There is a wide range of applications of DESs as solvents. The use in food analysis includes the extraction of phenolic compounds, flavonoids, dyes, toxins and metals. The extraction of tert-butylhydroquinone, the most cost-effective phenolic preservative in edible oils, has been described. The solvents previously used for extraction were not as effective as the solvents based on DESs (ChCl:ethylene glycol in a molar ratio of 1:2), which achieved an extraction rate of 100%. In addition to the extraction of synthetic phenols, the extraction of phenols and flavonoids from plant samples has also been described. Due to the contamination of edible oils with heavy metals, especially lead and cadmium, the use of DESs based on ChCl and urea in a molar ratio of 1:2, which have a high extraction rate, has been described as well (29). DESs are also used in organic synthesis and biocatalysis (30), as solvents for the extraction or isolation of compounds from biological samples (31) and in analytical chemistry (32–34).

The application of DESs as stationary phase modifiers and mobile phase additives in pharmaceutical analysis has not yet been reviewed. Acetonitrile and methanol have been essential components of mobile phases for 50 years. Considering how many HPLC analyses are performed worldwide in a single day, a considerable amount of waste is generated that is difficult to recycle and contributes to environmental pollution. Over the last two decades, scientists have been working to replace acetonitrile and methanol with biodegradable components in mobile phases. DESs have proven to be promising solvents for the replacement of acetonitrile and methanol. Studies have shown that their elution capacity is comparable to that of these organic solvents, that they reduce peak tailing and significantly improve resolution. However, their use is limited due to their high viscosity. Their use as mobile phases is restricted as they could potentially damage HPLC equipment. However, with the development of systems tailored to the physical properties of DESs, their use as mobile phases could be facilitated. So far, their concentration in the mobile phase ranges from 0.1% v/v to 60% v/v. It is important to consider the presence of water, as excessive dilution can lead to disruption of the DESs structure (35, 36).

Therefore, we aimed to critically evaluate the available literature data on the possibility of using DESs in drug quality control.

1. Deep Eutectic Solvents as Stationary Phase and Sorbent Modifiers

The stationary phase is one of the most important components of the chromatographic system. DESs are used as modifiers of stationary phases and thus influence the separation mechanisms. Although their use as stationary phase modifiers is less described in the literature than their use as mobile phase components, there are a few studies describing this application.

The paper by Yang et al. describes the use of DESs as stationary phase modifiers for the HPLC separation of nucleobases and amino acids. Namely, the synthesis of new stationary phases in which DESs replace the solvents usually used for synthesis (such as chloroform, methanol) and thus modify the stationary phase was described. The

modification of the stationary phase was carried out with three eutectic mixtures based on ChCl with ethylene glycol (Figure 3), urea and glycerol in a molar ratio of 1:2. The mixture was prepared at 60°C. The influence of various chromatographic conditions such as the ratio of acetonitrile, temperature and flow rate was investigated. It was found that a better separation was achieved compared to the classically synthesized stationary phases (37).

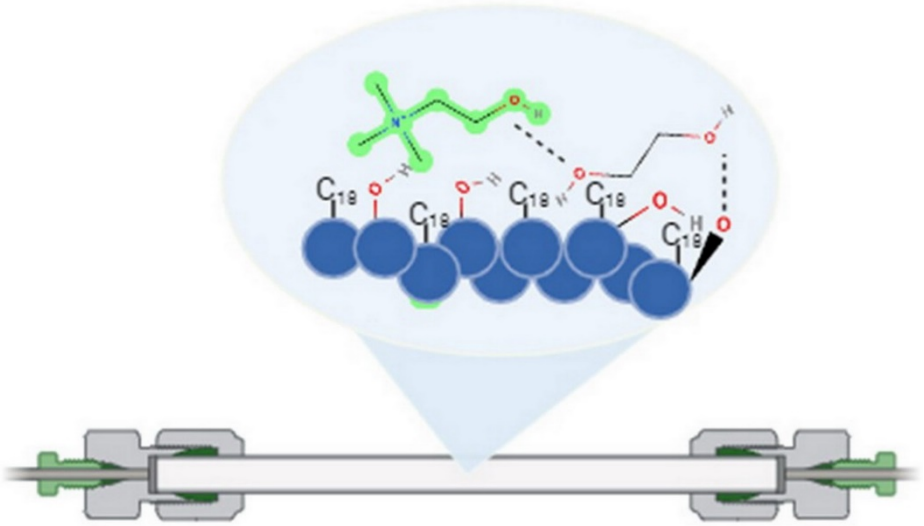

Figure 3. DESs ChCl : ethylene glycol (1:2, n/n) interactions with the stationary phase
Slika 3. Interakcije eutektičke smeše ChCl : ethylene glycol (1:2, n/n) sa stacionarnom fazom

In the study by Boczkaj et al., the use of DESs for the synthesis of the stationary phase in gas chromatography (GC) was described for the first time. A mixture of tetrabutylammonium chloride and heptadecanoic acid in a molar ratio 1:2 was used. A medium polarity column was synthesized, and it had a particular affinity for alcohols (1-hexanol and 1-heptanol) due to its ability to form hydrogen bonds. Although the abovementioned column showed good specificity and selectivity towards only one group of compounds, the authors emphasized that the use of DESs as a modifier for stationary phases opened new possibilities for the synthesis of new stationary phases with high targeted selectivity (38).

Very few studies address the use of DESs as modifiers of extraction sorbents. Makoś-Chełstowska and Gębicki investigated the use of DESs as modifiers of sorbents and emphasized the importance of DESs as modifiers due to their specific properties. The synthesis of modified sorbents and their application in solid phase extraction were described in detail in the study. In the conclusion, the authors emphasized the importance of sorbent modification, as it could lead to the creation of specific sorbents for a certain class of compounds or target analytes, which could speed up the analytical process and reduce the cost of routine analysis (39).

2.Deep Eutectic Solvents as Mobile Phase Additives

DESs as green solvents have found their application as mobile phase additives or modifiers in liquid chromatography. There have been an increasing number of studies in which the organic component of the mobile phase is completely or partially replaced with DESs.

The first study in which DESs were added as additives in mobile phases was published in 2016 by Tan et al. The mechanism of separation of quaternary alkaloids was investigated (coptisine chloride, sanguinarine, berberine chloride and chelerythrine) on C_{18} stationary phase in RP-HPLC system (40). During method development, parameters such as pH value, DESs concentration, types of DESs and acetonitrile amount were varied. The authors concluded that DESs were equally effective as additives, regardless of whether the mobile phase contained methanol or acetonitrile. The concentration of DESs significantly affected chromatographic separation. An increase in DESs concentrations led to increased retention time of analytes, contributing to better selectivity and sharper peaks, with higher symmetry. However, these factors varied depending on which HBAs and HBDs were used to form the eutectic mixture. In particular, the interaction of choline cation from ChCl with deprotonated silanol groups of the stationary phase significantly affected the chromatographic separation.

The same group of authors, Tan et al., also used DESs as additives in hydrophilic interaction liquid chromatographic (HILIC) analysis with silica stationary phase. Various concentrations of a DESs mixture of ChCl and ethylene glycol, in a molar ratio of 1:3, were added to the mobile phase containing acetonitrile. The chromatographic separation of a group of nucleoside bases was monitored. The authors concluded that the addition of DESs to the mobile phase improved the separation compared to the use of mobile phases without DESs. Interestingly, the addition of DESs led to a separation of cytidine and cytosine that could not be achieved with a mobile phase that was a mixture of water and acetonitrile. An increase in the column temperature, in combination with DESs as additives in mobile phases, resulted in decreased retention times of the analytes, and consequently shortened analysis times (41).

In addition, a eutectic mixture based on ChCl and ethylene glycol was investigated as an additive to optimize the chromatographic behavior of quercetin in an RP-HPLC system on C_{18} stationary phase. The influence of temperature, DES concentration, and the molar ratio of ChCl and ethylene glycol was investigated. Confirmation of DES formation was performed by FT-IR spectroscopy. Three mixtures of ChCl and ethylene glycol were prepared in different molar ratios and at different temperatures: 1:2 (60°C), 1:3 (80°C) and 1:4 (100°C). DESs were added to the mobile phase in three concentrations: 0.05, 0.10 and 0.15, v/v. The authors concluded that the best peak symmetry and the highest number of theoretical plates were obtained with the mixture of ChCl and ethylene glycol at a molar ratio of 1:2 and in concentration of 0.15, v/v in the mobile phase. The mechanism of interaction was also investigated. Enhanced interactions with the stationary phase and improved peak symmetry were obtained as the DES performed like a large ion

pair. It was concluded that DESs have great potential as an additive or substitute for organic solvents in the mobile phase (42).

Lil et al. investigated the use of DESs as an additive to optimize the chromatographic behavior of caffeic acid in RP-HPLC system at C_{18} stationary phase (43). The optimization of chromatographic conditions for the analysis of caffeic acid using an experimental design and a mixture of ChCl and glycerol as a mobile phase additive has been described. The authors used a Box-Behnken experimental design to simultaneously vary the factors corresponding to the preparation of DESs and concentration of DESs added to the mobile phase. The factors in the Box-Behnken experimental design were the molar ratio of glycerol to ChCl (2:1, 3:1, 4:1, n/n), the temperature (60°C, 80°C and 100°C), and the volume fraction of additive in the mobile phase (0.05, 0.1 and 0.15, v/v). The formation of DESs was confirmed by FT-IR spectroscopy. Optimal chromatographic behavior was achieved under the following conditions: molar ratio of glycerol to ChCl 3:1, temperature 80°C, and volume percent of the additive in the mobile phase 0.1, v/v. The authors compared the chromatograms obtained with those from previous studies in which the chromatographic conditions were optimized for the analysis of caffeic acid using phosphoric or acetic acid as additives. The conclusion of the study was that DESs are superior to the previous additives for the mentioned analysis (43).

It is already well-known that the use of eutectic solvents depends on their viscosity. Considering the publications on the application of DESs in pharmaceutical analysis, it is important to overcome this major drawback by preparing DESs with low viscosity. The mentioned physicochemical parameter is influenced by the preparation temperature and the molar ratio in which the components are mixed. For this reason, researchers have addressed this problem by the preparation of low-viscosity DESs from ChCl and carboxylic acids (citric acid, lactic acid, tartaric acid, malic acid, and malonic acid). In addition to the preparation described, the DESs were classified, for the first time, according to the number of functional groups and rheological properties (44). The preparation of a eutectic mixture based on ChCl and glycerol was also described in detail as another example of DESs in which the high viscosity was overcome (45).

Due to a very high viscosity, DESs are mainly used as mobile phase additives in liquid chromatography. Recently, Ray described the application of DESs solely as mobile phases in thin-layer chromatography (TLC) and HP-TLC on Si60 stationary phase for the qualitative and quantitative analysis of five alkaloids from the plant species *Chelidonium majus* (berberine, chelerythrine, chelidonine, coptisine, sanguinarine) (46). The composition of 17 DESs, labeled as natural deep eutectic solvents (NADESs) and used in the study as mobile phases, is shown in Table II. NADESs are mixtures of HBDs and HBAs isolated from plant biomass, an example of which is proline–malic acid (45). The author concluded that, compared to conventional TLC chromatography, the results obtained with DESs are representative and applicable. It was demonstrated that the viscosity of DESs is also a significant factor that must be considered during TLC method development. The dilution of the initially obtained DESs with methanol, acetone and 30%

of acetic acid led to a decrease in viscosity and resulted in improved chromatograms and separation (47).

Table II Composition of NADESs used as mobile phases for TLC separation of berberine, chelerythrine, chelidonine, coptisine and sanguinarine (46)

Tabela II Sastav eutektičkih smeša koje su korišćene kao mobilne faze za TLC razdvajanje berberina, heleritrina, helidonina, koprizina i sangvinarina (46)

Components	**Molar ratio (n/n)**
borneol + phenol	1:1
camphor + formic acid	1:1
camphor + phenol	1:1
camphor + phenyl salicylate	1:1
menthol + acetic acid	1:1
menthol + borneol	8:2
menthol + lactic acid	1:2
menthol + limonene	1:1
menthol + phenol	1:1
menthol + thymol	1:1
thymol + acetic acid	1:1
thymol + linalool	1:1
thymol + phenol	1:1
ChCl + lactic acid 1	1:1
ChCl + malic acid 1	1:1
ChCl + oxalic acid 1	1:2
ChCl + phenol 1	1:1

The micellar liquid chromatography (MLC) method was described for the separation and determination of four drugs (aspirin, metformin, metoprolol, and atorvastatin) from urine and human plasma using eutectic solvents as additives in the mobile phase on C_{18} stationary phases. Central composite design was employed to develop a new method for the analysis of these drugs, achieving better peak symmetry and shorter analysis times compared to previous methods. The following factors were tested: sodium dodecyl sulfate solution concentration (0.06, 0.07, 0.08, 0.09 and 0.1 mol

L^{-1}), butanol content (8, 9, 10, 11 and 12, v/ v), DESs content (2.0, 2.5, 3.0, 3.5 and 4.0, v/v) and the content of glacial acetic acid (2.5, 3.0, 3.5, 4.0 and 4.5, v/v). The optimal mobile phase composition was sodium dodecyl sulfate solution (0.09 mol L^{-1}) : butanol : DES : glacial acetic acid (83:10:3.5:3.5, v/v/v/v) (48).

A new cyclodextrin-electrokinetic chiral chromatographic method for the analysis of the antiepileptic drug lacosamide has been developed. In this method, DESs were used as additives to the mobile phase. The preparation of four different DESs was described, each in various molar ratios: ChCl : ethylene glycol 1:2; ChCl : urea 1:2; ChCl : D-glucose 2:1 and ChCl : D-sorbitol 1:1. The temperature range from 70°C to 80°C was used for the preparation of DESs. The best resolution was achieved using the eutectic mixture of ChCl : D-sorbitol in a 1:1 molar ratio as an additive to the mobile phase. The analysis was performed on an uncoated fused-silica capillary using 100 mM a borate buffer (pH 9.0) containing 18 mM of succinyl-β-cyclodextrin and 0.5 (w/v) of ChCl-D-sorbitol 1:1 (n/n) (49).

The use of DESs is not limited to LC and TLC; they have also been used as mobile phase additives in supercritical fluid chromatography (SFC). A new method for the separation of 10 isoquinoline alkaloids using SFC and DESs as “green” solvents has been described (50). The aim of the study was to develop a new method, since the existing methods severely damage the stationary phase, resulting in poor separation of the compounds. In order to achieve symmetrical peaks and good separation of the compounds, and to reduce the organic modifier content, DESs were added to the mobile phase as modifiers in the mobile phase. Sixteen new DESs were prepared at a temperature of 80°C (Table III). The characterization of the prepared DESs was carried out by IR spectroscopy. The influence of different DES on the separation of 10 isoquinoline alkaloids was investigated considering the effects of the molar ratio of the solvents used to prepare the DESs and the concentration of DESs as additives in the mobile phase. It was concluded that the best separation was achieved with ChCl as the HBA, while optimal separation and the most symmetrical peaks were obtained with a mixture of ChCl and glycerol (2:1, n/n) added to the mobile phase in a concentration of 0.25, v/v (50).

Table III Composition of DESs used for the separation of 10 isoquinoline alkaloids using SFC (50)

Tabela III Sastav eutektičkih smeša koje su korišćene za razdvajanje 10 izohinolinskih alkaloida primenom SFC (50)

Hydrogen Bond acceptor	**Hydrogen bond donor**	**Molar ratio HBA : HBD (n/n)**
Choline chloride	D-glucose	1:1
	Maltose	2:1
	Ethylene glycol	2:1
	Glycerol	2:1
	Laevulinic acid	1:2
	Oxalic acid	1:1
	Urea	1:2
	Acetamid	1:1
Betain	D-glucose	1:1
	Laevulinic acid	1:2
	Acetamid	1:1
	Glycerol	1:2
L-proline	D-glucose	1:1
	Glycerol	2:5
	Laevulinic acid	1:2
	Acetamid	1:1

Conclusion

This review article summarizes all existing knowledge on DESs and their application in drug analysis, either as modifiers for the stationary phase/sorbents or as mobile phase additives. Since the first description of DESs in 2004, there have been only a very limited number of studies regarding their use in LC for drug quality control. The use of DESs as stationary phase modifiers opened new possibilities for the synthesis of stationary phases with high targeted selectivity. In addition, DESs proved to be promising mobile phase additives in LC and TLC systems and substitutes for organic modifiers in mobile phases. DESs were found to be superior to the additives used in previous separation studies. Although DESs are increasingly being studied due to their unique properties and complex interactions, many questions remain unanswered. They have a specific viscosity, pH, and density that are different from those of the individual

components that constitute these mixtures. Since drugs can be acidic or basic, the use of eutectics in pharmaceutical analysis raises questions about the specific interactions between DESs and analytes, as well as the underlying separation mechanisms in chromatography.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Anđelija Malenović: Conceptualization, Supervision, Writing - review & editing; **Đorđe Vasilić**: Writing - original draft, **Marija Rašević**: Writing - original draft; **Mira Zečević**: Writing - review & editing; **Marija Čarapić**: Writing - review & editing.

References

1. Liu J, Li X, Ho Row K. Development of deep eutectic solvents for sustainable chemistry. J Molec Liq. 2022;362:119654.
2. El Achkar T, Greige-Gerges H, Fourmentin, S. Basics and properties of deep eutectic solvents: a review. Environ Chem Lett. 2021;19(4):3397–3408.
3. Abbott AP, Boothby D, Capper G, Davies DL, Rasheed RK. Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. J Am Chem Soc. 2004;126(29):9142–9147.
4. Smith EL, Abbott AP, Ryder KS. Deep Eutectic Solvents (DESs) and Their Applications. Chem Rev. 2014;114(21):11060-11082.
5. Płotka-Wasylka J, de la Guardia M, Andruch V, Vilková M. Deep eutectic solvents vs ionic liquids: Similarities and differences. Microchem J. 2020;159:105539.
6. Sharma A, Sharma RC, Thakur R, Singh L. An overview of deep eutectic solvents: Alternative for organic electrolytes, aqueous systems & ionic liquids for electrochemical energy storage. J Energy Chem. 2023;82:592-626.
7. Ijardar SP, Singh V, Gardas RL. Revisiting the Physicochemical Properties and Applications of Deep Eutectic Solvents. Molecules. 2022;27(4):1368.

8. Florindo C, Oliveira FS, Rebelo LPN, Fernandes AM, Marrucho IM. Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids. ACS Sustain Chem Eng. 2014;2(10):2416–2425.
9. Pandey A, Pandey S. Solvatochromic Probe Behavior within Choline Chloride-Based Deep Eutectic Solvents: Effect of Temperature and Water. J Phys Chem B. 2014;118(50):14652–14661.
10. Abbasi NM, Farooq MQ, Anderson JL. Investigating the Variation in Solvation Interactions of Choline Chloride-Based Deep Eutectic Solvents Formed Using Different Hydrogen Bond Donors. ACS Sustain Chem Eng. 2021;9(35):11970–11980.
11. Wang H, Liu S, Zhao Y, Wang J, Yu Z. Insights into the Hydrogen Bond Interactions in Deep Eutectic Solvents Composed of Choline Chloride and Polyols. ACS Sustain Chem Eng. 2019;7(8):7760–7767.
12. Shah PA, Chavda V, Hirpara D, Sharma VS, Shrivastav PS, Kumar S. Exploring the potential of deep eutectic solvents in pharmaceuticals: Challenges and opportunities. J Mol Liq. 2023;390:123171.
13. Tomé LIN, Baião V, da Silva W, Brett CMA. Deep eutectic solvents for the production and application of new materials. Appl Mater Today. 2018;10:30-50.
14. Jablonský M, Škulcová A, Šima J. Use of Deep Eutectic Solvents in Polymer Chemistry-A Review. Molecules. 2019;24(21):3978.
15. Li L, Wu Z, Liang J, Yu L. Application of deep eutectic solvents in lignocellulosic biomass processing. J For Eng. 2020;4:20-28.
16. Crema APS, Schaeffer N, Bastos H, Silva L, Abranches DO, Passos H, et al. New family of Type V eutectic solvents based on 1,10-phenanthroline and their application in metal extraction. Hydrometallurgy. 2022;215(6):105971.
17. Piao H, Jiang Y, Qin Z, Ma P, Sun Y, Wang X, et al. Application of an in-situ formulated magnetic deep eutectic solvent for the determination of triazine herbicides in rice. Talanta. 2021;222:121527.
18. Hu C, Feng J, Cao Y, Chen L, Li Y. Deep eutectic solvents in sample preparation and determination methods of pesticides: Recent advances and future prospects. Talanta. 2024;266:125092.
19. Zhang J, Zhang Z, Yao L, Qian M, Li Z, Han Y, et al. pH-responsive switchable deep eutectic solvents to mediate pretreatment method for trace analysis of triazole fungicides in peel wastes. Food Chem. 2023;411:135486.
20. Chen Z, Jacoby WA, Wan C. Ternary deep eutectic solvents for effective biomass deconstruction at high solids and low enzyme loadings. Bioresour Technol. 2019;279:281-286.
21. Meenu M, Bansal V, Rana S, Sharma N, Kumar V, Arora V, Garg M. Deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESs): Designer solvents for green extraction of anthocyanin. Sustain Chem Pharm. 2023;34:101168.
22. Omar KA, Sadeghi R. Physicochemical properties of deep eutectic solvents: A review. J Mol Liq. 2022;360:119524.
23. Zhang Q, De Oliveira Vigier K, Royer S, Jérôme F. Deep eutectic solvents: syntheses. properties and applications. Chem Soc Rev. 2012;41(21):7108.
24. Skulcova A, Russ A, Jablonsky M, Sima J. The pH behavior of seventeen deep eutectic solvents. Bio Res. 2018;13(3):5042-5051.

25. Hayyan M, Ali Hashim M, Hayyan AH, Al-Saadi MA, Al Nashef IM, Mirghani MES, Kola SO. Are deep eutectic solvents benign or toxic? Chemosphere. 2013;90:2193-2195.
26. Wen Q, Chen J, Tang Y, Wang J, Yang Z. Assessing the toxicity and biodegradability of deep eutectic solvents. Chemosphere. 2015;132:63-69.
27. Sharma A, Lee BS. Toxicity test profile for deep eutectic solvents: A detailed review and future prospects. Chemosphere. 2024;350:141097.
28. Azzouz A, Hayyan M. Are deep eutectic solvents biodegradable? PSEP. 2024;176:1021-1025.
29. Chen J, Yun L, Wang X, Liu W. Application of Deep Eutectic Solvents in Food Analysis: A Review. Molecules. 2019;24(4):4594.
30. Perna MF, Vitale P, Capriati V. Deep eutectic solvents and their applications as green solvents. Curr Opin Green Sustain Chem. 2020;21:27-33.
31. Taheri E, Yousefinejad S, Dehghani F. Deep eutectic solvents in biological monitoring of occupational exposures. TrAC. 2023;168:117299.
32. Gao F, Liu L, Tang W, Ho Row K, Zhu T. Optimization of the chromatographic behaviors of quercetin using choline chloride-based deep eutectic solvents as HPLC mobile-phase additives. Sep Sci Technol. 2018;53(2):397-403.
33. Ramezani MA, Ahmadi R, Absalan G. Designing a sustainable mobile phase composition for melamine monitoring in milk samples based on micellar liquid chromatography and natural deep eutectic solvent. J Chromatogr A. 2020;1610:460563.
34. Pérez-López LA, Cavazos-Rocha N, Delgado-Montemayor C, Waksman-Minsky N, Hernández-Salazar M, Portillo-Castillo OJ. A simple HPLC-DAD method for analysis of phenolic acids: Addition effect of a hydrophilic deep eutectic solvent to the mobile phase. Acta Chromatogr. 2023;35(2):204-216.
35. Płotka-Wasylka J, Rutkowska M, de la Guardia M. Are deep eutectic solvents useful in chromatography? A short review. J Chromatogr A. 2021;1639:461918.
36. Sutton AT, Fraige K, Mazzi Leme G, da Silva Bolzani V, Hilder EF, Cavalheiro AJ, et al. Natural deep eutectic solvents as the major mobile phase components in high-performance liquid chromatography – searching for alternatives to organic solvents. Anal Bioanal Chem. 2018;410:3705–3713.
37. Yang B, Cai T, Li Z, Guan M, Qiu H. Surface radical chain-transfer reaction in deep eutectic solvents for preparation of silica-grafted stationary phases in hydrophilic interaction chromatography. Talanta. 2017;175:256-263.
38. Momotko M, Łuczak J, Przyjazny A, Boczkaj G. First deep eutectic solvent-based (DES) stationary phase for gas chromatography and future perspectives for DES application in separation techniques. J Chromatogr A. 2012;1635:461701.
39. Makoś-Chełstowska P, Gębicki J. Sorbents modified by deep eutectic solvents in microextraction techniques, TrAC. 2024;172:117577.
40. Tan T, Zhang M, Wan Y, Qiu H. Utilization of deep eutectic solvents as novel mobile phase additives for improving the separation of bioactive quaternary alkaloids. Talanta. 2016;149:85-90.
41. Tan T, Qiao X, Wan Y, Qiu H. Deep eutectic solvent: a new kind of mobile phase modifier for hydrophilic interaction liquid chromatography. Chin J Chromatogr (Se Pu). 2015;33(9):934-937.

42. Gao F, Liu L, Tang W, Row KH, Zhu T. Optimization of the chromatographic behaviors of quercetin using choline chloride-based deep eutectic solvents as HPLC mobile-phase additives. Sep Sci Technol. 2017;53(2):397–403.
43. Li G, Zhu T, Lei Y. Choline chloride-based deep eutectic solvents as additives for optimizing chromatographic behavior of caffeic acid. Korean J Chem Eng. 2015;32(10):2103–2108.
44. Ninayan R, Levshakova AS, Khairullina EM, Vezo OS, Tumkin I, Ostendorf A, et al. Water-induced changes in choline chloride-carboxylic acid deep eutectic solvents properties. Colloids Surf A: Physicochem Eng Asp. 2023;679:132543.
45. Sazali AL, Al Masoud N, Amran SK, Alomar TS, Pa'ee KF, El-Bahy ZM, et al. Physicochemical and thermal characteristics of choline chloride-based deep eutectic solvents, Chemosphere. 2023;338:139485.
46. Raj D. Eutectic Thin-Layer Chromatography as a New Possibility for Quantification of Plant Extracts-A Case Study. Molecules. 2022;27(9):2960.
47. Liu Y, Friesen JB, McAlpine JB, Lankin DC. Chen SN, Pauli GF. Natural Deep Eutectic Solvents: Properties, Applications, and Perspectives. J Nat Prod. 2018;81(3):679-690.
48. Ramezani MA, Absalan G. Employment of a natural deep eutectic solvent as a sustainable mobile phase additive for improving the isolation of four crucial cardiovascular drugs by micellar liquid chromatography. J Pharm Biomed Anal. 2020;186:113259.
49. Salido-Fortuna S, Casado N, Castro-Puyana M, Luisa Marina M. Use of choline chloride-D-sorbitol deep eutectic solvent as additive in cyclodextrin-electrokinetic chromatography for the enantiomeric separation of lacosamide. Microchem J. 2021;160:105669.
50. Jiang Z-M, Liu W-J, Li Y, Liu J, Wang H-Y, Li P, Liu E-H. Eco-friendly deep eutectic solvents contribute to improving the separation of isoquinoline alkaloids in supercritical fluid chromatography. ACS Sustainable Chem Eng. 2020;36:13777–13783.

Mogućnost zamene konvencionalnih organskih rastvarača zelenim dubokim eutektičkim rastvaračima u analitici lekova

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Kratak sadržaj

Poslednjih godina, eutektički rastvarači privlače sve veću pažnju u različitim naučnim disciplinama. Njihova jedinstvena svojstva, kao što su niska toksičnost, biorazgradivost, niska cena i mogućnost primene u različitim oblastima, učinila su ih atraktivnom alternativom konvencionalnim organskim rastvaračima. U farmaceutskoj analizi, gde su sve veći zahtevi za ekološki prihvatljivim metodama, postavlja se pitanje da li eutektički rastvarači, kao „zeleni rastvarači“, mogu da zamene organske rastvarače. Ovaj rad istražuje potencijalnu primenu eutektičkih rastvarača u analizi lekova i ističe prednosti i izazove koje oni donose. Prikazani su primeri upotrebe eutektičkih rastvarača u hromatografiji i u ekstrakciji lekova iz različitih uzoraka. Pored toga, razmatrana je stabilnost i toksičnost eutektičkih rastvarača koji se koriste u analitičkim metodama. Na osnovu dostupnih podataka, može se zaključiti da eutektički rastvarači imaju značajan potencijal u pogledu upotrebe u analitičkim metodama u farmaceutskoj analizi i pružanja ekološki održive i efikasne alternative konvencionalnim organskim rastvaračima. Međutim, potrebna su dalja istraživanja kako bi se bolje razumela njihova svojstva i optimizovala njihova primena u ispitivanju lekova.

Ključne reči: eutektički rastvarači, zeleni rastvarači, tečna hromatografija, analitika lekova