Natural deep eutectic solvents and their application in natural product research and development

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Chapter 2

Liquefied mixtures of solids can extract natural products: application of ionic liquids and deep eutectic solvents to natural product research

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Abstract

Mixtures of solid chemicals may become liquids under certain conditions. The liquids are characterized by formation of strong ionic (ionic liquids) or hydrogen bonds (deep eutectic solvents). Due to their extremely low vapor pressure, these liquids are now widely used in polymer chemistry and synthetic organic chemistry, yet little attention has been paid to the extraction capacity of ionic liquids or deep eutectic solvents for natural products. This review summarizes the preparation of ionic liquids and deep eutectic solvents with natural product components, and recent progress in their application to the extractions and analysis of natural products, covering both primary and secondary metabolites, and enzymatic reactions alternative to conventional organic solvents. Additionally, the various factors affecting extraction features of ionic liquids and deep eutectic solvents, as well as potential technologies including microwave and supercritical fluid extraction to increase the extraction efficiency are discussed.

Key words: ionic liquids; deep eutectic solvents; natural products; herbal medicine; extraction; recovery; enzyme activity.

1. Introduction

Ionic liquids (ILs) are low melting point (＜100 ^{o}C) organic salts composed of organic cations and organic or inorganic anions. The well-accepted definition of a room temperature ionic liquid (RTIL) is "a salt that has a melting point lower than ambient temperature" (Welton, 1999). Deep eutectic solvents (DES) are a new class of solvents obtained by mixing solid compounds in certain Molar ratiosinto an eutectic mixture with a melting point that is much lower than either of the individual components (Abbott *et al*., 2004). In most cases, ILs have an asymmetrically substituted cation (e.g., imidazolium, pyrrolidinium, pyridinium, ammonium phosphonium) and a variety of anions, especially halogen-based anions (e.g., [Cl], [Br], [I], [BF_4], [$AlCl_4$], [PF_6]), as summarized in table 1. To overcome the potential toxicity of ILs with halogen-containing anions (Swatloski *et al*., 2003; Garcia-Lorenzo *et al*., 2008), these were replaced by biomaterial-derived products such as organic acids, amino acids, amines or sugars, creating a new safer type of ILs known as "Bio-ILs" or " green ILs" (Tao *et al*., 2006; Fukaya *et al*., 2007; Hu *et al*., 2007). There are also reports of DES made of various natural products that fit into this category (Abbott *et al*., 2003; 2004; Imperato *et al*., 2005; Choi *et al*., 2011).

Interest in ILs stems from this potential application as ''green solvents'' based on perhaps, their most important feature: nearly complete non-volatility at ambient condition. Besides, ILs have many attractive physicochemical properties, such as chemical and thermal stability, non-flammability, high conductivity and a good solubilizing capacity of various organic compounds (Welton, 1999). Both the anion and the length of the *n*-alkyl chain in the cation of the IL affect its physical properties (Zhang *et al*., 2008) and its viscosity and polarity, for example, can be tuned by changing the cation-anion combination. Thus, ILs are termed "tailor-made solvents" and offer a huge potential for practical applications (Visser *et al*., 2001). The same properties are also observed in DES, converting both these types of liquids into ideal substitutes of conventional organic solvents.

Recently, various applications of ILs and DES have been reported. In the case of natural product analysis, ILs have been used as a stationary phase or a mobile phase additive in chromatography (Berthod *et al*., 2008; Herrera-Herrera *et al*., 2009; Tian *et al*., 2010). Ionic liquids have been used for the extraction of compounds of diverse polarity from liquid samples, such as the extraction of polyunsaturated fatty acid methyl esters, organic acids, amino acids, phenols, alkaloids and proteins from aqueous solutions. In the case of solid matrix samples, ILs' applications have been limited, partly due to their high viscosity. However, when applied in combination with other technologies, such as microwave, ultrasonic treatment, or supercritical CO_2 extraction, the extraction process and subsequent separation of the components was greatly facilitated. For example, ILs have been successfully employed to extract different kinds of natural products, such as alkaloids, carbohydrates, polyphenolic compounds, and sesquiterpenes. After extraction, the resulting extracts were generally

diluted with water or organic solvents for further HPLC analysis (Du *et al*., 2007) and volatile compounds were evaporated for GC analysis (Bica *et al*., 2011). The lack of volatility of ILs makes it nearly impossible to directly concentrate and isolate non-volatile compounds from them, but the use of supercritical CO_2 extraction (Kroon *et al*., 2006), with the addition of anti-solvents (Lapkin *et al*., 2006), back extraction (Yu *et al*., 2007) and column separation30 have been successfully used to resolve this problem.

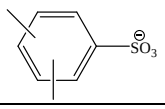
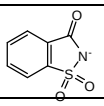
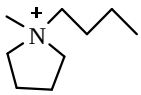
Depending on the structure of the cations and anions, ILs with different physical properties have been obtained. This means that it is possible to design an optimal IL for a specific compound, therefore allowing specific "object-oriented" or "task specific" ILs to be prepared. For example, some ILs with larger π values are expected to solubilize many π conjugated organic substances (Holbrey *et al*., 2008; Du *et al*., 2009). Also, ILs based on simple natural compounds can be synthesized for the extraction of certain kinds of natural products, such as amino acid-based ionic liquids (AAILs) that display enantioselectivity in the extraction of amino acids (Tang *et al*., 2010) and Gly-ChCl, which has been used to extract glycerol from biofuel (Hayyan *et al*., 2010). Similarly, the application of other types of ILs and DESs in the extraction of natural products should be investigated further in terms of their physical properties.

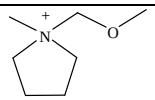
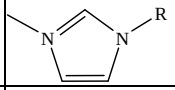
In the case of natural products, these solvents offer a prospect of being novel extraction methods, with a high efficiency and the yield of a greater variety of compounds given the high dissolving power of some ILs for cellulose (Swatloski *et al*., 2002). There are also other great advantages of ILs over conventional organic solvents. For instance, some ILs have been use to extract both essential oils and polar compounds in the same step (Ma *et al*., 2011). However, because of their high viscosity, most ILs are used as diluted aqueous solutions (Du *et al*., 2007). The extraction efficiency of other types of low-viscosity ILs, such as ILs made of natural products, which have been developed requires further exploration, since there are few reports of their applications.

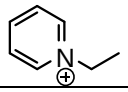
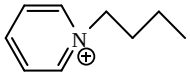
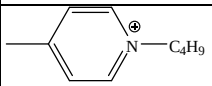
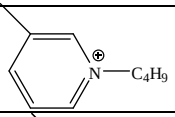
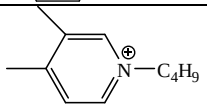
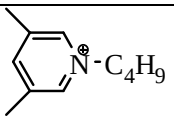
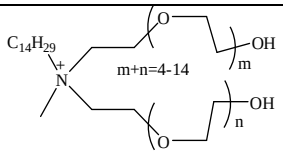
In this paper, all major aspects of ILs and DES concerning natural products will be reviewed, including the synthesis of "green" ILs and DES with natural products and their properties (table 2, 3), and their application to product extraction (table 4, 5), factors affecting the extraction efficiency of ILs, the combined use of ILs with other technologies, methods for the analysis of ILs extracts, and the recovery of compounds from ILs. In addition, enzyme reactions in ILs will also be reviewed (table 6). The problems and challenges that have arisen from the above applications will also be discussed.

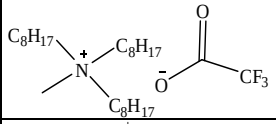
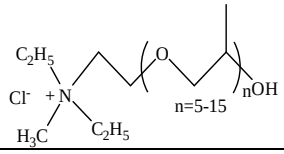
Table 1. The main anions and cations of ionic liquids and deep eutectic solvents.

Components	**Full name**	**Structure or molecular**
anion		

[BF_4]	tetrafluoroborate	BF_4^-
[DAC]	dicyanamide	$(CN)_2N^-$
[SCN]	thiocyanate	SCN^-
[Tf_2N]	bis(trifluoromethanesulfonyl) amide	$(CF_3SO_2)_2N^-$
[OAc]/[ACE]	acetate	CH_3COO^-
[Oct]	octanoate	$CH_3(CH_2)_5\ CH_2COO^-$
[lactate]	lactate	$CH_3CHOHCOO^-$
[salicylate]	2-hydroxybenzoic acid salt	$HOC_6H_5COO^-$
[SO_4]	sulfate	SO_4^{2-}
[$MeSO_4$]/[MS]	methylsulfate	$CH_3\text{-}SO_4^-$
[$EtSO_4$]	ethylsulfate	$C_2H_5\text{-}SO_4^-$
[OSO_4]	octylsulfate	$CH_3\text{-}(CH_2)_7\text{-}SO_4^-$
[MDEGSO4]/ [Meesu]	2-(2-methoxyethoxy) ethylsulfate	$CH_3OC_2H_5OC_2H_4SO_4^-$
[cetyl-PEG10-sulfate]	cetyl-PEG10-sulfate	$C_{16}H_{33}\text{-}PEG_{10}\text{-}SO_4^-$
[MeSO3]	methanesulfonate	$CH_3\text{-}SO_3^-$
[TfO]/[Tf]	trifluoromethane sulfonate	$CF_3\text{-}SO_3^-$
[TSO]	toluolsulfonate	$C_6H_5\text{-}SO_3^-$
[ABS]	alkylbenzenesulfonate	
[XS]	xylenesulfonate	
[H_2PO_4]	dihydrogenphosphate	$H_2PO_4^-$
[$MeHPO_4$]	methylphosphate	$(CH_3)\text{-}HPO_4^-$
[$PHPO_4$]	propylphosphate	$C_3H_7\text{-}HPO_4^-$
[Me_2PO_4]	dimethylphosphate	$(CH_3)_2\text{-}PO_4^-$
[$BHPO_4$]	butylphosphate	$C_4H_9\text{-}\ HPO_4^-$
[Sac]	saccharide	
cation		
[BMP]	1-butyl-1-methylpyrrolidinium	

[MMEP]	1-methyl-1-(-2-methoxyethyl) pyrrolidinium	
[Rmim]	1-R-3-methylimidazolium	
[Emim]	1-ethyl-3-methylimidazolium	R=CH_2CH_3
[Bmim]/[C_4mim]	1-butyl-3-methylimidazolium	R=$CH_2CH_2CH_2CH_3$
[Amim]	1-allyl-3-methyl imidazolium	R=$CH_2CH=CH_2$
[Pmim]	1-propyl-3-methylimidazolium	R=$CH_2CH_2CH_3$
[C_2OHmim]	1-hydroxymethyl-3-methylimidazolium	R=CH_2OH
[MOMmim]	1-methoxymethyl-3 -methylimidazolium	R=CH_2OCH_3
[MOEmim]	1-methoxyethyl-3-methylimidazolium	R=$CH_2CH_2OCH_3$
[EOEmim]	1-ethoxyethyl-3-methylimidazolium	R=$CH_2CH_2OCH_2CH_3$
[C_4SO_3mim]	1-(3-sulfopropyl)-3-methylimidazolium	R=$CH_2CH_2CH_2SO_3$
[Hmim]/[C_6mim]	1-hexyl-3-methylimidazolium	R=$(CH_2)_5CH_3$
[Omim]/ [C_8mim]	1-octyl-3-methylimidazolium	R=$(CH_2)_7CH_3$
[Nmim]/ [C_9mim]	1-nonyl-3-methylimidazolium	R=$(CH_2)_8CH_3$
[Bzmim]	1-benzyl-3-methylimidazolium	R=C_6H_5
[Btmsim]	1-butyl-3-trimethylsilyl imidazolium	R_1=$CH_2CH_2CH_2CH_3$ R_3=$(CH_3)_3Si$
[BBim]	1,3-dibutylimidazolium	R_1=R_3= $CH_2CH_2CH_2CH_3$
[C_5O_2mim]	1-[2-(2-methoxy ethoxy)-ethyl]-3-methyl-imidazolium	R= $CH_2CH_2OCH_2CH_2$ OCH_3

[C_2py]/ [Epy]	1-ethylpyridinium	
[Bpy]	1-butylpyridinium	
[BM_4Py]	1-butyl-4-methylpyridinium	
[BM_3Py]	1-butyl-3-methylpyridinium	
[$BdM_{3,4}$Py]	1-butyl-3,4-dimethylpyridinium	
[$BdM_{3,5}$Py]	1-butyl-3,5-dimethylpyridinium	
[TBP/P_{4444}]	*tetra*-*n*-butylphosphonium	$(CH_3CH_2CH_2CH_2)_4P^+$
[TEP/P_{2222}]	*tetra*-ethylphosphonium	$(CH_3CH_2)_4P^+$
[P_{66614}]	trihexyl(tetradecyl) phosphonium	$(C_6H_{13})_3P^+C_{14}H_{29}$
[TAA]	*tetra*-alkylammonium	$(C_nH_{2n+1})_4N^+$
[CPMA]	cocosalkyl pentaethoxy methyl ammonium	
[TMA/N_{1111}]	*tetra*-methylammonium	$(CH_3)_4N^+$
[TEA/ N_{2222}]	*tetra*-ethylammonium	$(CH_3CH_2)_4N^+$
[N_{4444}]	*tetra*-butylammonium	$(CH_3CH_2CH_2CH_2)_4N^+$
[BMOEA]	bis(2-methoxyethyl) ammonium	$(CH_3OCH_2CH_2)_2NH_2^+$
[DMEA]	*N*,*N*-dimethyl ethanolammonium	$(CH_3)_2NH^+CH_2CH_2OH$
[DMMOEA]	*N*,*N*-dimethyl(2-methoxyethyl) ammonium	$(CH_3)_2NH^+ CH_2CH_2OCH_3$
[Ch]/[$N_{111}(C_2OH)$]	choline/(2-hydroxyethyl) trimethylammonium	$HOCH_2CH_2N^+ (CH_3)_3$
anion+cation		

[MTOATFA]	methyltrioctylammonium trifluoroacetate	
[DIMCARB]	*N*'*N*'-dimethyl ammonium *N'N'*-dimethylcarbamate	$(CH_3)_2NH_2^+$ $^-OOCN(CH_3)_2$
[Amim110]	AMMOENGTM 110	
components of DES		
EAC	ethylammonium chloride	$C_2H_5NH_3^+$ Cl^-
ChCl	choline chloride	$(CH_3)_3$ $N^+CH_2CH_2OH$ Cl^-
U	urea	NH_2CONH_2
Gly	glycerol	$(HOCH_2)_2CHOH$
EG	ethyl glycol	$HOCH_2CH_2OH$
Acet	acetamide	CH_3CONH_2

2. Preparation of ionic liquids (ILs) and deep eutectic solvents (DES), their properties

2.1 Preparation of Ionic liquids with natural products

In recent times, there is a growing consensus on the acceptance of ILs as greener alternatives to volatile organic solvents, mainly due to their negligible vapor pressure. However, they fall short of complying with the 12 principles of green chemistry, casting doubt on the legitimacy of this claim (Anastas and Eghbali, 2010). Commonly used ILs based on halogenated anions and their derivatives are low melting point ILs, but their applications are obviously limited by toxicological (Swatloski *et al*., 2003), ecological and economic issues. ILs with the imidazolium cation are synthetic chemicals, even though some of them use amino acids as starting materials (Bao *et al*., 2002). Furthermore, some of these imidazolium-based ILs are also toxic (Garcia-Lorenzo *et al*., 2008).

In order to obtain ‘‘green’’ ILs, the first condition is that the starting materials at least must be non-toxic, whilst for a perfect solution, they should be renewable; secondly, the development of “green” ILs should require relatively low production costs, easy preparation and a green route; another requirement for “green” ILs is that the “designer solvent” properties of ILs be maintained, i.e., that the physicochemical properties of the obtained ILs can be adjusted by e.g. changing the side-chain attached to the main group, and even that the

natural properties of the materials be completely conserved. Bio-renewable natural compounds that have well characterized biodegradable and toxicological properties are ideal materials from both environmental and economical viewpoints. Recently, some kinds of bio-renewable natural products, including organic acids, amino acids, sugars and their derivatives, have been applied to prepare "green" ILs (table 2) and DES (table 3) (Imperato *et al*., 2007; Choi *et al*., 2011).

Table 2. The physical properties of some ionic liquids made of natural products.

ILs	T_m/ °C	T_g/ °C	T_{dec}/	viscosity/cP	Ref.
organic acids					
[Ch][acetate]	51	a	189		Fukaya *et al*., 2007
[Ch][glycolate]	38	-67	220		
[Ch][benzoate]	47	-51	202		
[Ch][propionate]	a	-74	184		
[Ch][succinate]	a	-62	192		
[Ch][malate]	99	-52	212		
[Ch][tartrate]	131	-40	210		
[Ch][maleate]	25	-6	203		
[Ch][fumarate]	80	-72	219		
amino acids					
[Emim][Gly]	b	-65		486	Fukumoto *et al*., 2005
[Emim][Ala]	b	-57			
[Emim][Met]	b	-57			
[Emim][Val]	b	-52			
[Emim][Ile]	b	-52			
[Emim][Leu]	b	-51			
[Emim][Ser]	b	-49			
[Emim][Lys]	b	-47			
[Emim][Thr]	b	-40			
[Emim][Phe]	b	-36			
[Emim][Trp]	b	-31			
[Emim][His]	b	-24			
[Emim][Tyr]	b	-23			
[Emim][Cys]	b	-19			
[Emim][Arg]	b	-18			

[Emim][Asn]	b	-16			
[Emim][Gln]	b	-12			
[Emim][Asp]	b	5			
[Emim][Glu]	b	6			
[Emim][Pro]	b	-48			
$[N_{4444}]$[Ala]	76	a	162		Kagimoto *et al*.,
$[N_{2226}]$[Ala]	a	-40	150		2006
$[N_{1111}]$[L-Ala]	42	a	219	a	Jiang *et al*., 2008
$[N_{2222}]$[L-Ala]	a	-80	185	81	
$[N_{1111}][\beta$-Ala]	a	-82	193	668	
$[N_{2222}][\beta$-Ala]	a	-85	184	132	
$[N_{4444}][\beta$-Ala]	a	-73	180	465	
$[N_{1111}]$[Gly]	a	-68	181	304	
$[N_{4444}]$[Gly]	16	-71	179	214	
$[N_{1111}]$[Val]	40	a	223	c	
$[N_{4444}]$[Val]	25	-69	185	660	
[Ch][Pro]	RT	-	159	-	Hu *et al*., 2007
[Gly][NO_3]	111		192		Tao *et al*., 2005
[Ala][NO_3]	159		168		
$[Ala]_2[SO_4]$	141		193		
[Val][NO_3]	134		169		
[Ile][NO_3]	105		167		
[Thr][NO_3]	b		147		
[Pro][NO_3]	b	-45	138	5140(30 ^oC)	
$[Pro]_2[SO_4]$	92		206		
$[GlyC_1]NO_3]$	44	-26	178	92(70 ^oC)	Tao *et al*., 2005
$[AlaC_1][NO_3]$	61	-34	186	104(80 ^oC)	
$[ValC_1][NO_3]$	74	-33	195	445(80 ^oC)	
$[LeuC_1]NO_3]$	75	-31	210	1550(80 ^oC)	
$[IleC_1][NO_3]$	-14	-36	172	-	
$[PheC_1][NO_3]$	92	-32	224	-	
$[ThrC_1][NO_3]$	-12	-32	156	-	
$[SerC_1][NO_3]$	105	-30	179	-	

[ProC$_1$][NO$_3$]	-16	-67	159	186(30 ^{o}C)	
[ProC$_2$][NO$_3$]	-17	-50	183	213(30 ^{o}C)	
[ProC$_3$][NO$_3$]	6	-71	208	398(30 ^{o}C)	
[ProC$_4$][NO$_3$]	-11	-70	163	275(30 ^{o}C)	
[AlaC$_1$][Sac]	-14	-27	184	1390(80 ^{o}C)	Tao *et al*., 2006
[ValC$_1$][Sac]	-16	-29	185	3040(80 ^{o}C)	
[LeuC$_1$][Sac]	7	-1	223	1050(80 ^{o}C)	
[IleC$_1$][Sac]	7	-8	177	14500(80	
[ThrC$_1$][Sac]	-1	-9	200	55900(80	
[ProC$_1$][Sac]	-19	-29	190	3320(80 ^{o}C)	
[ProC$_2$][Sac]	8	-42	212	3021(50 ^{o}C)	
[P$_{4444}$][Ala]	a	-70	286	344	Tao *et al*., 2006
[P$_{4444}$][Met]	a	-63.5		371	
[P$_{4444}$][Gly]	13.6	-63.7		415	
[P$_{4444}$][Leu]	30	-63.4		389	
[P$_{4444}$][Ile]	b	-60.7		605	
[P$_{4444}$][Ser]	b	-59.9		902	
[P$_{4444}$][Val]	26	-59.1	b	423	
[P$_{4444}$][Lys]	b	-58.8		779	
[P$_{4444}$][Pro]	25.4	-57.5		851	
[P$_{4444}$][Thr]	b	-56.1		965	
[P$_{4444}$][Phe]	8.1	-53.1		927	
[P$_{4444}$][Arg]	30.7	-36		c	
[P$_{4444}$][Trp]	b	-25.6		c	
[P$_{4444}$][Gln]	b	-25		c	
[P$_{4444}$][Glu]	101.7	-23.3		c	
[P$_{4444}$][Gys]	b	-20.7		3029	
[P$_{4444}$][Asp]	b	-7.6		c	
[P$_{4444}$][Tyr]	b	-6.5		c	
[P$_{4444}$][Asn]	83	-3.9		c	
[P$_{4444}$][His]	85.9	a		299	
sugars					
[C$_4$mim][Sac]	RT				Carter *et al*.,
[C$_6$mim][Sac]	RT				

[C_9mim][Sac]	RT				2004
[C_4mim][Ace]	RT				
[C_6mim][Ace]	RT				
[C_9mim][Ace]	RT				
[Ch][Sac]	69			328(70 ^{o}C)	Nockemann *et*
[Ch][Ace]	25			1072(25 ^{o}C)	*al*., 2007

T_m: melting point; T_g: glass transition point; T_{dec}: decomposition temperature.
[a]: not observed; [b]: no melting point; [c]: solid or glass at 25 ^{o}C.

Organic acids A series of ILs composed of choline and organic acids with strong hydrogen bonding characteristics were prepared (Fukaya *et al*., 2007). These ILs were prepared via a two-step anion exchange reaction. On the other hand, mixtures of organic acids and choline chloride (Table 3) were also reported to form DES by heating.

Sugars and sugar substituents were also considered for the preparation of green ILs. Fructose was used as the starting material of a new class of ILs that exhibited tunable solvent properties much like conventional imidazole-based ILs. The process, however, was not simple and included some toxic chemicals even though the starting material was green (Handy *et al*., 2003). Saccharin (Sac) and acesulfame (Ace) are widely used in foodstuffs as non-nutritive sweeteners and they have well-established toxicological profiles, compared with the common anions in ILs, [Cl] and [BF_4]. A series of ILs made of Sac and Ace as anions with common organic cations were prepared via metathesis (Carter *et al*., 2004). Two green and hydrophilic ILs, [Ch][Ace] and [Ch][Sac], were prepared by a silver-free metathesis reaction and ion-exchange. The ecotoxicity of these ILs in aqueous solution was very low in comparison to other types of ILs (Nockemann et al., 2007).

Amino acids (AAs) are molecules with both a carboxylic acid and an amino group. These groups are useful in their ability to introduce functional group(s) and so ILs based on amino acids may be natural "designer solvents". A family of 20 different natural amino acids ILs (AAILs) combined with an imidazolium cation was prepared via a one-step anion exchange reaction and a neutralization method (Fukumoto *et al*., 2005; Ohno and Fukumoto, 2007). Furthermore, as AAs are zwitterions, they can be used as either anions or cations. AAILs with amino acids as cations were synthesized through a one-step acidification without any poisonous by-products (Tao *et al*., 2005). Although 17 products were obtained, half of them were white solids. Fortunately, not only does esterification reduce the amount of hydrogen bounding, resulting in a significant decrease in the melting points of the salts, but it also provides the possibility of adjusting the properties of the resulting ILs, and the "designer solvent" character of ILs is therefore potentially enhanced. Furthermore, the incorporation of an ester group could increase the biodegradability of those

AAILs. A series of ILs with low melting points has been successfully obtained from amino acid esters, through a one- step esterification and the metathesis method. AAILs with other type of cations have been developed, such as [TBP][AA]s made of tetrabutylphosphonium (TBP) and amino acids (Kagimoto *et al*., 2006), and [TAA][AA]s composed of a tetraalkyl ammonium (TAA) cation and an amino acid (Jiang *et al*., 2008). With the objective of increasing the "greenness" level, [Sac] and [NO_3] were used as anions and a family of novel ILs from amino acids esters (AAE) has synthesized applying the metathesis method. The obtained ILs had the same characteristics as conventional imidazolium ILs and the same chirality as natural amino acids (Tao *et al*., 2006). The natural products choline chloride and proline, were applied to synthesize green solvents by a simple and relatively green route (Hu *et al*., 2007). Based on the above studies, choline hydroxide and five different amino acids (glycine, alanine, phenylalanine, threonine and histidine) were used to form natural AAILs through a one-step neutralization method (Moriel *et al*., 2010). Choline, amino acids, [Sac] and [NO_3], are non-toxic, with well-established toxicological profiles and produce pharmaceutically acceptable ions, so that the resulting ILs could qualify as "fully green solvents". In addition, these AAILs are also called "Chiral ionic liquids (CIL)", "functional or task-specific ILs" and "designer solvents" because they have chiral centers, functional groups and properties that can be adjusted by changing their side-chains. In all cases, the stereogenic center present in the amino acid is successfully conserved in the final CILs. Furthermore, AAILs have biodegradable characteristics and high biocompatibility. AAILs have already been used in the field of organic chemistry for asymmetric catalysis with excellent enantioselectivity and good diastereoselectivity (Winkel *et al*., 2008), as well as in the chiral liquid-liquid extraction of amino acids with high enantioselectivity (Tang *et al*., 2010) and in water containing solvents to enhance protease enantioselectivity (Zhao *et al*., 2006).

2.2 The properties of "green" Ionic liquids (Table 2)

The glass transition temperature (T_g), melting temperature (T_m), viscosity, thermal stability, and polarity are important properties for the application of ILs as extraction solvents; they are summarized in Table 2. The properties of AAILs depend greatly on the side groups of the amino acids involved (Ohno and Fukumoto, 2007). These AAILs, composed of an amino acid with some functional groups such as a hydrogen bonding group (-OH, -NH_2, -COOH), a charged group or an aromatic ring, exhibited an increased glass transition temperature (T_g) or melting temperature (T_m) and/or higher viscosity resulting from the additional interactions among ions, such as hydrogen bonding, stacking interaction, and electrostatic interaction. The introduction of -COOH had a much greater effect on the increase of T_m than that of an -OH and -NH_2. [Emim][AA]s and some [TBP][AA]s and [TAA][AA]s have no T_m reported but do have a T_g and most of ILs with amino acid as cations have both known T_g and T_m values. In general, the more symmetric the anion structure, the higher the crystal structure stability, and the higher the T_m. For [Emim][AA]s and

[TBP][AA]s, an increase of the alkyl side-chain length coincides with a gradual increase of T_g due to an increase of the Van der Waals force between alkyl side chains: [Emim][Gly] (-65 ^oC)< [Emim][Ala] (-57 ^oC)<[Emim][Val] (-52 ^oC), whereas the trend of T_g values for AAILs with different functional side groups, is: [Emim][Met] (-47 ^oC)<[Emim][Lys] (-47 ^oC)< [Emim][Gln] (-12 ^oC)< [Emim][Glu] (+6 ^oC), which might due to the difference in the intensity of hydreogen bond. ILs made of choline and organic acids have both T_g (from -6 to -72 °C) and T_m (from 25 to 131 °C) (Fukaya et al., 2007). [AAE][Sac] ILs have a higher T_g, a much higher viscosity and better thermal stability than the corresponding [AAE][NO_3] (Tao *et al*., 2006). There is a linear relationship between the T_g value and ionic conductivity for major amino acid ILs with [Emim] as a cation and the relationship between viscosity and the T_g for [TBP][AA]s is also linear. Viscosities of [AAE][NO_3] are comparable with those of conventional imidazolium ILs. Among AAILs with the same amino acid as the anion, and [Emim], [TBP] or [TAA] as the cation, [TAA]-based AAILs are the least viscous. This can be explained by the lower molecular weight and higher flexibility of the alkyl chain of [TAA] as compared to the [TBP] cation (Jiang *et al*., 2008). The lower viscosity of AAILs with [TAA] than with [Emim] indicates that [TAA]-based AAILs may be promising solvents as extraction media for natural products. As regards to thermal stability, all the obtained AAILs are stable up to 150 or 200 °C, a little below imidazolium based-ILs, but clearly above the minimum 100°C requirement described by Wasserscheid for chiral ILs (Wasserscheid *et al*., 2002). [TBP][AA]s have a higher decomposition temperature (T_m) (>300 ^oC) than [Emim][AA]s (170 to 200 ^oC) and [TAA][AA]s (Kagimoto *et al*., 2006). The properties of AAILs depend greatly on the side groups involved (Ohno and Fukumoto, 2007). The AAILs composed of an amino acid with some functional groups such as a hydrogen-bonding group (-OH, -NH_2, -COOH), a charged group or an aromatic ring, exhibit a high T_g or T_m and higher viscosity resulting from the additional interactions among ions, such as hydrogen bonding, stacking and electrostatic interaction. Esterification reduces the amount of hydrogen bonding, resulting in a significant decrease in the T_m and viscosity of the salts. The T_m decreases to -17 ^oC for [$AlaC_2$][NO_3] from 159 ^oC for [Ala][NO_3] (Tao *et al*., 2005).

Polarity is another important property of ILs, because the solubility of the solute, their miscibility with other solvents and even their extraction ability are influenced by their polarity. Compared to general ILs, AAILs have a stronger hydrogen bond basicity, equivalent hydrogen bond acidity and equivalent dipolarity. The β parameter, hydrogen-bonding basicity, introduced by Kamlet and Taft, has been established as a measure of the hydrogen-bond accepting ability of anions (Chiappe *et al*., 2005). [Emim][AA]s exhibit strong hydrogen-bond basicity (β between 0.88 and 1.38), above [Bmim][Cl] (β=0.95), which is the IL precisely chosen for its high β

values (Ohno and Fukumoto, 2007). ILs made of choline and an organic acid also have a polarity similar to [Bmim][Cl] (Fukaya *et al*., 2007). Thus, [Emim][AA]s and ILs made of organic acid and choline are expected to be polar solvents for scarcely soluble compounds.

2.3 Preparation of DES and their properties

To date, there are far less reports on DES as compared to the large amount of papers on ILs. Different kinds of natural products such as organic acids, sugars and polyalcohols with amine or choline chloride and sugars or amino acids with organic acids have been used to prepare DES in a simple step, heating and stirring (Table 3) (Abbott *et al*., 2003; Imperato *et al*., 2005). Abbott and co-workers reported that mixtures of urea or organic acids with quaternary ammonium salts could become liquid when heated to 80-100 ^{o}C (Abbott *et al*., 2003; 2004). In those liquids, two urea molecules or two carboxylic acid groups were found to be required to complex each chloride ion. With the intention of extending the variety of DES and exploring their application in organic reactions, König's group found different combinations of compounds that formed DES, including sugar-urea/dimethyl urea, polyalcohol-dimethyl urea, organic acid-dimethyl urea (Imperato *et al*., 2005; Gore *et al*., 2011), *l*-carnitine-urea (Ilgen and Koning, 2009), sugar-choline chloride, sugar-malonic acid (Ilgen *et al*., 2009). In addition, salts with a chloride ion, such as $CaCl_2$, NH_4Cl, NaCl could also be used as components in the previously mentioned DES. These DES have a melting point in the 50-100 ^{o}C range, mostly around 70 ^{o}C and some that were found to be stable at 95 ^{o}C for 4 h without any evident decomposition proved to be good solvents for some organic reactions (Imperato *et al*., 2005). DES can also be formed between glycerol and choline chloride in different ratios. Glycerol - choline chloride (3:1) was used as an electrolyte for dye-sensitized solar cells (Jhong *et al*., 2009), glycerol - choline chloride (1:1/2:1) for extracting glycerol from biofuel (Abbott *et al*., 2007) and for enzyme-catalyzed reactions (Gorke *et al*., 2008). All the above-mentioned DES were prepared by heating. Meanwhile, Gutiérrez *et al*. (2009) found that DES could also be made by freeze-drying aqueous solutions, such as urea-choline chloride (2:1) and thiourea-choline chloride (2:1), glycerol-choline chloride (2:1) and ethylene glycol-choline chloride (2:1) (Gutiérrez, *et al*., 2010). The reported freeze-drying method allowed the incorporation of bacteria into the DES in its pure state (Gutiérrez *et al*., 2010), the solubilization of DNA and RNA with secondary structures (Mamajanov *et al*., 2010), and the preparation of nanotubes (Gutiérrez *et al*., 2010).

Table 3. Composition and physical properties of deep eutectic solvents.

Components		**Molar ratio**	T_m/	**Ref.**
organic acid				
adipic acid	choline chloride	1:1	15	Abbott *et al.*,2004
benzoic acid	choline chloride	2:1	12	Abbott *et al.*,2004

citric acid	choline chloride		14	Abbott *et al.*,2004
malonic acid	choline chloride	1:1	13	Abbott *et al.*,2004
oxalic acid	choline chloride	1:1	19	Abbott *et al.*,2004
phenylacetic acid	choline chloride	2:1	77	Abbott *et al.*,2004
phenylpropionic	choline chloride	2:1	48	Abbott *et al.*,2004
succinic acid	choline chloride	1:1	18	Abbott *et al.*,2004
tricarballylic acid	choline chloride		15	Abbott *et al.*,2004
citric acid	dimethylurea	4:6[a]	65	Imperato *et al.*, 2005
acrylic acid	choline chloride	1:2	15	Mota-Morales, 2011
methacrylic acid	choline chloride	1:2		Mota-Morales, 2011
tartaric acid	dimethylurea	3:7[a]	70	Gore *et al.*, 2011
citric acid	choline chloride	1:2;1:3	-[b]	Choi *et al.*, 2011
malic acid	choline chloride	1:1; 1:2;1:3	-[b]	Choi *et al.*, 2011
maleic acid	choline chloride	1:1; 1:2;1:3	-[b]	Choi *et al.*, 2011
aconitic acid	choline chloride	1:1	-[b]	Choi *et al.*, 2011
polyalcohols				
sorbitol	urea+NH_4Cl	7:2:1[a]	67	Imperato *et al.*,2005
sorbitol	dimethylurea	4:6[a]	77	Imperato et al.,2005
glycerol	choline chloride	3:1	20	Jhong *et al.*, 2009
glycerol	choline acetate	2:3	13	Zhao *et al.*, 2011
glycerol	choline chloride	2:1	23	Guti érre *et al.*, 2010
glycerol	choline chloride	2:1		Abbott *et al.*, 2011
glycerol	choline acetate	1:1	20	Zhao *et al.*, 2011
glycerol	choline acetate	1.5:1	13	Zhao *et al.*, 2011
ethylene glycerol	choline acetate	2:1	23	Zhao *et al.*, 2011
sorbitol	urea	5:5[a]	70	Ilgen *et al.*, 2009
sorbitol	choline chloride	4:6[a]	70	Ilgen *et al.*, 2009
sorbitol	imidazole	3:7[a]	80	Ilgen *et al.*, 2009
sorbitol	4-methyl-imidazole	2:8[a]	50	Ilgen *et al.*, 2009
sorbitol	pyrazole	3:7[a]	60	Ilgen *et al.*, 2009
sorbitol	guanidinium HCl		-[b]	Ilgen *et al.*, 2009
sorbitol	dimethylurea+NH_4	7:2:1[a]	67	Gore *et al.*, 2011
sugars				

frutose	urea	6:4[a]	65	Imperato *et al.*, 2005
glucose	urea+$CaCl_2$	5:4:1[a]	75	Imperato *et al.*, 2005
maltose	dimethylurea+NH_4	5:4:1[a]	73	Imperato *et al.*, 2005
mannose	dimethylurea	3:7[a]	75	Imperato *et al.*, 2005
α-cyclodextrin	dimethylurea	3:7[a]	77	Imperato *et al.*, 2005
glucose	choline chloride	4:6[a]	80	Ilgen *et al.*, 2009
mannose	choline chloride	4:6[a]	50	Ilgen *et al.*, 2009
fructose	choline chloride	4:6[a]	70	Ilgen *et al.*, 2009
sucrose	choline chloride	5:5[a]	80	Ilgen *et al.*, 2009
isomaltose	choline chloride	4:6[a]	90	Ilgen *et al.*, 2009
glucosamine	choline chloride	1:9[a]	100	Ilgen *et al.*, 2009
mannose	malonic acid	5:5[a]	90	Ilgen *et al.*, 2009
fructose	malonic acid	7:3[a]	100	Ilgen *et al.*, 2009
sucrose	malonic acid	6:4[a]	80	Ilgen *et al.*, 2009
glucose	dimethylurea	3:7[a]	80	Ilgen *et al.*, 2009
mannose	dimethylurea	4:6[a]	80	Ilgen *et al.*, 2009
fructose	dimethylurea	4:6[a]	70	Ilgen *et al.*, 2009
isomaltose	dimethylurea	4:6[a]	90	Ilgen *et al.*, 2009
glucose	4-methyl-imidazole	2:8[a]	50	Ilgen *et al.*, 2009
mannose	4-methyl-imidazole	2:8[a]	50	Ilgen *et al.*, 2009
fructose	4-methyl-imidazole	2:8[a]	50	Ilgen *et al.*, 2009
sucrose	4-methyl-imidazole	4:6[a]	70	Ilgen *et al.*, 2009
isomaltose	4-methyl-imidazole	3:7[a]	70	Ilgen *et al.*, 2009
glucosamine	4-methyl-imidazole	3:7[a]	50	Ilgen *et al.*, 2009
glucose	pyrazole	5:5[a]	80	Ilgen *et al.*, 2009
mannose	pyrazole	4:6[a]	50	Ilgen *et al.*, 2009
fructose	pyrazole	5:5[a]	70	Ilgen *et al.*, 2009
sucrose	pyrazole	4:6[a]	60	Ilgen *et al.*, 2009
isomaltose	pyrazole	5:5[a]	70	Ilgen *et al.*, 2009
glucosamine	pyrazole	1:9[a]	90	Ilgen *et al.*, 2009
glucose	guanidinium HCl	4:6[a]	70	Ilgen *et al.*, 2009
mannose	guanidinium HCl	4:6[a]	80	Ilgen *et al.*, 2009
fructose	guanidinium HCl	4:6[a]	70	Ilgen *et al.*, 2009
isomaltose	guanidinium HCl	4:6[a]	80	Ilgen *et al.*, 2009

fructose	dimethylurea	7:3[a]	71	Gore *et al*., 2011
mannose	dimethylurea	3:7[a]	75	Gore *et al*., 2011
glucose	malic acid	1:1	-[b]	Choi *et al.*, 2011
fructose	malic acid	1:1	-[b]	Choi *et al.*, 2011
sucrose	malic acid	1:1	-[b]	Choi *et al.*, 2011
glucose	citric acid	1:2	-[b]	Choi *et al.*, 2011
sucrose	citric acid	1:1	-[b]	Choi *et al.*, 2011
trehalose	citric acid	2:1	-[b]	Choi *et al.*, 2011
glucose	fructose	1:1:1	-[b]	Choi *et al.*, 2011
amines				
urea	choline chloride	2:1	12	Abbott *et al*., 2003
urea	choline chloride	2:1	25	Morrison *et al*., 2009
urea	Choline acetate	2:1	18	Zhao *et al*., 2011
methyl urea	choline chloride	2:1	29	Abbott *et al*., 2003
1,3 dimethyl urea	choline chloride	2:1	70	Abbott *et al*., 2003
1,1 dimethyl urea	choline chloride	2:1	149	Abbott *et al*., 2003
thiourea	choline chloride	2:1	69	Abbott *et al*., 2003
acetamine	choline chloride	2:1	51	Abbott *et al*., 2003
benamide	choline chloride	2:1	92	Abbott *et al*., 2003
amino acid				
proline	citric acid	1:1;2:1;3:1	-[b]	Choi *et al*., 2011

[a]: ratio of weight; [b]: not detected.

Natural products are indeed a plentiful and ideal source of ILs and DES components due to their enormous chemical diversity, biodegradability, sustainability and pharmaceutically acceptable toxicity profile. Our group introduced the term Natural Deep Eutectic Solvents (NADES) for these liquids, which extends the composition of DES to natural products, such as citric acid and proline (Choi *et al*., 2011). In addition to contributing to the number of ILs and DES, the hypothesis of the existence of NADES in organisms could account for many biological processes that cannot be otherwise explained by the theory that water and lipids are the only media in organisms.

In the case of DES made of urea and quaternary ammonium, it was found that the amides with the greatest ability to form hydrogen bonds (i.e. urea and thiourea) exhibited the largest depression in T_m, e.g., choline chloride-urea (1:2) has a melting point of 12 C, while T_m (urea) =134 ^oC and T_m (choline chloride) >300 ^oC. The freezing point of DES with different choline salts decreased

according to their anion in the order $F^- > NO_3^- > Cl^- > BF_4^-$, suggesting some correlation with hydrogen bond strength. In the case of cations, when the symmetry of the cation decreases so does the T_m of the mixture, similarly to ionic liquids. The charge delocalization that occurs through hydrogen bonding between the halide anion and the hydrogen-donor moiety is responsible for the lower T_m of the mixture, as compared to the T_m of the individual component. Most DES have melting points above 50 ^oC (Table 3). DES made of choline salts with quaternary ammonium are highly conductive, confirming the presence of anionic species in the liquid that can move independently (Abbott *et al*., 2003). The conductivity of DES made of organic acids and choline chloride is in the range of 0.1-10 $mScm^{-1}$ changing with temperature and composition and is is similar to imidazolium-based ILs and DES made of urea and choline chloride. In general, conductivity increases significantly with temperature (Abbott *et al*., 2003; 2004). There is an inverse relationship between the viscosity of DES and temperature, as viscosity decreases with increasing temperature (Abbott *et al*., 2003; 2004). These data and their relationship with temperature are important for their applications.

3. Application of ILS and DES in extraction of natural products

3.1. Application of ILs and DES in liquid-solid extraction of natural products

Diverse ILs, covering the whole range of polarity were tested for their aptitude to extract natural products such as phenolic compounds, alkaloids, essential oils, lignins, and carbohydrates from different materials (Table 4). Most ILs were used in the form of aqueous solution because of their high viscosity in pure state.

Phenolic compounds The ILs-based microwave assisted extraction (IL-MAE) technique was first developed to extract different kinds of polyphenolic compounds. The structure of ILs components plays an important role in extraction. In the case of phenolic compounds, the efficiency has proven to be anion-dependant, with [Bmim][Br] being the best choice as compared to the other ILs tested (Du *et al*., 2007; 2009; Zeng *et al*., 2010). This can be attributed to its strong solvatation power and its multiple interactions, especially H-bonding, polarity, π-π, n-π and ionic/charge-charge (Anderson *et al*., 2002; Guo *et al*., 2007). On the other hand, for phenolic compounds with less hydroxyl groups, such as magnolol, honokiol, quercetin and *trans*-resveratrol, ILs with $[BF_4]$ showed a high extraction ability and [Bmim][TSO] also exhibited a high extraction efficiency because of the extra aromatic system.

The parameters that are generally used in an extraction process involving an IL are the following: its concentration (2.0-3.0 mol/L for liquids and 1:30-1:20 for solid/liquid ratio) and a temperature range of 60-70 ^oC. Additionally, the pH value of the solution may also affect the extraction efficiency (Zhang and wang, 2010).

Alkaloids The extraction of alkaloids is structure dependent and interactions such as H-bonding, *n–π*, ionic/charge-charge, are the driving forces for the

extraction. [Bmim][BF_4] showed efficient extraction ability for piperine (Cao, *et al*., 2009) and phenolic alkaloids (liensine, isoliensine, neferine, fangchinoline and tetrandrine) (Lu, *et al*., 2008; Zhang, *et al*., 2009), while [Hmim][Br] showed ca 40% higher extraction ability than [Bmim][BF_4] for *N*-nornuciferine, *O*-nornuciferine, and nuciferine (Ma, *et al*., 2010). The extraction of alkaloids thus depends on both the kind of IL and the structure of the alkaloids and should also take the extraction method into account. For example, [Hmim][BF_4] and [Bmim][BF_4] can reach the same extraction efficiency for the three phenolic alkaloids in IL-MAE (Ma, *et al*., 2010), while the extraction ability of [Bmim][BF_4] was 80% higher than that of [Hmim][BF_4] with ionic liquids based ultrasonic-assisted extraction (IL-UAE) (Cao, *et al*., 2009). This means that in IL-UAE, the viscosity of ILs has a big influence on the extraction efficiency, while the high temperature in IL-MAE decreases the viscosity differences between ILs caused by the alkyl chain length. As for the extraction parameters, the important and generally measured ones are the concentration of ILs (1.0-2.0 mol/L) and solid/liquid (1:30-1:10) and an extraction time of 60-90 s for MAE and 30 min for UAE.

Essential oils Most essential oil components are sensitive to high temperatures and will degrade causing undesirable effects in the properties of the oil. Shortening the general distillation time is a good way of avoiding the damage caused by high temperature. IL-MAE can provide a great advantage as it shortens the required extraction time significantly (from 2 h to 15 min). This is because the microwave absorption performance of ILs ([Bmim][PF_6]) is better than water, and this reduced exposure of the essential oils decreases the amount of oxy-compounds ratio in the fraction obtained with MAE as opposed to that obtained by distillation (Zhai, *et al*., 2009). Furthermore, ILs have advantages in terms of selectivity and high extraction efficiency over normal organic solvents. [Emim][Meesu], for example, demonstrated a high selectivity and efficiency (extraction ratio close to 1) in the extraction of linalool from citrus essential oil (Francisco, *et al*., 2010) and in the case of artemisinin, if compared to hexane extraction, [DMEA][Oct] gives a similar efficiency at a faster rate, while, [BMOEA][Tf_2N] is more efficient at the same rate (Lapkin, *et al*., 2006). Again, the selection of ILs for the extraction of essential oils depends on the extraction method. Some ILs with a long alkyl chain or with lower polarity anions such as [Tf_2N] were used directly in liquid-solid extraction, while a [Bmim][PF_6] aqueous solution with a relative higher polarity also proved to be highly efficient with IL-MAE.

Lignin Lignin is an aromatic polymer composed of phenylpropanoids and its extraction is anion-dependent. It is highly soluble in polar ILs with anions such as [XS], [ABS], [Cl], [MeSO4], [TfO], [OAc], while they are not soluble in less polar ILs with [BF_4], [PF_6] anions (Lee, *et al*., 2009; Tan, *et al*., 2009). [Bmim][PF_6] extracted the phenylpropanoids magnolol and honokiol very efficiently (Zhang and wang, 2010), but could not solubilize phenylpropanoid

polymers. Lignocellulose is a major source of lignin and a highly concentrated solution of chemically unmodified lignin was obtained from lignocelluloses with [Emim][OAc] (Lee, *et al*., 2009).

Carbohydrates Carbohydrates are only sparingly soluble in common organic solvents. However, ILs containing the dicyanamide anion ([dca]) are able to dissolve glucose in high concentrations (>100 gL^{-1}) (MacFarlane, *et al*., 2001) and di- and trisaccharides in considerable but unspecified amounts (Forsyth, *et al*., 2002; Forsyth and McFarlane, 2003). Sheldon proved [dca] based ILs to be highly effective, non-protic solvents, capable of dissolving carbohydrates from glucose to starch and even cellulose in large amounts (Liu, *et al*., 2005). Fort, *et al*. (2006) found that banana pulp at any stage can be completely dissolved in [Bmim][Cl] or a [Bmim][Cl]/DMSO-d_6 mixture while neat DMSO-d_6 under the same conditions led only to partial dissolution of the samples. Another example is the solubiliization of up to 10% (w/w) of starch in [Bmim][dca] or [Bmim][Cl] at 80 ^{o}C (Biswas, *et al*., 2006).

Cellulose is insoluble in water and most common organic liquids. Recent studies found, however, that cellulose could be dissolved, without derivitization, in some hydrophilic ILs such as [Bmim][Cl] and [Amim][Cl] (Swatloski, *et al*., 2002; Zhu, *et al*., 2006). Unfortunately, these ILs are toxic and have a high melting point, and viscosity, requiring energy for the dissolution. To overcome the above drawbacks, a series of ILs with alkylimidazolium carboxylates, such as acetate and formate salts with lower viscosity were applied to dissolve cellulose from wood biomass in high concentrations (Sun, *et al*., 2009). These ILs with carboxylates are not particularly thermally stable, however, which limits their use. In a further step, alkylimidazolium cations coupled with alkylphosphates, [Emim][H_2PO_4], [Emim][$MeHPO_4$], were found to be good solvents for cellulose, with low viscosity and thermal stability (Abe, *et al*., 2010). In general, ILs with various anions, such as [Cl], carboxylates or akylphosphates provide good solubility for cellulose.

Dissolution of cellulose is anion-dependent and is based on the disruption of inter- and intra-hydrogen bonding of cellulose and formation of new hydrogen bonds between the anions of ILs and the hydroxyl groups of carbohydrates (Resming, *et al*., 2006). Highly polar ILs from [Bmim][dca], [Bmim][Cl], [Bmim][OAC] to [Emim][H_2PO_4] in order of increasing polarity have a good solubilizing power of different carbohydrates, Further studies showed that the dissolution of cellulose increases with the hydrogen bond-accepting ability (hydrogen bonding basicity β) of its anions (Xu, *et al*., 2010) and among polar ILs with similar β values, the extraction ability of ILs for polysaccharides in a limited time is dependent on the viscosity of ILs (Abe, *et al*., 2010). Another important factor that affects the solubility of cellulose is the temperature. Cellulose did not dissolve in ILs under ambient conditions, but when heated to 100-110 ^{o}C dissolution occured at a slow rate in 3-methylimidazolium based ILs containing [Cl], [Br] or [SCN]. Moreover, microwave heating significantly accelerated the dissolution process, increasing the solubility of cellulose in [Bmim][Cl] up to a 25% (Swatloski, *et al*., 2002).

Cellulose can dissolve in [Emim][H_2PO_4] at room temperature but its solubility increases three-fold (up to 42%) when the temperature increases to 50 ^oC.

From the extraction studies mentioned above, it is clear that ILs are excellent solvents for all those types of compounds, showing faster or at least an equivalent rate of extraction and solubilizing ability for different kinds of target compounds when compared to classical solvents. Another aspect of extraction is the selectivity of solvents. Some studies have revealed differences between the selectivity of ILs and common organic solvents. For example, obvious differences in the components and in the content of one same compound have been observed in the chromatograms of essential oils extracted by distillation or ILMAE-([Bmim][PF_6]) (Zhai, *et al*., 2009). Furthermore, the latter also showed significant activities (Cieniecka-Roslonkiewicz, *et al*., 2007).

In all, if compared with the current industrial extraction methods based on volatile organic solvents, ILs are promising solvents for the extraction of active metabolites in terms of environmental, economicals and pharmaceutical aspects.

Table 4. Application of ionic liquids (ILs) and deep eutectic solvents (DES) in the liquid-solid extraction of different types of natural products.

extractants	ILs/DES	conclusions	Ref.
phenolic compounds			
trans-resveratrol	[Bmim][Cl], [Bmim][Br] [Bmim][BF_4]	1.Under optimized conditions the extraction efficiency of *trans*-resveratrol was 92.8% in a single-step extraction; 2.With the same cation, the extraction efficiency follows the trend: [Br](47.9%)>[Cl](35.8%)>[BF_4] (17.9%); 3.A comparison between the extraction behavior of [Bmim]Br and methanol showed higher extraction yield for [Bmim]Br than methanol (92.8% vs 88%) and these two solvents had different selectivity for the substances in the sample.	Du *et al*., 2007
gallic acid ellagic acid quercetin *trans*-resveratrol	[Bmim][Br] [Emim][Br] [Hmim][Br] [Bmim][Cl] [Bmim][BF_4] [Emim][BF_4] [Bmim][dca], [Bmim]$_2$[SO_4], [Bmim][H_2PO_4]	1. [Bmim]Br was the optimal IL; 2.The cations and anions influenced the extraction yields. For the [Bmim] based ILs, [H_2PO_4] and [Br] were more efficient than other anions for polyphenolic compounds from P. *guajava* leaves, and [Br] and [BF_4] were more efficient for *trans*-resveratrol and quercetin from S. *china* tubers. [Bmim]Br was more efficient than [Emim]Br, and [Hmim]Br in the MAE of polyphenolic compounds; ③The extraction efficiency follows the trends: [bPy]Cl>[Bmim]Cl>$(CH_3)_4$NCl because cations with electron-rich aromatic π-systems produced more interactions; 3.The extraction ratio increased with the increase in concentration of [Bmim]Br below 3 M ml^{-1}.	Du *et al.*, 2009

	[bPy][Cl] $[(CH_3)_4N][Cl]$	Important parameters to be optimized are sample size (0.45-0.5 mm for leaves and 0.9-0.45 for tubers), temperature (70 ^oC for leaves and 60 ^oC for tubers) and liquid/solid (20:1) ratio.	
rutin	[Bmim]Cl, [Bmim]Br, $[Bmim][BF_4]$, [Bmim][TSO],	1.With the same cations, the extraction ratio follows the trend: [TSO]=[Br] > Cl >$[BF_4]$; 2.Optimal conditions: for Flos Sophorae and Saururus, the concentration of [Bmim]Br (2.0 mol/L) and solid/liquid (1:30) are the same. The extraction temperature is 60 ^oC for Flos Sophorae and 70 ^oC for Saururus; 3. the extraction efficiency obtained by [Bmim]Br was quite close to to that obtained by methanol.	Zeng *et al*., 2010
magnolol and honokiol (IL-UAE[1])	$[Bmim][BF_4]$ $[Bmim][PF_6]$	1.An aqueous solution of $[Bmim][PF_6]$ was more efficient than the $[Bmim][BF_4]$ aqueous solution (48.6% and 45.9% higher, respectively) and the traditional ethanol reflux extraction (16.2% and 13.3% above, respectively) with a short extraction time; 2.The optimized conditions were 2.0 M $[Bmim][PF_6]$, sonication power: 200W for 30 min; 3.The change of pH had no effect on the extraction efficiency.	Zhang and wang, 2010
hydrolysable tannin	*N'N*'-dimethylammonium *N'N*'-dimethylcarbamate (DIMCARB)	1.The extraction efficiency was significantly higher compared to traditional extraction methods with water; 2.The ILs method was more selective for ellagic acid and total tannins; 3.The ILs extracted materials possessed good shelf life and fungal growth resistance.	Chowdhury *et al*., 2010
lignins			
lignin from wood	[Mmim][$MeSO_4$] [Emim][OAC] [Amim]Cl [Bmim] with anions ([Cl], $[BF_4]$, $[PF_6]$,][TfO]) [Bzmim]Cl	1. For lignin solubility [Mmim][$MeSO_4$], [Bmim][TfO] > [Emim][OAC], [Amim]Cl > [Bmim]Cl , [Bzmim]Cl; 2. For wood flour solubility: [Amim]Cl,[Bmim]Cl>[Bzmim]Cl>[Emim][OAC], while [Mmim],[$MeSO_4$] could not dissolve wood; 3.[Emim][OAC] selectively extracted lignin from wood flour and was easily reused, resulting in a highly concentrated solution of chemically unmodified lignin; 4.$[Bmim][BF_4]$ and $[Bmim][PF_6]$ were not effective at dissolving either lignin or cellulose.	Lee *et al*., 2009
lignin from sugarcane plant waste	[Emim][ABS], [Emim][XS],	1. An extraction yield exceeding 93% was attained at 170-190 ^oC; 2. The regenerated lignin showed a good preservation of structure and properties and the ILs could be recycled.	Tan *et al*., 2009
alkaloids			
phenolic alkaloids (liensine, isoliensine, neferine)	$[Emim][BF_4]$ [Bmim] with anions ([Cl], $[BF_4]$, [Br], $[PF_6]$)	1.The new method reduced extraction time (from 2 h to 90 s) and had remarkably higher efficiency (20-50% more) than the conventional heat-reflux extraction and regular MAE; 2.The anion had apparent effects on the overall extraction efficiency. [BF4] was more efficient than others for the target analytes. Increasing the alkyl chain length had no significant effect on the extraction	Lu *et al*., 2008

(IL-MAE[2])	[Hmim][BF_4] [Omim][BF_4]	efficiency; 3. Optimized conditions: 1.5 M [Bmim][BF_4] or 1.0 M [Hmim][BF_4], liquid/solid ratio 15:1for [Bmim][BF_4] and 10:1for [Hmim][BF_4], irradiation power 280 W, extraction time 90 s.	
piperine (IL-UAE[1])	[Hmim][BF_4] [C_4SO_3mim] [Bmim] with anions ([BF_4], [Br], [PF_6], [H_2PO_4])	1.Both the characteristics of anions and cations have remarkable effects on the extraction efficiency. The extraction ability of [Bmim][BF_4] was 60% higher than that of [Bmim][Br] and 80% than [Hmim][BF_4]; 2.With [Bmim] based ILs, the extraction ability followed the order: [BF_4]> [Br]> [H_2PO_4]> [PF_6]; 3.Optimized conditions: extraction time 30 min, 2.0 M [Bmim][BF_4], solid/liquid ratio 1:15.	Cao *et al*., 2009
N-nornucife rine, *O*-nornucife rine, nuciferine (IL-MAE[2])	[Omim][Br][Hmim][Br][E mim][Br] [Bmim] with anions ([Cl], [BF_4], [Br], [PF_6])	1.For extraction efficiency, the influence of anions was much greater than cations. The extraction efficiency of [Bmim]Br was improved by 80% compared with that of [Bmim][PF_6], whereas the difference between different cations was 30%; 2.With [Bmim] based ILs, the extraction ability followed the order: [Br]>[BF_4]>[Cl]>[PF_6]; 3.[Hmim][Br] solution has much stronger multi-interactions including H-bonding, n–π, ionic/charge-charge with alkaloids; 4.Optimized conditions: extraction time 60 s, 1.0 M [Hmim][Br], solid/liquid ratio 1:30, irradiation power 280W.	Ma *et al*., 2010
fangchino line and tetrandrin e (IL-UAE[1])	[Bmim][BF_4]	1.The proposed methods showed higher efficiency than ethanol- based UAE (ca 30% improved) and reflux ethanol extraction (ca 15% increased) in a shorter time; 2.Optimized conditions:1.5 M [Bmim][BF_4], sonication power 150 W for 40 min, pH 9.8.	Zhang *et al*., 2009
carbohydrates			
mono-, di- and poly-saccharides	[Bmim] with anionas([BF_4], [PF_6][dca]) [MOMmim] with anions ([Tf_2N], [BF_4], [dca], [TfO]) [MOEmim] with anions ([Tf_2N], [PF_6], [BF_4], [dca],], [TfO]) [EOEmim] with anions ([Tf_2N], [PF_6], [BF_4], [dca])	1.The solubility of glucose was influenced much more by the nature of the anion than that of the cation although the positive effect of the oxygenated side-chains could be observed: with the same anion, the solubility decreased in the order [dca]> [TfO]≥[BF_4]> [PF_6]> [Tf_2N]; 2.[dca] anion is highly effective, dissolving carbohydrates from glucose to starch and even cellulose in large amounts, attributed to the H-bond acceptor properties of the [dca] anion; 3. The solubility of sucrose in [dca] containing ILs was better than glucose; 4.The solubility of glucose in those ILs increased by a factor of 2.5-5 when the temperature increased from 40 ^oC (211 g L^{-1}) to 75 ^oC (405 g L^{-1}); 5. The solubility of glucose in ILs was much higher than in *tert*-butyl alcohol.	Liu *et al*., 2005

banana pulps	[Bmim]Cl	1. A clear 5 %(w/w) solution containing polyglucan, starch, amylopectin, sucrose, glucose and fructose in banana was obtained; 2.[Bmin]Cl showed higher solubility than Neat DMSO-d_6.	Fort *et al*., 2006
starch	[Bmim][Cl] [Bmim][dca], NH_4Cl-Urea, $CaCl_2$-Urea, DES made of ChCl (Urea, succinic acid, maleic acid, citric acid, oxalic acid, phenylacetic acid, $ZnCl_2$)	1.Starch was soluble at 80 ^oC in [Bmim]Cl, [Bmim][dca] in concentrations up to 10% (w/w); 2.Starch was also soluble in the following DES ChCl-oxalic acid, ChCl-citric acid, ChCl-$ZnCl_2$, ChCl-Urea and $CaCl_2$-Urea.	Biswas *et al*., 2006
cellulose	[Hmim]Cl [Omim]Cl [Bmim] with different anions(Cl, Br, [SCN], $[BF_4]$, $[PF_6]$)	1.On heating to 100-110 ^oC, cellulose slowly dissolved in the $[Cl]^-$, $[Br]^-$,and $[SCN]^-$containing ILs; 2.Microwave heating significantly accelerated the dissolution process, reaching a solubility of up to 25% in [Bmim]Cl;3. The solubility of cellulose was influenced by the nature of anions, in [Bmim] based ILs, the solubililizing power followed the trend: [Cl]>[Br], [SCN]	Swatloski *et al*., 2002
wood from southern yellow pine and red oak	[Emim][OAC] [Bmim][Cl] [Amim][Cl]	1.Both types of soft wood were completely dissolved in [Emim][OAC] by heating in an oil bath after mild grinding; 2.[Emim][OAC] is a better solvent than [Bmim][Cl] for dissolving wood; 3.Microwave and ultrasound can accelerate the dissolution. Variables such as type of wood, initial wood load, particle size, affect dissolution; 4.Carbohydrate-free lignin and cellulose-rich materials can be obtained by using proper reconstituted solvents.	Sun *et al*., 2009
microcrystalline cellulose	[Bmim] with different anions ($[CH_3COO]$ $[HSCH_2COO]$ $[HCOO]$ $[C_6H_5COO]$ $[H_2NCH_2COO]$ $[HOCH_2COO]$ $[CH_3CHOHCOO]$ $[N(CN)_2]$)	1.The solubility of cellulose in [Bmim] based ILs decreased in the order: $[CH_3COO]$> $[HSCH_2COO]$>$[HCOO]$>$[C_6H_5COO]$>$[H_2NCH_2COO]$>$[HOCH_2COO]$>$[CH_3CHOHCOO]$>$[N(CN)_2]$. Cellulose could dissolve in $[Bmim][CH_3COO]$ at 40 ^oC, but not in [Bmim][$N(CN)_2$] even at 70 ^oC;2. The addition of lithium salts increased the solubility of cellulose; 3.Cellulose was regenerated by adding water or ethanol and the regenerated cellulose had good thermal stability; 4. The solubility test was performed at 40-70 ^oC with stirring under argon atmosphere.	Xu *et al*., 2010
cellulose from bran	$[MeHPO_4]$ with cations ([Emim], [Amim], [Pmim],	1.The extraction efficiency of polysaccharides varied according to the cation (19% difference) and anion structures (23% difference); 2.The extraction efficiency depended on the viscosity of ILs within a limited time span, following the order: [Emim]>[allylmim]>[pmim]>[Bmim]	Abe *et al*., 2010

	[Bmin]) [Emim] with anions ([$EtHPO_4$] [$EtHPO_4$] [$pHPO_4$][$bHPO_4$][H_2PO_4])	([Me_2PO_4] as the anion); [PO_4]>[Et_2PO_4]> [pPO_4]> [bPO_4] ([Emim] as the cation) (the β values of those ILs were similar); 3.[Emim][H_2PO_4] with the lowest viscosity, extracted a series of polysaccharides including cellulose, hemicelluloses, and residual starch from bran without heating with a 40% extraction ratio within 10 min.	
wool keratin fibers	[C_4mim]with anions([Cl], [Br],[BF_4], [PF_6]) [C_2mim][Cl]	1. Temperature has a strong effect on the solubility; 2.Fibers showed a better solubility in ILs with [Cl] than [Br] and were not soluble in ILs with non-coordinating anions, such as [BF_4], [PF_6].	Xie *et al*., 2005
essential oils and sesquiterpenes			
essential oil from dried fruits	[Bmim][PF_6]	1. IL-MAE showed higher efficiency (15 min at the microwave power of 440 W), a lower ratio of oxidized compounds (decreasing from 57% in distillation method to 29%), compared with the water distillation method; 2.Obvious differences were observed in the content of identical compounds and in the type of constituents obtained with the two methods.	Zhai *et al*., 2009
linalool from citrus essential oil	[Emim][Meesu]	[Emim][Meesu] exhibited high selectivity and efficiency (extraction ratio close to 1).	Francisco *et al*., 2010
artemisinin	[DMEA][Oct] [BMOEA][Tf_2N] [DMMOEA][Pro]	1.[DMEA][Oct] and [BMOEA][Tf_2N] showed the best performance; 2.Optimized conditions: liquid/solid ratio 6.3:1 (w/w) for [DMEA][Oct] and 0.9:1 for [BMOEA][Tf_2N] at 25 ^oC in 30 min.	Lapkin *et al*., 2006
others			
active extracts	[Mmim][lactate](1); 1-methyl-3[(pentyloxy) methyl]-1Hmimidazol-3-ium[BF_4](2); 1,3-bis (butoxymethyl)-1H-imidazol-3-ium[BF_4](3); 1-[(nonyloxy)methyl]-1H-imidazol-3-ium	1.The type of ILs played a significant role in the yield and composition of the extract and the protic ionic liquid (4) was the most efficient extracting agent, being superior to classical solvents such as EtOAc or hexane; 2.The extracts showed high insecticidal activities of similar potency as the standard pesticides and significant activity against bacteria, yeast and moulds.	Cieniecka-Roslonkiewicz *et al*., 2007

3.2. Application of ILs and DES in liquid-liquid extraction of natural products

The use of solvents for liquid-liquid extraction depends on their physical properties, such as viscosity, density and miscibility. It is convenient to select solvents with low viscosity to facilitate mixing but with a large density

difference for the separation process (Huddleston, *et al*., 2001). The hydrophilicity of ILs is an important factor determining its water miscibility and extraction efficiency. ILs can be hydrophobic or hydrophilic depending on the structure of cations and anions, though the anion seems to be more important. Those based on lower H-bond accepting strength anions ($[PF_6]$, $[Tf_2N]$ and $[BF_4]$) are normally water immiscible, therefore, they are the solvents of choice for forming biphasic systems in most IL extraction applications (Wang, *et al*., 2005; Soto, *et al*., 2005; Katase, *et al*., 2007). However, hydrophilic ILs such as with [Cl] or [I], can be induced to form aqueous biphasic systems when in contact with concentrated solutions of the water-structuring salt, K_3PO_4. This new system can be utilized to recycle hydrophilic ILs from aqueous solutions (Gutowski, *et al*., 2003). The viscosity and hydrophobicity of ILs also increases with the length of the alkyl chain ILs-based aqueous two phase systems are great candidates for the replacement of volatile solutions in typical liquid-liquid extraction due to their high extraction efficiency for different kinds of compounds, as listed in table 5.

Phenolics The distribution ratios of phenolic compounds are influenced significantly by the pH of the aqueous phase, nature of the IL and chemical structure of the phenols among other factors. Most of the phenolic compounds were extracted quantitatively from an aqueous solution into ILs at pH< p*K*a, when they were in the neutral form. Furthermore, picric acid was recovered into the ILs phase at high pH values (Khachatryan, *et al*., 2005). In a basic solution, phenols are ionized, and ion-exchange occurs during the separation. The H-bonding between anions and phenols is the main force for the extraction of phenols from water to ILs and those made of [Cl] combined with long chain imidazolium derivatives are a good choice. The higher extraction efficiency observed with ILs with $[BF_4]$ instead of $[PF_6]$ (Vidal, *et al*., 2004; Fan, *et al*., 2008) is due to the fact that $[BF_4]$ contains a more effective negative charge than $[PF_6]$ (Tsunekawa, *et al*., 2003) and is thus more hydrophilic and less viscous (Vidal, *et al*., 2004). The partition ratio of phenols between IL and water increases with the increasing length of the side chain in imidazolium, the effect of cations on the separation ratio between the two phases can be ascribed to hydrophobic interactions (Fan, *et al*., 2008). The extraction efficiency of phenols from water by a given IL is also affected by the structure of phenols and decreases in the order: 4-nonylphenol>4-octylphenol>phenol. Other factors that affect the extraction ratio are the proportion ILs to aqueous solution and the initial concentration of the solute in aqueous solution.

Alkaloids and heteroaromatics In the case of alkaloids, the separation mechanism varies according to their basic or neutral nature. For alkaloids such as caffeine and nicotine, the interactions between the cations and solutes are the main force because of their H-bonding acceptor property (Freire, *et al*., 2010). On the other hand, interactions between anions and solutes are the main forces for the partitioning of neutral alkaloids such as carbazole, because of their H-bonding donor property (ILs with [Cl] are effective) (Xie, *et al*., 2008). Another mechanism to consider is the salt-in effect of anions that is more effective in the

extraction of alkaloids from aqueous solution to ILs than salt-out ions (Freire, *et al*., 2010). For other heteroaromatic compounds, such as dibenzothiophene and aromatic carbohydrates, the interactions between cations and solutes are the main force for partition. Pyridinium and imidazolium ILs are more efficient than ammonium ILs, although ammonium ILs show high selectivity for aromatic hydrocarbons (Arce, *et al*., 2009). Addition of an alkyl group to the pyridinium ring increases the partition ratio with water (Holbrey, *et al*., 2008).

Organic acids The extraction ratio of organic acids is strongly affected by the pH of the aqueous phase and the chemical structure of the ILs. Similarly to phenolic compounds, organic acids can be extracted quantitatively from an aqueous solution at a pH below their p*K*a. [Bmim][PF_6] is a good solvent for organic acids because it is less viscous than [Omim][PF_6] and more hydrophobic than ILs with [BF_4]. No obvious effects due to temperature and ionic strength of the aqueous phase were reported.

Amino acids The pH value of the aqueous solution has a great effect on the characteristics of amino acids, as their charge state changes with increasing pH of the medium from cationsto zwitterions and anions successively. The higher extraction efficiency corresponds with the range in which the cationic form of the respective amino acids dominates. Arg and Lys can form cationic and dicationic species, why the pH range of efficient extraction of these amino acids is much broader. Crown ethers play an important role as a complexing reagent and H-bonding occurs between the ammonium center of cationic amino acid and the polyether. Unlike conventional organic solvents, it is not necessary to add counter-ions in the presence of a crown ether with [Bmim][PF_6] as a partitioning solvent from aqueous solution (Smirnova *et al*., 2004). The complexes of AAILs with copper enabled the separation of racemic amino acids, which was achieved through a chiral ligand-exchange mechanism (Tang *et al*., 2010).

Proteins For the extraction of proteins, pH, extraction time, and the volume of the ILs affect the extraction efficiency. The hydrophobic interactions, the electrostatic interaction and salting-out effects are important driving factors for the extraction of proteins from aqueous solution. Different types of ILs can be used for the extraction of proteins from aqueous solution, such as imidazolium based ILs with [Cl], [Br], [PF_6] anions.

Esters For the extraction of esters from organic solvents, larger distribution ratios were obtained with long chain cations. With the same cation, [PF_6] was more efficient than [BF_4] because of the hydrophobic property of [PF_6]. For example, [Omim][PF_6] proved to be a good choice for fatty acid esters such as omega-3 fatty acid methyl esters (Li *et al*., 2009).

Glycerol Deep eutectic solvents made of glycerol and choline chloride mixtures were used to extract glycerol from biofuel. A 1:1 ratio was better than 1:1.5, 1:2 due to the fact that a DES with less glycerol have a greater tendency

to attract glycerol molecules to form a DES with a higher ratio of glycerol (Hayyan *et al*., 2010).

Overall, except in case of glycerol, the pH value of the aqueous solution, the structures of the ILs and the addition of salts play an important role in the distribution of solutes between an IL and an aqueous solution.

Table 5. Application of ionic liquids (ILs) and deep eutectic solvents (DES) in the liquid-liquid extraction of different types of natural products.

extractants	ILs/DES	conclusions	Ref.
phenolic compounds			
endocrine-disrupting phenols	$[C_n mim][BF_4]$ (n=6,8) $[C_n mim][PF_6]$ (n=4,6,8)	①In the range of pH<7, most of the endocrine-disrupting phenols were extracted quantitatively from aqueous solution into the ILs; ②In the same acidic conditions, the extraction efficiency for a given phenol decreased in the order: [C8mim]>[C6mim]>[C4mim]; [BF4]>[PF6]; with a given IL extraction decreased in the order: 4-nonylphenol>4-octylphenol>phenol; ③The distribution ratio decreased slightly with as the phase volume of water/ILs increased, the best being 1:1; ④The distribution ratio of phenols increased with the increasing concentration of salts, such as $ZnSO_4$, Na_2SO_4, $Al_2(SO_4)_3$ and $NaClO_4$ in the aqueous phase; ⑤Compared with benzene and dichloromethane, the distribution ratio of the endocrine-disrupting phenols into $[Omim][BF_4]$ was about 496 times as high as that into dichloromethane under the same conditions; ⑥ There was no influence from the temperature.	Fan, *et al*., 2008
p-hydroxybenzoic acid	$[C_n mim][BF_4]$ $[C_n mim][PF_6]$ (n=6-10)	ILs with the $[BF_4]$ anion were more efficient in extracting *p*-hydroxybenzoic acid from an aqueous phase than $[PF_6]$.	Vidal, 2004
vanillin	$[C_4 mim]$ with anions([Cl],[dca],[Br],$[CH_3SO_4]$,[TfO],$[CH_3SO_3]$,$[CH_3CO_2]$), [Cl] with cations ($[C_n mim]$(n=2-10), $[OHC_2 mim]$, $[C_7H_7 mim]$)	① The extraction ability of ILs (with [Cl] as anions) followed the order: $[C_6 mim]$>$[C_4 mim]$> $[C_7H_7 mim]$ >$[C_7 mim]$>$[C_2 mim]$>$[OHC_2 mim]$>$[C_{10} mim]$; the extraction ability of ILs (with $[C_4 mim]$ as the cation followed the order: [Cl] > [dca] > [Br] > $[CH_3SO_4]$ > [TfO] > $[CH_3SO_3]$=$[CH_3CO_2]$, though the influence of cations was lower than that of anions; ②Temperature greatly affected the vanillin partition, and room temperature was the best for $[C_4 mim]$Cl; ③The partition coefficient of vanillin increased with the increase of the initial concentration of vanillin.	Cláudio, *et al*., 2010.
phenols	[Bmim] $[PF_6]$	①4-nitrophenol, 2,4-dinitrophenol, 2,6-dinitrophenol, 1-naphthol, 2-naphthol,4-chlorophenol, were extracted nearly quantitatively from aqueous solutions into the ILs at pH<p*K*a; ②Some phenols showed a high recovery ratio even at a pH of up to 12; ③The recovery	Khachatryan, *et al*.,

		of pyrocathechol and resorcinol is lower (20-58%).	2005
heteromatic compounds			
Neutral nitrogen compounds	[Bmim][Cl]	[Bmim][Cl] was found to be a very promising candidate for the extraction of neutral *N*-containing aromatic compounds from diesel with a high selectivity and up to 50% of indole and carbazole obtained in one step.	Xie, *et al*., 2008
Caffeine and nicotine	[Emim]with anions([$MeSO_4$] [$EtSO_4$] [Cl] [OAC] [TfO]) [Bmim]with anions([Me SO_3][Br][Cl][TfO][CF_3CO_2]) [$C_n$mim]Cl(n=2-12) [C_4C_1min][Cl] [C_7H_7mim][Cl] [OHC_2mim][Cl	①Nicotine showed higher partition coefficients compared to caffeine in all tested ILs because of its lower polarity ; ②The difference in partition coefficients between the cations (100%) was larger than the anions (50-60%); ③[C_4C_1min][Cl], [C_7H_7mim][Cl], [OHC_2mim][Cl] were the most efficient ILs because of multi- interactions between cation-solutes; ④[Cl],] [TfO], [CF_3CO_2] and [OAC] were effective anions because of the salting-in effect.	Freire, *et al*., 2010
Dibenzothophene	[Bpy] with anions([NTf_2][BF_4]) [Bmim] with anions ([BF_4][$C_8H_{17}SO_4$][TfO] [PF_6][SCN][OAC][Tf_2 N]) [BM_4Py] with s([BF_4][TfO] [SCN][TfO][Tf_2N]) [BM_3Py] with ([BF_4] [SCN][TfO][Tf_2N])	① The partition ratio of dibenzothophene to the ILs with the same anion increased in the order: [dimethylPy]>[MPy]>[Py]=[imidazolium]= [pyrrolidinium]; ②There was no significant difference in partition ratio between different anions.	Holbrey, *et al*., 2008
Aromatic hydrocarbons (benzene)	[Tf_2N] with cations like ([C_2mim], [C_2py][$N_{111}(C_2OH)$], [P_{66614}])	① No difference in efficiency was observed between the [C_2mim] and the [C_2py]; ② [$N_{111}(C_2OH)$] presented high selectivity but a low distribution ratio.	Arce , *et al*., 2009
organic acids			
ferulic acid (FA) and caffeic acid (CA)	[Bmim][PF_6] [Hmim][PF_6]	①Changes in temperature and type of inorganic salts had no obvious influence on the extraction ratio; ②The pH value had a big influence on the extraction efficiency, the appropriate pH value for FA was below 3.67, and for CA below 3.71; ③[Bmim][PF_6] showed higher extraction efficiency than [Hmim][PF_6]; ④The extraction efficiency of FA was higher than that of CA.	Yu, *et al*., 2007
3-indole butyric acid	[Bmim][PF_6] [Hmim][PF_6]/ [BF_4]	①Quantitative extraction was achieved with a pH below the p*K*a of IBA, ②With [Bmim][PF_6], higher extraction efficiency was achieved; ③No obvious effect from	Absalan, *et*

(IBA)	[Omim][PF_6]/ [BF_4]	temperature and ionic strength of aqueous phase was observed.	*al*., 2008
benzoic acid	[Bmim][PF_6]	With acetonitrile as a dispersing solvent, a high extraction ratio and a 96.6-99.3% recovery rate of benzoic acid from aqueous solution were obtained.	Zhang, *et al*., 2010
amino acids			
Trp, Gly, Ala, Leu	[Bmim][PF_6]	①Amino acids were extracted efficiently at low pH values of 1.5-4.0 (Lys and Arg at pH of 1.5-5.5); ②The most hydrophilic amino acid ,i.e., Gly was extracted as efficiently as the less hydrophilic amino acids (92-96%); ③Extraction of amino acids into ILs without crown ether is rather low, 5-10%, while quantitative extraction obtained with 0.05-0.1molL^{-1} crown ether was very high; ④No counter ions were needed.	Smirnova, *et al*., 2004
D/L-phe, racemic (D,L-Phe, D,L-Tyr, D,L-His)	Amino acid based ILs (AAILs) [$C_n$mim][L-Pro](n=4,6,8)	AAILs were efficient extraction solvents, showing enantioselectivity in amino acids extraction; more L-enantiomers of amino acids were extracted in the ILs phase than D-enantiomers, with a 50% enantiomeric excess value in a single-step extraction.	Tang, *et al*., 2010
proteins			
proteins from body fluids	[Bmim][Cl] with K_2HPO_4	①A distribution ratio of 10 was obtained with [Bmim][Cl]; ②Addition of an appropriate amount of K_2HPO_4 to the aqueous phase increased the distribution ratio ytwo-fold; ③No chemical (bonding) interaction was observed between ILs and protein and the electrostatic potential difference between the two phases and salting out effects facilitated the partition.	Du, *et al*., 2007
cytochrome C(Cyt-c)	[Bmim][PF_6]	①At pH 1, an extraction efficiency of 85% for Cyt-c from the aqueous solution was achieved; ②The high extraction yield was based on the exposure of hydrophobic groups of heme and a vacant position from the cleavage of the sixth coordinating bond of the ion at low pH value; ③ pH, extraction time, volume of the ILs affect extraction efficiency.	Cheng, *et al*, 2008
hemoglobin	[BTmsim][PF_6] [BBim][PF_6] [Bmim][PF_6]	① [btmsim][PF_6] showed high selectivity and efficiency in quantitative extraction of hemoglobin from an aqueous phase in the presence of other proteins at pH 7; ②The high selectivity and extraction ability were attributed to the interaction between the iron atom in the heme group of hemoglobin and the cation in ILs as the *penta*-coordinated ferrous atom provides a vacant binding site for the cationic [btmsim] moiety; ③A back extraction efficiency ca 80% for hemoglobin in ILs was achieved with sodium dodecyl sulfate solution as stripping reagent.	Cheng, *et al*., 2008

bovine serum albumin, trypsin, cytochrome C and γ-globulin	[Bmim]Br, [Hmim]Br, [Omim]Br	①It is noted that 75-100% of the protein could be extracted into the ILs phase in a single-step extraction; ②The extraction efficiency of protein increased with increasing temperature and the alkyl chain length of cation in ILs following the trend: [Omim]Br>[Hmim]Br>[Bmim]Br; ③The conformation of the protein was not affected after extraction into the ILs phase and 88-90% of the enzyme activity was maintained; ④ Hydrophobic interaction was the main driving force, although the electrostatic interaction and salting-out effects were also important factors.	Pei, *et al*., 2009
ester			
omega -3 polyunsaturated fatty acid methyl esters (PUFAM	[$C_n$mim] [BF_4] [$C_n$mim] [PF_6] (n=2,4,6,8)	①The extraction ratio of PUFAMEs increased significantly with an increase in the degree of unsaturation of PUFAMEs; ②The extraction ratio reached the optimum with [C_8mim][PF_6], which is about 5 times higher than [C_8mim][BF_4] and 2 times higher than [C_4mim][PF_6].	Li, *et al*., 2009
lovastatin and simvastatin	[C_6min][PF_6]	①The established ultrasound assisted ILs disperse extraction method from spiked aqueous solutions was applied to three water samples with recoveries in the range of 80.5-112.0%; ②The volume ratio of IL to aqueous solution played an important role in the extraction efficiency, 1:100 being the best; ③The extraction efficiencies reached an optimum witha pH value of the aqueous solution of 6.0.	Mao, *et al*., 2009

3.3. Extraction mechanism (Features of ILs that affect their extraction efficiency)

Considering information provided above, in this section some more general aspects of ILs as extraction solvents will be discussed. First, as in all dissolution processes, the interaction between solutes and solvent are the main driving force for extraction. Hydrogen bonding, misfit and Van der Waals interaction energy have been shown to effectively characterize the complex multiple interactions in the IL system. Thus the chemical structures of ILs (including the kind of anion, cation, and the alkyl chain length of the cation) have a significant influence on the extraction yield of analytes, owing to their distinct multiple interactions (Anderson *et al*., 2002). Second, the viscosity of ILs has to be considered as it has a great effect on the diffusion of solutes. Lastly, both the pH of the aqueous phase and the salting-out/in effect have to be taken into account since they could play an important role in liquid-liquid extraction.

Anion The extraction efficiency of most compounds is anion dependent. Solutes are strongly solvated, principally by forming H-bonds with the anions of the ILs (Hanke *et al*., 2002). COSMO-RS computation simulation revealed that the solubility of flavonoids in the same class of ILs was strongly anion-dependent (Guo *et al*., 2007). The above-mentioned experiments showed [Br], [BF_4], [Cl], [H_2PO_4], [SO_4], [DCA], [OAC] to be the most commonly used anions in the extraction of phenolic compounds, alkaloids, lignin and carbohydrates, all of which are hydrogen-bond donors. In the extraction of lignin, cellulose and starch from biomass, the H-bonding between solute and solvent molecules should be strong enough to break down inter- and intra-molecular H-bonds in the biomass and only ILs with anions with a strong H-bond accepting ability work. ILs with anions such as [Cl], [OAC], [alkylHPO_4] with a β value of around 1 are efficient solvents, whereas ILs with [Br], [BF_4] are inefficient. The same applies to DES formed between choline chloride and oxalic acid/citric acid, which were able to dissolve starch (Biswas *et al*., 2006). In the liquid-liquid extraction of neutral alkaloids and phenols from water, the H-bonding donor interactions between anions and solutes are the prevailing forces.

Cation The cation species also has an effect on the extraction efficiency. ILs which have cations with an electron-rich aromatic π-system produce stronger interactions with polarizable solute molecules, such as π–π and n–π interactions (Anderson *et al*., 2002; Crowhurst *et al*., 2003). The extraction yield of polyphenolic compounds was a little higher for [BPy]Cl than [Bmim]Cl, and lowest for $(CH_3)_4NCl$, because the *N*-butylpyridinium cation has a more aromatic character than the imidazolium based ILs, whereas the ammonium cation does not give π–π and n–π interactions (Du *et al*., 2009).

In the ILs aqueous two-phase systems, the extraction efficiency for heteroatomic compounds depends more on the nature of the cations than that of anions. ILs with the cations like imidazolium and pyridinium were effective in extracting heteroatomic compounds such as caffeine and nicotine from water. This was found to be driven by different factors including: 1) π-π interaction between the aromatic part of the solutes and the cations; 2) hydrogen-bonding interaction between solutes (the non-bonded electron pair in oxygen and nitrogen atoms) and acidic hydrogen atoms present in the cations of the ILs; and 3) dispersion-type interactions between alkyl groups of the solutes and the alkyl side chain of the cations. The influence of such factors are supported by the high extraction ability of [C_7H_7mim][Cl], [OHC_2mim][Cl], [C_4C_1mim][Cl] for caffeine and nicotine (Freire *et al*., 2010) and the increasing extraction capability of ILs with cations in the sequence[Py> [MePy]methylpyridinium > [dimethylPy] for dibenzothiophene (Holbrey *et al*., 2008). Furthermore, the aromaticity factor plays a more important role than the ring size; pyridinium and immidazolium based ILs showed the same extraction efficiency in the isolation of dibenzothiophene from dodecane (Holbrey *et al*., 2008).

The alkyl chain of the cation The melting point of ILs can be tuned by changing the length and symmetry of the alkyl chain of cations. Low symmetry

in substitution and a longer alkyl chain within a certain range leads to a low melting point. In the case of imidazolium cations, C_8 gives the lowest melting point (Zhang *et al*., 2008). Thus, ILs applied to the extraction of natural products have ([$C_n$mim], n = 2-8) cations, and and C_8 gives the lowest T_m (Zhang *et al*., 2009). The alkyl chain length of the cation affects the extraction yield of some natural products as its hydrophobicity increases with the increasing alkyl chain length. Apart from the hydrophobicity, viscosity also increases, so that while the longer chain length benefits the extraction of some middle to less polar compounds the corresponding increased viscosity limits the diffusion of compounds. ILs with a cation such as [Bmim] are most often used for mid-polar compounds and [Hmim] for non-polar compound (Du *et al*., 2009; Cao *et al*., 2009; Fan *et al*., 2009; Li *et al*., 2009).

The viscosity of ILs is strongly influenced by the cation and anion species. The longer the substituted chain of the cation, the more viscous the ILs. The high viscosity is one of the greatest obstacles for the application of ILs in natural products extraction. Two efficient ways are used to decrease their viscosity: increasing the temperature through heating or microwaves (Swatloski *et al*., 2002; Zhai *et al*., 2009) and dilution with water or organic solvents such as ethanol. To date, the most common solution has been the latter. The extraction ratio of compounds can be increased by 60-90% by adjusting the concentration of ILs (Cao *et al*., 2009; Du *et al*, 2007; 2009) showing that this factor is crucial since it balances not only the viscosity but also the extraction ability of the ILs solutions. The optimal concentration of ILs differs according to the type of ILs, the targeted compounds and the biomass (Lu *et al*., 2008; Zeng *et al*., 2010).

pH In liquid aqueous two- phase systems, the pH of the aqueous phase is an important factor since it can influence the existing form of ionic or ionizable compounds affecting thus, the extraction efficiency of organic acids, phenolic compounds, amino acids and proteins among others (Tzeng *et al*., 2008; Cheng *et al*., 2008). For example, ILs easily extracted organic acids or phenolic compounds in the neutral form (Yu *et al*., 2007; Fan *et al*., 2008) and amino acids as cations (Smirnova *et al*., 2004) from an aqueous phase.

Salting-out/in effect Another important factor affecting the extraction efficiency in liquid aqueous two-phase systems is the concentration of some inorganic ions, which can be explained by a salting-out effect. When salts are dissolved in water, they use up molecules of water for their solvation, reducing the concentration of free water, and increasing the relative concentration of solutes in the aqueous solution allowing them to be preferably extracted into the ILs phase. The stronger the hydration ability of a salt, the stronger its salting-out effect. The distribution ratio of phenols between ILs and aqueous phase increases as the concentration of salt present in the aqueous phase increases (Fan *et al*., 2008).

Concerning the influence of the ILs anion, there seems to be a similar salting-in/out behavior of the anions from ILs. Generally, salting-in inducing anions (low charge density ions) are more efficient at extracting solutes from a second liquid phase than salting-out (high charge density ions) inducing anion. For salting-in inducing anions the tendency to form hydration complexes is marginal and thus they tend to stabilize the solutes in solution by specific ion binding to the solute; On the other hand, salting-out inducing ions have a great tendency to form a hydration complex, and thus do not have interaction with solutes (Freire *et al*., 2009). With the same cation, ILs with [Cl], [Br], $[CH_3SO_4]$, $[CF_3SO_3]$ are more efficient than ILs with $[CH_3SO_3]$, $[CH_3CO_2]$ in extraction of vanillin (Cláudio *et al*., 2010), and ILs with [Cl], $[CF_3SO_3]$, $[CF_3CO_2]$ are more efficient than ILs with $[CH_3SO_3]$, $[CH_3SO_4]$ in the extraction of alkaloids (Freire *et al*., 2010).

The observations are important to achieve an improved understanding of the ILs solvation properties and reflect their versatility and their potential for tailoring the composition of IL for the efficient extraction of the desired natural compounds.

3.4. Combination of ILs with other technologies

3.4.1 Microwave-assisted extraction

ILs can efficiently absorb and transfer microwave energy (Hoffmann *et al*., 2003), and thus be employed to rapidly heat solvents and co-solvents in microwave-assisted extraction (MAE). MAE is rapid and effective compared to traditional extraction techniques (Zeng *et al*, 2010) and easy and cheap compared with other modern extraction techniques. Recently, microwave coupled with ILs aqueous solutions or pure ILs were evaluated for the extraction of some polyphenolic compounds (Du *et al*., 2007; 2009; Zeng *et al*., 2010), alkaloids (Lu *et al*., 2008; Ma *et al*., 2010) and essential oils (Zhai *et al*., 2009) from medicinal plants. Compared to conventional heat-reflux extraction (HRE), MAE had the advantage of remarkably higher efficiency (20–50% higher) and shorter extraction time (from 2 h to 90 s) (Lu *et al*., 2008).

The important parameters of the MAE procedure include the sample size, liquid-solid ratio, extraction time and temperature. The extraction rate of phenolic alkaloids with $[Hmim][BF_4]$ increased when the liquid-solid ratio was raised from 5:1 to 10:1 and decreases dramatically when the ratio further increases to 20:1. An extraction time of around 8-12 min was sufficient to obtain high extraction yields of polyphenolic compounds from 0.2-0.5 g of plant powder with 20-35 mL of a 1.5-2.0 mol/L [Bmim][Br] aqueous solution (Zeng *et al*., 2010). Another aspect to be considered is thermal stability. Phenolic compounds were found to be stable up to 100 ^{o}C during MAE (Liazid *et al*., 2007), but with ILs as the solvent the stability temperature decreased to 60-80 ^{o}C and compounds with a larger number of hydroxyl substituents were more readily degraded under the extraction conditions (Du *et al*., 2009).

MAE is highly efficient as an extraction method due to its unique mechanism. In MAE extraction, the direct interaction of the microwaves with the IL solution and free molecular water present in the cell, results in the rupture

of the cells and release of intracellular products into the solvent. The surface of the plant material was observed to be greatly destroyed and the structure of the cell walls ruptured after IL-MAE (Du *et al*., 2009; Zeng *et al*, 2010). Moreover, ILs improved the transfer of energy from the microwaves to the sample, which increased the speed of energy transfer and thus extraction efficiency (Eskilsson and Björklund, 2000).

3.4.2 Ultrasound-assisted ionic liquid extraction

IL-based ultrasound-assisted extraction (IL-UAE) methods have been developed for the effective extraction of alkaloids (Cao *et al*., 2009; Zhang *et al*., 2009) and phenolic compounds (Zhang *et al*., 2010) from plant material. The extraction time is one of the most important factors and optimal extraction efficiency can be achieved in 30-40 min. For example, the extraction efficiency of the optimized IL-UAE approach increased the yield of piperine by ca. 30-45% as compared with UAE but with a conventional solvent (ethanol) (Cao *et al*., 2009).

4 Analysis methods for extraction by ionic liquids

4.1 HPLC (high-performance liquid chromatography)

In general, the sample preparation for the HPLC analysis of an IL extract is similar to that required for conventional solvent extracts, involving its dilution with either water or the HPLC mobile phase (Fu *et al*., 2006) or alternately its injection as such (Fan *et al*., 2009), after filtration through a 0.45μm filter. A reversed-phase HPLC method using a C_{18} column and acetonitrile and 0.6% acetic acid aqueous solution as the mobile phase was applied to analyze the polyphenolic compounds in IL extracts of medicinal plants, with no interference from the ILs being observed (Du *et al*., 2007; 2009). The method showed a good repeatability, precision and accuracy, and no degradation of the target compounds. The presence of ILs did not affect usual chromatographic parameters such as peak resolution, peak shape, elution order and retention times in the separation of three phenolic alkaloids (Lu *et al*., 2008). Cao *et al*. (2009) came to the same conclusion when analyzing a non-diluted IL piperine extract with UPLC and a C_{18} column. In another case, HPLC-DAD-ELSDA with a C_{18} was used to determine the esterification of flavonoids in ILs (Lue *et al*., 2008). All these studies show that ILs can be used as extraction solvents for the analysis of natural products from a biological matrix, without the need to eliminate the ILs opening interesting novel opportunities for natural products extraction for quantitative analysis.

4.2 NMR (nuclear magnetic resonance spectroscopy)

^{13}C NMR spectroscopy was applied to analyze carbohydrates extracted with ILs to detect their content, conformation and interactions. The direct measurement of IL banana pulp extracts, gave well-resolved signals in the ^{13}C NMR spectra corresponding to different sugars in the anomeric carbon region

that could be used for quantitative analysis (Fort *et al*., 2006). Solid-state ^{13}C NMR spectroscopy was used to monitor the extraction of lignin from lignocellulose material (Tan *et al*., 2009). It is known that ^{13}C NMR spectroscopy is a very useful tool in the study of the conformational preferences of repetitive polysaccharides (Swalina *et al*., 2001) but a drawback for its application to ILs extracts is their high viscosity because it reduces molecular tumbling, resulting in low resolution and sensitivity. However, the spectrum recorded at 90 ^{o}C showed baseline resolution for most signals (Moulthrop *et al*., 2005). NMR relaxation measurements of the ILs ^{13}C and $^{35/37}Cl$ nuclei provided a better understanding of the mechanism of solvation of cellulose, the destruction of β-(1→4)-linked glucose oligomers (Moulthro *et al*., 2005) and the formation of H-bonds between the carbohydrate hydroxyl protons and the ILs chloride ions (Remsing *et al*., 2006; Xu *et al*., 2010). Two-dimensional heteronuclear overhauser enhancement (HOESY) spectrum of choline fluoride and urea showed intense cross-correlation between the fluoride ion and the primary amine protons of urea revealing the existence of hydrogen bonding in the liquid (Abbott *et al*., 2003). Thus, IL extracts can be analyzed by NMR spectroscopy, but the various signals of the protons in the IL constituent will, in fact, overlap with sample signals in certain spectral regions.

4.3 IR (infrared spectra)

FTIR spectra can provide useful information for identifying the presence of certain functional groups, and chemical bonds in a molecule, or interactions in a system. It has been applied in the rapid and nondestructive identification and quantification of medicinal plants, the analysis of interactions between ILs and proteins or DNA (Wang *et al*., 2007; Zhuo *et al*., 2007) and the identification of some secondary metabolites, including lignin (Tan *et al*., 2009) and polysaccharides (Fort *et al*., 2007). FT-IR is a useful tool to provide a characteristic fingerprint, reflectance peaks between 1800 and 700 cm^{-1} were selected as representative peaks for known carbohydrate compounds.

4.4 Others

Circular dichroism (CD) spectroscopy measures differences in the absorption of left- and right-handed circularly polarized light as a function of the wavelength. It occurs in structurally asymmetric molecules when a chiral (optically active) chromophore is in an asymmetric environment. CD spectroscopy is particularly good for demonstrating the conformational change of structure of proteins (Cheng *et al*., 2008) and DNA (Mamajanov *et al*., 2010).

Scanning electron microscope (SEM) allows the observation and characterization of heterogeneous organic and inorganic materials at a nano- to micrometer scale. The popularity of SEM stems from its capacity of obtaining three-dimensional-like images of the surfaces of a very wide range of materials. XL-30 SEM spectroscopy was applied to identify the microstructure of plant material after MAE using a [Bmim]Br solution as a solvent and SEM images shows that the structure of the cell walls was ruptured and the microstructure of leaves and tubers was greatly destroyed (Fig. 1) (Du *et al*., 2009).

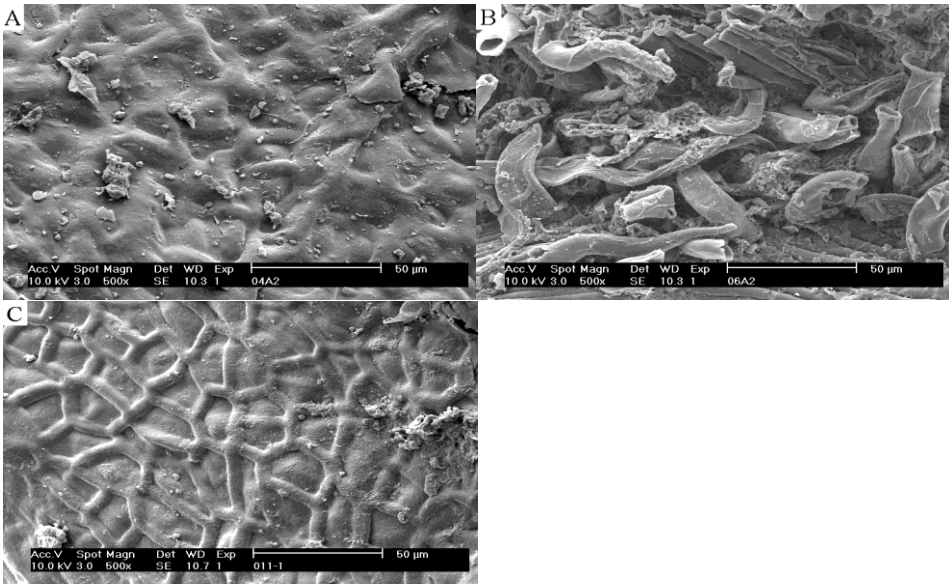


Fig. 1 Scanning electron micrographs of *Psidium guajava* leaves: (**A**) untreated *Psidium guajava* leaves; (**B**) after MAE for 10 min in [Bmim]Br and (**C**) heating extraction for 4 h. Image magnification is 500 for A, B and C (Du *et al*., 2009).

5 Recovery of compounds from ILs

The low volatility of ILs that on one hand makes them 'green' creates challenges for product separation and recovery. For volatile products, a back-distillation may be used to recover the product from ILs. In the case of a hydrophilic product in a hydrophobic IL, water may be used to remove the product from the ILs (Huddleston *et al*., 1998), but the biggest problem is to recover non volatile or thermally labile products from ILs. A number of methods have been used for the recovery of diverse compounds (carbohydrates, tannins, N-compounds, phenolic compounds) from diverse ILs (normal ILs, distillable ILs and DES).

5.1 Supercritical Carbon Dioxide

The low volatility of ILs that on one hand makes them "green" creates challenges for product separation and recovery. For volatile products, a back-distillation may be used to recover the product from ILs. In the case of a hydrophilic product in a hydrophobic IL, water may be used to remove the product from the ILs (Huddleston amd Rogers, 1998), but the biggest problem is to recover poorly volatile or thermally labile products from ILs.

Experiments using another type of "green" solvent, supercritical carbon dioxide (SC-CO_2), for product recovery from ILs solution have been reported. The volatile $scCO_2$ is insoluble in the non-volatile and polar ILs and they form two-phase systems. The principle of product recovery with these biphasic

systems is based on the solubility of the compounds in CO_2. During the process, high volatility and low polarity will favor the solubility of a solute in CO_2. Naphthalene was extracted from [Bmim][PF_6] using sc$CO_2$with recoveries of up to 94-96%, a near-quantitative recovery without detectable [Bmim][PF_6] in the extract (Blanchard *et al*., 1999). A variety of aromatic and aliphatic compounds were also quantitatively recovered from this IL with scCO_2without any IL contamination in the recovered product (Blanchard and Brennecke, 2001). In contrast, a mixture of CO_2 with conventional organic solvents results in a significant amount of the solvent in the CO_2-rich phase, due to their solubility. Diverse compounds such as *N*-acetyl-(*S*)-phenylalanine methyl ester and epoxides of olefins were extracted from ILs with scCO_2 by optimizing pressure, temperature and the type of IL, especially from enzyme-catalyzed reactions (Bortolini *et al*., 2003; Kroon *et al*., 2006). For example, 2-methylbutanoic acid that results from the asymmetric hydrogenation of tiglic acid in [Bmim][PF_6] was extracted with scCO_2. Moreover, the solution of the catalyst in [Bmim][PF_6] could be recycled without significant losses of both enantioselectivity and conversion (Brown *et al*., 2001).

The removal of a product from ILs by extraction with CO_2, however, only works for products that have a sufficiently high solubility in CO_2. For compounds that are insoluble in CO_2, crystallization from ILs with CO_2 as an anti-solvent has been explored. The addition of CO_2 lowers the solubility of the product in the ILs, thereby creating supersaturation (Kroon *et al*., 2006). With this method, removal of even solid, inorganic salts from [Hmim][Tf_2N] was achieved with CO_2 at 25 ^oC (Saurer *et al*., 2006). Strong hydrogen-bonding interactions between the ILs and the solutes make it more difficult to induce a separation. Further studies found that CO_2 acts primarily by disrupting the nonspecific interactions in the IL/organic mixtures (Mellein and Brennecke, 2007) and that the ability of CO_2 to act as an anti-solvent is highly dependent on the solubility of CO_2 in the IL/organic mixtures. Kroon *et al*. (2008) found that methyl-(*Z*)-*α*-acetamido cinnamate could be recovered from [Bmim][BF_4] by either shifting to a higher CO_2 concentration at a constant temperature (anti-solvent crystallization) or changing to lower temperatures at constant CO_2 concentration, showing the importance of exploring the effects of pressure, temperature and CO_2 concentration in each case.

The applicability of scCO_2 for extraction of solutes from an IL relies heavily on the phase behavior of the binary IL-CO_2 system. Solubility of CO_2 depends on the substituents on the cation and nature of the anion (Blanchard *et al*., 2001). Furthermore, experimental and molecular simulation studies found that the anion of the IL dominates the interaction with CO_2, with the cation playing a secondary role (Cadena *et al*., 2004). The solubility of CO_2 in the IL-rich phase was greater for IL with fluorinated anions, following the trend of [Bmim][PF_6] and [C_8mim][PF_6] > [C_8mim][BF_4] > [*N*-bupy][BF_4] > [Bmim][NO_3] > [Emim][$EtSO_4$] (Blanchard *et al*., 2001). The highest solubility of CO_2 is in ILs with anions containing fluoroalkyl groups, [TfO], [Tf_2N] while the least soluble in ILs with non-fluorinated anions, [NO_3] and [dca] (Aki *et al*., 2004). For the cations, CO_2 was more soluble in [Hmim][BF_4] than [Bmim][BF_4],

[Bmim][PF_6] than [Emim][PF_6] (Shariati *et al*., 2005). The solubility of CO_2 increases with the alkyl chain length at all pressures, with the increase being more apparent at higher pressure (Aki *et al*., 2004). In addition, the pressure and temperature also affect the solubility of CO_2 in ILs. The solubility of CO_2 in imidazolium-based ILs increases with increasing pressure and decreases with increasing temperature (Aki *et al*., 2004). Thus, by varying the substituents on the cation and nature of the anion, changing the pressure and temperature, one should be able to design ILs to achieve the desired phase behavior characteristics.

5.2 Anti-Solvents

In general, adding anti-solvent with agitation may result in the separation of solutes from ILs. Some examples use H_2O, ethanol, or acetone to isolate cellulose from [Bmim][Cl] or [Bmim][CH_3COO] (Swatioski *et al*., 2002; Xu *et al*., 2010), or H_2O, methanol, or acetone to isolate wool keratin [Bmim][Cl] (Xie *et al*., 2005), or mixtures of acetone-water (1:1) to isolate cellulose and lignins from [Emim][OAC] (Sun *et al*., 2009). Lignin was also separated by precipitation from [Emim][XS] by acidification to pH 2 at room temperature after which, the IL was recovered by neutralization, removal of water at reduced pressures and drying at 70 ^oC under high-vacuum conditions (Tan *et al*., 2009).

5.3 Re-crystallization

Re-crystallization by cooling or adding anti-solvent can be used to separate solution and target molecules. The addition of 1-butanol to [Gly][ChCl] with extra glycine and then subcooling to -20 ^oC, allowed the separation of choline chloride (Hayyan *et al*., 2010). Water was used as an anti-solvent (3:1 v/v H_2O to ILs) to partition and then recrystallize artemisinin from ILs; crystallization yielded 82% of the total extracted artemisinin and a 95% concentration of anhydrous artemisinin (Lapkin *et al*., 2006).

5.4 Back Extraction

Organic acids, *nitrogen-containing* compounds, phenolic compounds, and amino acids were removed from ILs by back extraction with aqueous solutions, making the necessary pH adjustments and the ILs were then quantitatively recovered after neutralization and evaporation of the solvent. For example, neutral *nitrogen-containing* compounds were recovered from [Bmim][Cl] by back extraction using water or methanol (Xie *et al*, 2008) and a 94-98% recovery of ferulic acid from [Bmim][PF_6] was achieved with a 0.02 M NaOH aqueous solution (Yu *et al*., 2007). In another case, 99% of phenols present were removed from [Bmim][PF_6] after back extraction with 0.1 mol L^{-1} NaOH aqueous solution (Fan *et al*., 2008) and amino acids were recovered from AAILs by adjusting the pH of the solution to 7.0 with HCl, thus neutralizing the amino group (Tang *et al*., 2010).

5.5 Chromatographic Techniques

In addition, some column chromatographic techniques have been developed for the separation and purification of compounds from ILs extracts. An anion-exchange resin has been successfully used in the isolation and purification of shikimic acid from [Bmim][Cl] extract with a 87% yield (Usuki *et al*., 2011). High speed counter current chromatography has been used in the separation and purification of three isoflavones from [C_8min][Br] extract with a purity above 95% (Sun et al., 2011). Non-porous membranes with a selective layer of hydrophilic or hydrophobic polymers ave been applied for the quantitative and selective recovery of solutes with different physicochemical properties from [Bmim][PF_6] (Schafer et al., 2001).

Thus, although ILs cannot be removed from extracts by evaporation, diverse methods have been employed succesfully to isolate compounds from ILs as described above. The solvents used in these processes are also green solvents such as SC-CO_2, water, or ethanol, ensuring the green extraction and isolation of natural products from live material with ILs. Furthermore, the ILs can also be reused several times decreasing the cost of the whole process.

6. Enzyme reactions in ILs and DES (factors affecting the enzyme activity in ILs and DES)

There have been many reports on enzyme reactions in ILs (table 6). Enzymes may exhibit enhanced activity, stability and selectivity in ILs, depending on the structure of ILs, properties, the concentration, water activity of the ILs, ILs coating effect and so on. Some enzyme reactions in DES have also been reported, as mentioned below.

6.1IL structure

The enzyme activity was found to be dependent on the type of anions, cations and their spatial configuration (Itoh *et al*., 2001). The enzymatic activity, stability and selectivity are greatly affected by the constituents of the ILs. [Bmim][BF_4] combined with acetone showed eight fold higher yields and three times faster reaction rates for the lipase-catalyzed (CaL B) synthesis reactions of ester of nucleoside drugs. However, the yield decreased drastically when the cationic part and anionic part were replaced, namely [Bmim][PF_6]and [Emim][BF_4] (Liu *et al*., 2006). The immobilized CaL B (*i*-CaL B) displayed enhanced activity when the alkyl chain of [$C_n$mim][BF_4] increased in length (n = 4-8) and no acylation reaction occurred in [C_4mim]Cl or [C_4mim]Br (Li *et al*., 2006). The mushroom tyrosinase treated with [Bmim][BF_4] retained a higher activity than that in [Bmim][PF_6] and [Bmim][$MeSO_4$](Yang *et al*., 2008). In addition, ILs with [PF_6] and [alkyl-SO_4] are also good solvents for some enzyme reactions. [Bmim][PF_6] and [Hmim][PF_6] were suitable reaction media for esterification of isoamyl acetate by CaL B (Fehér *et al*., 2008). The best synthetic activity was obtained when free CaL B was assayed in [CPMA][$MeSO_4$] (De Diego *et al*., 2009). [Emim][$MDEGSO_4$] was the most promising IL for laccase compared with [Emim][$EtSO_4$] and [Emim][$MeSO_3$] (Tavares *et al*., 2008).

Table 6. Examples of enzyme activities in ionic liquids (ILs) and deep eutectic solvents (DES).

enzymes	ILs/DES	reaction	conclusions	Ref.
lipase from *Candida Antarctica* (CaL)	[Bmim][PF_6]	acylation of L-carnitine with conjugated linoleic acid	the conversion in [Bmim][PF_6] was 2.13 and 1.56 times higher than that in acetonitrile and in solvent-free system, respectively.	Tian *et al*., 2010
	[$C_n$mim][dca], [$C_n$mim][Cl] (n=2,4,8) [$C_n$mim][PF_6] (n=4,6,8) [$C_n$mim][BF_4] (n=4,6) [$C_n$mim][Tf_2N] (n=2,4,6,8) [Bmim] with anions ([OSO_4], [$MDEGSO_4$] [NO_3][OAC])	synthesis of butyl butyrate by transesterification	1.CaLB exhibited greater stability in water-immiscible ILs than in water-miscible ILs. The highest activity was observed in [Omim][PF6]; 2.The activity of CaLB decreased following the trends: water-immiscible ILs(ILs with anions like [Tf_2N] and [PF_6]>hexane> water -miscible ILs (ILs with anions like [BF_4], [dca], [NO_3], [OAC], [OSO_4], [$MDEGSO_4$]), with the [Bmim] cation, the enzyme activity in [Tf_2N] is nearly 430 times higher than that in [OSO4]; 3.For ILs with the same anion, the synthetic activity was gradually enhanced by increasing alkyl length in cation: the enzyme activity in [Omim][PF_6] is nearly 6 times higher than that in [bmim][PF_6].	De Los Rios *et al*., 2007
	[Bmin][BF_4]/ [PF_6] [Emim][BF_4]	synthesis reactions of ester of nucleoside	Eight fold higher yields and three times faster reaction rates using a mixture solvent system composed of 90% acetone and 10% [Bmim][BF_4].	Liu *et al*., 2006
Immobilized Candida antarctica lipase B (i-CaLB)	[Emim][BF_4] [Bmim] with anions([HSO_4] [Cl] [Br] [NO_3] [BF_4])	enantioselective hydrolysis Of D,L-phenylglycine methyl ester	[Bmim][BF_4] is the most suitable IL for the reaction with the highest initial rate and enantioselectivity, while the reaction became much less active and enantioselective in the systems with [Bmim][HSO_4].	Lou *et al*., 2006
	Cation: $C_n$mim (n=4-8), Anion: BF_4, PF_6, Cl, Br	regioselective acylation of 1-*β*-D-arabinofuranosylcytosine	10% (v/v) [C_4mim][PF_6]-tetrahydrofuran gave the highest initial rate and substrate conversion.	Li *et al*., 2006
	[Bmim] with anions([PF_6][BF_4] [Cl]) [Hmim][PF_6] [Emim][tosy] [Mmim][$(Me)_2PO_4$]	esterfication of isoaml acetate	1. Both [Bmim][PF_6] and [Hmim][PF_6] are suitable reaction media because their hydrophobic properties and they can form two phase system with the substrates; 2.Water as a by-product of the esterification reaction can shift the equilibrium towards the direction of the hydrolysis; 3.The best results were obtained with 1 to 2% (w/w)	Fehér *et al*., 2008

			initial water content, while with 10% the reaction ratio approached to zero.	
	[Bmim][PF_6]	Esterification geraniol	1.The maximum initial reaction rate was found at aw=0.6; 2.The average value of reaction equilibrium constant was 12-20 fold lower than that in hexane.	Barahona *et al*., 2006
	27 ILs with different anions and cations	transesterification	1.Under microwave irridation, enhanced enzyme activities were observed with a layer of water; 2.The initial reaction rates bear no direct relationship with the viscosity and polarity of Ils, but have a loose correlationship with the hydrophobicity of ILs.	Zhao *et al*., 2009
lipases from (CaL A ,CaL B), *Thermomyces lanuginosus Rhizomucor miehei*	[Bmim][PF_6]/[BF_4],[Omim][PF_6]/[BF_4][Hmim][BF_4] [Bdmim][PF_6] [Bdmim][BF_4][Bmim][MS] [CPMA][MS]	transesterification	1.enzyme activities were clearly dependent on the nature of the ions, and the results improved as the alkyl chain length of the imidazolium cation increased; 2.The best synthetic activity was obtained when free CaL B were assayed in [CPMA][MS].	De Diego *et al*., 2009
lipases (CaL B, *i*-CaL B, CaL A and PcL)	ChCl-Acet, ChCl-malonic acid, ChCl-U, EAC:Acet, EAC-Gly, ChCl-EG, EAC-EG, ChCl-malonic acid	Transesterification of ethyl valerate with 1-butanol	1.In ChCl:Gly, all the lipases showed conversions comparable to that in toluene; 2. *i*-CaL B also showed conversions comparable to that in toluene for ChCl:EG, ChCl:U and EAC:Gly; 3.*i*-CaL B had the highest initial specific transesterification activity in EAC:Gly, which was twice as high as in [Bmin][Tf_2N] and 7 times higher than the activity in [Bmin][BF4]; 4. DES are also suitable as co-solvents for reaction in aqueous solution, increasing activity up to 20 fold.	Gorke *et al*., 2008
pseudomonas capaci lipase (PcL)	[*i*-C_4mim][PF_6] [C_4mim][PF_6] hexane	Transesterification reaction 2-phenylethanol with vinyl acetate	[*i*-C_4mim][PF_6] was the best among the three solvents. The initial reaction rate, the equilibrium conversion of 2-phenylethanol and the half-lifetime of the lipase in [*i*-C_4mim][PF_6] medium were about 1.5, 1.2 and 3-fold that obtained in [C_4mim][PF_6] medium, respectively.	Shan *et al*., 2008
lipase from *Candida rugosa* (CrL)	[Bmim][PF_6]	enantioselective esterification	the enantioselective esterification of (-)-menthol exceeded 53%.	Zhang 2008
	[Bmim]/[MMEP]with anions([PF_6] [NO_3][OAC]), [Bmim] with	transesterification of methyl methacrylate	1.The enzymatic activity was 1.5 times in [Bmin][PF_6] faster than that in hexane. However, no detectable activity was observed in all the "hydrophilic" ILs studied; 2.No lipase	Kaar *et al*., 2003

	anions ([PF_6][NO_3][OAC][TfO][CF_3CO_2][CH_3SO_3])		activity was observed in all ILs with [Bmin][PF_6] and more hydrophilic ILs; 3.Lipase exhibited greater stability in ILs than in organic solvents including hexane.	
CrL *Burkholderia cepacia* lipase (BcL)	[BDmim][cetyl-PEG10-sulfate] [Bmim][cetyl-PEG10-sulfate] [Bmim][BF_4] [BDmim][BF_4][BDmim][PF_6]	transesterification of 1-phenylethanol	A remarkable acceleration was accomplished by coating the lipase with IL in an *i*Pr_2O solvent system, reaching a 500-600 fold acceleration for some substrates.	Itoh *et al.*, 2006
i-BcL	[Bmim][PF_4] [Bmim][PF_6], 4-methyl-N-butylpyridinium [BF_4], and methyltrioctyl ammonium trifluoroacetate	butanolysis of triolein	1.Only methyltrioctylammonium trifluoroacetate was miscible with 1-butanol and triolein; 2.The addition of 80% methyltrioctylammonium trifluoroacetate to the reaction system gave the best result in terms of alcoholysis rate.	Miyawaki *et al*., 2008
Mucor javanicus lipase(MjL)	[Bmim][PF_6] [Emim][Tf_2N] [Bmim][BF_4] [Emim][BF_4]	the hydrolysis reaction	1.The activities of lipase pretreated with [Bmim][PF_6], [Emim][Tf_2N], [Bmim][BF4] and [Emim][BF_4] were 1.81, 1.66, 1.56 and 1.60 times higher than that of untreated lipase, respectively; 2.Activities of lipase in ILs were well maintained after 7 days of incubation in ILs at 60 ^oC, while untreated lipase in phosphate buffer was fully inactivated only after 12 h of incubation at the same temperature.	Dang *et al*., 2007
A feruloyl esterase (AnFaeA)	[Bmim][PF_6], [Omim][PF_6], [C_2OHmim][PF_6] [C_5O_2mim][PF_6]	the esterification of glycerol with sinapic acid	the esterase was able to catalyse both transesterification and esterification only in [C_2OHmim][PF_6] and [C_5O_2mim] [PF_6] with a high conversion yield around 72.5 ±2.1%.	Vafiadi *et al*., 2009
esterases from *Bacillus stearothermophilus*	[Bmim][Tf_2N] [Bmim][PF_6] [Bmim] [BF_4]	transesterification of 1-phenylethanol	1.Highest specific activity was obtained in n-hexane for both enzymes, while the specific activity was similar in organic solvents and in the ILs; 2.The enantioselectivity was independent of the solvent.	Persson, 2003
Protease (subtilisin)	[Emim][Tf]	esterification *N*-acetyl-*L*-phenyl alanine	The activity was improved 9-fold in [Emim][Tf] compared to acetonitrile and 3-fold to octane.	Noritomi *et al*., 2007

horseradish peroxidase (HRP)	[Bmim][BF_4]		1.No detectable activity exhibited in anhydrous [Bmim][BF4], while HRP was active in the presence of a small amount of water (4.53%, v/v); 2.The *i*-HRP was very sensitive and stable in H_2O-containing [Bmim][BF_4].	Wang *et al*., 2007
	water-in-hydrophobic [C_8mim][Tf_2N] (w/IL) microemulsions	oxidation of pyrogallol	1.The HRP-catalyzed oxidation of pyrogallol by hydrogen peroxide in water/IL microemulsions is much more effective than in general water/isooctane microemulsion; 2.HRP retained almost 70% of its initial activity after incubation at 28 ^oC for 30 h.	Monir uzzaman *et al*., 2008
Chloroperoxidase from *Caldariomyces fumago*	citrate buffer/ILs mixtures,	oxidation of 1,2-dihydronaphthalene	The enzyme activity is retained for 24 h, but it falls to 3 h using non-ionic organic solvents such as *t*-BuOH or acetone.	Sanfilippo *et al*., 2004
mushroom tyrosinase	[Bmim][PF_6], [Bmim][BF_4] [Bmim][$MeSO_4$]	Oxidation of 4methylcatechol to 4-methyl-*O*- quinone.	1.The three ILs were able to trigger the enzyme activity; 2.The enzyme treated with [Bmim][BF4] retained a higher activity than that in the other two; 3.The enzyme could be stabilized by addition of $KMeSO_4$ and $NaBF_4$.	Yang *et al*., 2008
laccase (DeniLite base)	[Emim][$MDEGSO_4$] [Emim][$EtSO_4$] [Emim][$MeSO_3$]		1.[Emim][$MDEGSO_4$] was the most promising IL for laccase with an activity loss of about 10% after 7 days of incubation; 2.The enzyme maintained activity at pH 9.0 for all tested ILs.	Tavares *et al*., 2008
Alcohol dehydrogenase glucose dehydrogenase	[BMP][Tf_2N] a water immiscible IL	ketone reductions	1.Employing 10% (v/v) [BMP][Tf_2N], facilitated conversion of 50 gL^{-1} ketone to the chiral alcohol in less than 24 h; 2.The initial rate of reaction was improved more than 4 times with 10% (v/v) [BMP][Tf_2N] compared with organic solvents and aqueous buffer.	Hussain *et al*., 2008
cellulase	[Emim] [Me_2PO_4]	enzymatic saccharification	1.When the volume of IL to water was greater than 3: 2(v/ v), little cellulase activity was observed; 2.An IL to water ratio of 1: 4 resulted in over 70% of the starting amount of cellulose being converted to glucose and cellobiose.	Kamiya *et al*., 2008

The cations and, particularly, the anions of ILs have a significant effect on the selectivity of enzyme reaction except for reaction activity. [Bmim][BF_4] gave the highest initial rate and enantioselectivity among various ILs examined for the hydrolysis of D,L-phenylglycine methyl ester, while [Bmim][HSO_4] makes the reaction much less active and enantioselective (Lou *et al*., 2006). In [Bmim][BF_4], best selectivity towards the formation of the monoester (74% at 1 h) over the diester (0% at 1 h) was obtained (Yang *et al*., 2008). Nevertheless,

in [Bmim][PF_6] a higher yield was obtained, though no selectivity was obtained with any substrate.

The spatial configuration of ILs is also considered a key factor affecting the behavior of the enzyme in ILs. The [*i*-C_4mim][PF_6] has good biocompatibility, showing superiority in the initial reaction rate, the equilibrium conversion and the half-lifetime of the lipase over [*i*-C_4mim][PF_6] and hexane (Shan *et al*., 2008). So [*i*-C_4mim][PF_6] can be used as green solvent in various biocatalytic reactions to improve the activity and stability of enzyme.

6.2 Properties of ILs

The effect of ILs on the enzyme can be largely attributed to their interaction with the enzyme, substrate and water, depending on their hydrophobicity, H-bonding basicity, ion's kosmotropoc and nucleophilicity (Yang *et al*., 2008). Kosmotropic is the ability of solutes to increase the stability of intermolecular forces in water-water interactions.Kosmotropic ions contribute to the stability and structure of an enzyme and tend to be small or having high charge density, e.g. SO_4^{2-}, Mg^{2+}. However, none of these properties are solely responsible for the enzyme functions in ILs and multiple factors have to be considered.

Hydrophobicity was considered a key parameter for analyzing the behavior of enzymes in ILs. In general, enzymes are more stable and exhibit higher activity in hydrophobic ILs than hydrophilic ILs. The initial reaction rates of lipase B have a loose correlation with hydrophobicity value of ILs (Zhao *et al*., 2009). The hydrophobic property is anion dependent and ILs with anions like [PF_6], [Tf_2N], [BF_4] are hydrophobic. The enzymatic activity of free lipase (Kaar *et al*., 2003) and lipase B (De Los Rios *et al.*, 2007) are higher in [Bmim][PF_6] or [Omim][PF_6] than in hexane, while no detectable or lower activity was observed in all the "hydrophilic" ILs studied. On the other hand, for the ILs based on the same anion, the hydrophobicity increased with increasing length of the alkyl group on the cation (Ropel *et al*. 2005). With [PF_6] or [dca] as anions, the activity of lipase B increases in the trend: [Bmim] > [Hmim] >[Omim], which can be attributed to the gradually enhanced hydrophobicity (De Los Rios *et al* 2007). The initial lipase activity in [Amim110][dca] is the same as in [Emim][Tf_2N], [BuPy][Tf_2N] and more than 7 times higher than that in [Bmim][dca]. In addition, the lipase has activity even in [Amim110][Cl]. These can be attributed to the hydrophobicity of [Amim110] cation (Zhao *et al*., 2009). However, there are some exceptions. With [PF_6] as the anion, the enzyme is active in ILs with cations like [C_2OHmim] and [C_5O_2mim] and inactive in [Bmim] and [Omim] based ILs, which can be correlated with the amphiphilic properties caused by the hydrophobic anions [PF_6] and hydrophilic cations [C_2OHmim] and [C_5O_2mim] (Vafiadi *et al*., 2009). Moreover, in DES with polarity equal to methanol to glycerol, lipases also show conversions comparable to that in toluene (Gorke *et al*., 2008). In all, not only the hydrophobic ILs are suitable solvents for enzyme activity, some

hydrophilic ILs are also good solvents depending on the type of enzyme and solvents.

H-bonding basicity (the H-bond acceptor strength) could have a considerable impact on the enzyme stabilization in some ILs. The strength of anion coordination is dependent on and consistent with the H-bonding basicity of the anions (β), listed in an increasing order (Henderson, 2007), [Tf_2N] < [PF_6], [BF_4] < [Br] < [Cl]. This order represents the strength of interactions between the anions and the charged surface of macromoleculars. [Bmim][BF_4], [Bmim][PF_6] and [Bmim][Tf_2N] have similar H-bonding basicities, which are much lower than that of [Bmim][Cl] (Anderson *et al*., 2002). The enzyme is less active in ILs with [Cl] than [dca], which can be attributed to the stronger H-bond accepting ability of [Cl] than [dca] (Zhao *et al*., 2009).

6.3 Concentration of ILs

The concentration of ILs or the water content in ILs is an important factor affecting the efficiency and selectivity of enzyme biocatalysis. The enzyme activity of *i*-CaL B in hydrolysis of D,L-phenylglycine methyl was strongly dependent on [Bmim][BF_4] content in the co-solvent system and the favorable content of the IL was 20% (v/v) (Lou *et al*., 2006). The volume ratio of methyltrioctylammonium trifluoroacetate (MTOATFA) in the solution with 1-butanol had an effect on the production rate of lipase-catalyzed *Burkholderia cepacia* lipase (BcL) butanolysis of triolein: a small amount of IL seemed inhibitory, a large amount of IL accelerated the reaction and the addition of 80% MTOATFA gave the best result in spite of the reduction in substrate concentration (Miyawaki and Tatsuno, 2008). Increasing the IL content in aqueous solution results in a decrease of the laccase activity and at high IL content (75%, v/v) the enzyme precipitates (Tavares *et al*., 2008). For horseradish peroxidase (HRP), no detectable activity was found in anhydrous [Bmim][BF_4], but in the presence of a small amount of water (4.53%, v/v) it was active (Wang *et al*., 2007). The water content in [Emim][Tf] affected the activity of subtilisin in the esterification of *N*-acetyl-(L)-phenylalanine and the initial rate with 0.5% water is enhanced 2.4-fold compared to that with 0.2% water (Noritomi *et al*., 2007). For cellulase, decreasing the volume ratio markedly enhanced enzymatic activity: an IL to water ratio of 1: 4 (v/ v) resulted in over 70% of the starting amount of cellulose being converted to glucose and cellobiose (Kamiya *et al*., 2008).

10% (v/v) [Bmim][PF_6]-tetrahydrofuran gave the highest initial rate and substrate conversion in the regioselective acylation of 1-beta-D-arabinofuranosylcytosine (ara-C) catalyzed by *i*-CaL B (Li *et al*., 2006). 10% (v/v) [BMP][Tf_2N] facilitated stereoselective conversion of ketone to the chiral alcohol with alcohol dehydrogenase and improved the initial rate of the reaction more than four times compared to no co-solvent (Hussain *et al*., 2008). Gervaise *et al*. (2009) found that the regioselectivity of lipase from *Candida cylindracea* (LCC) can be influenced by the proportion of [Bmim][PF_6]-phosphase buffer. The influence on the regioselectivity of the enzyme reaction can be explained

by the ILs-cosolvent mixture having an impact on the surface of enzyme and consequently leading a conformational change.

6.4 Water content

Water content (α_w) is known to play an important role in affecting enzyme activity in non-aqueous environments and the thermodynamic water activity is an important variable affecting the activity of enzymes in non-aqueous solvents. The maximum initial reaction ratio was observed at α_w=0.6 in the conversion of geraniol with CaL B (Barahona *et al*., 2005). In the regioselective acylation of 1-beta-D-arabinofuranosylcytosine, catalyzed by *i*-CaL B in [C_4mim][PF_6], at α_w=0.07, the initial rate, substrate conversion and the regioselectivity were 94.0 mM h^{-1}, 98.5% and 99%, respectively. However, when α_w = 0.85, the reaction rate was near to zero (Li *et al*., 2006).

6.5 ILs-coating effect

The activity and stability of *Mucor javanicus* lipase (MjL) pretreated with ILs (such as [Bmim][PF_6], [Emim][Tf_2N], [Bmim][BF_4] and [Emim][BF_4]) were higher than those of untreated lipase for the hydrolysis reaction in an aqueous medium; activities of lipase in ILs were well maintained even after 7 days of incubation, while untreated lipase in phosphate buffer was fully inactivated already after 12 h of incubation at the same temperature (Dang *et al*., 2007). A remarkable acceleration was accomplished by the lipase coated with IL in transesterification of 1-phenylethanol catalyzed by the *Burkholderia cepacia* lipase (BcL) (Itoh *et al*., 2006). Lipase coated by [PPmim][PF_6] (PPmim 1-(3´-phenylpropyl)-3-methyllimidazolium) showed higher enantioselectivity than that of commercial lipase in toluene (Lee and Kim, 2002). With ILs as the coating materials, the activity, stability and enantioselectivity might be enhanced due to a change in the structure of the enzyme coated with ILs.

In addition, there are also some factors affecting the enzyme activity in ILs, such as pH (Ren *et al*., 2008), temperature (Zhang *et al*., 2008), microwaves (Zhao *et al*., 2009), which should be taken into consideration when optimizing the conditions for enzyme reaction in ILs. Enzymes in ILs maintain their activity and also their structure, over a much longer period than in organic solvents and often even at a much higher temperature. Van Rantwijk and Sheldon (2007) suggested that the underlying cause of this stabilizing effect is the high viscosity of ionic liquids, which slows the migration of protein domains from the active conformation into the inactive one.

6.6 Deep eutectic solvents

There are a few applications of DES in the extraction of natural products. This is probably due to their high viscosity and most are in nearly solid state at room temperature. However, DES made of glycerol and choline have relative low viscosity and are good solvents for enzyme reaction. Hydrolases show good catalytic activity in DES including choline chloride-acetamide, choline

chloride-glycerol, choline chloride-urea. In those DES, lyophilized *Candida antarctica* lipase B shows higher activity than that in the conventional solvent, toluene. Particularly, in choline chloride-glycerol, the conversion is much higher than in toluene (22% *vs* 5%) with *lyophilized Burkholderia cepacia* lipase. Furthermore, the enzyme activity of lyophilized *Candida antarctica* lipase B did not decrease in choline chloride-urea at 60 ^{o}C over 90 min (Gorke *et al*., 2008). The high activity and stability of hydrolases in DES show the promise of DES as solvents in biotransformation. The enzymatic transesterification of Miglyol oil 812 catalyzed by Novozym 435, a commercial immobilized *Candida antarctica* lipase B, showed high reaction rates in choline acetate:glycerol (1:1.5) at 50 ^{o}C. The high conversion rate of up to 97% ensures the further exploration of DES as potential solvents in enzyme reactions (Zhao *et al*., 2011).

7. Conclusions and Perspective

ILs are promising solvents for natural products research basically due to their high extraction efficiency of a wide range of metabolites and their low environmental impact. They are compatible with HPLC analytical procedures and the compounds can be recovered from the ILs. Green ILs-AAILs- have already been used in the chiral liquid-liquid extraction of amino acids displaying high enantioselectivity. All these results show their great potential for further applications in natural products-related areas such as food additives and pharmaceuticals or cosmetics.

ILs, with their special properties, constitute a new and green type of solvents. They are of great importance for applications in all areas where general organic solvents are used. For natural products, they offer a perspective of novel extraction processes, with high efficiency and yielding a greater variety of compounds given their high dissolving power. This feature allows ILs to be considered as a new kind of solvent capable of extracting all metabolites for total metabolomics, for example. Most ILs that have been used in the extraction of natural products, however, were imidazolium-based, and only a few of them were suitable for certain extraction applications. The application of other types of ILs and DES in natural products extraction should be further investigated, especially regarding their physical properties.

All natural products are soluble in ILs, although, as mentioned before, when used to extract compounds from plant material, high viscosity may be a problem. There are a number of reports of the efficient extraction of certain types of natural products using aqueous ILs solutions combined with microwaves. Some thermally unstable natural products, such as flavonoids, however, should be extracted at lower temperatures. The combination of ILs/supercritical CO_2 might also be a good choice for the extraction of natural products especially for thermally unstable compounds.

ILs might be applied to extract natural products without polluting the environment. Obviously, for certain applications of natural ILs, the extract maybe used as such, e.g. as food additives and in pharmaceutical or cosmetic

products. How to isolate compounds from ILs, however, is still an important challenge. Some recovery methods with supercritical CO_2 have been established for simple organic model compounds as a means of exploring the recovery mechanism. It may also be useful to develop a liquid/solid separation using different column materials to separate target solutes and ILs. Further studies are needed to optimize the recovery of natural products from ILs.

Finally, considering all the facts mentioned above, our group has recently postulated that ILs and DES also occur in nature (Choi *et al*., 2011). The existence of Natural Deep Eutectic Solvents (NADES) would explain the biosynthesis of the water insoluble macromolecules cellulose, starch, lignin, the biosynthesis of small water insoluble molecules, drought and cold resistance of all kinds of organisms, the germination of an almost dry seed, etc. Where could they occur? In our view, they are attached to cell membranes, like being caught on an ion-exchanger, but in a dynamic equilibrium with the water phase. In fact this hypothesis would explain many biological phenomena at the level of the cell, the tissues and the whole organism. Once more, nature has probably already invented ILs and DES in ancient times, if not already in the very beginning, developing self-organizing fluids as the start of life.

It seems that with the natural ILs and DES we are at the beginning of something very fundamental which on the one hand may explain many basic biological cellular processes and on the other hand will generate many applications in the field of extractions and enzymatic reactions. For example, most biosynthetic enzymes have been characterized in an aqueous environment. The finding that in certain ILs enzymes are more active than in the classical solvents shows that in nature the biosynthetic pathways also may be controlled by e.g. water contents and water cativity, the presence of NADES. The green chemistry has opened a realm of pandora.

Reference

Abbott, A. P., Capper, G., Davies, D. L., Rasheed, R. K., Tambyrajah, V. *Chem. Commun.* 2003, 70-71.
Abbott, A. P., Boothby, D., Capper, G., Davies, D. L., Rasheed, R. K. *J. Am. Chem. Soc.*, 2004,126, 9142-9147.
Abbott, A. P., Cullis, P. M., Gibson, M. J., Harris, R. C., Raven, E. *Green Chem.*, 2007, 9, 868-872.
Abbott, A. P., Harris, R. C., Ryder, K. S., D'Agostino, C., Gladden, L. F., Mantle, M. D. *Green Chem.*, 2011, 13, 82-90.
Abe, M., Fukaya, Y., Ohno, H. *Green Chem.*, 2010, 12, 1274-1280.
Absalan, G., Akhond, M., Sheikhian, L. *Talanta*, 2008, 77, 407.
Aki., S.N.V.K, Mellein, B.R., Saurer, E.M., Brennecke, J.F. J. Phys. Chem. B 2004,108, 20355-65.

Ana Paula Mora Tavares, O. R. E. A. M. *Biotechnol. Bioeng*. 2008, 101, 201-207.
Anastas, P., Eghbali, N. *Chem. Soc. Rev*. 2010, 39, 301-312.
Anderson, J. L.; Ding, J.; Welton, T.; Armstrong, D. W. *J. Am. Chem. Soc*. 2002, 124, 14247-14254.
Arce, A., Earle, M. J., Rodriguez, H., Seddon, K. R., Soto, A. *Green Chem*., 2009, 11, 365-372.
Arzhantsev, S., Jin, H., Baker, G. A., Maroncelli, M. *J. Phys. Chem. B*, 2007, 111, 4978-4989.
Barahona, D.,Pfromm, P. H., Rezac, M. E. *Biotechnol. Bioeng*. 2006, 93, 318-324.
Bao, W., Wang, Z., Li, Y. *J. Org. Chem*., 2002, 68, 591-593.
Berthod, A., Ruiz-angel, M. J., Carda-Broch, S. *J. Chromatogr. A*, 2008, 1184, 6-18.
Biswas, A., Shogren, R. L., Stevenson, D. G., Willett, J. L., Bhowmik, P. K. *Carbohydr. Polym.*, 2006, 66, 546-550.
Blanchard, L. A., Brennecke, J. F. *Ind. Eng. Chem. Res*., 2001, 40, 287-292.
Blanchard, L. A., Hancu, D., Beckman, E. J., Brennecke, J. F. *Natur*e, 1999, 399: 28-29.
Bortolini, O., Campestrini, S., Conte, V., Fantin, G., Fogagnolo, M., Maietti, S. *Eur. J. Org. Chem.*, 2003, 2003, 4804-4809.
Brown, R. A., Pollet, P., McKoon, E., Eckert, C. A., Liotta, C. L., Jessop, P. G. *J. Am. Chem. Soc*., 2001, 123, 1254-1255.
Cadena, C., Anthony, J.L., Shah, J.K., Morrow, T.I., Brennecke, J.F., Maginn, E.J. *J. Am. Chem. Soc.* 2004, 126, 5300-08.
Cao, X., Ye, X., Lu, Y., Yu, Y., Mo, W. *Anal. Chim. Acta*, 2009, 640, 47-51.
Carter, E. B., Culver, S. L., Fox, P. A., Goode, R. D., Ntai, I., Tickell, M. D., Traylor, R. K., Hoffman, N. W., Davis, J. H., *Chem. Commun*., 2004, 630-631.
Cheng, D. H., Chen, X. W., Shu, Y., Wang, J. H., Chinese *J. Anal. Chem*., 2008, 36, 1187-1190.
Cheng, D. H., Chen, X.W., Shu, Y., Wang, J. H. *Talanta*, 2008, 75, 1270-1278.
Chiappe, C., Pieraccini, D. *J. Phys. Org. Chem*., 2005, 18, 275-297.
Choi, Y. H., van Spronsen, J., Dai, Y., Verberne, M., Hollmann, F., Arends, I. W. C. E., Witkamp, G.J., Verpoorte, R. *Plant Physiol*., 2011, 156, 1701-1705.
Chowdhury, S. A., Vijayaraghavan, R., MacFarlane, D. R. *Green Chem*., 2010, 12, 1023-1028.
Cieniecka-Rosłonkiewicz, A., Sas, A., Przybysz, E., Morytz, B., Syguda, A., Pernak, *J. Chem. Biodivers*. 2007, 4, 2218-2224.
Cláudio, A. F. M.; Freire, M. G.; Freire, C. S. R.; Silvestre, A. J. D.; Coutinho, J. A. P. *Sep. Purif. Technol*., 2010, 75, 39-47.
Crowhurst, L., Mawdsley, P. R., Perez-Arlandis, J. M., Salter, P. A., Welton, T. *PCCP*, 2003, 5, 2790-2794.
Dang, D. T., Ha, S. H., Lee, S. M., Chang, W. J., Koo, Y. M. *J. Mol. Catal. B: Enzym*. 2007, 45, 118-121.
De Diego, T., Lozano, P., Abad, M. A., Steffensky, K., Vaultier, M., Iborra, J. L. *J. Biotechnol*. 2009, 140, 234-241.

De Los Rios, A. P., Hernandez-Fernandez, F. J.,Martinez, F. A., Rubio, M., Villora, G. *Biocatal. Biotransform*. 2007, 25, 151-156.
Du, F.Y., Xiao, X.H., Li, G.K. *J. Chromatogr. A*, 2007, 1140, 56-62.
Du, F.Y., Xiao, X. H., Luo, X.J., Li, G.K. *Talanta*, 2009, 78, 1177-1184.
Du, Z., Yu, Y. L., Wang, J. H. *Chem. Eur. J*., 2007, 13, 2130-2137.
Eskilsson, S. C., Bjö rklund, E. *J. Chromatogr. A*, 2000, 902, 227-250.
Fan, J., Fan, Y., Pei, Y., Wu, K., Wang, J., Fan, M. *Sep. Purif. Technol*., 2008, 61, 324-331.
Fan, Y., Chen, M., Shentu, C., El-Sepai, F., Wang, K., Zhu, Y., Ye, M. *Anal. Chim. Acta*, 2009, 650, 65-69.
Fehé r, E., Illeov, V., I., K. H.,Bé lafi-Bak, K., Polakovic, M., Gubicza, L., *J. Mol. Catal. B: Enzym.* 2008, 50, 28-32.
Forsyth, S. A., MacFarlane, D. R. *J. Mater. Chem*., 2003, 13, 2451-2456.
Forsyth, S. A., MacFarlane, D. R., Thomson, R. J., von Itzstein, M. *Chem. Commun*., 2002, 714-715.
Fort, D. A., Swatloski, R. P., Moyna, P., Rogers, R. D., Moyna, G. *Chem. Commun*., 2006, 714-716.
Francisco, M., Lago, S., Soto, A., Arce, A. Fluid Phase Equilib., 2010, 296, 149-153.
Freire, M.G., Carvalho, P.J., Silva, A.M.S., Santos, L.M.N.B.F., Rebelo, L,P.N., Marrucho, I.M. *J. Phys.Chem. B* 2009,113, 202-11.
Freire, M. G., Neves, C. M. S. S., Marrucho, I. M., Canongia Lopes, J. N., Rebelo, L. P. N., Coutinho, J. A. P. *Green Chem*., 2010, 12, 1715-1718.
Fu, X., Dai, S., Zhang, Y. *Chin. J. Anal. Chem*., 2006, 34, 598-602.
Fukaya, Y., Iizuka, Y., Sekikawa, K., Ohno, H. *Green Chem*., 2007, 9, 1155-1157.
Fukumoto, K., Ohno, H. *Chem. Commun*., 2006, 3081-3083.
Fukumoto, K., Yoshizawa, M., Ohno, H. *J. Am. Chem. Soc.*, 2005,127, 2398-2399.
Garcia-Lorenzo, A., Tojo, E., Tojo, J., Teijeira, M., Rodriguez-Berrocal, F. J., Gonzalez, M. P., Martinez-Zorzano, V. S. *Green Chem*., 2008, 10, 508-516.
Gervaise, C., Daniellou, R.,Nugier-Chauvin, C.,Ferriè res, V. *Tetrahedron Lett*. 2009, 50, 2083-2085.
Gore, S., Baskaran, S., Koenig, B. *Green Chem*., 2011, 13, 1009-1013.
Gorke, J. T.; Srienc, F.; Kazlauskas, R. J. *Chem. Commun*., 2008, 1235-1237.
Guo, Z., Lue, B.M., Thomasen, K., Meyer, A. S., Xu, X. *Green Chem*., 2007, 9, 1362-1373.
Gutiérrez, M. a. C., Ferrer, M. a. L., Mateo, C. R., del Monte, F. *Langmuir*, 2009, 25, 5509-5515.
Gutiérrez, M. C., Rubio, F., del Monte, F. *Chem. Mater*., 2010, 22, 2711-2719.
Gutowski, K. E., Broker, G. A., Willauer, H. D., Huddleston, J. G., Swatloski, R. P., Holbrey, J. D., Rogers, R. D. *J. Am. Chem. Soc*., 2003,125, 6632-6633.
Handy, S. T., Okello, M., Dickenson, G. *Org. Lett*., 2003, 5, 2513-2515.

Hanke, C. G., Atamas, N. A., Lynden-Bell, R. M. *Green Chem*., 2002, 4, 107-111.
Hayyan, M., Mjalli, F. S., Hashim, M. A., AlNashef, I. M. *Fuel Process.Technol*., 2010, 91, 116-120.
Henderson, W. A., *Macromolecules* 2007, 40, 4963-4971
Herrera-Herrera, A. V., Hernandez-Borges, J., Rodriguez-Delgado, M. A. *J. Chromatogr. A*, 2009, 1216, 7281-7287.
Hoffmann, J., Nuchter, M., Ondruschka, B., Wasserscheid, P. *Green Chem*., 2003, 5, 296-299.
Holbrey, J. D., Lopez-Martin, I., Rothenberg, G., Seddon, K. R., Silvero, G., Zheng, X. *Green Chem*., 2008, 10, 87-92.
Hu, S. Q., Jiang, T., Zhang, Z. F., Zhu, A. L., Han, B. X., Song, J. L., Xie, Y., Li, W. J. *Tetrahedron Lett*., 2007, 48, 5613-5617.
Huddleston, J. G., D. Rogers, R. *Chem. Commun*., 1998, 1765-1766.
Huddleston, J. G., Visser, A. E., Reichert, W. M., Willauer, H. D., Broker, G. A., Rogers, R. D. *Green Chem*., 2001, 3, 156-164.
Hussain, W.,Pollard, D. J., Truppo, M., Lye, G. J. *J. Mole. Catal. B: Enzym*. 2008, 55, 19-29.
Ilgen, F., Konig, B. *Green Chem*., 2009, 11, 848-854.
Ilgen, F., Ott, D., Kralisch, D., Reil, C., Palmberger, A., Konig, B. *Green Chem*., 2009, 11, 1948-1954.
Imperato, G., Eibler, E., Niedermaier, J., Konig, B. *Chem. Commun*., 2005, 1170-1172.
Imperato, G., Konig, B., Chiappe, C. *Eur. J. Org. Chem*., 2007, 1049-1058.
Itoh, T., Akasaki, E., Kudo, K., Shirakami, S. *Chem. Lett*. 2001, 262-263.
Itoh, T.,Matsushita, Y., Abe, Y., Han, S. H., Wada, S., Hayase, S.,Kawatsura, M., Takai, S., Morimoto, M.,Hirose, Y., *Chem. Eur. J*. 2006, 12, 9228-9237.
Jhong, H. R., Wong, D. S. H., Wan, C. C., Wang, Y. Y., Wei, T. C. *Electrochem. Commun*., 2009, 11, 209-211.
Jiang, Y. Y., Wang, G. N., Zhou, Z., Wu, Y. T., Geng, J., Zhang, Z. B. *Chem. Commun*., 2008, 505-507.
Kaar, J. L., Jesionowski, A. M., Berberich, J. A., Moulton, R., Russell, A. J. *J. Am. Chem. Soc.* 2003, 125, 4125-4131.
Kamiya, N., Matsushita, Y., Hanaki, M., Nakashima, K., Narita, M.; Goto, M., Takahashi, H., *Biotechnol. Lett*. 2008, 30, 1037-1040.
Kagimoto, J., Fukumoto, K., Ohno, H. *Chem. Commun*., 2006, 2254-2256.
Katase, T., Murase, K., Hirato, T., Awakura, Y. *J. Appl. Electrochem*., 2007, 37, 339-344.
Khachatryan, K. S., Smirnova, S. V., Torocheshnikova, I. I., Shvedene, N. V., Formanovsky, A. A., Pletnev, I. V. *Anal. Bioanal. Chem*., 2005, 381, 464-470.
Kroon, M. C., Toussaint, V. A., Shariati, A., Florusse, L. J., Spronsen, J. V., Witkamp, G. J., Peters, C. J. *Green Chem*., 2008, 10, 333-336.
Kroon, M. C., van Spronsen, J., Peters, C. J., Sheldon, R. A., Witkamp, G. J. *Green Chem*., 2006, 8, 246-249.
Lapkin, A. A., Plucinski, P. K., Cutler, M. *J. Nat. Prod.*, 2006, 69, 1653-1664.

Lee, S. H., Doherty, T. V., Linhardt, R. J., Dordick, J. S. *Biotechnol. Bioeng*., 2009, 102, 1368-1376.
Lee, J. K.,Kim, M. J., *J. Org. Chem*. 2002, 67, 6845-6847.
Li, M., Pittman Jr, C. U., Li, T. *Talanta*, 2009, 78, 1364-1370.
Li, X. F.,Lou, W. Y., Smith, T. J., Zong, M. H.,Wu, H., Wang, J. F. *Green Chem*. 2006, 8, 538-544.
Liazid, A., Palma, M., Brigui, J., Barroso, C. G. *J. Chromatogr. A*, 2007, 1140, 29-34.
Liu, Q., Janssen, M. H. A., Rantwijk, F. v., Sheldon, R. A. *Green Chem*., 2005, 7, 39-42.
Liu, B. K., Wang, N., Chen, Z. C., Wu, Q., Lin, X.F. Bioorg. *Med. Chem. Lett*. 2006,16, 3769-71.
Lou, W. Y., Zong, M. H., Liu, Y. Y.,Wang, J. F. *J. Biotechnol*. 2006, 125, 64-74.
Lu, Y., Ma, W., Hu, R., Dai, X., Pan, Y. *J. Chromatogr. A*, 2008, 1208, 42-46.
Lue, B. M., Guo, Z., Xu, X. *J. Chromatogr. A*, 2008, 1198-1199, 107-114.
Ma, W., Lu, Y., Hu, R., Chen, J., Zhang, Z., Pan, Y. *Talanta,* 2010, 80, 1292-1297.
MacFarlane, D. R., Golding, J., Forsyth, S., Forsyth, M., Deacon, G. B. *Chem. Commun*., 2001, 1430-1431.
Miyawaki, O., Tatsuno, M. *J. Biosci. Bioeng*. 2008, 105, 61-64.
Mamajanov, I., Engelhart, A., Bean, H., Hud, N. *Angew. Chem. Int. Ed.*, 2010, 49, 6310-6314.
Mao, T., Hao, B., He, J., Li, W. L., Li, S. Q., Yu, Z. N. *J. Sep. Sci*., 2009, 32, 3029-3033.
Mellein, B. R., Brennecke, J. F. *J. Phys. Chem. B*, 2007, 111, 4837-4843.
Moniruzzaman, M., Kamiya, N., Goto, M. *Langmuir* 2008, 25, 977-82.
Moriel, P., Garcia-Suarez, E. J., Martinez, M., Garcia, A. B., Montes-Moran, M. A., Calvino-Casilda, V., Banares, M. A. *Tetrahedron Lett*, 2010, 51, 4877-4881.
Morrison, H. G., Sun, C. C., Neervannan, S. *Int. J. Pharm*., 2009, 378, 136-139.
Mota-Morales, J. D., Gutierrez, M. C., Sanchez, I. C., Luna-Barcenas, G., del Monte, F. *Chem. Commun.*, 2011, 47, 5328-5330.
Moulthrop, J. S., Swatloski, R. P., Moyna, G., Rogers, R. D. *Chem. Commun*., 2005, 1557-1559.
Nockemann, P., Thijs, B., Driesen, K., Janssen, C. R., Van Hecke, K., Van Meervelt, L., Kossmann, S., Kirchner, B., Binnemans, K. *J. Phys. Chem. B*, 2007, 111, 5254-5263.
Noritomi, H., Nishida, S., Kato, S., *Biotechnol. Lett*. 2007, 29,1509-1512.
Ohno, H., Fukumoto, K. *Acc. Chem. Res*., 2007, 40, 1122-1129.
Pei, Y., Wang, J., Wu, K., Xuan, X., Lu, X. *Sep. Purif. Technol*., 2009, 64, 288-295.
Persson, M., Bornscheuer, U.T. *J. Mol. Catal. B* 2003, 22, 21-27.
Remsing, R. C., Swatloski, R. P., Rogers, R. D., Moyna, G. *Chem. Commun*., 2006, 1271-1273.

Ren, M. Y., Bai, S., Zhang, D. H.,Sun, Y. J. Agr. *Food Chem*. 2008, 56, 2388-2391.
Ropel, L.,Belvèze, L. S., Aki, S. N. V. K., Stadtherr, M. A., Brennecke, J. F. *Green Chem*. 2005, 7, 83-90.
Sanfilippo, C., D'Antona, N., Nicolosi, G. *Biotechnol. Lett*. 2004, 26,1815-19.
Saurer, E. M., Aki, S. N. V. K., Brennecke, J. F. *Green Chem*., 2006, 8, 141-143.
Schafer, T., Rodrigues, C. M., Afonso, C. A. M., Crespo, J. G. *Chem. Commun*., 2001, 1622-1623.
Shan, H.,Li, Z., Li. M., Ren, G., Fang, Y. *J. Chem. Technol. Biotechnol*. 2008, 83, 886-891.
Shariati, A., Gutkowski, K., Peters, C. J. *AIChE J*. 2005, 51, 1532-1540.
Smirnova, S., Torocheshnikova, I., Formanovsky, A., Pletnev, I. *Anal. Bioanal. Chem*., 2004, 378, 1369-1375.
Soto, A., Arce, A., Khoshkbarchi, M. K. *Sep. Purif. Technol*., 2005, 44, 242-246.
Sun, N., Rahman, M., Qin, Y., Maxim, M. L., Rodriguez, H., Rogers, R. D. *Green Chem*., 2009, 11, 646-655.
Swalina, C. W., Zauhar, R. J., DeGrazia, M. J., Moyna, G. *J. Biomol. NMR*, 2001, 21, 49-61.
Swatloski, R. P., Holbrey, J. D., Rogers, R. D. *Green Chem*., 2003, 5, 361-363.
Swatloski, R. P., Spear, S. K., Holbrey, J. D., Rogers, R. D. *J. Am. Chem. Soc*., 2002, 124, 4974-4975.
Tan, S. S. Y., MacFarlane, D. R., Upfal, J., Edye, L. A., Doherty, W. O. S., Patti, A. F., Pringle, J. M., Scott, J. L. *Green Chem*., 2009, 11, 339-345.
Tang, F., Zhang, Q., Ren, D., Nie, Z., Liu, Q., Yao, S. *J. Chromatogr. A*, 2010, 1217, 4669-4674.
Tao, G. H., He, L., Liu, W. S., Xu, L., Xiong, W., Wang, T., Kou, Y. *Green Chem.*, 2006, 8, 639-646.
Tao, G.h., He, L., Sun, N., Kou, Y. *Chem. Commun*., 2005, 3562-3564.
Tavares, A. P. M., Rodriguez, O.,Macedo, E. A. *Biotechnol. Bioeng*. 2008, 101, 201-207.
Tian, M., Yan, H., Row, K. H. *Anal. Lett*., 2010, 43, 110-118.
Tsunekawa, S., Ito, S., Kawazoe, Y., Wang, J. T. *Nano. Lett*., 2003, 3, 871-875.
Tzeng, Y.P., Shen, C.W., Yu, T. *J. Chromatogr. A*, 2008, 1193, 1-6.
Usuki, T., Yasuda, N., Yoshizawa-Fujita, M., Rikukawa, M. *Chem.Commun.* 2011, 47, 10560-10562.
Vafiadi, C., Topakas, E., Nahmias, V. R., Faulds, C. B., Christakopoulos, P. *J. Biotechnol*. 2009, 139, 124-129
Van Rantwijk, F.; Sheldon, R. A. *Chem. Rev*. 2007, 107, 2757-2785.
Vidal, S. T. M., Correia, M. J. N., Marques, M. M., Ismael, M. R., Reis, M. T. A. *Sep. Sci. Technol*., 2004, 39, 2155-2169.
Visser, A. E., Swatloski, R. P., Reichert, W. M., Mayton, R., Sheff, S., Wierzbicki, A., Davis, J. J. H., Rogers, R. D. *Chem. Commun*., 2001, 135-136.
Wang, J., Pei, Y., Zhao, Y., Hu, Z. *Green Chem*., 2005, 7, 196-202.
Wang, S. F., Chen, T., Zhang, Z. L., Pang, D. W. *Electrochem. Commun*. 2007, 9, 1337-1342.

Wasserscheid, P., Bosmann, A., Bolm, C. *Chem. Commun*., 2002, 200-201.
Welton, T. *Chem. Rev*. 1999, 99, 2071-2084.
Winkel, A., Reddy, P. V. G., Wilhelm, R. *Synthesis-Stuttgart*, 2008, 999-1016.
Xie, H., Li, S., Zhang, S. *Green Chem*. 2005, 7, 606-608.
Xie, L.L., Favre-Reguillon, A., Pellet-Rostaing, S., Wang, X. X., Fu, X., Estager, J., Vrinat, M., Lemaire, M. *Ind. Eng. Chem. Res*. 2008, 47, 8801-8807.
Xu, A., Wang, J., Wang, H. *Green Chem*., 2010, 12, 268-275.
Yang, Z., Yue, Y. J., Xing, M., *Biotechnol. Lett*. 2008, 30, 153-158.
Yu, Y.Y., Zhang, W., Cao, S.W. *Chinese J. Anal. Chem*., 2007, 35, 1726-1730.
Zeng, H., Wang, Y., Kong, J., Nie, C., Yuan, Y. *Talanta*, 2010, 83, 582-590.
Zhai, Y., Sun, S., Wang, Z., Cheng, J., Sun, Y., Wang, L., Zhang, Y., Zhang, H., Yu, A. *J. Sep. Sci*., 2009, 32, 3544-3549.
Zhang, H., Cheng, M., Jiang, X. *Chromatographia*, 2010, 72, 1195-1199.
Zhang, L. J., Geng, Y. L., Duan, W. J., Wang, D. J., Fu, M. R., Wang, X. *J. Sep. Sci*., 2009, 32, 3550-3554.
Zhang, D., Kovach, I. M., Sheehy, J. P. *Biochim. Biophs. Acta Proteins Proteomics* 2008, 1784, 827-833.
Zhang, L., Wang, X., *J. Sep. Sci*., 2010, 33, 2035-2038.
Zhang, S., Lu, X., Zhang, Y., Zhou, Q., Sun, J., Han, L., Yue, G., Liu, X., Cheng, W., Li, S. *Struc. bond*, 2008, 143-191.
Zhao, H., Baker, G. A., Holmes, S. *Org. Biomol. Chem*., 2011, 9, 1908-1916.
Zhao, H., Baker, G. A., Song, Z., Olubajo, O., Zanders, L., Campbell, S. M. *J. Mol. Catal. B: Enzym.* 2009, 57, 149-157.
Zhu, S., Wu, Y., Chen, Q., Yu, Z., Wang, C., Jin, S., Ding, Y., Wu, G. *Green Chem*., 2006, 8, 325-327.
Zhuo, D., Yu, Y.L., Wang, J. H. *Chem. A Eur. J*. 2007, 13, 2130-2137.