Application of Ionic Liquids in Separation and Fractionation Processes

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Article Outline

Glossary

Aqueous biphasic systems Liquid-liquid systems formed by two water-soluble components dissolved in water, which above given concentrations form two phases.

Biorefinery Facility with integrated processes to convert biomass into energy, fuels, materials, and commodity and value-added chemicals.

Green chemistry Concept addressing the design of chemical products and processes aiming at reduce or eliminate the use and generation of hazardous substances.

Ionic liquids Low melting temperature salts, composed of organic cations and organic/inorganic anions.

Natural products Compounds that can be obtained from biomass.

Solid-liquid extraction Process in which a solvent is added to solid biomass samples in order to extract target compounds.

Definition of the Subject

Research on the extraction and separation of value-added products from biomass has been a hot topic in the framework of biorefinery aiming a sustainable conversion of biomass into chemicals, materials, energy, and fuels. However, a complete use of the biomass potential is still limited by the lack of cost-effective extraction and separation processes. In the last years, a large number of studies on the application of ionic liquids (ILs) as alternative solvents for the extraction and separation of several bioactive compounds, for instance, alkaloids, antioxidants, phenolic/polyphenolic compounds, saponins, anthraquinones, and isoflavones from biomass, have been reported. Based on an extended compilation and analysis of the data hitherto reported, this entry provides an overview on the use of ILs in the extraction and separation of value-added compounds from natural sources. An overview on the use of solid-liquid and liquid-liquid extraction techniques for such purposes is outlined, highlighting and discussing the most relevant works in this area. New insights and directions to follow in IL-based processes within the biorefinery framework are suggested.
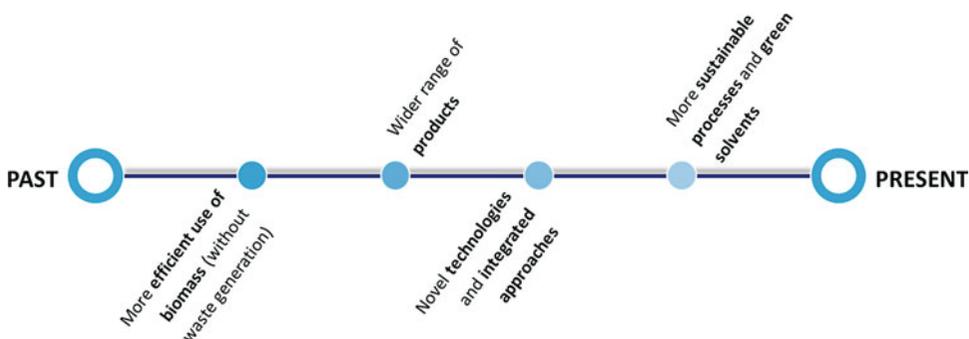
Introduction

The efficient use of low-cost high-volume agricultural and forest biomass, as well as of biomass residues or by-products, for the production of energy, materials, and commodity and value-added chemicals plays an important role in the advent of a sustainable society [1–3]. In this context, biorefinery plays a central role as an integrated plant that processes various biological nonfood feedstocks, converting them into a range of useful products, as represented in Fig. 1 [4].

Despite being a recent topic of research, biorefinery is not a novel concept. The novelty shown in the last decade is related to a more
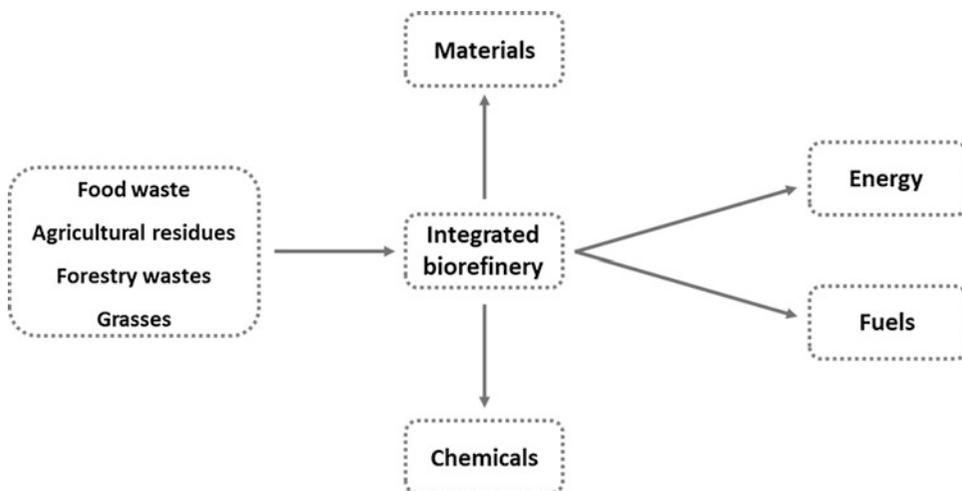
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 1 Biorefinery concept, with the generation of chemicals, energy, fuels, and materials

Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 2 Advances introduced in the biorefinery concept up to date

efficient use of biomass (without waste generation) and to the production of a wider range of products using novel technologies and processes (physical, chemical, biochemical and thermal) in integrated approaches [5]. The major challenge that biorefinery faces nowadays is the use of sustainable processes and "green" solvents to ensure maximum extraction efficiencies and yields, along with minimal waste generation. In Fig. 2 it is provided a scheme on the biorefinery concept novelty/evolution through time.

There are several large-scale (industrial) biorefinery schemes, which primarily differ in their biomass feedstock source. In particular, the lignocellulosic feedstock biorefinery has shown to be very promising, as it has the potential to

accommodate a wide range of low-cost feedstocks (e.g., straw, reeds, grass, wood, paper-waste) that could yield competitive products over the petrochemical-dominated market [2], in addition to be able to generate chemicals with application in food, nutraceutical, cosmetic, and pharmaceutical industries.

The fractionation of biomass into its core constituents is an important step aiming the development of biorefining technologies. However, the development and application of biomass fractionation processes comprising cost-effective and sustainable technologies and solvents is still on-going, with bottlenecks that require fundamental and applied research to be overcome. For instance, most of the hemicellulose found in

traditional kraft pulping is dissolved in the black liquor in the form of saccharified mono-sugars, along with lignin and inorganic pulping chemicals [2]. This black liquor is traditionally burned for power generation and hemicellulose is not properly valued [6]. If hemicellulose and lignin could be efficiently separated prior to combustion (fractionation of biomass), a more significant use for hemicellulose could be derived, such as for the production of fuels (e.g., ethanol) or high value chemicals (e.g., polyesters) [6].

The choice of the feedstock and final products are quite important criteria in the design of a biorefinery scheme taking into account the large-scale production implications. Both the initial feedstock availability and its potential use in multiple production streams need to be considered [7]. Lignocellulosic matrices, such as hardwood, softwood, and agriculture residues, are the main raw materials from renewable feedstocks, representing an abundant carbon source [1]. In general, biomass is composed of cellulose, hemicelluloses, and lignin; yet, a large variety of extractable compounds are also present in its composition, such as proteins, lipids, vitamins, antioxidants, and other natural compounds [8–13]. Natural products, commonly designated as "secondary metabolites," can be highlighted as examples of value-added compounds that can be obtained from biomass [14]. In fact, biomass is a rich source of biologically active natural products that play an important role in the prevention and treatment of several disorders. The complex chemical structures of such natural compounds make difficult their acquirement by other pathways; thus, in most cases, their isolation from natural sources is the most viable option. Of the 1,135 new drugs approved from 1981 to 2010, 50% were of natural origin (comprising also their derivatives and analogues) [15, 16]. For instance, several medicinal compounds derived from biomass display analgesic (e.g., morphine), antitussive (e.g., codeine), antihypertensive (e.g., reserpine), cardiotonic (e.g., digoxin), antineoplastic (e.g., vinblastine and paclitaxel), or antimalarial (e.g., artemisinin) properties [17], as depicted in Fig. 3. Furthermore, some plant-derived compounds, such as Taxol/Paclitaxel (*Taxus brevifolia*), vinblastine and vincristine (*Catharanthus roseus*), topotecan and irinotecan (*Camptotheca acuminata*), and etoposide and teniposide (*Podophyllum peltatum*), were approved by the Food and Drug Administration (FDA) for clinical use due to their anticancer potential.

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Fig. 3 Summary of some therapeutic properties displayed by natural compounds
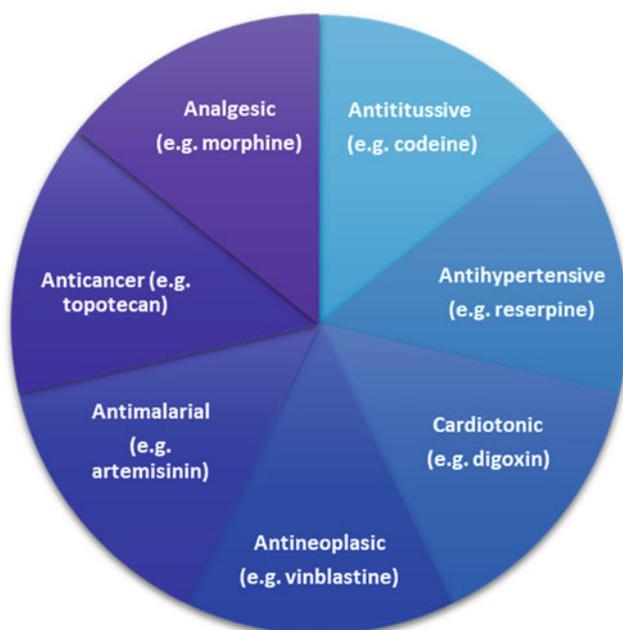
The first stage of an integrated biorefinery scheme should be the extraction of valuable secondary metabolites, which is usually performed using volatile organic compounds (VOCs) [18, 19, 5]. Nevertheless, these solvents raise some concerns, such as the danger of handling large volumes of volatile and combustible solvents, with human risks and safety issues, and serious environmental impact [18, 19]. Furthermore, strict limitations on nonhazardous solvents exist when dealing with the processing of natural products, particularly when envisaging their use in food and nutraceutical industries. Therefore, there is a crucial need of using "greener" nonhazardous solvents and on the development of cost-effective and sustainable extraction and separation processes [20, 19].

Green chemistry focuses on the design of chemical products and processes by reducing or eliminating the use and generation of hazardous substances to human health and environment [21]. The design of such environmentally benign products, solvents, and processes may be guided by the 12 Principles of Green Chemistry established by Anastas and Warner [22]: (1) prevention, (2) atom economy, (3) less hazardous chemical syntheses, (4) design for safer chemicals, (5) safer solvents and auxiliaries, (6) design for energy efficiency, (7) use of renewable feedstocks, (8) reduction of derivatives, (9) catalysis, (10) design for degradation, (11) real-time analysis for pollution prevention, and (12) inherently safer chemistry for accident prevention. These principles are a categorization of the fundamental steps that should be considered to fulfill the green chemistry goals to obtain and develop sustainable products and processes. They have been used as guidelines and design criteria, changing the way by which academia and industry design chemical processes and select solvents [23]. In this context, the minimization of the environmental and health impacts of volatile organic solvents, commonly used in biomass processing and biorefinery, became a priority in the past years. Novel solvents, such as solvents produced from renewable resources, water, supercritical CO_2, deep eutectic solvents (DES), and ionic liquids (ILs), have been proposed in the past decades as "greener" alternatives [24].

Ionic liquids (ILs) belong to the molten salts group, being defined as salts with melting temperatures below 100 °C. They are usually composed of a large organic cation and an organic or inorganic anion. The large dimensions of their ions lead to charge dispersion and do not allow their easy organization in a crystal structure; therefore, they are liquid at lower temperatures than conventional inorganic salts. Furthermore, due to their ionic character, ILs display a set of unique features, such as high ionic conductivity, negligible flammability and volatility, high thermal and chemical stability, and a strong solvation capability for a large variety of compounds [25, 26]. They are frequently designated as "green solvents" mainly due to their negligible flammability and volatility and also as "designer solvents" since there is a large number of cation/anion combinations allowing to tune their physic-chemical properties, their biodegradability, and toxicological impact, as well as their selective extractive potential for extractions/separations [27–29]. Unlike traditional molecular solvents, ILs can be regarded as nano-segregated media composed of a polar network permeated by nonpolar domains [30]. These domains allow ILs to selectively interact with different types of solutes/solvents, and improved properties and applications are thus envisioned.

The first synthesized IL (ethylammonium nitrate – melting point 12 °C) dates from 1914, when Paul Walden was testing new explosives for the replacement of nitroglycerin [31]. Later, several patents concerning the use of ILs were filled [32]. Nevertheless, it was just in the beginning of the twenty-first century with the proposal of water and air stable ILs that the research on the synthesis and application of novel ILs significantly increased. Since then, the most studied ILs are composed of imidazolium-, piperidinium-, pyridinium-, pyrrolidinium-, phosphonium-, and ammonium-based cations, combined with anions such as chloride (Cl^-), bromide (Br^-), acetate ($[CH_3CO_2]^-$), bis(trifluoromethylsulfonyl)imide ($[NTf_2]^-$), hexafluorophosphate ($[PF_6]^-$), and tetrafluoroborate ($[BF_4]^-$). Nonetheless, the research on ILs as alternative solvents is moving fast from these hydrophobic and fluorinated anions

(such as $[PF_6]^-$ and $[BF_4]^-$) with very poor water stability [33], towards less toxic and more biodegradable alternatives derived from natural sources, such as those based on carboxylic acids, amino acids, and mandelic acid derived anions [34, 35], often combined with the cholinium cation [36, 37]. The synthesis of novel biodegradable and low-toxicity ILs has considerably increased in the last few years, and several ILs are classified as biodegradable (at least 60% of the IL is biodegraded within 28 days) [38].

ILs have been investigated as alternative solvents in several applications, such as in synthesis and (bio)catalysis [39, 40], analytical applications [41], electrochemistry [42], among others. In addition to these applications, they have demonstrated a high performance in extraction and separations processes of value-added compounds from biomass [43], a trend that results from their designer solvents characteristic. Usually, ILs lead to higher extraction yields and to a higher selectivity, obtained at moderate conditions (time of extraction, temperature, and pressure), than those achieved with conventional VOCs [23].

In this chapter, potential strategies proposed in the past few years for the extraction and separation of value-added compounds from biomass/natural sources using ILs are reviewed and discussed. Both solid-liquid and liquid-liquid approaches are considered, including their integration. Simultaneously, this chapter focuses on the change and evolution of the type/family of ILs used in these processes, as well as on the challenges that remain to be accomplished and future developments. In Fig. 4, an outline of the present chapter is presented.

Solid–Liquid Extraction of Value-Added Compounds from Biomass

In biorefinery solid-liquid extraction processes are employed when envisioning the recovery of value-added compounds from solid biomass samples. These consist on the addition of a solvent to the solid biomass under a set of operating conditions to obtain an extract rich in the target value-added compound [44]. In this subchapter the most
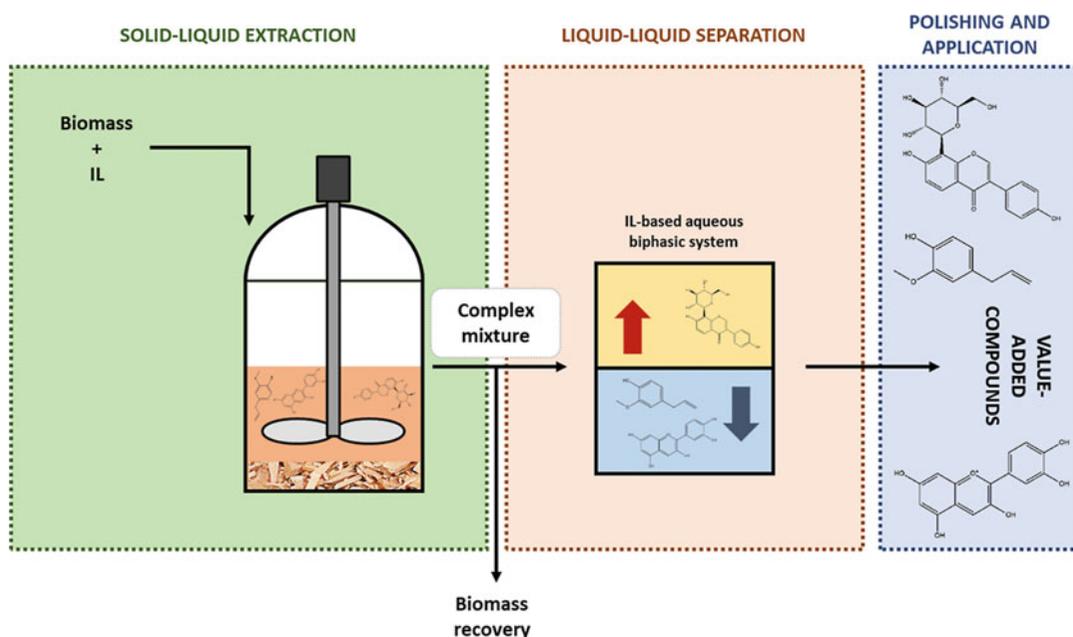
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 4 Outline of the information presented in the present chapter, focused on the use of ionic liquids in: (i) solid-liquid extraction processes and (ii) liquid-liquid separation processes of value-added compounds from biomass

relevant studies concerning the use of ILs in this type of approach are reviewed. Unless the solvent is completely selective, which is seldom the case, these extracts are complex and need to be further fractionated, separated, or purified by other procedures, such as by the use of adsorbents [45] (out of the scope of this chapter), or by liquid-liquid extraction, namely, by IL-based aqueous biphasic systems (ABS) that are discussed in the next subchapter.

The pioneering work on the use of ILs as alternative solvents for solid-liquid extractions of value-added compounds from biomass was reported in 2007, by Du et al. [46], who demonstrated the successful application of IL aqueous solutions in the microwave-assisted extraction (MAE) of trans-resveratrol from a Chinese medicine herb. After this evidence on the potential of ILs as alternative solvents for extracting value-added compounds from biomass, the number of related works increased significantly [23]. ILs, neat, or in aqueous or alcohol solutions, have been the most studied solvents. Although there is a large number of possible cation-anion combinations in ILs, imidazolium-based ILs remain the most widely investigated. Within this IL family, the most commonly applied anions are halogen- and $[BF_4]$-based ones [23]. Although it is well established that $[BF_4]$-based ILs are not water stable [33], even at room temperature, this anion remains the selected choice of many authors – a tendency that in an era of natural-derived ILs urgently needs to be reversed. Biodegradable and biocompatible ILs can nowadays be synthesized at low cost, meaning that advances in the biorefinery field need to comprise these more attractive alternatives. Among the high-value compounds extracted from biomass using ILs and their solutions, alkaloids, flavonoids, and terpenoids have the largest representation (circa 50%) of all fine chemicals [23].

In the past years it has been demonstrated the potential of IL-assisted extractions of bioactive compounds from several natural sources, overcoming some major drawbacks associated to conventional techniques that are energy-intensive and time-consuming and often use high amounts of VOCs, such as heat-reflux extraction (EHRE),

Soxhlet extraction (ESE), and maceration extraction (EME). Furthermore, it has been reported that the selectivity, yield of extraction, and purity levels can be improved by applying ILs. These levels are further improved if combined with microwave- [47, 48], ultrasonic- [49, 50], or enzyme-assisted [51, 52] extractions (IL-MAE, IL-UAE, or IL-EAE, respectively). Table 1 summarizes the works that are discussed in this subchapter, comprising the compounds extracted and their yield, biomass and IL-based solvent used, and operating conditions applied. Table 2 lists the ILs that have been investigated and are discussed in this subchapter, including their names, acronyms, and chemical structures.

Table 1 clearly reinforces the potential of ILs as biomass extraction solvents to obtain lignin [53–55], cellulose [56], alkaloids [47, 49, 50, 58], proanthocyanidins [48], phenolic/polyphenolic compounds [57, 52, 51], essential oils [59], hydroxymatairesinol [60], and triterpenic acids [61] from natural sources.

Figure 5 depicts the chemical structure of some of these value-added compounds, extracted by means of solid-liquid processes herein reviewed. Figure 6 summarizes the distribution of the number of works dealing with solid-liquid extractions using IL-based solvents.

ILs have been used in solid-liquid extraction processes for the recovery of lignin from biomass samples [53–55]. Zakaria et al. [55] investigated aprotic $[C_2mim]$- and $[C_4mim]$-based ILs, combined with acetate and chloride anions, for lignin extraction from coconut shell, showing that the dissolution of biomass can be achieved in 6 h at 110 °C, and in just 2 h at 150 °C, under a nitrogen atmosphere. Using this approach, 10% of regenerated lignin was obtained using $[C_2mim]$[Ac], where the main parameters affecting the extraction efficiency are temperature, time, particle size and IL type. In the same line, Ma et al. [53] extracted lignin from corn stalk using pyrrolidonium-based ILs. In this work, the authors [53] applied a pretreatment step using N-methyl-2-pyrrolidonium chloride ([Hnmp]Cl) and N-methyl-2-pyrrolidonium methanesulfonate ([Hnmp][CH_3SO_3]) during 30 min at 90 °C, leading to an yield of lignin of 85.94% and 56.02% of

Application of Ionic Liquids in Separation and Fractionation Processes, Table 1 Compounds extracted and their yield, biomass, and IL-based solvent used and operating conditions applied in the solid-liquid extraction of value-added compounds from biomass

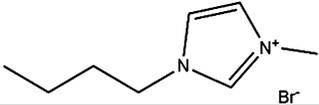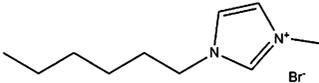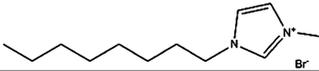| Biomass	Target compound	Solvent	IL	Operating conditions	Yield	Ref.
Corn stalk	Lignin	IL + H$_2$O	[Hnmp]Cl and [Hnmp][CH$_3$SO$_3$]	Extraction time: 30 min Temperature: 90 °C	85.94% and 56.02% of the lignin content	[53]
Miscanthus x giganteus	Lignin	IL + H$_2$O	[N$_{0222}$][HSO$_4$]	Extraction time: 8 h Temperature: 120 °C	80% of the lignin content	[54]
Cocos nucifera L. (coconut shell)	Lignin	Pure IL	[C$_2$mim][Ac]	Extraction time: 6 h/ 2 h Temperature: 110 °C / 150 °C Nitrogen atmosphere	10% of regenerated lignin	[55]
Arachis Hypogea (peanut shells) and *Castanea sativa* (chestnut shells)	Cellulosic material	Pure IL	[C$_4$mim]Cl and [C$_2$mim][Ac]	Extraction time: 12 h Temperature: 90 °C	75% and 95% of cellulosic material from peanut and chestnut shells, respectively	[56]
Wheat straw	Lignin, cellulose and hemicellulose Phenolic compounds	Pure IL + microporous resins	[C$_2$mim][Ac]	Extraction time: 6 h Temperature: 120 °C Solid/liquid ratio: 1:20	42, 114 and 86 wt% of lignin, cellulose and hemicellulose, respectively 75.5% of phenolic compounds in the final of the process	[57]
Glaucium flavum Crantz (Papaveraceae)	S-(+)-glaucine	IL + H$_2$O	[C$_4$mim][Ac]	–	–	[58]
Camptotheca acuminata samara	Alkaloids	IL-MAE	[C$_8$mim]Br	Soak time: 2 h Irradiation power: 385 W Extraction time: 8 min Solid/liquid ratio: 1:12 IL concentration: 0.80 M 2 cycles	674.5 µg·g^{-1} (CPT) 104 µg·g^{-1} (HCPT)	[47]

(continued)

Application of Ionic Liquids in Separation and Fractionation Processes, Table 1 (continued)
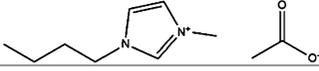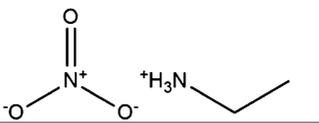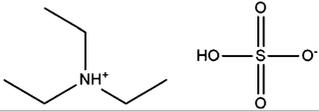
Biomass	Target compound	Solvent	Il	Operating conditions	Yield	Ref.
Camptotheca acuminata samara	Alkaloids	IL-UAE	[C$_8$mim]Br	Ultrasonic power: 239.42 W Extraction time: 34.58 min Solid/liquid ratio: 1:12 IL concentration: 0.50 M	100% (maximum extraction efficiency with 0.50 M of [C$_8$mim]Br aqueous solutions defined as 100%)	[49]
Phellodendron amurense Rupr	Alkaloids	IL-UAE	[C$_4$mim]Br	Ultrasonic power: 100 W Extraction time: 75 min Solid/liquid ratio: 1:14	106.7% (extractions by heat reflux defined as 100%)	[50]
Larix gmelini bark	Proanthocyanidins	IL-MAE	[C$_4$mim]Br	Soak time: 3 h Irradiation power: 230 W Extraction time: 10 min Solid/liquid ratio: 1:20 IL concentration: 1.25 M 2 cycles	114.86 mg·g^{-1}	[48]
Flos Lonicera Japonicae	Chlorogenic acid	IL-EAE	[C$_6$mim]Br	Temperature: 70 °C pH: 4.0 Pectinase concentration: 1 mg·mL^{-1} IL concentration: 0.75 M Extraction time: 40 min	6.06 wt%	[51]

| Source	Target compound	System	IL	Conditions	Yield	Ref
Eucommia ulmoides leaves	Chlorogenic acid	IL-EAE	[C$_6$mim]Br	Temperature: 50 °C; pH: 3.0; Cellulase concentration: 2 mg·mL⁻¹; IL concentration: 0.50 M; Extraction time: 2 h	8.32 mg·g⁻¹	[52]
Cinnamomum cassia bark	Essential oil (enriched in coumarin)	Mixture of two ILs	[N$_{0002}$][NO$_3$] [C$_4$mim]Cl	Extraction time: 15 min; Temperature: 100 °C; Solid/liquid ratio: 1:10	4.6 wt%	[59]
Picea abies knots (Norway spruce knots)	7-hydroxymatairesinol	IL + H$_2$O	[(C$_2$)$_3$NC$_2$]Br	Extraction time: 280 min; Temperature: 25 °C; Solid/liquid ratio: 1:100; IL concentration: 1.50 M	9.45 wt%	[60]
Apple peels	Triterpenic acids	IL + H$_2$O	[C$_{14}$mim]Cl	Extraction time: 60 min; Temperature: 80 °C; Solid/liquid ratio: 1:10; IL concentration: 0.50 M	2.62 wt%	[61]
Herba Artemisiae Scopariae	Rutin Quercetin Scoparone	IL + methanol	[C$_4$mim]Br	Extraction time: 60 min; Temperature: 60 °C; Solid/liquid ratio: 1:120; IL concentration: 0.50 mg·mL⁻¹	10275.92 µg rutin·g⁻¹; 899.73 µg quercetin·g⁻¹; 554.32 µg scoparone·g⁻¹	[62]

Application of Ionic Liquids in Separation and Fractionation Processes, Table 2 ILs investigated, their names, acronyms, and chemical structures

IL	Acronym	Chemical structure
Imidazolium-based ILs		
1-butyl-3-methylimidazolium bromide	[C$_4$mim]Br	
1-hexyl-3-methylimidazolium bromide	[C$_6$mim]Br	
1-octyl-3-methylimidazolium bromide	[C$_8$mim]Br	
1-butyl-3-methylimidazolium chloride	[C$_4$mim]Cl	
1-tetradecyl-3-methylimidazolium chloride	[C$_{14}$mim]Cl	
1-ethyl-3-methylimidazolium acetate	[C$_2$mim][Ac]	
1-butyl-3-methylimidazolium acetate	[C$_4$mim][Ac]	
Ammonium-based ILs		
Ethylammonium nitrate	[N$_{0002}$][NO$_3$]	
Triethylammonium hydrogen sulfate	[N$_{0222}$][HSO$_4$]	
Pyrrolidonium-based ILs		
N-methyl-2-pyrrolidonium chloride	[Hnmp]Cl	
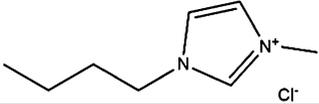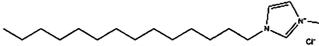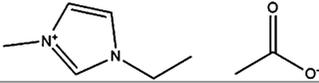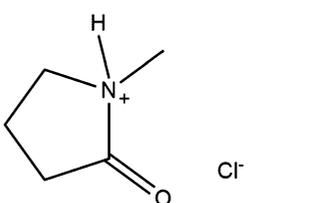
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Application of Ionic Liquids in Separation and Fractionation Processes, Table 2 (continued)

IL	Acronym	Chemical structure
N-methyl-2-pyrrolidonium methanesulfonate	[Hnmp][CH$_3$SO$_3$]	
Analogues of glycine-betaine ILs		
Triethyl[2-ethoxy-2-oxoethyl]ammonium bromide	[(C$_2$)$_3$NC$_2$]Br	
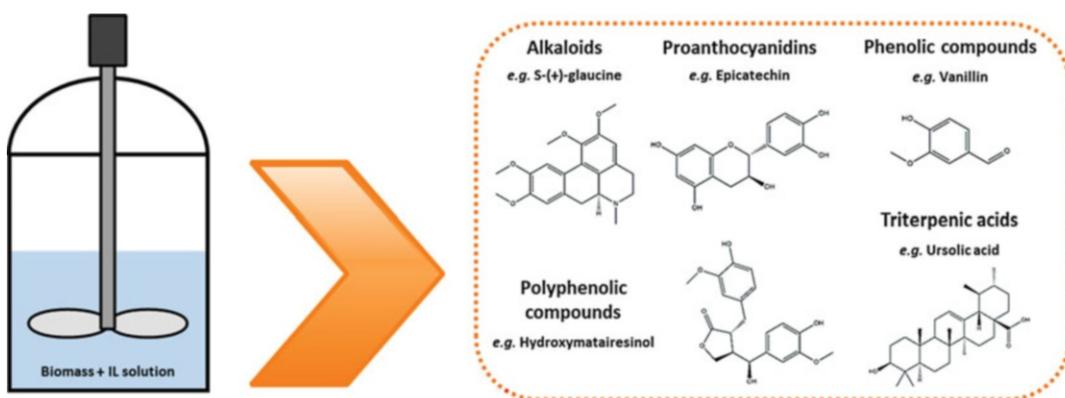
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 5 Schematic representation of the solid-liquid extraction processes reviewed, and the chemical structures of some compounds extracted

the original lignin content, respectively. Finally, through enzymatic hydrolysis, the authors [53] obtained a high yield of reducing sugars from the regenerated cellulosic feedstock (91.81%). The pyrrolidonium-based ILs combined with anions with strong hydrogen bond basicity used by the authors were considered to effectively disrupt the intricate cellulose network, thus leading to improved results in lignin extraction. In addition, the morphology of untreated corn stalk was assessed by the authors [53] by SEM, revealing a compact ordered and rigid fibril structure, which suffers pronounced changes when pretreated with adequate ILs. Lignin was regenerated from the IL

using acetone/deionized water at room temperature.

Gschwend et al. [54] went further and developed a fractionation process for deconstructing the lignocellulosic matrix into a cellulose-rich pulp, a lignin fraction, and an organic distillate from the grass *Miscanthus x giganteus*, using low cost ILs. The authors [54] synthesized a low-cost IL, triethylammonium hydrogen sulfate ([N$_{0222}$] [HSO$_4$]), which can be produced at bulk scale for $1.24/kg, a cost similar to that of common organic solvents, such as acetone or toluene. Using this IL-based approach, the authors showed the recovery of the solid pulp from biomass and the
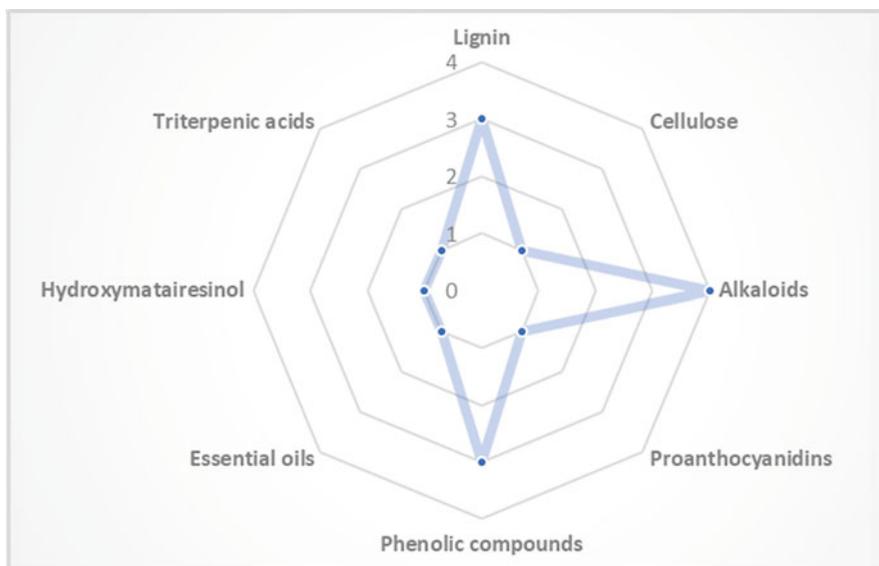
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 6 Number of articles discussed in this subchapter using ILs for the extraction of natural compounds from biomass

recovery of lignin from the IL liquor by precipitation with water. In addition, the authors [54] also investigated the IL complete recovery and reuse, showing that there are no losses on the solvent extraction efficiency. Through this approach, and by applying economic analysis, the authors [54] classified their process as efficient and economically viable. In the same line, Carneiro et al. [56] showed the potential of imidazolium-based ILs for biorefining purposes, by using [C$_4$mim]Cl and [C$_2$mim][Ac] to dissolve and fractionate peanut shells (*Arachis Hypogea*) and chestnut shells (*Castanea sativa*). Under the optimized operating conditions, up to 7 wt% of raw biomass can be dissolved, allowing an extraction of 87% of the cellulosic material. After a precipitation with an acetone/water mixture, the regeneration of the cellulosic material and the IL recovery was achieved, with a recovery of 75 and 95% for peanut and chestnut shells, respectively.

Mehta et al. [59] developed an efficient processing scheme for the extraction of essential oils enriched in coumarin from *Cinnamomum cassia* bark using a protic IL – [N$_{0002}$][NO$_3$] – through dissolution and further creation of a biphasic system with diethyl ether. The process was boosted in terms of biomass dissolution and essential oil

yield by the addition of aprotic [C$_4$mim]-based ILs, combined with chloride or acetate anions. The authors [59] demonstrated that the addition of these ILs allow to tailor the viscosity, solvation ability, and extraction efficiency of ILs, described as a synergic effect of IL ions (protic + aprotic). With the mixture of [N$_{0002}$][NO$_3$] and [C$_4$mim][Ac], an yield of 4.7 wt% of essential oil was obtained, although some degradation of the IL was observed. However, when [C$_4$mim]Cl was used, a similar yield was obtained (4.6 wt%) without any degradation. After the extraction of the essential oil, the cellulosic material and free lignin were regenerated from the biomass-IL solution by the addition of a mixture of acetone and water.

In addition to more complex structures and matrices such as lignin, cellulose, hemicellulose, and essential oils, there has also been a large interest on the study of ILs as alternative solvents to extract value-added chemicals from biomass. Bogdanov et al. [58] reported the successful substitution of methanol (a widely used solvent in industry to extract value-added molecules from natural sources) by a [C$_4$mim][Ac] aqueous solution for the extraction of the biologically active alkaloid S-(+)-glaucine from the aerial parts of *Glaucium flavum* Crantz (Papaveraceae), proving

that a higher extraction efficiency can be obtained using the IL-based solvent. The authors [58] performed consecutive extractions of biomass fresh samples using the same solvent up to saturation, avoiding the need of the IL recycling, while also contributing to a reduction of the process cost. The authors [58] finally showed the recovery of glaucine from the IL by a back-extraction step with chloroform that unfortunately decreases the "green" character of the proposed process. Ma et al. [62] optimized the extraction of bioactive compounds (rutin, quercetin, and scoparone) from *Herba artemisiae scopariae*, using IL-methanol solutions. Using 0.5 mg·mL^{-1} of [C$_4$mim]Br, at a reflux temperature of 60 °C and during 60 min of extraction, 10275.92 μg rutin/g, 899.73 μg quercetin/g, and 554.32 μg scoparone/g could be extracted from the natural source, corresponding to significant improvements when compared to the values obtained with pure methanol. The use of [C$_4$mim]Br at low concentrations has the advantage of tuning the polarity of the extraction solvent while keeping a low viscosity. Lopes et al. [57] published an interesting work concerning the development of a sustainable process for the valorization of biomass, by exploring the fractionation of wheat straw and extraction and separation of high value phenolic compounds. Although the main goal of their work was the separation and purification of phenolic compounds through adsorption with specific polymeric resins, they firstly developed a scalable [C$_2$mim][Ac]-based pretreatment and fractionation process of biomass. Taking only in consideration the step of interest to this subchapter, the authors found optimal conditions using a solid/liquid ratio of 1:20, a temperature of 120 °C, and continuous stirring during 6 h, yielding 75.5% of total phenolic compounds.

Faria et al. [61] investigated the potential of aqueous solutions of hydrotropes or surface-active ILs to improve the solubility and extraction of triterpenic acids from apple peel, a major residue of food industries. The authors [61] combined solubility and extraction studies to better understand the molecular-level mechanisms and ILs chemical structures which improve the extraction

efficiency. The best conditions of the process were found to be an extraction time of 60 min, a temperature of 80 °C, a solid/liquid ratio of 1:10, and a concentration of [C$_{14}$mim]Cl of 0.50 M. In such conditions, a total extraction yield of triterpenic acids of 2.62 wt% was attained, larger than the values obtained with volatile organic solvents, such as chloroform or acetone in similar conditions. The authors found an increase in the solubility of ursolic acid of 8 orders of magnitude in IL aqueous solutions when compared to pure water and thus proposed the use of water as an anti-solvent to recover the target biomolecules from the IL aqueous solution.

Recently, Ferreira et al. [60] reported on a more sustainable and greener extraction-recovery process, both by using biocompatible ILs and by reducing the number of steps required to recover the target compound/extract. The authors used analogues of glycine-betaine (naturally occurring and low cost amino acid, AGB) ILs for the extraction of 7-hydroxymatairesinol (HMR) from Norway spruce knots (*Picea abies*). Several operating conditions were optimized, namely, the IL concentration, extraction time, and solid–liquid ratio, for which a response surface methodology was applied. The best conditions were obtained with an aqueous solution of 1.5 M of [(C$_2$)$_3$NC$_2$]Br, with a solid-liquid ratio of 1:100, and during 280 min at 25 °C, in which extraction yields of 9.46 wt% of HMR were obtained. The authors then proposed the use of the IL-water-extract for cosmetic, food, and nutraceutical applications, without requiring an additional step for the target product recovery [60]. The IL aqueous solution enriched in hydroxymatairesinol demonstrated no cytotoxicity towards a macrophage cell line as well as a higher anti-inflammatory potential than the recovered extracts (no IL present). If suitable ILs are chosen, it is thus possible to carry out the extraction and the direct application of the natural extracts, without requiring a step for product recovery. Figure 7 summarizes the integrated process proposed by the authors [60], starting with the solid-liquid extraction step, followed by a filtration step, and finishing it with the direct use of the IL-extract in industry applications. This work represents a huge advance in biorefinery
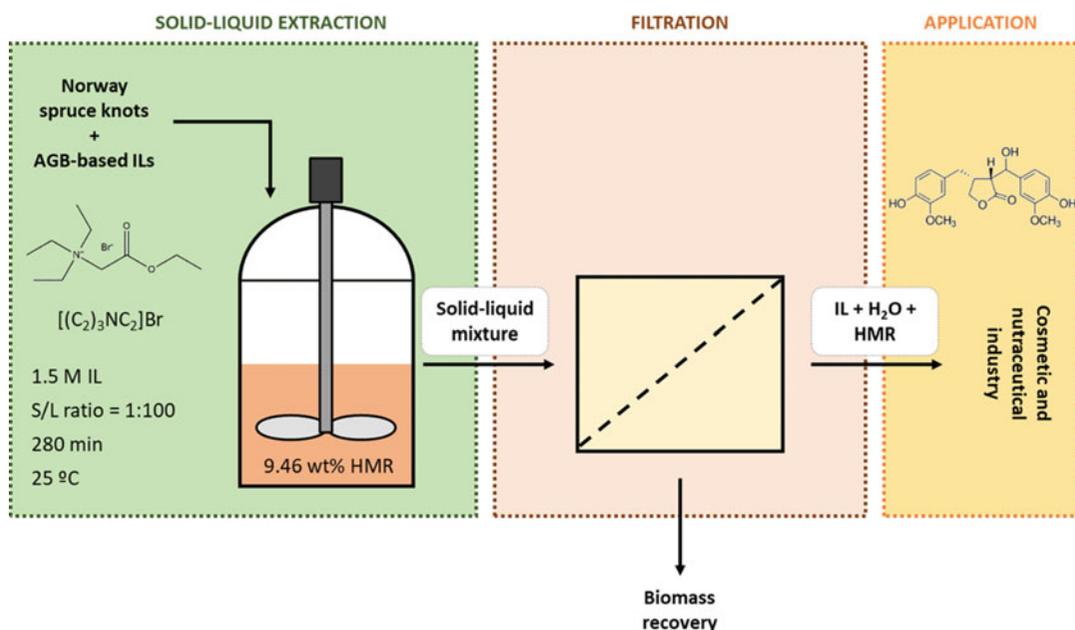
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 7 Process proposed by Ferreira et al. [60] for the extraction of 7-hydroxymatairesinol (HMR) from Norway spruce knots using aqueous solutions of glycine-betaine analogues (AGB) ILs

approaches using ILs, since it comprises biocompatible ILs, a moderate temperature, and the effectiveness and cost of the process were improved.

In addition to the works previously described carried out only with inputs of temperature and agitation, microwave- [47, 48], ultrasonic- [49, 50], and enzyme-assisted [51, 52], processes combined with ILs as solvents were also proposed. In fact, there are several roles that ILs play in these kind of processes, for instance, leading to more efficient extraction by reducing the mass transfer barrier, or by enhancing the activity of enzymes. Up to date, only imidazolium-based ILs were studied for the extraction of alkaloids, proanthocyanidins, and chlorogenic acid from biomass. All of these studies were performed using halogen-based ILs, most of the times with the bromide anion. The use of more benign and sustainable ILs was still not investigated in this type of strategies. Although some improvements could be achieved in terms of extraction yield, it should be remarked that these processes require an extra step, the use of more sophisticated equipment, and/or higher energetic inputs.

In summary, ILs and their mixtures with alcohols or water are remarkable extraction solvents for value-added compounds from biomass. Neat ILs may act as solvents and as pretreatment tools of biomass that usually presents a compact ordered and rigid structure, inducing pronounced changes in the morphological structure of biomass (usually visualized by SEM) and a better access of the solvent to the target compounds [56]. When using a coupled extraction technology, for example, an enzymatic one, morphological changes in biomass are also observed, thus leading to higher extraction efficiencies. In fact, the chemical structures of ILs have an important role to deconstruct biomass when pure ILs are employed in the process, where ILs comprising anions with high hydrogen bond basicity are the most efficient. On the other hand, aqueous or alcoholic solutions of ILs are not expected to destroy the lignocellulosic part of biomass. These solutions are thus more selective to extract target compounds, while also leading to a reduced IL consumption and to a viscosity decrease, ultimately resulting in improvements

in the mass transfer phenomenon. Large increments in the solubility of the target natural value-added compounds in this type of mixtures involving ILs have also been reported, which can occur by a micelle-mediated or by hydrotropic phenomena [61, 63]. Both phenomena are highly dependent on the IL chemical structure (surfactant-like or hydrotropic ILs, respectively) and on the target compounds chemical structure and hydrophobicity. In the cases where the micelle-mediated phenomenon prevails, it is frequently noticed a higher performance of ILs composed of longer alkyl side chains to solubilize and extract bioactive compounds, following their critical micellar concentration (CMC) trend. On the other hand, the formation of solute–hydrotrope complexes has been to occur with ILs, leading to significant enhancements in the solubility of poorly soluble compounds in aqueous media [61, 63].

Based on the exposed, the high efficiency of ILs and ILs solutions in the extraction of bioactive compounds from biomass is not only related with their ability to disrupt of the biomass structure, but also with the enhanced solubility of the target compounds in these solvents. This possibility also opens doors for the development of simpler recovery procedures, e.g., by using water as antisolvent to induce the precipitation of the extracted molecules and to recover the IL solvents. Although the recovery of value-added compounds and ILs regeneration are crucial factors in the process development, both are scarcely explored in the literature. The extracts obtained by IL-based solid-liquid extraction approaches are usually complex mixtures enriched in the desired products, thus requiring further fractionation and purification steps. These can be achieved, for instance, by means of liquid-liquid approaches (that are discussed in the next subchapter). The process proposed by Ferreira et al. [60] should be however highlighted in this field, since the authors designed ILs with high performance to extract 7-hydroxymatairesinol (HMR) from Norway spruce knots and capable of being directly used in the final extract for the envisioned applications.

Separation of Value-Added Compounds Using Liquid–Liquid Extraction (LLE) Techniques

Most of the works discussed in the previous subchapter refer to the use of aqueous solutions of ILs to extract value-added compounds from biomass. However, no significant attempts have been carried out to separate and purify the valuable compounds extracted, and as such no information on the purity degree of the target compounds is usually given. The use of aqueous solutions presents several advantages, such as the increase in the solubility of the target compounds by a hydrotropic or micelle-mediated solubilization phenomena and the decrease of the high viscosity of most ILs, enhancing the mass transfer and reducing energetic inputs. Furthermore, the most green and low cost solvent (water) is added to the process, also contributing to increase the selectivity for target chemicals.

All the extracts obtained by solid-liquid extractions using IL aqueous solutions are enriched in the desired chemicals, but they are usually complex mixtures unless completely selective solvents have been used, which is seldom the case. Therefore, depending on the target application, further fractionation and purification steps are required. Since almost all ILs used in solid-liquid extractions from biomass are completely miscible with water, they can be used to form aqueous biphasic systems (ABS). ABS are two-phase systems that can be used for liquid-liquid extraction. They are formed by water and two water-soluble components, such as two polymers, a polymer and a salt, or two salts. Recently, IL-based ABS have been proposed [64] by the combination of ILs with a wide variety of compounds, such as salts, polymers, carbohydrates, and amino acids in aqueous solution [26, 65, 66]. Above given concentrations, these systems form two aqueous phases, allowing therefore the partition of different compounds between them [67]. Due to the wide range of IL cation-anion combinations, plus the mixture with different salts, polymers, carbohydrates, and amino acids, IL-based ABS allow the tailoring of the phases' polarities and affinities, and thus selective and effective
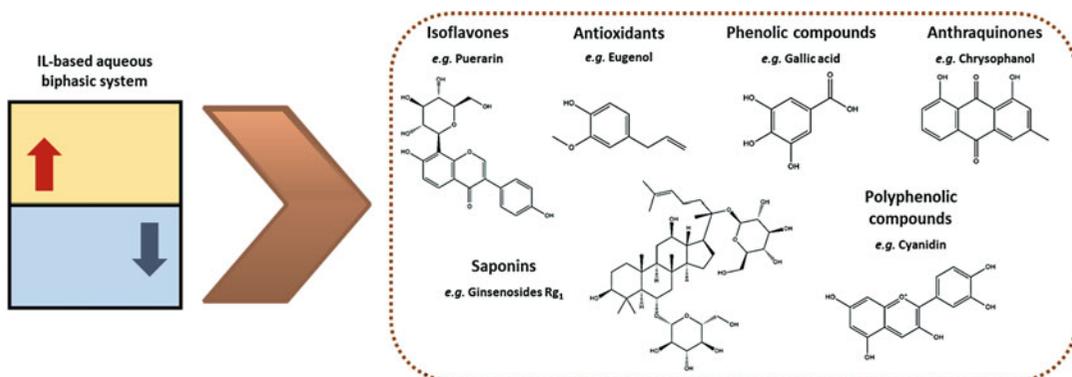
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 8 Schematic representation of the liquid-liquid extraction approach herein reviewed based on ABS, and chemical structures of the compounds fractionated/separated
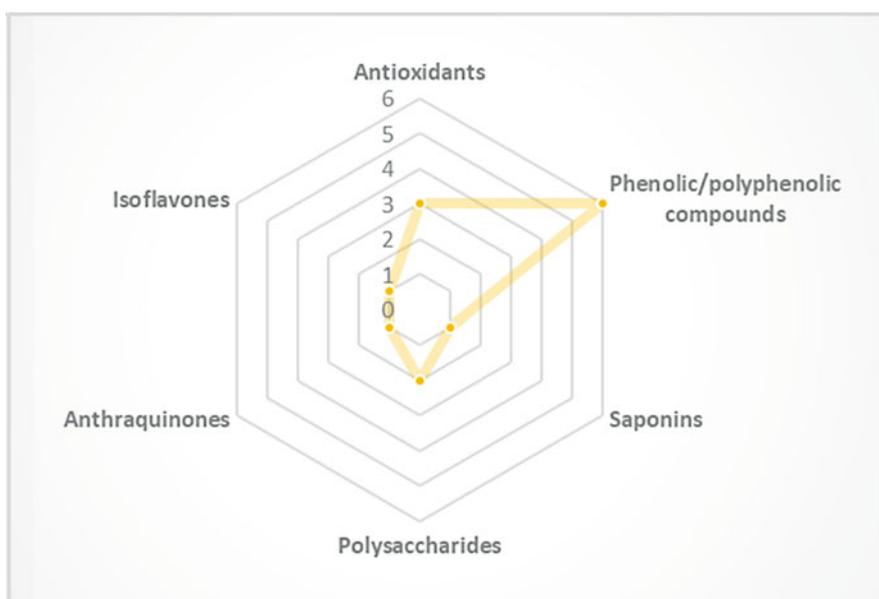
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 9 Number articles discussed in this chapter regarding the use of IL-based ABS for the separation of natural compounds

extractions of target natural compounds [68]. In this subchapter, the most recent and relevant studies concerning the application of IL-based ABS for the separation and fractionation of value-added compounds are reviewed and discussed.

IL-based ABS have been investigated for the separation of antioxidants [69–71], phenolic/polyphenolic compounds [72–77], saponins [78, 79], polysaccharides [80], anthraquinones [81], and isoflavones [82], which are reviewed herein.

Figure 8 provides a schematic representation of the ABS-based strategies herein reviewed, as well as the chemical structures of some value-added compounds investigated. Figure 9 depicts the distribution of the articles using this approach for the separation and purification of natural compounds.

Table 3 summarizes the works that are discussed below, including the value-added compounds studied, ILs and additional phase-forming components investigated, as well as the extraction
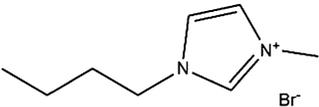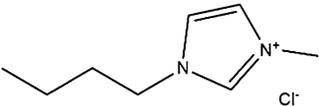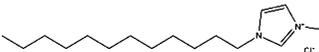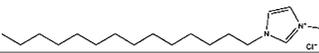
Application of Ionic Liquids in Separation and Fractionation Processes, Table 3 Compounds fractionated/separated and their extraction efficiency/recovery yield, IL-based solvent used, and ABS phase-forming components applied in the separation of value-added compounds from biomass

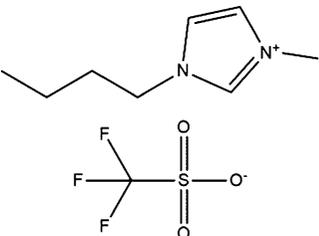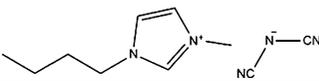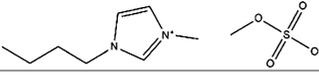| Value-added compound	IL	Abs phase-forming components	Extraction efficiency/ recovery yield	Ref.
Eugenol Propyl gallate	$[C_nmim]Cl$, $[C_4mpip]Cl$, $[C_4mpyr]Cl$ and $[N_{4444}]Cl$	IL + $C_6H_5K_3O_7$/ $C_6H_8O_7$, pH 7 PEG + K_2HPO_4/ KH_2PO_4 + IL as adjuvant, pH 7	47.1% – 100%	[69]
Gallic acid Vanillic acid Eugenol Nicotine Caffeine	$[C_4mim]Cl$, $[C_4mpyr]Cl$, $[C_4mpip]Cl$, $[N_{4444}]Cl$ and $[P_{4444}]Cl$	PEG + $C_6H_5K_3O_7$/ $C_6H_8O_7$ + IL as adjuvant	–	[76]
Gallic acid	$[C_nmim][CF_3SO_3]$, $[C_4mim]Br$, $[C_4mim][CH_3SO_4]$, $[C_4mim][C_2H_5SO_4]$, $[C_4mim][OctylSO_4]$, $[C_nmim]Cl$ and $[C_4mim][N(CN)_2]$	IL + K_3PO_4 IL + K_2HPO_4/ KH_2PO_4 IL + Na_2SO_4	Up to 98.80%	[72]
Gallic acid Vanillic acid Syringic acid	$[C_4mim][CF_3SO_3]$ and $[C_4mim][N(CN)_2]$	IL + Na_2CO_3 IL + Na_2SO_4	73–99%	[71]
Caffeic acid Vanillic acid Gallic acid Vanillin Syringaldehyde	$[C_{12}mim]Cl$ and $[C_{14}mim]Cl$	PEG + NaPA + IL as electrolyte	40.73–82.52%	[73]
Capsaicin	$[Ch]Cl$, $[Ch][Bit]$ and $[Ch][DHC]$	IL + acetonitrile	90.57%	[75]
Phenolic acids Ferulic acid p-coumaric acid	$[Ch][DHC]$	IL + tween 20	97% of total phenols 89% of ferulic acid 93% of p-coumaric acid	[74]
Anthocyanins	$[C_2mim][Ac]$	IL + K_2CO_3	31.90%	[77]
Triterpene saponins	$[C_{2\text{-}5}Tr]Br$ and $[C_{2\text{-}6}Qn]Br$	IL + K_3PO_4, K_2HPO_4, K_2CO_3, $Na_3C_6H_5O_7$, or $K_3C_6H_5O_7$	> 99%	[78]
Polyphenols Saponins	$[Ch]Cl$	IL + K_3PO_4	35–70%	[79]
Aloe polysaccharides	$[C_4mim][BF_4]$	IL + NaH_2PO_4	93.12%	[80]
Aloe anthraquinones	$[C_4mim][BF_4]$	IL + Na_2SO_4	92.1%	[81]
Puerarin	$[C_4mim]Br$	IL + K_2HPO_4	> 99%	[82]

performance of the process. It should be remarked that although many of these studies were carried out with model aqueous solutions comprising the compounds of interest [69, 73, 76, 72, 71], eight works have been found on the use of ABS to fractionate and purify value-added compounds from crude biomass extracts [75, 74, 77–79, 81, 80]. A list of the ILs employed in these works is provided in Table 4, including their names, acronyms, and chemical structures.

Application of Ionic Liquids in Separation and Fractionation Processes, Table 4 ILs investigated, their names, acronyms, and chemical structures

IL	Acronym	Chemical structure
Imidazolium-based ILs		
1-butyl-3-methylimidazolium bromide	[C$_4$mim]Br	
1-butyl-3-methylimidazolium chloride	[C$_4$mim]Cl	
1-dodecyl-3-methylimidazolium chloride	[C$_{12}$mim]Cl	
1-tetradecyl-3-methylimidazolium chloride	[C$_{14}$mim]Cl	
1-ethyl-3-methylimidazolium acetate	[C$_2$mim][Ac]	
1-butyl-3-methylimidazolium tetrafluoroborate	[C$_4$mim][BF$_4$]	
1-butyl-3-methylimidazolium trifluoromethanesulfonate	[C$_4$mim][CF$_3$SO$_3$]	
1-butyl-3-methylimidazolium dicyanamide	[C$_4$mim][N(CN)$_2$]	
1-butyl-3-methylimidazolium methylsulfate	[C$_4$mim][CH$_3$SO$_4$]	
1-butyl-3-methylimidazolium ethylsulfate	[C$_4$mim][C$_2$H$_5$SO$_4$]	
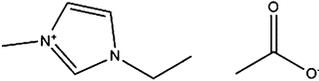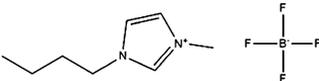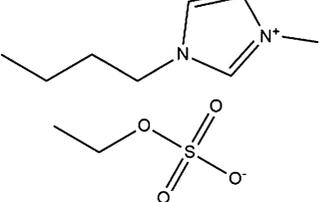
(continued)

Application of Ionic Liquids in Separation and Fractionation Processes, Table 4   (continued)

IL	Acronym	Chemical structure
1-butyl-3-methylimidazolium octylsulfate	[C4mim] [OctylSO4]	
Piperidinium-based ILs		
1-butyl-1-methylpiperidinium chloride	[C4mpip]Cl	
Pyrrolidinium-based ILs		
1-butyl-1-methylpyrrolidinium chloride	[C4mpyr]Cl	
Ammonium-based ILs		
Tetrabutylammonium chloride	[N4444]Cl	
Cholinium chloride	[Ch]Cl	
Cholinium bitartrate	[Ch][Bit]	

(*continued*)

Application of Ionic Liquids in Separation and Fractionation Processes, Table 4 (continued)

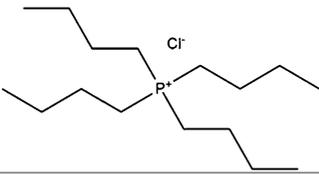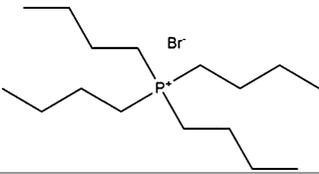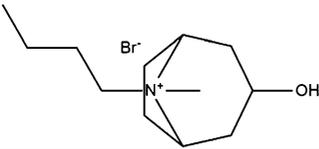| IL	Acronym	Chemical structure
Cholinium dihydrogen citrate	[Ch][DHC]	
Phosphonium-based ILs		
Tetrabutylphosphonium chloride	$[P_{4444}]Cl$	
Tetrabutylphosphonium bromide	$[P_{4444}]Br$	
Tropinium-based ILs		
n-alkyl(ethyl to pentyl)-tropinium bromide	$[C_{2-5}Tr]Br$	(Example for $[C_4Tr]Br$ that lead to the best results in the work)
Quinolinium-based ILs		
n-alkyl(ethyl to hexyl)-quinolinium bromide	$[C_{2-6}Qn]Br$	(Example for $[C_4Qn]Br$)
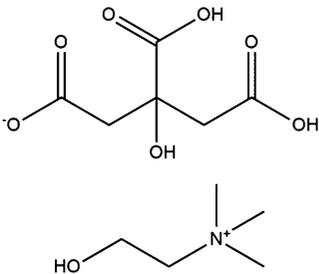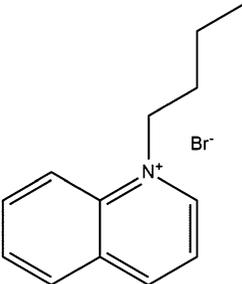
Santos et al. [69] studied the potential of IL-based ABS to separate antioxidants from aqueous solutions. In this work, ILs were studied in two ways: as main phase-forming components of IL-based ABS, combined with potassium citrate ($C_6H_5K_3O_7/C_6H_8O_7$) at pH 7, and as adjuvants in more conventional systems composed of polyethylene glycol (PEG) and potassium phosphate (K_2HPO_4/KH_2PO_4) at pH 7. In the first approach, the authors [69] studied imidazolium-,

piperidinium-, pyrrolidinium-, and ammonium-based ILs, and in the second case, only imidazolium-based ILs were studied as adjuvants. Using both types of ABS, the authors were able to attain a 100% extractive performance of these systems for the natural antioxidants eugenol and propyl gallate. In general, higher extraction efficiencies were achieved using the IL + salt ABS (from 75.9 to 100%) than with PEG + salt + IL ABS (between 47.1% and 100%). The poorer efficiency of polymer-based ABS was suggested to be a result of less tuned and nonspecific interactions, contrarily to what is observed in systems where the ILs are present. π-π stacking interactions (between ILs aromatic cations and antioxidants aromatic rings) and other antioxidant–IL interactions may be decisive for the success of the separation process. Almeida et al. [70] also demonstrated the relevance of IL-solute interactions in the separation of antioxidants carried out in polymer + salt ABS with 5 wt% of IL (as adjuvant). These results were further interpreted in more detail by Sousa et al. [76], who studied a larger range of ABS, ILs, and natural compounds. In general, the addition of ILs as adjuvants to polymer-based ABS changes the coexisting phases' characteristics and thereby modifies the partition of biomolecules. An increase in the extraction of more hydrophobic biomolecules is observed when using ILs as adjuvants in PEG-salt systems, whereas IL + salt ABS perform better in the extraction of more hydrophilic biomolecules. In summary, the favorable partition of more hydrophilic biomolecules in IL + salt ABS seems to be ruled by specific solute-IL interactions, while the favorable partition of more hydrophobic biomolecules in PEG + salt and PEG + salt + IL seems to be governed by the phases hydrophobicity/polarity.

Cláudio et al. [72] focused their studies in gallic acid, a phenolic compound present in relatively high concentrations in a large number of biomass sources presenting antioxidant, anti-inflammatory, antifungal, and antitumoral properties [83]. The authors [72] aimed at the development of an efficient purification process based on ABS formed by a wide variety of imidazolium-based ILs and Na_2SO_4, K_3PO_4 or K_2HPO_4/

KH_2PO_4. In general, the partition of the phenolic compound to the IL-rich phase decreases in the order: Na_2SO_4 (pH ca. 3–8) > > K_2HPO_4/ KH_2PO_4 (pH ca. 7) > K_3PO_4 (pH ca. 13), demonstrating a strong dependence of the partition of the target compound between the phases with the medium pH. For pH values below the pKa of gallic acid (pKa = 4.4), the uncharged molecule preferentially partitions to the IL-rich phase, whereas for higher pH values the preferential partition to the salt-rich phase is observed. These results led the authors to propose a subsequent work on the back-extraction of antioxidants present in biomass, as well as on the recovery and reuse of the IL-rich phases without loss of extraction efficiency [71]. Two types of IL-based ABS were studied for the extraction of phenolic acids (gallic, syringic, and vanillic acids), namely, IL + Na_2CO_3 and IL + Na_2SO_4, in order to tailor the pH values of the coexisting phases. From the several [C_4mim]-based ILs investigated, the most promising were [C_4mim][CF_3SO_3] and [C_4mim] [$N(CN)_2$], which were used in sequential two-step cycles (comprising both the product and IL recoveries). In four sequential partitioning experiments involving phenolic acids, extraction efficiencies ranging between 73% and 99% were attained, while allowing the regeneration of the IL to be further reused. In Fig. 10, it is depicted a scheme with the IL-based approach proposed by the authors for the extraction and recovery of gallic acid, and subsequent recovery of the IL-rich phase. This work supports the development of greener cost-effective IL-based ABS with a substantial reduction in the environmental footprint and economic issues.

In a recent work, Santos et al. [73] proposed an ABS approach to fractionate five phenolic compounds resulting from lignin depolymerization, namely, caffeic acid, vanillic acid, gallic acid, vanillin, and syringaldehyde. ABS formed by sodium polyacrylate (NaPA 8000) and polyethylene glycol (PEG 8000) were used, in which cationic and anionic commercial surfactants and ionic liquids with surface-active nature ([C_{12}mim]Cl and [C_{14}mim]Cl) were used as electrolytes at concentrations below 1 wt%. The recovery, partition coefficients, and selectivity of
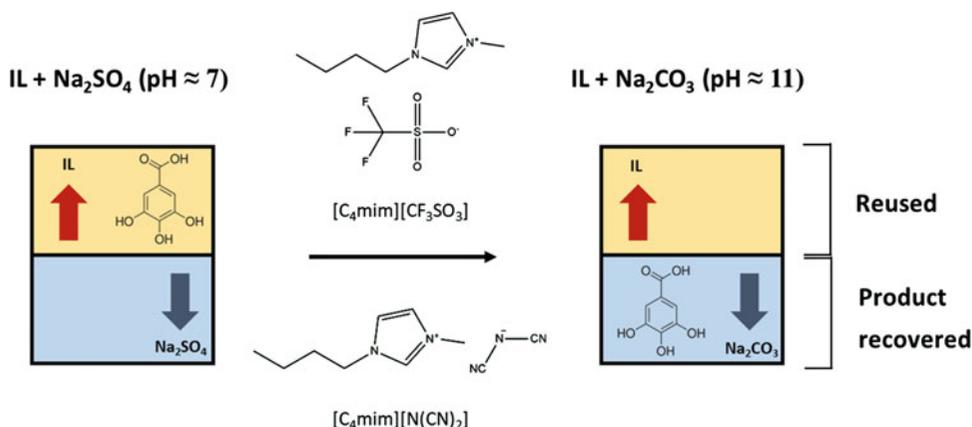
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 10 Approach proposed by Cláudio et al. [71] for the extraction and recovery of gallic acid, comprising the reuse of the IL-rich phase
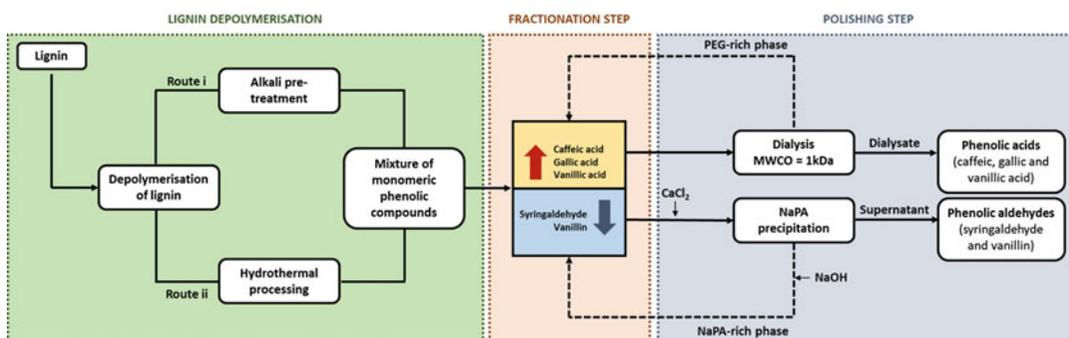
Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 11 Overview of the integrated process proposed by Santos et al. [73] for the fractionation/ separation, isolation, and recovery of phenolic compounds obtained from lignin depolymerization, and recycling of the phase-forming components

each system were evaluated, and recoveries ranging between 40.73% for syringaldehyde and 82.52% for caffeic acid were reported. The investigated systems were finally used in the design of an integrated process comprising the fractionation of phenolic compounds, their isolation, and recycling of the phase-forming components. Figure 11 represents a schematic overview of the integrated process proposed by the authors [73] for the fractionation/separation, isolation, and recovery of the phenolic compounds and recycling of the phase-forming components.

In addition to the previously works focused on the separation of phenolic compounds, yet only carried out with model aqueous solutions comprising the target solutes, Santos et al. [75] went further and reported the extraction and recovery of capsaicin, a bioactive phenolic compound with different therapeutic properties (anticancer, antioxidant, antiobesity) directly from pepper *Capsicum frutescens*, followed by the use of ABS for its separation. ABS consisting of acetonitrile and cholinium-based ILs ([Ch]Cl, [Ch][Bit] and [Ch][DHC]) were investigated. First, the solid-liquid extraction of the target bioactive compound from the natural biomass was conducted using mixtures of water and acetonitrile. By using a mixture of 40% of water and 60% of acetonitrile, the maximum extraction yield of capsaicin was 0.146 $mg \cdot g^{-1}$. The water-acetonitrile-extract mixture was then used to create ABS with cholinium-based ILs. An ABS composed of 30% of acetonitrile + 35% of [Ch]Cl allowed the successful recovery and purification of capsaicin to

the acetonitrile-rich phase, with an extraction efficiency of 90.57% and a purification factor of 3.26. In the end, an integrated process for the extraction and purification of capsaicin from biomass was proposed, combining the extraction step from biomass, the purification stage with ABS, and the recycling of the phase-forming components. More recently, Xavier et al. [74] reported a more environmentally friendly and competitive strategy aiming at the extraction of ferulic and *p*-coumaric acids from wheat straw biomass. In this approach, the biomass was pretreated by an acid hydrolysis and a subsequent alkaline delignification. Afterwards, the authors used an ABS composed of polyoxyethylene (20) sorbitan monolaurate (Tween 20) and a biocompatible IL – choline dihydrogencitrate ([Ch][DHC]) – to optimize the extraction of phenolic compounds from the alkaline hydrolysate samples. The surfactant content revealed to be a key parameter in ruling the extraction performance, where higher surfactant concentrations lead to higher extraction levels of phenolic acids: 97% total phenolic acids, 89% of ferulic acid, and 93% of *p*-coumaric acid. Moreover, by using this biocompatible approach, no bioactivity impairment was reported by the authors, as addressed by the free radical scavenging capacity and trolox equivalents antioxidant capacity.

Other type of (bio)molecules that can be found in biomass are anthocyanins, water-soluble bioflavonoids (polyphenolic compounds) presenting a vast range of applications in human health, mainly as inhibitors of lipid peroxidase, as anticancer and anti-inflammatory compounds, and as neuro- and cardioprotector agents. Based on these features, Lima et al. [77] developed a process for the extraction and purification of anthocyanins from biomass using IL-water mixtures. The strategy reported by the authors includes a solid-liquid extraction step of anthocyanins from the agricultural waste product grape pomace using aqueous solutions of [C$_2$mim][Ac] and a sequential purification step using ABS formed by the addition of a salt. Under optimized conditions for the solid-liquid extraction, the authors reported an yield of 3.58 mg of anthocyanins *per* g of biomass. Then, using the ABS, the undesired flavonoids are separated from the anthocyanins, with the best purification factor (16.19 fold) achieved with an ABS composed of 29.38% of [C$_2$mim][Ac] + 29.40% of K$_2$CO$_3$ at 35 °C.

Natural saponins have immunopotentiation, cardio-protective, antidiabetic, and anthypnotic effects, motivating He et al. [78] to develop a method for the extraction of ginseng saponins from the root of *Panax ginseng* C. A. Mey. The novel approach proposed comprises the use of two IL + salt ABS, using *n*-alkyl-tropinium and *n*-alkylquinolinium bromide ILs ([C$_{2-5}$Tr]Br/[C$_{2-6}$Qn]Br). The authors evaluated initially the ability of these ILs to induce aqueous phase separation in presence of several inorganic salts, followed by the exploration of the generated ABS to separate genosides (Rg$_1$, Re, Rd, and Rb$_1$) from crude extracts of ginseng roots. At the best conditions (35% [C$_4$Tr]Br + 20% NaH$_2$PO$_4$ + 3% ginseng extracts + 42% water for 60 min at room temperature), extraction efficiencies higher than 99% were achieved, although no major discussions on the purification factors and selectivity of the systems were provided (Fig. 12).
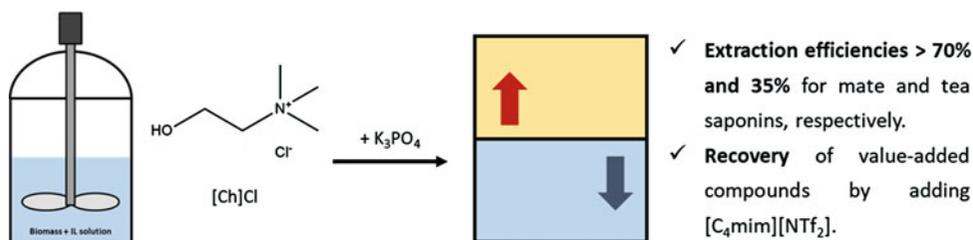

Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 12 Overview of the process proposed by He et al. [78] for the extraction of ginseng saponins

Ribeiro et al. [79] studied the extraction of saponins and polyphenols from dried leaves and aerial parts of Tea (*Camellia sinensis*) and Mate (*Ilex paraguariensis*) using aqueous solutions of ILs. A wide range of ILs were studied by the authors, ranging from imidazolium-based ILs differing in their alkyl side chain length to more sustainable ones, such as cholinium based. At the solid-liquid extraction, most of the ILs studied allowed higher extraction yields than those obtained using a 30% ethanol aqueous solution, commonly applied in such processes. Then K_3PO_4 and Na_2CO_3 were added to the IL aqueous solution of [Ch]Cl aqueous solution enriched in the extract in order to form ABS to further fractionate and purify the compounds of interest. Yet, only with K_3PO_4 an ABS is formed. In general, interesting results were obtained for both tea and mate matrices, since partition coefficients two orders of magnitude higher were obtained for saponins and phenolic compounds than with ethanolic solutions, with extraction efficiencies around 70% and 35% achieved for mate and tea saponins, respectively. Moreover, after the two-phase separation, a nonwater miscible IL – $[C_4mim][NTf_2]$ – was added to the [Ch]Cl-rich phase resulting in the formation of two new phases: one containing the two ILs and the other an aqueous phase containing saponins and phenols, allowing the value-added compounds recovery from the IL-rich phase. Figure 13 depicts a schematic representation of the process developed by the authors.

Tan et al. [80, 81] published two works regarding the extraction and separation of value-added compounds from *Aloe vera L.* (Liliaceae) using IL-based approaches. The *Aloe vera* contains

75 potentially active substances [84, 85], but two major bioactive compounds were targeted, namely, aloe polysaccharides present in the aloe fillet and aloe anthraquinones in leaves. Aloe polysaccharides are responsible for the pharmacological activities of wound healing, anti-inflammation, and immunomodulating properties [86], while aloe anthraquinones have antibacterial, anti-inflammation, hemostatic, and antispasmodic features [87]. In both studies, the authors started by the pretreatment of the plant material, soaking aloe peel powder in an aqueous solution containing 60% of ethanol. For the aloe polysaccharides work, the colloid was dried and used [80]. For the anthraquinones [81], sulfuric acid and chloroform were added into the extract, refluxed, and the chloroform finally removed. After evaporation, a yellowish-brown colloid was used as the crude extract and dissolved in methanol as the stock solution [81]. In a strategy similar to that adopted in the previous works, the stock solutions containing the compounds of interest were used in the formation of IL-based ABS, composed of $[C_4mim][BF_4]$ and sodium-based salts, and the fractionation and purification of the value-added components investigated. Based on the gathered results, the authors shown that an ABS composed of 18.52% $[C_4mim][BF_4]$ and 25.93% NaH_2PO_4 is able to retain the aloe polysaccharides into the salt-rich phase (93.12%), while the other proteins and impurities are extracted to the IL-rich phase (extraction efficiency of 95.85%). The purity of the final product was demonstrated by thermogravimetric analysis (TGA) [80]. Concerning the aloe anthraquinones, a maximal extraction efficiency of 92.1% of anthraquinones into the IL-rich phase was obtained under

Application of Ionic Liquids in Separation and Fractionation Processes, Fig. 13 Overview of the integrated process proposed by Ribeiro et al. [79] for the extraction and fractionation/purification of saponins and polyphenols

optimum conditions, using an ABS composed of [C$_4$mim][BF$_4$] and Na$_2$SO$_4$ at 25 °C and pH 4.0 [81]. In the end, a last step for the IL recovery was proposed using reverse extraction experiments, carried out by taking the IL-rich phase containing anthraquinones and forming a new ABS by adding a salt with alkaline characteristics. The alkaline medium led to the speciation of the molecules of interest and consequent migration into the salt-rich phase, allowing the IL-rich phase to be recovered and reused in the extraction procedure, being in agreement with the back-extraction procedure proposed by Cláudio et al. [71] for phenolic acids. Although these works demonstrate the potential of IL-based ABS to separate and purify specific value-added compounds from natural sources, the authors focused on ABS formed by [C$_4$mim][BF$_4$], which has some drawbacks in terms of IL toxicity, stability in water and cost, strengthening the notion that more benign ILs should be explored in the development of sustainable bioprocesses. Moreover, the authors [80, 81] performed the extraction of the target compounds from biomass using a common organic solvent, and the IL-based strategy was only applied for the fractionation and purification. Thus, based on the high performance of aqueous solutions of IL for the extraction of value-added compounds from biomass, as discussed in the previous subchapter, an integrated process could be designed, in which the extraction of the target compounds from the biomass can be carried out with an IL aqueous solution that could be directly used in the formation of ABS for the fractionation and purification steps, in a similar strategy as that proposed by Lima et al. [77] and He et al. [78] for anthocyanins and saponins, respectively.

Fan et al. [82] investigated ILs as phase-forming components of ABS for the extraction and isolation of puerarin from *Radix Puerariae Lobatae* extracts. Puerarin is an important isoflavone with beneficial effects concerning the treatment of hypertension, arteriosclerosis, and diabetes mellitus. In this work, the separation/purification step was optimized using pure puerarin, and some important key factors affecting the extraction process were appraised, namely, the IL nature, the presence of short-chain

alcohols, the salting-out ability of the salt, and the pH of the medium. When applying the optimized system (ABS composed of [C$_4$mim]Br and K$_2$HPO$_4$) to *Radix Puerariae Lobatae* extracts, the authors [82] achieved an extraction efficiency higher than 99% to the IL-rich phase. However, no information was provided by the authors regarding the purity levels afforded by using this IL-based ABS approach.

Based on the aforementioned information and discussion, and on the summary given in Tables 1 and 3, most of the studies carried out hitherto on the use of IL-based strategies for extraction, separation, and purification purposes within a biorefinery framework applied imidazolium-based ILs combined with a limited number of anions (e.g., chloride, bromide, acetate, dicyanimide, and tetrafluoroborate). Nevertheless, in the past few years, some promising works appeared reporting successful results in terms of extraction, fractionation and purification processes using ammonium-, phosphonium-, cholinium-, and betaine-glycine-based ILs. However, there is still a wide range of IL ions that can be explored for the solid-liquid extraction and further isolation of value-added compounds from natural sources. Also, most works addressing the application of IL-based ABS were carried out using model solutions of target value-added compounds; therefore, more studies are needed by employing more complex and real biomass extracts in the separation and purification steps, as well as on the development of combined and integrated extraction-separation processes to fulfill the requirements of a sustainable biorefinery.

Conclusions and Future Directions

In the past decade, ILs have demonstrated their potential for the extraction, fractionation, and purification of value-added compounds from natural sources. The most relevant property of ILs behind such successful results is their "designer solvent" ability, allowing to tailor the extraction and purification performance of target compounds. In this chapter, it was overviewed the potential and suitability of IL-based solvents for

solid-liquid extractions from biomass, followed by IL-based separation processes by the application of ABS. IL-based processes have been used to extract, separate, and purify a wide range of value-added bioactive compounds, such as antioxidants, phenolic/polyphenolic compounds, saponins, polysaccharides, anthraquinones, and isoflavones, in addition to more complex structures such as lignin, cellulose and hemicellulose.

Imidazolium-based ILs are still the preferred choice for most researchers working on solid-liquid extraction and liquid-liquid separation approaches. This may be due to the well-established knowledge on their properties and phase behavior. However, recent works are moving into a different direction by using quaternary ammonium-based ILs, such as cholinium- and glycine-betaine-based. In the coming years, it is expected that more biocompatible and benign ILs will start to be comprehensively investigated, without forgetting the compromise between the IL chemical structure and the performance of the extraction and separation processes. It is interesting to note that the solid-liquid and liquid-liquid strategies discussed in this chapter can complement each other since the development of an integrated process can be foreseen, e.g., by extracting value-added compounds from biomass using IL aqueous solutions, which can be further used to form IL-based ABS for separation and purification purposes. In fact, some pioneering studies on the development of this type of integrated processes appeared in the recent years and were discussed in this chapter, demonstrating that sustainable and efficient IL-based processes can be developed and applied in biorefinery.

Based on the promising evidences reported and discussed herein concerning the use of ILs in biorefinery processes, some challenges still needed to be addressed. In summary, the ILs community working in this area is already aware of the potential of IL-based extraction and separation processes, but more progress is required, mainly on the replacement of the widely used imidazolium-based by more biocompatible ILs, on the development of integrated processes or reduction of the number of steps involved, and on decreasing the cost of the processes.

Furthermore, it is of high importance to explore and develop processes for both the target compounds recovery and IL recycling, as well as to demonstrate the potential of scaling-up such processes. Finally, both economic and life cycle analyses are of high relevance to evaluate and guide the design of IL-based processes, so that they can become an industrial reality in biorefinery plants.

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