



Review

Application of ionic liquid for extraction and separation of bioactive compounds from plants

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ABSTRACT

In recent years, ionic liquids (ILs), as green and designer solvents, have accelerated research in analytical chemistry. This review highlights some of the unique properties of ILs and provides an overview of the preparation and application of IL or IL-based materials to extract bioactive compounds in plants. IL or IL-based materials in conjunction with liquid–liquid extraction (LLE), ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), high performance liquid chromatography (HPLC) and solid-phase extraction (SPE) analytical technologies etc., have been applied successfully to the extraction or separation of bioactive compounds from plants. This paper reviews the available data and references to examine the advantages of IL and IL-based materials in these applications. In addition, the main target compounds reviewed in this paper are bioactive compounds with multiple therapeutic effects and pharmacological activities. Based on the importance of the targets, this paper reviews the applications of ILs, IL-based materials or co-working with analytical technologies. The exploitation of new applications of ILs on the extraction of bioactive compounds from plant samples is expected to increase.

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1. Introduction

An ionic liquid (IL) is an organic salt in the liquid state that consists of an organic cation paired with an organic or inorganic anion. ILs are widely recognized solvents owing to their excellent properties, such as poor conductors of electricity, non-ionizing (e.g. non-polar), highly viscous, low vapor pressure, low combustibility, excellent thermal stability, wide liquid regions and favorable solvating properties for a range of polar and non-polar compounds [1,2]. Accordingly, ILs are known as “designer solvents”, and have developed rapidly from their initial use as electrolytes in battery applications since the 1970s [3]. Currently, ILs are being applied in every branch of chemistry including organic chemistry, inorganic chemistry, biochemistry, physical chemistry and analytical chemistry [4–8]. The interest of ILs in analytical chemistry has grown quickly over many years and a wide range of applications of ILs in analytical chemistry has been reported [9–14].

Studies of ILs in analytical chemistry focused mainly on the following: ILs as solvents for extraction in conventional liquid–liquid extraction (LLE), microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), liquid-phase microextraction (LPME) and solid-phase microextraction (SPME); as a mobile phase additive in high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and countercurrent chromatography (CCC); and as a modifier of the sorbent or stationary phase in HPLC, SPE, gas chromatography (GC), and SPME [13–22]. ILs, as extraction solvents, can promote interactions between the analyte and solvent because of their unique chemical functional groups. As a mobile phase additive in HPLC, ILs can decrease band tailing by removing the deleterious effects of free silanols on the retention of basic analytes [23,24]. In recent years, many new IL-based silica materials and porous polymers have been synthesized as sorbents or stationary phases in a range of separation processes. These IL-based materials have been applied widely because of their unique properties, such as polarity and low volatility associated with non-polar and ionic interactions [15], as well as their different chromatographic performance attached to different functional groups on their surfaces. These materials interact with analytes through anion–exchange, hydrogen bonding, π – π , or hydrophobic interaction etc. Ho et al. [10], Han and Row [14], Chen and Qi [21], Qiu et al. [25], Vidal et al. [26] and Pino and Afonso [27] published excellent reviews on ILs co-working with these analytical technologies. These reviews discussed the technologies applied in many fields.

IL-based technologies have the capability of enriching and separating a range of organic and inorganic compounds. Therefore, they have been widely applied in food hygiene, drug testing, environment monitoring, biological analysis and other areas [28,29]. Among the biological application fields, bioactive compounds in plants have attracted considerable attention because of their direct effects on living organisms depending on the substance, dose or bioavailability. These bioactive compounds are important substances for the biosome include amino acids, erythromycin, penicillin, lysozyme, hemoglobin, cytochrome and DNA etc. [30–37]. Many researchers have used IL-based technologies to extract and separate bioactive compounds from plants. This paper reviews the applications of IL-based analytical technologies to the extraction and separation of bioactive compounds in plants.

Several authors included the use of IL phases in analytical technologies as a part of their review articles regarding ILs in the extraction and separation of a wide range of compounds and samples [9,10,38], or IL-based materials [16,39]. This review highlights ILs and IL-based materials by describing their preparation, and combined use with analytical technologies in the extraction and separation of bioactive compounds from plant samples.

2. Ion liquid solvent

2.1. Unique properties of IL

In recent years, the unique physical and chemical properties of ILs have attracted considerable attention in analytical chemistry. Tables 1 and 2

list the ILs popularly applied in analytical chemistry, and the commonly desired properties of ILs in these technologies, respectively.

The favorable solvating properties of ILs are generally understood to be due to their ability to undergo a multitude of solvation interactions. The extraction and separation of bioactive compounds is truly a hallmark of ILs because these interactions can be tailored by structural tuning of the IL to meet the specific requirements. As an example in a Row study, tanshinone, as a class of bioactive compounds, is non-polar and beneficial in terms of the extraction efficiency and sensitivity to impart non-polar functionality to 1-oxyl-3-methylimidazolium chloride ([OMIM][Cl]) [40]. Moreover, amino acids as hydrophilic and polar bioactive compounds are difficult to enrich by conventional solvent extraction. Smirnova et al. used the room temperature IL, 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF₆]), with dicyclohexano-18-crown-6 (CE) to efficiently extract the amino acids, Trp, Gly, Ala and Leu, from an aqueous solution at pH 1.5–4.0 [31]. As ILs have a dual nature, they can undergo interactions with both polar and non-polar molecules, and be useful as extraction media for LLE, MAE, UAE, LPME and SPME.

For MAE, LPME and SPME, the thermal stability of ILs as extraction media is an important parameter that governs the suitability and long-term stability of extraction media. As the extraction media in these technologies, ILs should be sufficiently stable to withstand the operating temperatures of the heating samples. The thermal stability is dependent on both the cation and anion of the IL. Ngo et al. reported that imidazolium-based cations exhibited higher thermal stability than tetraalkylammonium cations based on thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) [41]. In addition, imidazolium-based ILs have thermal stability that increase in the following order: [Cl][−], [Br][−], [I][−] < [BF₄][−] < [CF₃SO₃][−] < [NTf₂][−] < [PF₆][−] [2,41,42]. Handy reported that the component part of the anion generally plays an important role in the thermal stability of ILs [43]. Furthermore, McEwen reported that IL-based organic anions have higher thermal stability than those based on inorganic anions [41].

The viscosity of ILs is also essential to their applications in the extraction or separation of bioactive compounds from plants. Normally, the viscosity of ILs decreases with increasing temperature. Therefore, ILs as LPME extractants or SPME sorbent coatings should be able to resist significant changes in viscosity when exposed to higher temperatures. The viscosity of ILs is affected by a range of intermolecular interactions, such as electrostatic, van der Waals forces and hydrogen bonding interactions [44]. The viscosity of ILs generally decreases with increasing asymmetry of IL anions. For example, with imidazolium cationic species, the viscosity can be increased by increasing the alkyl substituent chain length or branching as a result of the higher van der Waals interactions between the ions themselves [43–46].

2.2. Preparation of IL solvent

Many ILs are commercially available or can be prepared simply by a reaction of the appropriate cation and anion bodies. Two basic methods are used in the preparation of ILs: metathesis of a halide salt with for example, silver, a group 1 metal or the ammonium salt of the desired anion; and acid–base neutralization reactions [47].

Table 1
The structures of the ILs applied in extraction and separation.

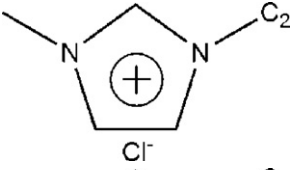
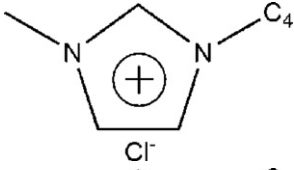
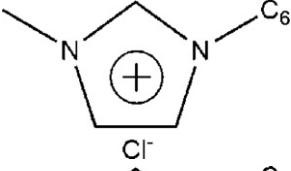
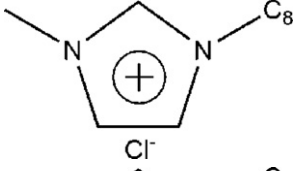
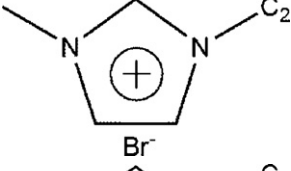
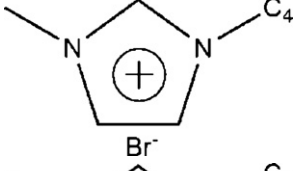
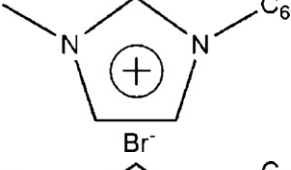
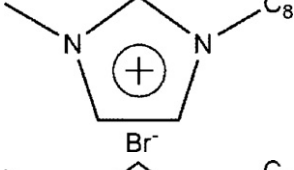
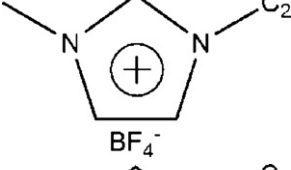
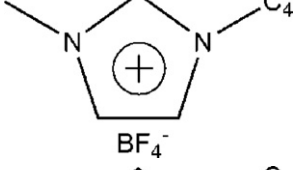
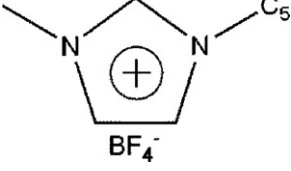
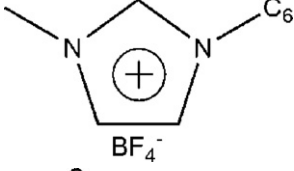
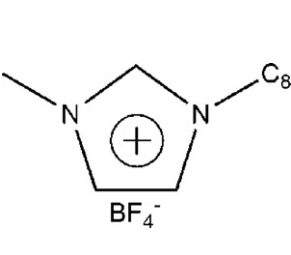
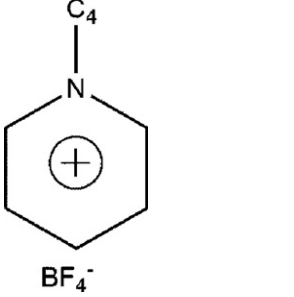
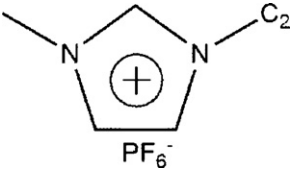
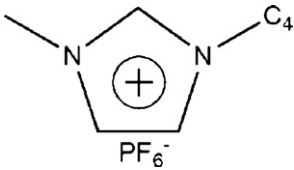
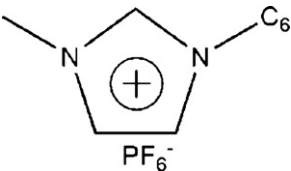
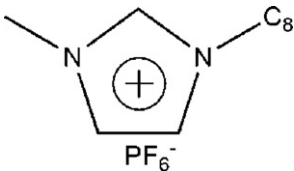
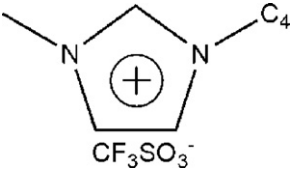
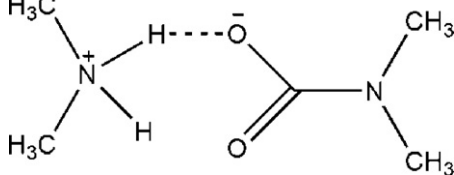
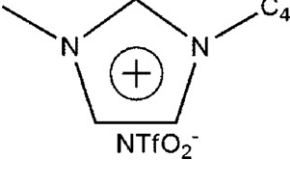
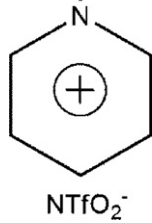
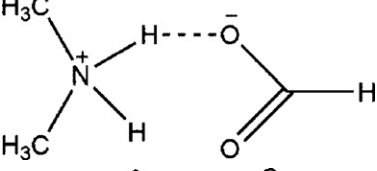
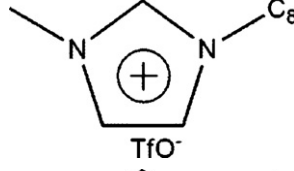
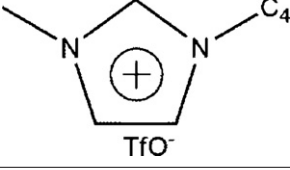
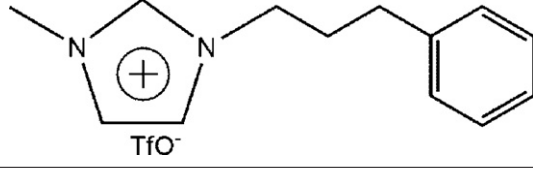
IL	Structure	IL	Structure
[EMIM][Cl]		[BMIM][Cl]	
[HMIM][Cl]		[OMIM][Cl]	
[EMIM][Br]		[BMIM][Br]	
[HMIM][Br]		[OMIM][Br]	
[EMIM][BF ₄]		[BMIM][BF ₄]	
[AMIM][BF ₄]		[HMIM][BF ₄]	
[OMIM][BF ₄]		[BPyrr][BF ₄]	
[EMIM][PF ₆]		[BMIM][PF ₆]	
[HMIM][PF ₆]		[OMIM][PF ₆]	

Table 1 (Continued)

IL	Structure	IL	Structure
[BMIM][CF ₃ SO ₃]		N,N-dimethylammonium N',N'-dimethylcarbamate (DIMCARB)	
[BMIM][NTfO ₂]		[MPPyr][NTfO ₂]	
[C ₂ H ₅ NH ₃][HCOO]		[OMIM][TfO]	
[BMIM][TfO]		[Phromim][TfO]	

Many alkylammonium halides are prepared simply by a reaction of the appropriate halogenoalkane and amine. The preparation of pyridinium and imidazolium halides can be achieved in a similar manner [48,49]. For halogenoalkanes, the low boiling points leads to preparations requiring either a sealed tube, such as in the synthesis of 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl], where [EMIM] is the 1-ethyl-3-methylimidazolium

cation), or an elaborate reaction [50]. Therefore, the use of salts with longer chain substituents, such as 1-butyl-3-methylimidazolium chloride ([BMIM][Cl], where [BMIM] is the 1-butyl-3-methylimidazolium cation), which can be prepared in conventional glassware by heating under reflux, has become popular [51]. In 1992, Wilkes and Zaworotko prepared a new IL, 1-butyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF₄]), by

Table 2
Physical properties of ILs in extraction and separation technology.

ILs		Density (g mL ⁻¹), 25 °C	Viscosity (cP), 25 °C	Decomposition temperature (°C, under air)	Solvent miscibility
Cation	Anion				
[EMIM] ⁺	[BF ₄] ⁻	1.248	66	No data	No data
	[PF ₆] ⁻	1.373	450	No data	No data
[BMIM] ⁺	[BF ₄] ⁻	1.208	233	No data	No data
	[PF ₆] ⁻	1.373	400	No data	Miscible with water. Immiscible with toluene, hexane, and other non-polar solvents
	[Br] ⁻	1.134	Solid	No data	Soluble in water
	[Cl] ⁻	1.120	Solid	232	Soluble in methanol. Miscible with water
	[CF ₃ SO ₃] ⁻	1.290	90	No data	No data
	[(CF ₃ SO ₃) ₂ N] ⁻	1.420	52	No data	No data
	[NTfO ₂] ⁻	1.404	48	No data	No data
[AMIM] ⁺	[BF ₄] ⁻	1.213	321	No data	No data
[HMIM] ⁺	[BF ₄] ⁻	1.075	211	No data	No data
	[PF ₆] ⁻	1.304	800	No data	No data
[OMIM] ⁺	[BF ₄] ⁻	1.11	440	No data	No data
	[Cl] ⁻	1.000	16,000	No data	Soluble in water
[MPPyr] ⁺	[NTfO ₂] ⁻	1.44	39	No data	No data
[C ₂ H ₅ NH ₃] ⁺	[HCOO] ⁻	0.99	11.5	No data	No data
[BMPyrrol] ⁺	[NTfO ₂] ⁻	1.4	71	No data	No data

the metathesis of 1-ethyl-3-methylimidazolium iodide ([EMIM][I]) with $\text{Ag}[\text{BF}_4]$ in methanol [50]. This salt had a low melting point and could be prepared economically using ammonium tetrafluoroborate ($[\text{NH}_4][\text{BF}_4]$) in acetone [52]. 1-ethyl-3-methylimidazolium hexafluorophosphate ([EMIM][PF₆]) was prepared by a reaction of [EMIM][Cl] with fluorophosphoric acid ($[\text{H}][\text{PF}_6]$) [53]. This salt is slightly less attractive than the tetrafluoroborate ($[\text{BF}_4]$) salt because of its higher melting point, which is undesirable for room temperature work. Nonfluorobutanesulfonate ($[\text{NfO}]^-$), tris(trifluoromethyl)sulfonylmethide ($[(\text{CF}_3\text{SO}_2)_3\text{C}]^-$), trifluoroacetate ($[\text{CF}_3\text{CO}_2]^-$), bis(trifluoromethyl)sulfonyl imide ($[\text{Tf}_2\text{N}]^-$), heptafluorobutanoate and thiocyanate ($[\text{SCN}]^-$) salts have been prepared by metathesis reactions [45,54,55]. The above metathesis reactions are good candidates for preparing new ILs but a small quantity of halide ions from the ILs can react with the solute materials [56]. Tetraalkylammonium tetraalkylborides are normally prepared by a metathesis reaction of the tetraalkylammonium bromide and lithium tetraalkylboride [57,58]. Reactions to produce appropriate quaternary amines have been employed to prepare a number of 1-alkyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][TfO]) ILs in the metathesis reaction [45]. Monoalkylammonium nitrate ILs are best prepared by neutralizing aqueous solutions of the amine with nitric acid [54,59].

2.3. Application of IL solvent

As some ILs are immiscible with water (which allows the formation of biphasic systems) and the organic species have high solubility in these ILs, they are suitable as solvents for the extraction most of bioactive compounds from plants and as mobile phase modifiers to improve LC separation for bioactive targets. As solvents, these ILs have been applied in conventional methods including LLE, UAE, MAE, HPLC, CE, SPME, LPME and CCC. The extraction of bioactive compounds from plants using ILs has great promise, which can alleviate the environmental pollution and improve the selectivity and extraction yields of interesting compounds in sample pretreatment processes compared to conventional organic solvents. Table 3 lists these applications. The popular and typical methods are discussed term by term in the following sections.

2.3.1. LLE

Auxins are well-known plant growth hormones involved in a variety of plant activities [82]. ‘Synthetic’ auxin 3-indole-butyric acid (IBA) was reported to promote rooting and is now used commercially worldwide to root many plant species [83–86]. IBA also occurs naturally in a number of plant species ranging from maize (*Zea mays*) to pea (*Pisum sativum*) and arabisopsis [87–89]. Absalan et al. used [BMIM][PF₆] as a solvent in the conventional LLE method to extract IBA from pea plants [75]. In Absalan’s study, imidazolium-based ILs, [BMIM][PF₆], 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF₆]), 1-oxy-3-methylimidazolium hexafluorophosphate ([OMIM][PF₆]), 1-hexyl-3-methylimidazolium tetrafluoroborate ([HMIM][BF₄]) and 1-oxy-3-methylimidazolium tetrafluoroborate ([OMIM][BF₄]), were evaluated as extracting solvents for the removal of IBA from aqueous media with subsequent determination by HPLC. They reported that the extraction efficiency of IBA was strongly affected by the pH of the aqueous phases and the chemical structures of the ILs. The extraction of IBA was quantitative at $\text{pH} < \text{pK}_a$ of IBA. A change in the 1-alkyl group of $[\text{C}_n\text{MIM}][\text{PF}_6]$ and $[\text{C}_n\text{MIM}][\text{BF}_4]$ to a long chain improved the extraction efficiency of IBA. They suggested that this effect is consistent with the affinity of IBA to more hydrophobic ILs. The anionic part of the ILs affected the extraction efficiency of IBA. For example, the percentage extraction of IBA into $[\text{C}_n\text{MIM}][\text{BF}_4]$ was higher than that of $[\text{C}_n\text{MIM}][\text{PF}_6]$ under similar experimental

conditions. The reason is that the hydroxyl groups of the IBA molecules exhibited stronger H-bonding with the $[\text{BF}_4]$ anion than the $[\text{PF}_6]$ anion. Therefore, the extraction efficiency of IBA was highest in $[\text{OMIM}][\text{BF}_4]$ than in the other ILs examined. Although the percentage extraction of IBA from aqueous solutions into $[\text{OMIM}][\text{BF}_4]$ was highest among the ILs, the percentage stripping of the analyte from this IL phase into basic solutions was low. [BMIM][PF₆] exhibited higher extraction and stripping efficiencies of IBA than the other ILs studied. The capacity of [BMIM][PF₆] was 17.6×10^{-4} mmol IBA per 1.0 mL of IL. The ionic strength of the aqueous phase and temperature had no serious effects on the extraction efficiency of IBA. A preconcentration factor of 100 and a relative standard deviation of 1.16% were obtained. The IL was reusable almost five times for extraction/stripping purposes. Auxin 3-indole acetic acid was also obtained as an interferential analyte in the extraction process.

Para Red and Sudan dyes were extracted from chili powder and chili oil using the IL-based LLE reported by Fan et al. [76]. In their work, two ILs, [BMIM][PF₆] and [OMIM][PF₆], were compared as extraction solvents. The latter exhibited higher recovery for each analyte. Good reproducibility of the extraction performance was achieved under optimal conditions with relative standard deviations (RSDs) ranging from 2.0% to 3.5%. The detection limits of Sudan dyes and Para Red (LOD, $S/N=3$) ranged from $7.0 \mu\text{g kg}^{-1}$ to $8.2 \mu\text{g kg}^{-1}$ for chili powder and $11.2\text{--}13.2 \mu\text{g L}^{-1}$ for chili oil. The recoveries ranged from 76.8% to 109.5% for chili powder and 70.7–107.8% for chili oil.

The amino acid Gly was extracted successfully from pharmaceutical samples into the room temperature IL, [BMIM][PF₆], with dicyclohexano-18-crown-6 in LLE [31].

A distillable IL, *N,N*-dimethylammonium *N,N*-dimethylcarbamate (DIMCARB), was used in LLE to extract the hydrolyzable tannins from *Acacia catechu* and *Terminalia chebula* plants sources [80]. The extraction efficiencies were 85%, which were significantly higher than the traditional extraction methods that utilize large quantities of water. Moreover, higher concentrations of ellagic acid were extracted from these plants. Microbiological tests revealed the IL extracted materials to resist fungal growth.

2.3.2. UAE

Tanshinones are abietanoid diterpenes found in the traditional medicinal herb, *Salvia miltiorrhiza* Bunge (SMB), and have been used to treat many diseases owing to their powerful antibacterial, anticancer, anti-inflammatory, antioxidant and hepatic-protection activities [90–96]. The ultrasonication-assisted extraction of tanshinones from SMB using ILs was reported in a previous study [40]. SMB was first oven-dried, sliced and crushed. The material was then mixed with different aqueous IL solutions, such as 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]), [BMIM][Cl], 1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]) and [OMIM][Cl]. The extraction conditions were optimized systematically. The extraction suspensions were filtered through a filter, pre-concentrated, and injected into the liquid chromatograph. Cryptotanshinone, tanshinone I and tanshinone IIA from SMB; 0.233 mg g^{-1} , 0.695 mg g^{-1} and 0.682 mg g^{-1} , respectively, were extracted and pre-concentrated successfully using [OMIM][Cl]. This method has potential applicability with other bioactive medicinal compounds.

Phenolic compounds are found widely in algae, and possess antioxidant, antimicrobial and antiviral activities that are important against DNA damage and cell death [97–103]. In this research group, Han and Row applied an IL-based UAE method to the effective extraction of phenolic compounds from *Laminaria japonica* Aresch [14]. Compared to the conventional solvent, the optimal approach resulted in the highest extraction efficiency within the

Table 3
Application of ILs for extraction and separation.

ILs	Method	Bioactive compound	Natural plant	Ref.
[BMIM][BF ₄]	UAE	Phenolic compounds	<i>L. japonica</i>	[60]
	UAE	Fangchinoline, tetrandrine	<i>S. tetrandrae</i>	[61]
	UAE	Piperine	White pepper	[62]
	LLE	Tocopherol homologues	Mixed Tocopherol	[63]
	MAE	Phenolic alkaloids	<i>N. nucifera</i> Gaertn.	[64]
	HPLC	Matrine, oxymatrine, sophoridine, sophocarpine	<i>S. flavescens</i> Ait.	[65]
	CE	Verticine, verticinone	<i>B. fritillariae</i>	[66]
	CE	Kaempferol-3-O-glucoside, 2,7-hydroxy-8-methoxycoumarin, 3,8-hydroxycoumarin-7-O-glucoside	<i>S. tangutica</i>	[67]
	CE	Anthraquinones	Chinese herb	[68]
	CE	Eastside, cinnamic acid and ferulic acid	<i>Euphrasia regelii</i>	[69]
[EMIM][Cl]	UAE	Tanshinones	<i>S. miltiorrhiza</i> Bunge	[40]
[HMIM][Cl]	UAE	Tanshinones	<i>S. miltiorrhiza</i> Bunge	[40]
[OMIM][Cl]	UAE	Tanshinones	<i>S. miltiorrhiza</i> Bunge	[40]
[BMIM][Br]	MAE	Polyphenolic compounds	<i>P. guajava</i> Linn. leaves and <i>S. china</i> tubers	[70]
	MAE	Phenolic alkaloids	<i>N. nucifera</i> Gaertn.	[64]
	MAE	<i>Trans</i> -resveratrol	<i>R. polygona cuspidata</i>	[71]
[HMIM][Br]	MAE	<i>N</i> -nornuciferin, <i>O</i> -nornuciferine, and nuciferine	Lotus leaves	[72]
[BMIM][PF ₆]	MAE	Essential oils	Fruits of <i>I. verum</i> Hook. f. and <i>C. cyminum</i> L.	[73]
	MAE	Phenolic alkaloids	<i>N. nucifera</i> Gaertn.	[64]
	LLE	Ferulic acid, caffeic acid	–	[74]
	LLE	3-indole butyric acid	Pea plants	[75]
	LE	Chilli powder, chilli oil, food additive	Para Red and Sudan dyes	[76]
	LE	Amino acids	Pharmaceutical samples	[31]
	CCC	Neomangiferin, mangiferin	<i>R. anemarrhenae</i>	[22]
[BMIM][CF ₃ SO ₃]	LLE	Tocopherol homologues	Mixed Tocopherol	[63]
[EMIM][BF ₄]	MAE	Phenolic alkaloids	<i>N. nucifera</i> Gaertn.	[64]
	HPLC	Octopamine, synephrine, tyramine	Citrus herbs	[77]
	CE	Kaempferol-3-O-glucoside, 2,7-hydroxy-8-methoxycoumarin, 3,8-hydroxycoumarin-7-O-glucoside	<i>S. tangutica</i>	[67]
[HMIM][BF ₄]	MAE	Phenolic alkaloids	<i>N. nucifera</i> Gaertn.	[64]
	LLE	3-indole butyric acid	Pea plants	[75]
[OMIM][BF ₄]	MAE	Phenolic alkaloids	<i>N. nucifera</i> Gaertn.	[64]
	LLE	3-indole butyric acid	Pea plants	[75]
[HMIM][PF ₆]	LLE	Ferulic acid, caffeic acid	–	[74]
	LLE	3-indole butyric acid	Pea plants	[75]
	DLLME	Thiophanate-methyl, carbofuran, carbaryl, tebuconazole, iprodione, oxyfluorfen, hexythiazox, fenazaquin	Table grape and plum samples	[78]
[BMIM][Cl]	LLE	Tocopherol homologues	Mixed Tocopherol	[63]
	MAE	Phenolic alkaloids	<i>N. nucifera</i> Gaertn.	[64]
	MMAE	Lycorine, lycoramine, galanthamine	<i>Lycoris radiata</i>	[79]
[OMIM][PF ₆]	LLE	3-indole butyric acid	Pea plants	[75]
	LE	Chilli powder, chilli oil, food additive	Para Red and Sudan dyes	[76]
N,N-dimethylammonium N',N'-dimethylcarbamate (DIMCARB)	SPME	Tannin	Catechu and myrobolan	[80]
1-alkyl-3-methylimidazolium-based ILs	CE	Polyphenols	Grape seed	[81]

shortest extraction time. The mean recoveries of phenolic compounds ranged from 75.5% to 88.3% under optimized conditions.

Radix Stephaniae tetrandrae, the dry root of *S. tetrandrae* S. Moore, has been used in Chinese traditional medicine and is listed in the Chinese Pharmacopoeia. Among the known biologically active components of the roots, two main alkaloids, fangchinoline and tetrandrine, exhibit pharmacological activities including anti-allergic, anti-inflammatory, anti-cancer and hypotensive effects, which promote phagocytosis, coronary artery dilation, and reduced myocardial oxygen consumption [104–107]. Traditional reflux solvent extraction has been used to extract fangchinoline and tetrandrine from *S. tetrandrae*. Compared to traditional reflux solvent extraction, Zhang et al. developed an IL-based ultrasound-assisted extraction technique for the effective extraction of fangchinoline and tetrandrine from *S. tetrandrae*

[61]. The effects of some ultrasound-assisted extraction parameters including the concentration of 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄]), pH, ultrasonic power and time were examined to optimize the ultrasound-assisted extraction conditions. Compared to regular ultrasound-assisted extraction and traditional reflux extraction, the proposed [BMIM][BF₄]-based UAE offered shorter extraction times (from 6 h to 40 min) as well as remarkably higher efficiencies (approximately 30% higher), highlighting the suitability of the proposed approach.

White pepper is used as a spice worldwide and is obtained from the fully ripe berries of the pepper plant. Piperine, the main constituent of white pepper, exhibits a range of pharmacological activities, e.g. antifungal, antidiarrhoeal and anti-inflammatory, as well as 5-lipoxygenase and cyclooxygenase-1 inhibitory activities [108–112]. Cao et al. developed an IL-based UAE (ILUAE)

method for the effective extraction of piperine from white pepper powder [62]. The extraction efficiency of a series of 1-alkyl-3-methylimidazolium ILs with different alkyl chains and anions were evaluated. The results suggested that the characteristics of both anions and cations have remarkable effects on the extraction efficiency. Compared to conventional heat-reflux extraction (HRE) and regular UAE, the optimized approach has attracted the highest extraction efficiency (from 1.950% to 3.577%) within the shortest extraction time (from 2 h to 30 min).

2.3.3. MAE

Psidium guajava Linn. (*P. guajava*) leaves and *Smilax china* (*S. china*) tubers are two popular and important herbal medicines. The extracts have multiple therapeutic effects and pharmacological activities, such as antioxidant, anti-inflammatory, anti-microbial and anti-tumor effects, which are related to polyphenolic compounds including gallic acid, ellagic acid, quercetin and trans-resveratrol [113–117]. Du et al. reported the MAE of polyphenolic compounds from the two plants using an IL solution as solvent in 2009 [70]. The IL concentration and MAE conditions were examined to extract polyphenolic compounds effectively from plant samples. The results suggested that the anions and cations of ILs affected the extraction of polyphenolic compounds. Moreover, ILs with an electron-rich aromatic π -system enhanced the extraction ability. Under optimized conditions, the extraction yields of the targets ranged from 79.5% to 93.8% with only one extraction step, and the recoveries ranged from 85.2% to 103% with RSDs <5.6%. Compared to conventional extraction procedures, the report suggested that the proposed method was an effective alternative for the extraction of polyphenolic compounds from the two plants. In addition, the extraction mechanisms and structures of the samples before and after extraction were also examined.

Three aporphine alkaloids, N-nornuciferine, onornuciferine and nuciferine, are the major phytochemicals present in lotus leaves (*Nelumbo nucifera* Gaertn.). The total alkaloids have been found to have biological functions by lowering the hyperlipemia and cholesterol level, resisting karyokinesis as well as exhibiting antimicrobial activity and anti-HIV activity [118–121]. Currently, the alkaloids from this medicinal plant are extracted mainly by HRE, which is a time-consuming and laborious process [122–124]. The aim of Pan's work was to develop a rapid, effective, validated and environmental friendly ILs-based MAE of the three alkaloids from this herbal medicine [72]. Seven types of 1-alkyl-3-methylimidazolium ILs were assessed with a 1-hexyl-3-methylimidazolium bromide ([HMIM][Br]) solution used as the solvent. The proposed approach exhibited higher efficiency (0.9–43.7% enhanced) and shorter extraction time (from 2 h to 2 min) than regular MAE and conventional HRE, indicating IL MAE to be an efficient, rapid and simple sample preparation technique.

Zhai et al. used [BMIM][PF₆] as microwave absorption medium and applied it to the extraction of essential oils from the dried fruits of the *Illicium verum* Hook. f. and *Cuminum cyminum* L. by MAE [73]. The constituents of the essential oils obtained by their method and hydrodistillation were the same. The phenolic alkaloids present in samples of the plant, *N. nucifera* Gaertn., were extracted effectively using [BMIM][BF₄] and [HMIM][BF₄]-based MAE according to Pan's study [64]. In Li's paper, the application of 1-n-butyl-3-methylimidazolium-based ILs as solvents in MAE was first developed for the extraction of trans-resveratrol from *Rhizma polygoni cuspidati*.

2.3.4. HPLC

Citrus herbs and their extracts, e.g. *Citrus aurantium* L, have been used in the treatment of obesity [125,126]. Adrenergic amines, such as octopamine (Octo), synephrine (Syne) and tyramine (Tyra), are the major active compounds in these herbs [127]. The separation

and quantification of adrenergic amines in different samples, such as mouse central nerves and *C. aurantium* crude drugs, by HPLC with an electrochemical detector or UV detector have been reported [128–134]. These amine compounds, however, can interact strongly with the free silanols on the surface of the stationary phase. This causes band tailing of the analytes, which affects the resolution and quantitative analysis. In Yao's study, aqueous solutions of ILs were first proposed as mobile phase additives for the separation of adrenergic amines from Citrus herbal extracts by LC [77]. Aqueous solutions of ILs were used successfully as 'green' mobile phases to separate the targets by LC without volatile organic solvents. The problems of the adrenergic amines separation, such as band tailing, low retention and low resolution were solved using an IL. The effect of [EMIM][BF₄] was the best of the six ILs investigated. The concentration of [EMIM][BF₄], mobile phase pH and column temperature, which influenced the chromatographic behavior of the targets, were investigated in detail. The change in retention factors caused by a pH shift was suppressed by [EMIM][BF₄]. The sensitivity, accuracy and reproducibility of this method were satisfactory. The contents of adrenergic amines in the Citrus herbs extracts were determined simultaneously using this 'green' chromatographic method.

Sophora flavescens Ait (SFA) has been used as a traditional Chinese herb since ancient times to treat a range of diseases. Sophora alkaloids, including matrine (MT), oxymatrine (OMT), sophocarpine (SC), sophoridine (SRI) and others are the chief active components [135]. Studies of their pharmacological effects have shown that SRI has wide range of pharmacological effects including anti-arrhythmic, anti-tumor, immunological enhancement, immunosuppressant, antiseptic and central nervous system excitation properties [136–140]. On the other hand, the similar chemical structures of these four alkaloids simultaneously make it difficult to separate. Therefore, Tian et al. examined the effects of three ILs, [HMIM][BF₄], [BMIM][BF₄] and [OMIM][BF₄], as mobile phase additives for the HPLC separation of OMT, SRI, SC and MT from SFA [65]. Excellent separation conditions of the four compounds was found in methanol/water (45:55, v/v) containing 0.1 mmol/L [HMIM][BF₄] as the additive at pH 11.3. Li used the same IL ([HMIM][BF₄]) as a mobile phase additive for the HPLC separation of chlorogenic acid, caffeic acid, rutin and scoparone from *Herba artemisiae scopariae* [141]. The results showed that a mobile phase with [BMIM][BF₄] enabled optimum separation of the four target compounds.

2.3.5. CE

Bulbus fritillariae is a famous Chinese crude herb, which has been used as one of the most important antitussive and expectorant drugs for thousands of years. Pharmacological studies revealed verticine and verticinone in *B. fritillariae* to be the primary active ingredients responsible for the antitussive activity [142,143]. Gao et al. used a CE/Ru(bpy)₃²⁺ electrochemiluminescence (ECL) system with the assistance of ILs for the sensitive determination of verticine and verticinone in *B. fritillariae* [66].

Sinacalia tangutica has long been used as a traditional medicine for expectorant, anti-cough, antihistamine, antiradical and cathartic purposes [144]. Qi et al. developed a method for the separation and determination of kaempferol-3-O-glucoside, 2,7-hydroxy-8-methoxycoumarin and 3,8-hydroxycoumarin-7-O-glucoside from *S. tangutica* by CE [67]. In the CE system, [EMIM][BF₄] and [BMIM][BF₄] were used as modifiers to improve the separation efficiency and selectivity. The research showed that increasing the applied electric field strengths not only reduced the analysis time, but also did not induce excessive Joule heating in the capillary when ILs were used as modifiers.

Phenolic mixtures are derived from a range of food substances associated with many health benefits in humans, including anticarcinogenic, anticariogenic, and hepatoprotective activities

[145–148]. Naturally occurring antioxidants in the form of polyphenols might be responsible for some of this therapeutic effect. In general, polyphenols are encountered in a range of biological samples as complex mixtures of homologues and isomers with differing degrees and sites of polymerization and thus represent a truly challenging separation problem. Most methods, such as HPLC, GC and CE, were employed to separate polyphenols [149–152]. On the other hand, few of these methods provided complete resolution of all the polyphenols in complicated samples. Yanes et al. first reported the use of [EMIM][BF₄] as the main running electrolyte in the CE to successfully determine the polyphenols in grape seed extracts [81].

From above discussion, ILs can be used in CE as running electrolytes or modifiers to separate bioactive compounds from plants.

2.3.6. SPME, LPME and CCC

Recently, some researchers have used silica coated with ILs as a sorbent for the SPME of volatile organic compounds, such as benzene, toluene, ethylbenzene and xylenes, followed by GC analysis with flame ionization detection (FID) in some samples except for plant samples [153–158]. The IL prevents the direct coupling of IL-based LPME with GC because the injection of ILs into GC can contaminate the GC column. More research on IL-based SPME and LPME of bioactive compounds from plants is needed.

As a unique liquid–liquid partition chromatography method, CCC has made great progress in the preparative isolation and purification of active components from natural products. ILs can form biphasic liquid systems with numerous solvents, including water, which makes them possible candidates in CCC [159]. Previous studies reported that ILs cannot be used directly in CCC due to the high viscosity, so very little work on the use of ILs in CCC has been reported [160]. Liu et al. examined the potentiality of ILs in CCC, and used CCC successfully to isolate neomangiferin and mangiferin from *Rhizoma anemarrhenae* using [BMIM][PF₆] as a modifier of the two-phase solvent system [22]. Although few studies associating ILs with CCC have been reported, the potential of the promising analytical technology still needs to be explored.

3. IL-based materials

In recent years, many IL-based materials have been applied in analytical technologies. Researchers have also reported these materials in some reviews papers, such as Ho et al. [10], Chen and Qi [21], Vidal et al. [26] and Pino and Afonso [27]. In those papers, the properties of many IL-based materials were introduced, the application of these materials in different analytical technologies, such as SPE, HPLC, CCC, GC and CE etc., were reviewed. On the other hand, only a few studies of IL-based silica or IL-based polymers applied in the separation of bioactive compounds in plants have been published in recent years. The present paper describes IL-based materials and the co-working of these materials with technologies applied in the analysis of bioactive compounds in plants. Among these IL-based materials, IL-based silica and IL-based polymer were reviewed. The two types of materials-based technologies, such as SPE and HPLC are the main points reviewed.

3.1. IL-based silica

IL-based silica materials have been developed extensively for analytical applications owing to their high surface areas, narrow pore size distributions, and adjustable mesopore sizes. The high surface area facilitated a uniform dispersion in the materials, and the IL molecule shape could control every type of pore size, which are the main concerns when using these IL-based silica materials. In this study, the preparation of IL-based silica materials, and the

co-working of these materials with technologies were reviewed systematically.

3.1.1. Preparation of IL-based silica

Silica is an almost ideal supporter of an extraction sorbent or chromatographic stationary phase because of its favorable physical and chemical characteristics but it still has some drawbacks. This has encouraged the modification of silica with new functional chemicals. Owing to their fascinating properties, ILs have been selected the most to modify silica in recent years. Based on the effect of IL as a modifier on silica, Moreira and Gushikem first synthesized IL-based silica to absorb and pre-concentrate metal ions from ethanol solutions in 1985 [161]. They used silica gel functionalized with 3(1-imidazolyl)propyl groups to form a new sorbent. In 2002, Valkenberg et al. reported methods to immobilize ILs on a silica support [162]. Liu et al. first reported the utilization of IL-based silica as a stationary phase for HPLC in 2004 [163]. A group of IL-based silica materials was synthesized to separate bioactive compounds from plants. Table 4

lists the main reactants and IL-based silica synthesized.

The IL-based silica materials listed in Table 4 have been used in a larger number of studies [168,169]. In Afonso's review article, the authors summarized and classified four approaches of the synthesis of IL-based silica [27]. These syntheses can be divided into two areas. The first is to prepare silica gel or alkylpropylsilica gel. Silica is added to a hydrochloric acid solution. The mixture is stirred gently and heated to approximately 100 °C. After a setting time, the silica is filtered and dried under vacuum. The dried silica is dispersed in toluene, and 3-alkoxy silane is added under N₂. The temperature is increased to the setting degree, and the mixture is heated under reflux for approximately half a day in a N₂ atmosphere. The product obtained was filtered and washed several times with toluene and dried at approximately 50 °C under vacuum for several hours.

In the second section of the synthesis of IL-based silica, the silica gel or alkylpropylsilica gel reacted with imidazole or pyridine as the main structure of the desired functional group. The desired functional group on the silica surface was imidazole or pyridine as a precursor compound, or an IL cation. Among the imidazole or pyridine-based ILs, halide ions, [PF₆]⁻, [BF₄]⁻, bis(trifluoromethylsulfonyl)imide ([INTF₂]⁻), trifluoromethanesulfonate ([TfO]⁻) and [NfO]⁻ etc. are popular anions. Briefly, dry gel was placed in a reaction flask containing anhydrous toluene and a large excess of the desired functional group. The mixture was heated under reflux with constant stirring for one day. Subsequently, the reaction was stopped and the modified silica was cooled to room temperature, transferred to a vacuum glass filter and washed sequentially with toluene, ethanol and methanol. The silica chemically bonded with the desired functional group was dried under vacuum for several hours.

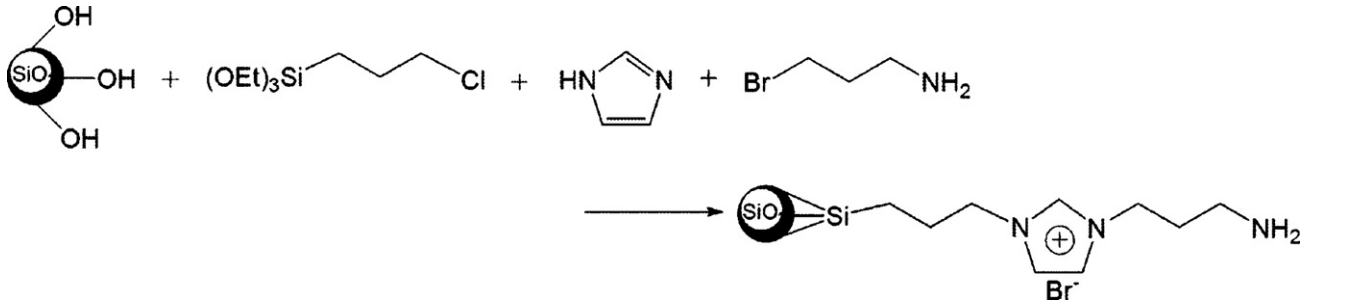
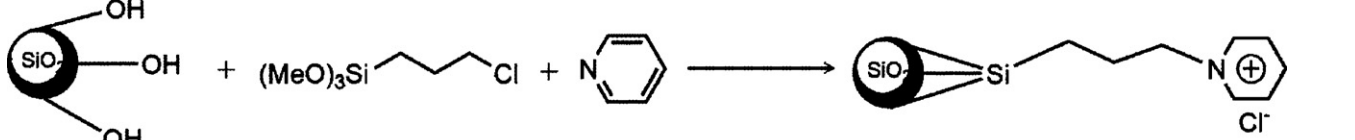
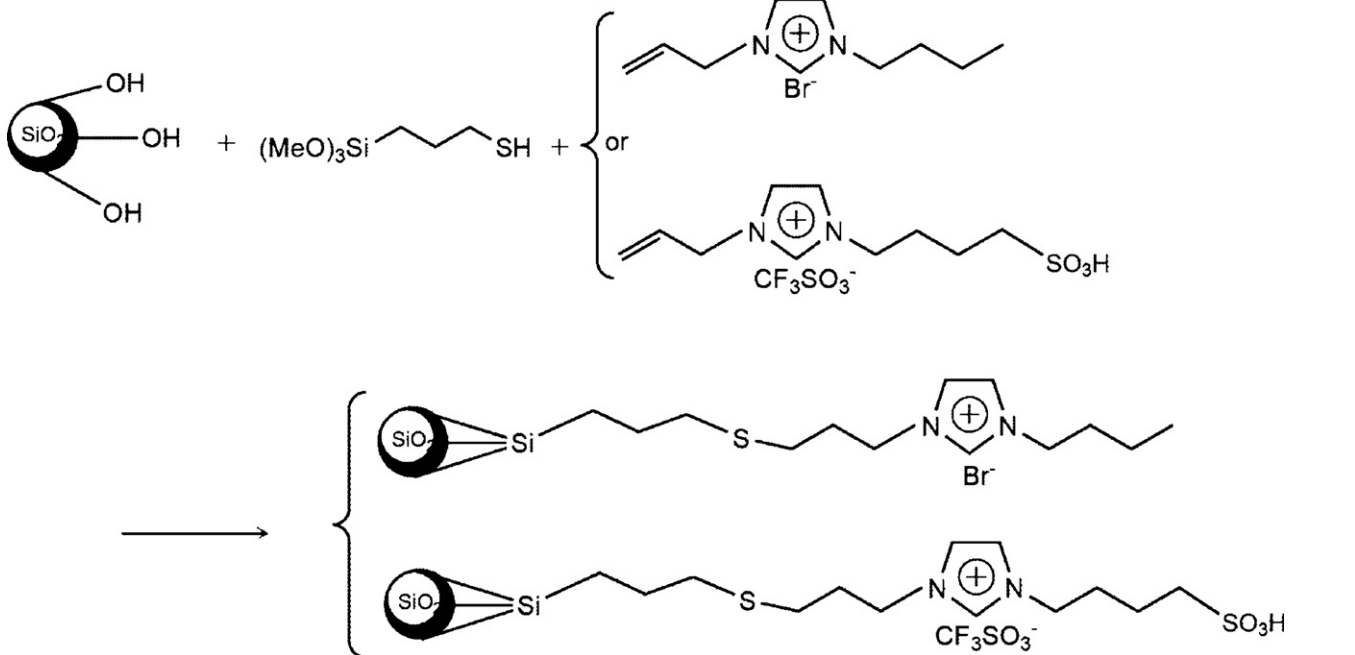
In 2004, Liu et al. reported the preparation of a new stationary phase based on 1-allyl-3-hexyl imidazolium tetrafluoroborate ([AHIM][BF₄]) that was confined on the silica gel, and the application of this stationary phase for the separation of ephedrine in HPLC [163]. [AHIM][BF₄] contains long alkyl chains and an allyl group as the anchoring group, and activated silica was modified with a silane-coupling agent, 3-mercaptopropyltrimethoxysilane (MPS). [AHIM][BF₄] was reacted with MPS-modified silica in the presence of azodiisobutyronitrile (AIBN) as an initiator via radical chain transfer addition.

For the chiral separation of (S)-naproxen and (R)-naproxen, Wang et al. report a novel methodology for the preparation of porous, IL-based silica hybrid molecularly imprinted polymer (MIP) monoliths for chiral recognition that overcomes the shortcomings associated with hydrolytic sol–gel synthesis and organic-polymer-based MIP systems [176]. The preparation of monoliths was based on the reaction of methacrylic acid (MAA), which is a

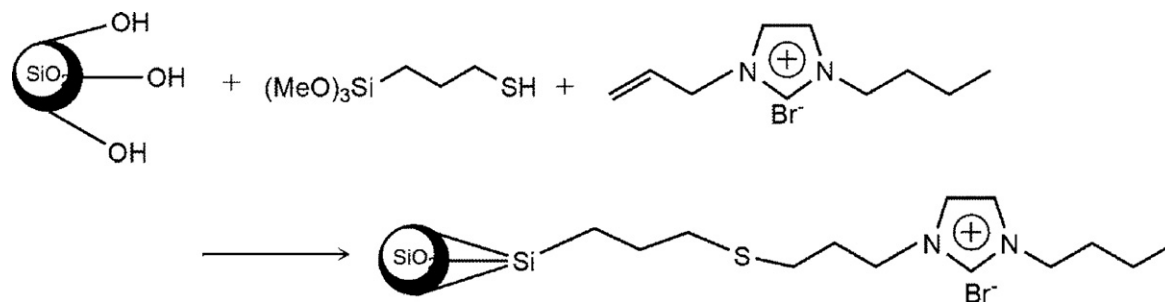
Table 4
Preparation of IL-based silica.

IL-based silica	Reaction	Ref.
N-Hexylimidazolium tetrafluoroborate based silica		[163]
Imidazolium chloride based silica		[164]
2-ethyl-4-methylimidazole chloride based silica		[165,166]
N-Methylimidazole chloride based silica		[18,167-169]

Table 4 (Continued)

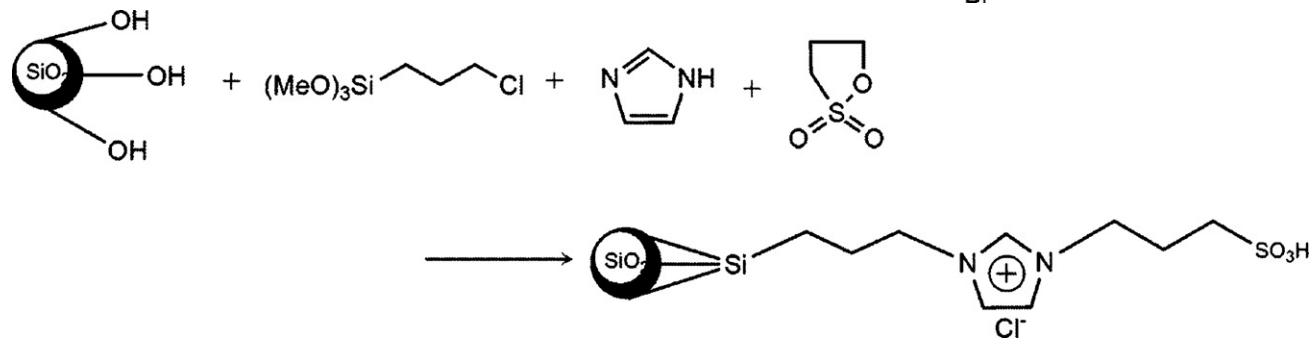
IL-based silica	Reaction	Ref.
N-(Propyl-3-amino)imidazolium bromide based silica		[170,171]
Pridinium chloride based silica		[172]
N-Butylimidazolium bromide based silica N-(butyl-4-sulfonate)imidazolium trifluoromethanesulfonate based silica		[173]

N-Butylimidazolium bromide
based silica



[174]

N-(Propyl-3-sulfonate)
imidazolium chloride based silica



[175]

functional monomer, and methacryloxypropyltrimethoxysilane (MPTMS), which acts as a crosslinker. As a carboxylic acid, MAA catalyzes the non-hydrolytic sol–gel condensation of MPTMS, and the Si–O–Si framework is formed according to Green et al.'s work [177]. The polymerization of carbon–carbon double bonds of MAA and MPTMS was initiated thermally in a water bath at 54 °C by the radical initiator, AIBN. [BMIM][PF₆], a hydrophobic room temperature IL, which was synthesized using a literature procedure [178], was used to reduce the level of gel shrinkage and act as a pore template. As a result, a silica-based network was formed, and the specific recognition site for (S)-naproxen was generated after removing the template molecule.

3.1.2. Application of IL-based silica on bioactive compounds

Ionic liquid-modified silica, which consists of bulky organic cations that are combined with inorganic or organic anions, has recently been developed as a new sorbent material. This media has attracted considerable attention because of its excellent properties and potential applications in many fields of analytical chemistry. IL-based silica has been applied successfully to the separation of bioactive analytes from plants, and is listed in Table 5. HPLC and SPE are the main technologies that employ IL-based materials.

3.1.2.1. HPLC. Bioethanol can be produced by the fermentation of monosaccharides from enzymatically hydrolyzed feedstocks, such as sugar cane, bagasse, corn, algae and other carbon-based sources [188]. The residues of crops and forest are preferred because of their low cost [189]. The enzymatic hydrolysis products of xylan and cellulose, which are the main polysaccharides found in these plant cell walls, are xylose and glucose. Xylose cannot be converted efficiently to ethanol in industrial quantities in the presence of glucose [190]. On the other hand, xylose is a raw material for the production of xylitol [191], which is a high value sweetener in the biomedical sector [192]. Therefore, xylose and glucose, as the main hydrolyzed products of the plant cell wall, were separated using IL-based silica as the stationary phase in HPLC [179]. Five different stationary phases were synthesized and characterized. Instead of using a traditional –NH₂ column, the imidazolium stationary phases exhibit excellent retention to xylose and glucose. The retention factor and resolution of the monosaccharides decreased with decreasing acetonitrile concentration. In addition, the effects of the IL cations and anions on the retention of xylose and glucose, as well as the adsorption behavior of these two monosaccharides on the stationary phases were investigated. The mobile phase and temperature were optimized, and applied successfully to separate xylose and glucose from the solution of enzymatically hydrolyzed *Water Hyacinth*. Another study by the same first author reported the separation of three alkaloids, caffeine, theophylline and theobromine, on the [EMIM] based silica stationary phase of HPLC from green tea extractions [193].

Wang et al. first synthesized 2-ethyl-4-methylimidazole chloride based silica as a HPLC stationary phase and used Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), and ¹³C nuclear magnetic resonance (NMR) spectroscopy to determine the performance of the new stationary phase [166]. They demonstrated that the IL synthesized was bond-linked to the silica surface and that the IL-based silica was more effective in separating tanshinone I and tanshinone IIA in Danshen herb than unmodified silica.

3.1.2.2. SPE. SMB is a well-known traditional medicinal herb used in the treatment of a range of diseases, particularly cardiovascular diseases including coronary heart disease, hypertension and chronic heart failure [194,195]. Tanshinones, including tanshinone I, tanshinone IIA and cryptotanshinone, are the major active constituents in SMB and all have anticancer effects. Among these tanshinones, tanshinone IIA is the most effective form and has

been used as a quality controller in some medicines [196]. The traditional extraction of tanshinones from SMB by organic solvents had been reported by several authors with subsequent analysis by HPLC [197–199] and spectrophotometry [200]. A new method was developed to simplify the operating procedure and increase the amounts extracted [186]. They synthesized a new IL-based commercial silica sorbent and used it successfully in a SPE process to isolate cryptotanshinone, tanshinone I and tanshinone IIA from SMB. Different washing and elution solvents, such as water, methanol and methanol–acetic acid (90/10, v/v), were evaluated. A comparison of IL-based silica cartridges and a traditional silica cartridge revealed higher recovery using IL-based silica sorbents. Quantitative analysis was conducted by HPLC using a C₁₈ column (5 μm, 150 mm × 4.6 mm) with methanol–water (78:22, v/v, and containing 0.5% acetic acid) as the mobile phase. Good linearity was obtained from 0.5 × 10^{−4} to 0.5 mg/mL (*r*² > 0.999) with relative standard deviations of <4.8%.

Another study highlighted the application of a two-step extraction method for the extraction and separation of oxymatrine from a *S. flavescens Ait* extract using IL-based silica as the sorbent [182]. The optimized IL-based silica was first mixed with the plant extract to adsorb oxymatrine. Some interference, such as matrine, was removed. The obtained suspension was then added to a cartridge for solid-phase extraction. Through these two steps, the target compound was separated sufficiently from interference with 93.4% recovery. Compared to traditional solid-phase extraction, this method accelerates the loading and reduces the use of organic solvents during washing. Moreover, optimization of the loading volume was simplified by optimizing the solid/liquid ratio. 2-ethyl-4-methylimidazolium chloride based silica was synthesized and used as a special sorbent in a SPE process to isolate liquiritin and glycyrrhizic acid from licorice [165].

Different IL-based silica materials were also prepared for SPE of three phenolic acids (protocatechuic, ferulic, and caffeic) from *Saliconia herbaces L* [201]. The recovery yields of purified protocatechuic acid, ferulic acid, and caffeic acid were 94.69%, 79.09% and 87.32%, respectively.

3.1.2.3. GC and CE. IL-based silica co-working with GC and CE were applied in many fields to analyze the targets. Poole and Poole summarized the development of IL-based silica as stationary phases for GC to characterize the selectivity differences compared to the usual stationary phases [202]. IL-based silica capillary in CE was discussed in Chen and Qi's review [21]. Many research papers have been published on these applications. Throughout these applications, the applications related to bioactive compounds in plants are introduced as follow.

Essential oils consist of a large number of compounds with widely ranging physicochemical properties [203]. The increasing demand for essential oils in the pharmaceutical, cosmetic and food industries is being driven by consumer preferences that favor natural over synthetic substances. Consequently, requirements for novel and efficient GC stationary phases for quality control of these substances are on the rise. In Qi's work, a dicationic IL, 1,9-di(3-vinylimidazolium)nonane bis[(trifluoromethyl)sulfonyl]imidate [abbreviated as C₉(vim)₂-NTf₂], was used as a mixed stationary phase with a traditional polysiloxane stationary phase [183]. Three different samples of essential oils from herbal plants (fennel seeds, cinnamon bark and nutmeg seeds) were employed for this purpose and effectively analyzed by GC–mass spectrometry (MS) on IL-based silica stationary phases.

Table 5
Application of IL-based material.

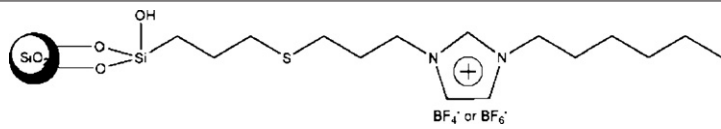
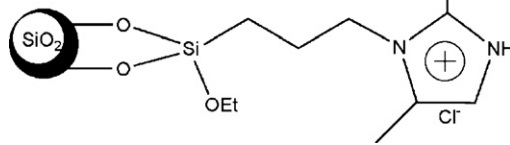
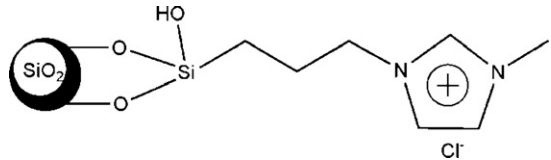
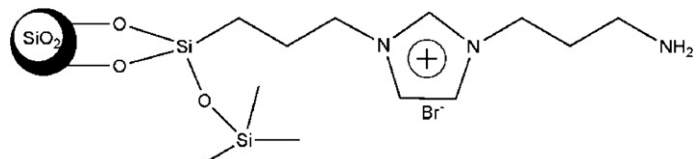
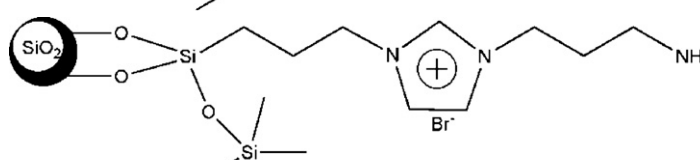
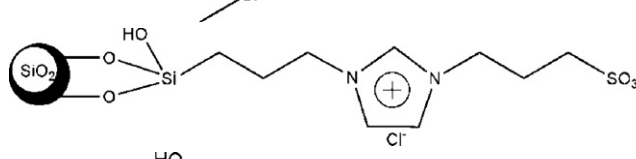
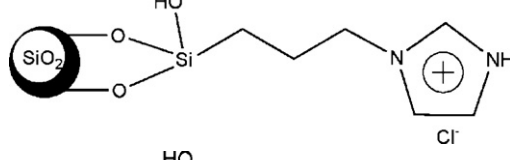
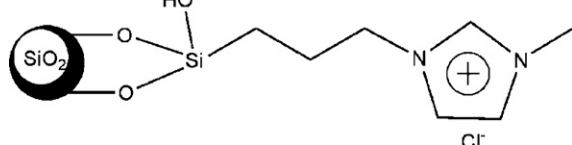
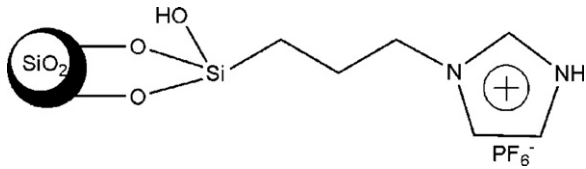
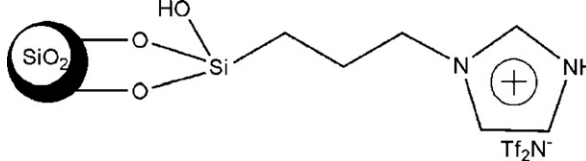
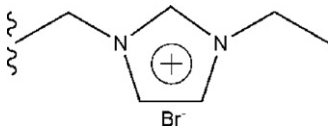
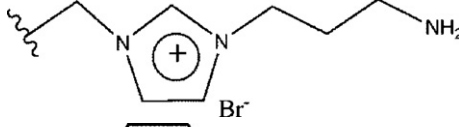
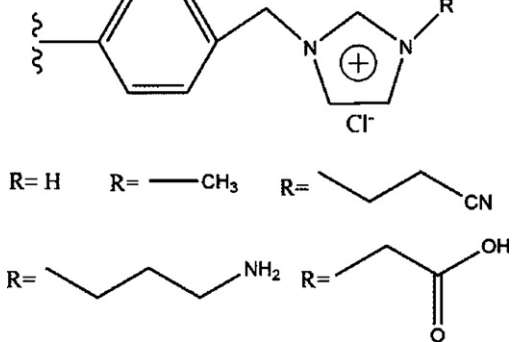
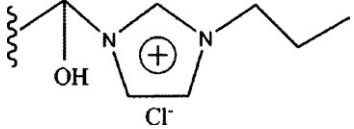
ILs based material	Structure	Method	Bioactive compound	Natural plant	Ref.
N-Hexylimidazolium tetrafluoroborate/hexafluorophosphate based silica		HPLC	Alkaloids	Mixtures of standards	[163]
2-ethyl-4-methylimidazolium chloride based silica		HPLC	Tanshinones	SMB	[166]
N-Methylimidazolium chloride based silica		HPLC	Nucleotides	Mixtures of standards	[168]
N-(Propyl-3-amino)imidazolium bromide based silica		HPLC	Caffeine, theophylline, theobromine, tanshones	Mixtures	[170]
N-(Propyl-3-amino)imidazolium bromide based silica		HPLC	Organic acids	<i>H. artemisiae scopariae</i>	[171]
N-(Propyl-3-sulfonate)imidazolium chloride based silica		HPLC	Vitamins	Mixtures of standards	[175]
Imidazolium chloride based silica		HPLC	Caffeine, theophylline	Green tea	[178]
1-methylimidazolium chloride based silica					

Table 5 (Continued)

ILs based material	Structure	Method	Bioactive compound	Natural plant	Ref.
2-ethyl-4-methylimidazolium chloride based silica					
Imidazolium tetrafluoroborate based silica		HPLC	Xylose, glucose	Water Hyacinth	[179]
Imidazolium bis(trifluoromethanesulfonyl) amide based silica					
Imidazolium chloride based silica		SPE	Lactic acid	Fermentation Broth	[20]
N-methylimidazolium chloride based silica					
2-ethyl-4-methylimidazolium chloride based silica					
Imidazolium chloride based silica		SPE	Tanshinones	<i>S. miltiorrhiza</i> Bunge	[164]

2-ethyl-4-methylimidazolium chloride based silica		SPE	Liquiritin, glycyrrhizic acid	Licorice	[165]
1-methylimidazolium chloride based silica		SPE	Tanshinones	SMB	[167]
Imidazolium chloride based silica	<p data-bbox="553 782 1234 949"> $R_2 = H, R_3 = H, \text{SilprIm}; (b) R_1 = \text{CH}_3, R_2 = H, R_3 = H$ </p>	SPE	Three phenolic acids	<i>Saliconia herbacea</i> L.	[180]
Imidazolium chloride based silica	<p data-bbox="553 1204 1202 1228"> $R_1 = H, R_2 = H, R_3 = H (b) R_1 = \text{CH}_3, R_2 = H, R_3 = H (c) R_1 = H, R_2 = \text{CH}_2\text{CH}_3, R_3 = \text{CH}_3$ </p>	SPE	Astaxanthin	<i>Saccharina japonica</i>	[181]
Imidazolium tetrafluoroborate based silica		SPE	Oxymatrine	<i>S. flavescens</i> Ait	[182]

Table 5 (Continued)

ILs based material	Structure	Method	Bioactive compound	Natural plant	Ref.
Imidazolium hexafluorophosphate based silica					
Imidazolium bis(trifluoromethanesulfonyl)amide based silica					
1,9-di(3-vinylimidazolium) nonane bis[(trifluoromethyl)sulfonyl] imidate based silica	–	GC	Essential oils	Herbal plants	[183]
Poly (1-allyl-3-ethylimidazolium bromide) ethylene		SPE	Phenolic acids	<i>S. herbacea</i>	[184]
Poly (3-aminopropyl imidazole bromide hydrobromide) 4-(chloromethyl) styrene		SPE	Matrine, oxymatrine, sophocarpin, sophoridine	SFA	[185]
Poly (imidazole chloride) 4-(chloromethyl) styrene		SPE	Tanshinones	SMB	[186]
Poly (1-propylimidazolium chloride) ethylene glycol dimethacrylate		HPLC	Caffeine, theophylline	Drug	[187]

3.2. IL-based polymer

The facile tunability of the physicochemical properties of ILs has led to intense interest in ILs as an alternative to conventional organic solvents in the synthesis of polymers. On the other hand, there have been a small number of studies on IL-based polymers co-working with analytical technologies. The present review summarizes the preparation and application of IL modified polymers from related references, and provides data for the development of novel IL-based polymers that can be co-worked with analytical technologies that are used widely in biological sample analysis.

3.2.1. Preparation of IL-based polymer

The special properties of ILs have prompted increasing interest in using ILs as solvents for chemical reactions over the last decade [204]. In recent years, ILs have also attracted the attention of polymer chemists. Although research on the use of ILs in polymer systems is still in the early stages, several interesting possibilities have already emerged. ILs have been used as solvents in a range of polymerization processes, and indeed affect the course of a chemical reaction in several polymerization systems [205,206]. ILs, as components of polymeric systems, have also attracted attention for the synthesis of microporous polymers [207–209]. Table 6 lists some of our previous studies on IL-based polymers.

An IL-based polymer can be synthesized using a monomer, crosslinker, porogen and initiator, and be prepared by thermal-initiated polymerization. The synthesis of IL-based polymers has been reported [185,213,214]. For example, Bi et al. reported the preparation of several poly (imidazole choride) 4-(chloromethyl) styrenes in detail [185]. Briefly, after dissolving polyvinylpyrrolidone (PVP) in ethanol in a flask, 4-(chloromethyl)styrene (monomer), divinylbenzene (crosslinker), heptanes (porogen), and AIBN (initiator) were added under a nitrogen atmosphere with rapid stirring. The emulsion solution was then heated for approximately 1 day to allow polymerization. After complete polymerization, the blank polymer obtained was filtered and washed several times with ethanol to remove any coagulated and soluble impurities. The blank polymer and different IL modifiers were added to ethanol. After heating under reflux for 12 h, the modified polymers obtained were cooled to room temperature. After washing sequentially with toluene, ethanol and methanol, the polymers were oven-dried for further use.

Bi et al. also synthesized IL-based MIP by thermal-initiated polymerization [213]. In the synthesis process, there were two sections. First, the IL monomers were prepared as described previously [215]. 1-allylimidazole, alkyl halide and 2,6-di-tert-butyl-4-methylphenol (DBMP, inhibitor) were added to a flask. The reaction mixture was stirred at a setting temperature under nitrogen. The resulting viscous liquid was washed with excess ethyl ether, and dried under vacuum at room temperature to give the purified product as a transparent viscous liquid. The chemical structures of these IL monomers were prepared. Second, phenolic acid as a template, IL monomers, ethyleneglycol dimethacrylate (EDGMA, crosslinker) and AIBN were dissolved in the appropriate porogen (alcohol and water mixture) to prepare the desired MIP. The solution was sonicated, sparged with helium to remove oxygen, and polymerized in a water bath. After polymerization, the polymers were ground and suspended repeatedly in acetone to remove small particles. The polymer particles were dried under vacuum, placed into a glass column, and washed with ethanol and ethanol-HCl to remove the templates. After balancing with methanol and drying in a drying oven, the particles were stored at ambient temperature. Non-imprinted blank polymers (NIP, without a template) were similarly prepared.

Tian et al. synthesized IL-based polymers in a monolithic column [186]. The authors first synthesized the IL using the above

methods for IL preparation, and then prepared an IL-based monolithic column by in situ polymerization. A mixture of MAA, glycidyl methacrylate (GMA), EGDMA, dodecanol, cyclohexanol, ILs and AIBN were purged with helium gas. The stainless steel column sealed at the bottom was then filled with a polymerization mixture and sealed at the top. After polymerization, the column was flushed with methanol to remove the porogen and other soluble compounds present in the polymer rod. Finally, the column was washed with an acetate buffer followed by deionized water until the eluent was neutral.

3.2.2. Application of IL-based polymer

Functional porous polymers have been used as the stationary phase in HPLC separation to improve the selectivity, but their main limitation is the low column efficiency. These polymers can be modified using ILs as better alternatives to conventional stationary phases to increase the column efficiency [14]. Some of the disadvantages of IL-based silica, such as low coverage of the functional groups and the limited pH stability, might limit its applications in SPE [26]. These shortcomings can be overcome using IL-based polymers with high selectivity and a wide operating pH range. IL-based polymers are developed sorbents applied in separation technologies. Nevertheless, the properties of IL-based polymers used as HPLC and SPE materials also need to be improved. Table 5 lists the IL-based polymers applied in the separation of bioactive analytes from plants. The following subsections give an explanation of some representative applications.

3.2.2.1. HPLC. Caffeine is a methylxanthine that occurs naturally in some beverages and is also used as a pharmacological agent because it stimulates the central nervous system, relaxes the smooth muscle, and causes agitation. Theophylline is a member of the xanthine family with structural and pharmacological similarities to caffeine [216]. They are distributed widely in some plants, such as tea, coffee, cocoa, etc., and are clinical drugs used under a range of brand names for the treatment of respiratory diseases, such as asthma [217–219]. Caffeine and theophylline were separated and determined on a new monolithic column, namely, the 1-propylimidazolium chloride based polymer monolithic column synthesized by Tian et al. [186]. The characteristics of the monolithic column were examined by field emission scanning electron microscopy (FE-SEM). FE-SEM revealed this monolithic column to have a porous structure. When the mobile phase contained $0.06 \text{ mol L}^{-1} \text{ Na}_2\text{HPO}_4$ (pH 9.0) and the flow rate was 0.7 mL min^{-1} , a good linear relationship was observed at caffeine and theophylline concentrations ranging from $0.1 \text{ } \mu\text{g mL}^{-1}$ to $60.0 \text{ } \mu\text{g mL}^{-1}$. The two compounds showed better resolution on the ILs-based monolithic column than the non-ILs monolithic column, and the recoveries ranged from 97.40% to 108.00%. The inter-day and intra-day relative standard deviations were <5%.

3.2.2.2. SPE. Phenolic acids are secondary metabolites found in many plant species, and have excellent antioxidant activities against reactive oxygen, higher than those of vitamins C and E [219]. Therefore, they have been used to prevent or treat diseases associated with oxidative damage, such as coronary heart disease, stroke and cancer [220–223]. Currently, they are isolated using chromatographic methods including HPLC, ultra-performance liquid chromatography (UPLC) and SPE [224,225]. Typically, the stationary phases or sorbents used in chromatography include C_{18} , anion-exchange resin and MIP. These materials interact with the target compounds via hydrophobic, anion-exchange and hydrogen bonding interactions, respectively. On the other hand, singly interacting materials cannot effectively separate phenolic acids without interference. IL-based materials with multiple interactions have been proposed as alternative stationary phases or sorbents for

Table 6
Preparation of IL-based polymer.

ILs based polymers	Structure	Monomer	Crosslinker	Porogen	Initiator	Ref.
Poly (3-aminopropyl imidazole bromide hydrobromide) 4-(chloromethyl) styrene		4-(chloromethyl) styrene	Divinylbenzene	Ethanol	AIBN	[184]
Poly (imidazole chloride) 4-(chloromethyl) styrene		4-(chloromethyl)styrene	Divinylbenzene	Heptanes	AIBN	[185,210]
Poly (1-propylimidazolium chloride) ethylene glycol dimethacrylate		Methacrylic acid	Glycidyl methacrylate	Chlorobutane	AIBN	[186,211]
Poly (1-methyl imidazole) epoxide		Glycidyl methacrylate	Ethylene glycol dimethacrylate	Dodecanol, cyclohexanol	AIBN	[212]
Poly (butyl pyridine chloride) divinylbenzene		4-vinylpyridine	divinylbenzene	–	AIBN	[213]

separation [183]. In Bi's work, IL-based polymers were synthesized and co-worked with a molecular imprinting technique to reduce the non-directional ion–ion interactions during anion-exchange as well as other interactions with substances that can decrease the selectivity. The developed IL-based molecularly imprinted anion-exchange polymer (IMAP) achieved high recovery rates by the SPE of phenolic acids from *Salicornia herbacea* L. extract: 90.1% for protocatechuic acid, 95.5% for ferulic acid and 96.6% for caffeic acid. Moreover, the phenolic acids could be separated from each other by repeated solid-phase extraction.

Molecular imprinting in IL-based porous polymer was also applied to the separation of three tanshinones from SMB [185]. The author synthesized five IL-based porous polymers with different imidazolium-based functional groups, and a molecular imprinting technique was introduced to form ordered functional groups in the porous structure of polymers. The obtained polymer was then applied as a sorbent in the SPE process to separate the target compounds from the methanol extract. The loading volume of the extract solution on the sorbent was determined using an adsorption isotherm equation and practical test. Under optimized washing and elution conditions, 0.35 mg g⁻¹ of cryptotanshinone, 0.33 mg g⁻¹ of tanshinone I, and 0.27 mg g⁻¹ of tanshinone IIA were obtained from the plants by quantitative HPLC analysis.

4. Conclusions

ILs have attracted considerable attention as solvents in the extraction and separation of bioactive compounds from plants owing to their excellent properties. Furthermore, IL-based silica and polymers that can enhance the extraction/separation of targets compounds have been synthesized by a large number of research groups. Although only a few studies have examined the extraction and separation of bioactive compounds, IL and IL-based materials have been applied successfully in analytical chemistry.

ILs have been used successfully for the separation and extraction of a wide range of analytes (organic ions, inorganic ions, metal ions and organic compounds) with different properties, thereby solving some previously difficult problems. On the other hand, more targeted IL-based materials are needed for the extraction and separation of bioactive targets compounds from plants.

IL-based materials with SPE have attracted less attention than HPLC. Hence, there is still a wide range of areas to be investigated. This review highlights the need to evaluate wider combinations of IL or IL-based materials as well as analytical technologies for applications to the extraction and separation of bioactive compounds from plants.

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