

Are deep eutectic solvents useful in chromatography? A short review

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Abstract

A literature update has been done concerning Deep Eutectic Solvents (DES) use in chromatography applications. The literature survey was based on the period from 2010 till 2020 and manuscripts reported in the data bases Web of Science and Scopus. The use of DES as mobile phase and mobile phase additives, stationary phases and solid phase modifiers and the use of DES as reaction solvents for chromatography use, were evaluated. Emphasis was placed on the differentiation of DES and Ionic Liquids (ILs) and the advanced green characteristics of the new solvents as compared with traditional organic solvents and ILs with a look into the drawbacks and future perspectives in the field of separation methods.

Keywords

Deep eutectic solvents; chromatography; green analytical chemistry; stationary phases; mobile phases; additives

1. Introduction

New approaches to green analytical chemistry are very often based on the use of new solvents to replace common organic solvents, which are toxic and highly volatile. In the last two decades, ionic liquids (ILs) have gained much scientific attention, due to their specific physicochemical properties and their applicability in very different areas [1]. However, the widespread use of ILs is limited by their high cost. Additionally, the green character of the ILs is often questioned, especially because of their poor biodegradability, biocompatibility and production sustainability [2]. A new generation of solvents, called deep eutectic solvents (DES) offer a more environment-friendly alternative than ILs. DES are defined as a mixture of two or several components, which may be liquid or solid and that at a particular composition present a high melting point depression staying liquids at room temperature [3]. DES may also be ionic but mainly consist of a mixture of organic compounds with a melting point significantly lower than that of any of the individual components. DES are easily available, inexpensive and biodegradable and can therefore be a viable eco-friendly substitute for conventional organic solvents [4]. Details on the advantages of the DES are shown in Figure 1.

Figure 1. The main advantages that characterize DES. Summarized from references [2,5–9].

44 The most popular DES are a combination of choline chloride (ChCl), carboxylic acids and
 45 other hydrogen bonds donors such as urea, citric acid, succinic acid and glycerol. ChCl is a
 46 low-costs, biodegradable and non-toxic quaternary ammonium salt that can be extracted from
 47 biomass or easily synthesized from fossil resources (4). DES formation can be done under
 48 simple operation conditions by directly mixing the components [6]. In 2007, DES was defined
 49 using a general formula of $R_1R_2R_3R_4N^+X^- \cdot Y^-$ including in case a metal ion, M, and a Z anion
 50 and on this basis a classification of four types of these substances was proposed (Table 1)
 51 [10,11].

52

53 Table 1. Types of deep eutectic solvents

Type of DES	Formula $R_1R_2R_3R_4N^+X^- \cdot Y^-$	Description	Example
DES Type I	Y = MCl _x M = Zn, Sn, Fe, Al, Ga	Combination of metal and organic salts	ZnCl ₂ + ChCl
DES Type II	Y = MCl _x · yH ₂ O M = Cr, Co, Cu, Ni, Fe	A hydrate of metal salt and organic salt	CoCl ₂ · 6H ₂ O + ChCl
DES Type III	Y = R ₅ Z Z = -CONH ² , -COOH, -OH	The hydrogen bond donor and organic salt	ChCl + urea
DES Type IV	-	Combination of metal chloride with a compound being the donor of a hydrogen bond	MCl _x + urea/ethylene glycol/ acetamide

54

55 DES can be synthesised as natural primary metabolites and therefore the toxicity of these
 56 systems should be significantly lower compared to ILs. The chemical structures of several
 57 compounds with the ability to form NDES are shown in Figure 2. After 2010, it was
 58 discovered that primary metabolites in many plants changed their state from solid to liquid
 59 when mixed in appropriate proportions. This led to the hypothesis that natural deep eutectic
 60 solvents (NADES) play a role as an alternative to water in living organisms and consequently
 61 to test a wide range of natural products, resulting in the discovery of over 100 NADES of
 62 natural origin [12].

63

64 Figure 2. Chemical structures of several of the most commonly used compounds (apart from
 65 ChCl) with the ability to form natural deep eutectic solvents.

66

67 2. Brief story of DESs

68 The first reports in the literature on the use of eutectic solvents date back to the end of
 69 20th and the early 21st century. Several manuscripts published in the 1990s referred to some
 70 specific applications of these liquid mixtures. In 1994 eutectic mixtures were reported as
 71 substrates for enzymatic reactions [13,14], whereas in 1998 scientific developments in this
 72 field were described, as the use of heterogeneous eutectic mixtures for enzymatic synthesis
 73 [15]. It was one of the first studies in this area. In the following years DESs have been gaining
 74 more and an increasingly interest from scientists. A considerable amount of literature has
 75 been published on DES, their innovative solutions and their wide range of applications as

76 greener alternatives to organic solvents and ILs. In Figure 3, the landmarks of DES
77 development are graphically depicted [3,16–18].

78

79 Figure 3. Landmarks of deep eutectic solvents development.

80

81 **2.1. DESs as green medium**

82 Over the past 20 years, green chemistry has rapidly expanded in almost every field of
83 chemistry. Nowadays, green technology is one of the key issues in the field of chemistry, as it
84 aims at minimizing environmental impact as well as reducing costs and improving safety and
85 health of operators. With the introduction of the principles of green chemistry, the main
86 efforts of many scientists have focused on the search and development of solvents that would
87 have the highest possible greenness index - they would be the least toxic. Therefore, four
88 directions towards ecological solvents have been developed: replacement of hazardous
89 solvents with better environmental, health and safety solvents; application of "bio-solvents"
90 produced from renewable sources; replacement of organic solvents with environmentally
91 friendly supercritical liquids or application of ILs characterized by low vapour pressure [19].
92 To overcome the drawbacks of ILs, DESs have emerged as a new type of green solvents and
93 natural origin products (NADES) are even more environmentally friendly compared to DES
94 due to their easy renewal [20].

95

96 **2.2. Application in analytical chemistry area**

97 Due to their many advantages and innovative properties, DESs are widely used in
98 many chemical fields [21] (Figure 4) including applications as valuable alternative solvents
99 for Green Analytical Chemistry [8]. DES and NADES generally provide a network of
100 hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) species, thereby enhancing
101 the dissolution of target analytes [22]. The application of DES in analytical chemistry can be
102 considered in several categories [9]. (I) (micro)extraction of target analytes from matrices
103 with complex composition followed by determination using analytical instruments; (II)
104 modification of nanoparticles, silica and other sorbents in order to increase the extraction
105 efficiency; (III) dissolution or digestion of solid samples; (IV) eluent after a dispersive solid-
106 phase extraction (DSPE) and further described in this review (V) chromatography as an
107 additive or modifier of the mobile phase.

108

109

110 Figure 4. Chemistry fields where DES is applied.

111

112 DES-based (micro)extraction procedures are considered as a specific type of
113 homogeneous liquid-liquid extraction, in which, after the addition of DES, a homogeneous
114 solution is formed. In order to obtain a turbid solution, an emulsifying solvent must be added
115 and phase separation is usually achieved by centrifugation [23]. On the other hand, in the
116 DES-based DSPE extraction, the sorbent containing the analytes is collected by an external
117 magnet, the aqueous phase is decanted and the analytes are eluted by a suitable solvent [24].
118 The combination of this method with gas chromatography (GC) with micro electron capture
119 detector (ECD) was used for the ultra-trace analysis of organochlorine pesticides (OCP) in

120 environmental water samples obtaining low LOD values [25]. High selectivity and high
 121 sensitivity was characterized by the procedure using DES-based DSPE to extract dopamine,
 122 epinephrine and norepinephrine from biological samples prior to high-performance liquid
 123 chromatography (HPLC) determination [26]. There are also reports of air-assisted liquid-
 124 liquid micro-extraction based on DES for pre-concentration of methadone in water and
 125 biological samples, in combination with GC-FID (flame ionization detector) [27], liquid-
 126 liquid microextraction method for the extraction of polycyclic aromatic hydrocarbons from
 127 aqueous samples before determination with HPLC and diode array detection (DAD) [28] or
 128 DES-based dispersive liquid-liquid microextraction for the extraction of pesticide residues in
 129 fruit and vegetable juices preceded by a determination using GC-FID [29]. DES-based micro-
 130 extraction is also used to analyse solid samples. Among others, a method of DES-based
 131 headspace solid-phase microextraction, in combination with GC-FID has been proposed for
 132 determination of bioactive terpenoids [30] and microwave assisted DES extraction combined
 133 with head space-solid phase microextraction (HS SPME) followed by GC with mass
 134 spectrometry detection (MS) was used to determine volatile compounds in tobacco [18] [9].
 135 Table 2 lists additional examples of DES applications in chemical analysis of solid and liquid
 136 samples. Both in the analysis of solid and liquid samples, ChCl is most commonly used as a
 137 HBA for the formation of DES in most of the described approaches. Whereas mainly organic
 138 acids, phenol and its derivatives, glycerol and urea were used as HBD for DES preparation.
 139 After extraction, the DES phase containing the target analytes was injected without pre-
 140 dilution directly into GC-MS, GC-FID or HPLC systems coupled with different types of
 141 detectors (UV, MS, FID, FLD, UV-Vis). Water is added to the mixture or heating is used to
 142 reduce the viscosity of the DES, especially when analysing solid samples. After extraction of
 143 the samples, filtration of the centrifuged suspensions is usually carried out before the
 144 determination [9].

145 There are literature reports which confirm that DES have great potential for routine
 146 analysis of trace metals in biological samples, which are characterized by a very complex
 147 matrix composition. DES were used, for example, during the determination Cu, Fe, and Zn in
 148 fish samples with the use of flame atomic absorption spectrometry (FAAS), where the
 149 extraction recovery of the metal elements in the DES was greater than 95% [31].

150 In the past few years, DES, as a new class of green solvents, has become very popular
 151 in many fields of science and technology. The use of DES in analytical chemistry is still in its
 152 infancy, but a significant increase in the number of scientific publications on this topic can be
 153 observed.

154
155

156 Table 2. Examples of use DESs for chemical analysis of solid and liquid samples.

Type of sample	Analyte	Sample matrix	DES composition	Detection method	LOD, [μgL^{-1}]	References
Liquid samples	BTE and PAHs	Water	ChCl:phenol	HPLC-UV	0.02–6.8	[32]
	Malachite green	Water	ChCl:phenol	UV-VIS	3.6	[33]
	Methadone	Water,	ChCl:TNO	GC-FID	0.7	[34]

		urine and plasma				
	Organochlorine pesticides	Water	ChCl:urea	GC- μ ECD	0.0004 – 0.0027	[35]
	Phenolic acids	Vegetable oils	ChCl:ethylene glycol	HPLC-UV	0.39 – 0.63	[36]
	Plant growth regulators	Edible oils	Tetramethylammonium chloride:ethylene glycol	HPLC-UV	5 – 7.5	[37]
	Phenolic compounds	Virgin olive oil	ChCl:xylitol	HPLC-UV	-	[38]
	Total phenolic content Virgin	Virgin olive oil	Lactic acid:glucose:water	UV-Vis	-	[39]
	Cr(III/VI)	Water	ChCl:phenol	FAAS	550	[40]
	Co	Pharmaceutical supplement and tea samples	ChCl:phenol	FAAS	1.1	[17]
Solid samples	As, Cr, Mo, Sb, Se, V	Agricultural soil	ChCl:oxalic acid	ICP-OES	0.009–0.1	[41]
	Fe	Sheep, bovine and chicken liver	ChCl:lactic acid (1:1)	FAAS	0.026	[42]
	Mn	Vegetable samples	ChCl:tartaric, or oxalic or citric acids	ICP-OES	0.0034–0.0123	[43]
	Bioactive terpenoids	<i>Chamaecyparis obtusa</i> leaves	ChCl:ethylene glycol	GC-FID	0.02–0.03	[30]
	Flavonoids	<i>Chamaecyparis obtusa</i>	ChCl:1,4-butanediol, 30% water	HPLC-UV	0.07–0.09	[19]
	Ochratoxin A	Wheat and derived products	ChCl:urea, 40% water	HPLC-FLD	0.0009	[44]
	Phenolic compounds	<i>Pyrola incarnata</i> Fisch	ChCl:1,4-butanediol, 30% water	HPLC-UV	0.04 – 0.14	[45]
	Phenolic	<i>Cajanus cajan</i> leaves	ChCl:maltose, 20% (v/v) water	UPLC-UV	0.06 – 0.13	[46]
	Volatile compounds	Tobacco	ChCl:ethylene glycol	GC-MS	-	[18]
	Phenolic acids	<i>Herba Artemisiae Scopariae</i>	Tetramethyl ammonium chloride:urea mixed with methanol/water (60:40, v/v)	HPLC-UV	0.07–0.11	[47]

			ChCl:ethylene			
	PAH	Marine biological samples (fish)	ChCl:oxalic acid	HPLC-FLD	0.005 – 0.03	[16]

157

158 3. Application of DESs in chromatography

159 DESs are mostly applied in the research on the stationary and mobile phases as well as
 160 mobile phase additives. Due to the role of DES in chromatography, there are three groups of
 161 applications, which are presented in the Figure 5. It also need to be mentioned, that DESs are
 162 pupular in counter current chromatography. All of these applications are briefly described
 163 with examples in this Section.

164

165 Figure 5. Application of DESs in chromatography.

166

167 3.1. DESs as mobile phases or mobile phase additives

168 Green solvents are applied widely to reduce the environmental problems connected
 169 with the application of conventional solvents in chemical production as well as to minimize
 170 cost and to improve safety and health. DESs as “eco-friendly” solvents are one of the most
 171 important subjects of the so-called “Green Chemistry” and they play a key role in
 172 environmentally friendly analytical techniques including their application as mobile phases or
 173 mobile phase additives. In such role, DESs can be used in liquid chromatography (LC) and
 174 HPLC.

175 Solvent selection rules for HPLC were developed over the past several decades, and
 176 acetonitrile as well as methanol have been permanently adopted as the most popular and
 177 applied organic modifiers in reversed phase RP-HPLC [48]. However, due to the growing
 178 environmental awareness brought about by the introduce of the “green chemistry” paradigm,
 179 new researches have been performed to find replacements to acetonitrile as well as other
 180 traditional organic solvents, as they are undesirable solvenst from a sustainable point of view
 181 [49,50]. Several problems associated with LC when green assessment is taken into
 182 consideration occur. Among them there is a large consumption of the organic solvents and
 183 other components and waste generation. It has been calculated that about 200 000 LC
 184 instruments are in use. With the simple assumption model when the typical column of 15 - 25
 185 cm in length, 4,6 mm of i.d. packed with 5 µm particles is used, with the flow rate at the level
 186 of 1 mL/min, one can realize, that it creates around 0.5-1 L of waste per day, giving around
 187 26,000,000–52,000,000 L of waste per year [51]. Due to this fact, green strategies and
 188 techniques targeted to the LC improvement are focused on solvent issue, including finding
 189 alternatives for organic solvents in HPLC. Other approaches applied to minimize organic
 190 solvents have been the introduction of surfactants or additives to the mobile phase [49]. Some
 191 of these additives have included DES (e.g. ChCl-Glycerol, ChCl-Ethylene Glycol), most of
 192 which can be environmentally considered as “green” solvents.

193 Only several works report the application of DESs as mobile phase in
 194 chromatography. The proposed mechanism is presented in Figure 6.

195

196 Figure 6. Schematic of application of DES as the mobile phase in RPLC.
197

198 The rare application of DESs as mobile phase in LC is due to the fact, that these
199 solvents are characterized by a high viscosity and such parameter disqualifies DESs to be
200 applied without dilution as mobile phase [52]. From the other side, addition of water impact
201 on the decomposition of DESs into their constituent compounds. Such a process affects
202 chromatography conditions. In this sense it must be mentioned that these effects do not
203 concern DESs but the aqueous solutions of their components [52]. Additionally, there are
204 other problems with application of DESs as mobile phase as presented in Figure 7. Due to
205 these reasons, application of DESs as mobile phase in chromatography have not yet brought
206 significant improvements. But what is worth to note, natural DESs, due to their
207 characteristics, show greater potential in this area. Such application was presented by Sutton
208 et al. [51]. Natural DES are shown to give chromatography performances in between those
209 observed for ACN and MeOH when eluotropic strength, resolution, and peak capacity were
210 taken into consideration, notwithstanding, the best overall performance for the mixtures tested
211 in that study was presented by acetonitrile. However, it would be worth to investigate the
212 addition of water as well as application of temperature which can impact on the decreasing
213 viscosity as well as the eluotropic strength of the new mobile phases. Such solution could also
214 result in fine tuning selectivity. Authors of presented work payed attention to the future
215 research and stayed that the development of appropriate technologies must be considered
216 essential before natural DES can be routinely used in HPLC analysis [51]. These
217 improvements include: an improved pump system to generate high back-pressure, high-
218 temperature durable instrument, and stationary phases that allow the chromatographic
219 analysis to operate at a high temperature to reduce the viscosity of DES.
220

221 Figure 7. Challenges to the use of DES as mobile phase
222

223 Although, the application of DESs as mobile phase is not successful yet, these solvents are
224 widely used as mobile phase additives (Figure 8). Additives of mobile-phase are sometimes
225 applied in order to optimize the chromatographic behaviours such as eliminating band tailing
226 and increasing the number of theoretical plates [53]. It has been shown that even at a small
227 amount, DESs can drastically improve the chromatographic performance in aspects of peak
228 tailing, band broadening and resolution. It also need to be mentioned that the separation
229 mechanism of DESs as mobile phase additives could be attributed to the combined effect of
230 hydrogen acceptors as well hydrogen-bond donors [54]. In the reserach performed by Tan et
231 al. [54], the following relationship has been shown: the single addition of hydrogen-bond
232 donor cannot suppress peak tailing, while the single addition of hydrogen-bond acceptor
233 showed a positive effect. Furthermore, the changes in the type of hydrogen-bond acceptor in
234 DES impact on the alteration of retention ability of the chromatography column. In addition,
235 DESs concentration was found to be a significant factor in chromatographic separation.
236

237 Figure 8. Schematic representation of the application of DES (in the example of ChCl/EG) as
238 the mobile phase additive in an RPLC system.
239

240 In another work [53], it is noticed that DESs can act as mobile phase additives, and their
 241 components can simultaneously work as ions and play anion pairing role. The DESs cation
 242 would compete with the polar group of target analytes for the free silanol groups on the
 243 surface of stationary phase by the specific electrostatic interactions. The cation might be
 244 helpful for creating the ion pairs with the anion solutes. Contrary, depending on the anion, it
 245 could have less localized charge, lower degree of hydration and high polarizability (e.g.
 246 [Cl⁻]). In such case, the anion can disrupt the sheath of water molecules around the analytes
 247 by hydrogen bond, and the hydrophobicity of the analytes can increase noticeably [53,55].
 248 Information on the application of DESs as mobile phase/mobile phase additives are presented
 249 in Table 3.

250

251 Table 3. Information on the application of DESs as mobile phase/mobile phase additives

Matrice	Analyte(s)	DES (m.r.)	%DES in m.p.	Separation conditions	Analytical methodology	Ref.
Herbal oral solution	Quaternary alkaloids	ChCl-EG (1:3)	1	Column: C18 Mobile phase: ACN:HCl (32:68 v/v); pH 3.3	HPLC-UV	[54]
Standard mixture	Quercetin	ChCl-EG (1:2)	0.2	Column: C18 Mobile phase: MeOH:water (60:40 v/v/)	HPLC-UV-VIS	[53]
Standard mixture	Caffeic acid	ChCl-glycerol (1:3)	0.1	Column: C18 Mobile phase: MeOH:water (18:82 v/v/)	HPLC-UV-VIS	[55]
Urine	Cardiovascular drugs	ChCl-EG-based NADES (2:1)	3.5	Mobile phase: SDS:buthanol:NADES:GA C (83:10:3.5:3.5 v/v/v/v/)	MLC-UV	[56]
Plasma	Cardiovascular drugs	ChCl-EG-based NADES (2:1)	3.5	Mobile phase: SDS:buthanol:NADES:GA C (83:10:3.5:3.5 v/v/v/v/)	MLC-UV	[56]
Milk	Melamine	ChCl-EG-based NADES (1:2)	4	Mobile phase: SDS:NADES:GAC	MLC-UV	[57]
Standard mixture	Nucleobases, nucleosides	ChCl-EG (1:3)	65	Column: C18 Mobile phase: Water:EtOH:NADES (30:5:65 v/v/v/)	UHPLC-DAD	[51]
Standard mixture	Caffeine, vanillin, coumarin, carvone, β -ionone, and β -carotene	L-menthol-levulinic acid (1:1)	5	Column: teflon Mobile phase: n-heptan: MeOH:DES	CPC	[58]
Standard mixture	Tocopherol mixture	ChCl-1,4-butanediol (1:1)	30	Mobile phase: heptane/EtOH/DES (30/40/30 wt/wt/wt)	CPC	[59]
Chelidonium maiusroot extract	Alkaloids	Menthol-phenol (1:1)	65	TLC Si60 plates; Mobile phase: DES/MeOH (65/35 wt/wt)	TLC	[60]
Standard mixture	Benzenes, PAHs, nucleosides, alkaloids	ChCl-IA: (2:1)	-	Electrolyte solution: ACN: phosphates buffers Column: Silica capillary:DES	CEC	[61]

ACN, acetonitrile; CEC, capillary electrochromatography; ChCl, choline chloride; ClChCl, chlorocholine chloride; CPC, centrifugal partition chromatography; EG, ethylene glycol; EGDMA, ethylene glycol dimethacrylate; GAC, glacial acetic acid; HI, hydrophilic interaction; HPLC, high performance liquid chromatography; IA, itaconic acid; MeOH, methanol; MLC, micellar liquid chromatography; NADES, natural deep eutectic solvent; PAHs, polycyclic aromatic hydrocarbons; SDS, sodium dodecyl sulphate; TLC, thin layer chromatography

m.p., mobile phase; m.r., molar ratio

252

253

254 3.1.1. *Application of DESs in countercurrent and centrifugal chromatography*

255 Countercurrent chromatography (CCC) is a separation technique based on the partition
256 of solutes between two different liquid phases. The technique is created by a multi-solvent
257 biphasic system, in which stationary phase is kept in the column with the aid of a centrifugal
258 field, while the mobile phase is pumped through the column. In practice, the CCC biphasic
259 system consists of three/four solvents which differ in polarity. In CCC, target analytes are
260 separated by application of changes in the ratio of the biphasic system components to adjust
261 their partition coefficients between both phases [62].

262 For the first time, DES were evaluated as solvents in CCC in 2016 [62]. In that research,
263 DESs have been shown to be a promising new class of solvents in CCC or CPC. They can be
264 used to substitute the water in biphasic systems composed of water and organic solvents.
265 From that time, several other studies have been published [58,59].

266

267 3.1.2. *Application of DESs in thin layer chromatography (TLC)*

268 In 2020, the results of a preliminary investigation of DES being employed as mobile phases in
269 thin layer chromatography (TLC) were published for the first time [60]. The work was
270 focused on the use of eutectic liquids allowing chromatographic separation of mixtures of
271 natural compounds, with particular regard to alkaloids. For this purpose several NADES
272 eutectic solvents were selected. In most of the tested modifications at least partial separation
273 of target analytes was achieved. The most successful mobile phase which enabled separation
274 of all the tested alkaloids was this where DES was diluted with the equimolar mixture of
275 menthol and phenol with a 35% addition of methanol. The study presents high potential of
276 DESs as mobile phase in TLC. However, future studies are highly recommended to explore
277 new methodological solutions and application possibilities.

278

279 3.2. *DESs as stationary phases or surface modifiers*

280 Nowadays, the stabilization of DES on an appropriate matrix to act as chromatography
281 stationary phase is attempted. However only few papers focused on the application of DESs
282 as stationary phase are available [61,63,64]. Considering results presented in these papers it
283 can be concluded that the modification of DES stability on matrix seems to be a key problem
284 in chromatography application. This is mainly due to the fact that although the hydrogen bond
285 acceptor can be covalently grafted to the matrix, the stabilization of hydrogen bond donor is
286 mainly based on the hydrogen bonding with HBA which can be easily break up by the rinse of
287 aqueous sample or elution [7]. Several methods could be used to regulation of the stability of
288 DES based stationary phase. In such way, several procedures have been proposed: i)

289 application of hydrophobic DES as stationary phase and elution with aqueous solvent, ii) co-
290 polymerization of polymerizable HBAs and HBDs onto matrix, or iii) application of
291 hydrophilic DES stationary phase in normal phase liquid chromatography.

292 A novel monolithic column, based on the copolymerization of ethylene dimethacrylate and a
293 DES composed of ChCl and itaconic acid, was developed for capillary electrochromatography
294 (CEC) by Wang et al. [61]. The basic research present a porous monolithic structure with
295 good permeability. The developed column exhibited excellent performance for the separation
296 of neutral compounds, phenols, toluidines, nucleosides, nucleotide bases and alkaloids. It was
297 stated by the author that the potential retention mechanism might be attributed to synergistic
298 effect of hydrophobic interaction, hydrogen bond interaction and electronic interaction [61].
299 The DES-based monolithic column was characterized by good analytical repeatability. The
300 success of the research team of Wang indicates that organic polymer monolithic columns with
301 DESs as functional monomers are a promising stationary phases in chromatography.

302

303 *3.3. DESs as the reaction solvent or solvent additive for the preparation of* 304 *chromatographic materials*

305 DESs are widely used as substitute to traditional solvents in organic synthesis [65,66]
306 and enzymatic reactions [67,68]. These successful applications inspired chromatography
307 researchers to evaluate the possibility of DESs application as solvent for preparative
308 chromatography. Preparation of stationary phases using DES as solvent is the most often
309 performed reaction in this area (Table B), including: silanization reaction [69], surface radical
310 chain-transfer reaction [70], and epoxy ring-opening reaction [71] (Table 4).

311 The first report on the silylation of silica particles was performed by Gu et al. using a
312 classic DES of ChCl/urea and successful preparation a glucamine-modified silica stationary
313 phase in DES [69]. The silica suspension can be maintained for 24 h in DES. However, it can
314 be maintained only for less than 5 min in toluene. Fine dispersibility is probably due to the
315 strong hydrogen bonding between hydrogen bond acceptor (ChCl) and hydrogen bond donor
316 (silanol on the silica surface) as well as the appropriate viscosity of DES. It need to be
317 mentioned that the stable suspension of silica is an advantage for the uniform modification of
318 its surface.

319 A new stationary phase based on poly(itaconic acid)-grafted silica (Sil-PIA) was
320 synthesized in DESs and characterized in detail [70]. Itaconic acid was homopolymerized on
321 silica via surface radical chain-transfer using DESs as a green solvents. The results were
322 compared with previous reported poly(acrylic acid)-grafted silica (Sil-PAA) stationary phase
323 with satisfactory results. Sil-PIA provided shorter retention time but similar or higher
324 selectivity for the separation of most polar compounds; such as bases, nucleosides, amino
325 acids, and saccharides in hydrophilic interaction chromatography. In addition, Sil-PIA
326 presented very good performance in the separation of eleven ginsenosides using isocratic
327 elution in hydrophilic chromatography.

328 In another work, two homopolymerized and one copolymerized silica-based stationary
329 phases were successfully prepared in DESs by the surface radical chain-transfer reaction by
330 Yang et al. [72]. Through investigating the effects of different chromatographic conditions on
331 retention of three stationary phases, it can be found that the retention of solutes on three
332 stationary phases were based on both, partitioning mechanism and adsorptive interactions as

333 electronic interaction and hydrogen bonding. In comparison with the conventional solvents
 334 as chloroform and methanol, higher surface coverage was found in the case of DESs. In
 335 addition, similar results of elemental analysis were found for the materials prepared
 336 repeatedly in DESs. However, because of the application of some aliquote of other
 337 compounds (methanol and CCl₄) during column packing, the proposed method reduced the
 338 use of toxic solvents but it is still not 100% green method. However, this study presents the
 339 huge potential in the area of micro- or nano-materials preparation that could be used in
 340 separation science and other research fields.

341 Zgand et al. [73] published very interesting results focused on application of N-doped
 342 carbon dots (NCDs) modifiers of the spherical porous silica surface in DESs. The appropriate
 343 density and hydrophibility of DESs guaranteed the fine dispersibility of silica particles and
 344 NCDs, provided a homogeneous and thin layer of immobilized NCDs. In comparison with
 345 conventional organic solvents (DMF and THF), increased surface coverage was obtained in
 346 the DES medium, proving its feasibility as a new kind of alternative solvent for hydrophilic
 347 nanomaterial-based surface modification of silica spheres. The new NCDs-silicaparticles (Sil-
 348 NCDs) were packed into chromatographic columns to study their initial feasibility as
 349 adsorbent material for LC. The results were satisfactory, and the new column presented a
 350 selective behavior for polar compounds in HILIC mode. It can be stated that the new Sil-
 351 NCDs stationary phases greatly broaden the application of carbon dots and give a typical
 352 example to design novel nano-on-micro materials in DESs. Moreover, this kind of NCDs
 353 decorated silica spheres are very promising to be used in other chemical and engineering
 354 fields.

355 After considering above mentioned studies, it must be stated that the high viscosity of
 356 DESs helps to create a stable dispersion of silica gel in comparison with organic solvents.
 357 Moreover, this parameter prevents particle aggregation, leading to an improved efficient
 358 modification.

359 It need to be also mentioned that no work has yet been presented on the application of
 360 DESs as stationary phases in GC. This is probably due to the fact that in GC high
 361 temperatures applied can weaken the hydrogen bonds in DESs, leading to their
 362 decomposition. However, it is still recommended to focus on this area as DESs are quite
 363 thermally stable compounds.

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365 Table 4. Preparation reactions of stationary phases using DES as solvent

Reaction solvent	Reaction type	Stationary phase	Application	Methodology	Ref.
ChCl: EG (1:3)	Surface radical chain-transfer reaction	Sil-PIA	Nucleosides, nucleobases, amino acids, saccharides, ginsenosides	HILIC	[70]
ChCl:urea (1:2)		Sil-PIm;	Nucleosides, saccharides, nucleobases, amino acids	HILIC	[72]
ChCl:glycerol (1:2)		Sil-PAA; Sil-PIm-PAA			

ChCl: EG (1:3)	Epoxy ring- opening reaction	Sil-PEI; Sil-PEICDs	Nucleosides, nucleobases, ginsenosides	HILIC	[71]
ChCl:EG (1:3)		Sil-NCDs	Tryptophan, nucleosides, nucleobases, saccharides	HILIC	[73]
ChCl:urea (1:2)	Silanization reaction	Sil-N-Glu	Sulfamides	HPLC	[69]

EG, ethylene glycol; ChCl, choline chloride; HILIC, hydrophilic interaction chromatography; NCDs, N-doped carbon dots; PEI, polyethyleneimine; PEICDs, PEI-functionalized carbon dots; Sil-N-Glu, N-methyl-glucamine-modified silic; Sil-PAA, poly(acrylic acid)-grafted silica; Sil-PIA, poly(itaconic acid)-grafted silica; Sil-PIm, poly(1-vinylimidazole)-grafted silica; Sil-PIm-PAA, poly(1-vinylimidazole-co-acrylic acid)-grafted silica

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4. Conclusions and future perspectives

Promising perspectives of the use of DES in Chromatography are based on the easy availability of DES components and the fact that many of these are of natural origin and thus renewable. So, from the Green Analytical point of view these new solvents offer non-toxic sustainable alternatives to traditional organic solvents and ILs. However, the relative high viscosity, low volatility strong UV absorption and lack of HPLC grade purity of many of DES components create drawbacks on their use as mobile phases in LC or general use in GC. However, promising perspectives focused on the modification of stationary and mobile phases for GC or HPLC, additionally on the use of DES for sample preparation previously to their chromatography determination. So, it can be expected that in the next years the use of DES could be extended to the development of new robust and high performance chromatography methods. In this way it must be explored the effect of temperature and the presence of small amounts of water on the DES systems.

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