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Separation of phenolic compounds by centrifugal partition chromatography†

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Phenolic compounds are ubiquitous biomolecules exhibiting a wide range of physiological properties, with application in the pharmaceutical and nutraceutical fields. In this work, aqueous biphasic systems (ABS) formed by polyethylene glycol and sodium polyacrylate, and inorganic salts or ionic liquids as electrolytes, were applied for the purification of caffeic, ferulic and protocatechuic acids (CA, FA, and PA, respectively), vanillin (VN) and syringaldehyde (SA), followed by the use of centrifugal partition chromatography (CPC) to reinforce the fractionation process scale-up. In single-step experiments in ABS, high selectivities and adequate partition coefficients (K_{CA} = 2.78 ± 0.20; K_{PA} = 0.44 ± 0.04; K_{FA} = 0.23 ± 0.01; K_{VN} = 1.12 ± 0.05 and K_{SA} = 1.23 ± 0.02) were achieved using ABS formed by sodium chloride as the electrolyte. This system was further applied in CPC, allowing an efficient separation of the five phenolic compounds after the optimization of the equipment operational conditions, while demonstrating the potential of polymer-based ABS to be used in liquid–liquid chromatography. Finally, the recovery of the phenolic compounds (between 65 and 87%) with high purity from the ABS phases was demonstrated, allowing the reuse of the ABS phase-forming components, which was proved to be of low environmental impact. In fact, in a scenario where the polymeric phases are reused, the carbon footprint is decreased to 36%, as the consumption of new chemicals and water reduces considerably.

Introduction

Phenolic compounds are relevant biomass building blocks. They are considered as one of the most versatile and important industrial organic chemicals,[1] widely used in the food[2,3] (*e.g.* as dyes and food additives), pharmaceutical[4,5] (*e.g.* natural antioxidants or raw materials for producing medical drugs like aspirin), chemical[6–9] (*e.g.* resins, plastics and polycarbonates) and cosmetic[3,10] (*e.g.* natural additives) industries. Due to their wide range of applications, these products are economically attractive[11] when compared with petrochemical phenolic compounds. In 2015, the price of synthetic phenols achieved values around 1000$ *per* tonne,[11] while the price of phenolic compounds derived from the lignocellulosic biorefinery ranged from 1000–12 000$ *per* tonne[12] depending on the phenolic compound (*e.g.* 12 000$ *per* tonne for vanillin; 4500$ *per* tonne for eugenol; and 2000$ *per* tonne for syringols/conyferols/guaiacol). Their high price is a result of significant drawbacks regarding the lignocellulosic biorefinery processing, especially considering the need for more effective purification methods and downstream processes. The alkaline oxidative process[13,14] and the hydrothermal processing[15] of lignin are the conventional depolymerisation platforms used to produce monomeric aromatic compounds, mainly due to their greener and cheaper characteristics. Nevertheless, one of the main concerns is the difficulty to fractionate the heterogeneity of phenolic compounds resulting from the lignin depolymerisation process.[16] In literature, supercritical carbon dioxide extraction, ionic liquid (IL) extraction, and adsorption in specific polymeric resins are the main fractionation techniques already described for lignocellulosic products (*e.g.* vanillin, syringaldehyde and *p*-hydroxybenzaldehyde).[17] However, the high cost and difficult scale-up are the main disadvantages of these techniques. Therefore, there is an effective and crucial need to develop more efficient, yet scalable, fractionation processes, operating under mild conditions with minimal waste formation, in order to selectively separate and purify each phenolic compound produced by lignin depolymerisation, in which aqueous biphasic systems (ABS) can be envisioned as a

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promising alternative. Previous successful studies used ABS to purify structurally similar biomolecules,[18–20] including phenolic compounds.[21] However, none of these studies reported the scale-up of the technique.

Two polymers, a polymer and a salt or two salts dissolved in water, are the most common combinations to form ABS. Polymer-based ABS are commonly composed of polyethylene glycol (PEG) and dextran.[22–24] However, due to the high cost of dextran and the high viscosity that PEG-dextran-based systems present,[25] other polymer combinations have been proposed.[26–28] Amongst these, sodium polyacrylate (NaPA)-PEG-based ABS appeared as a promising combination of phase-forming components that have been successfully applied in the purification of a wide variety of biomolecules.[19,29,30] These novel polymer-based aqueous systems have some important advantages when compared to other polymer combinations, namely a lower viscosity and a faster separation rate, beneficial to reduce energetic inputs and improve mass transfer.[28] Moreover, PEG-NaPA-based ABS exhibit a remarkable high water content, with phase separation occurring at very low concentrations of the polymer (3–5 wt% of each polymer).[28] These two polymers are biocompatible, relatively inexpensive, and easy to recycle and reuse.[21,28] PEG is an uncharged polymer whereas NaPA is a polyelectrolyte. To form an ABS at reasonable polymer concentrations, a minimum amount of an electrolyte is however required.[28] Various authors have evaluated the effect of different electrolytes, principally inorganic salts,[27,28] and more recently, ionic liquids[31] and surfactants,[21] to induce the phase separation. Some of these authors suggested that the entropy penalty upon compartmentalization of the counter ions present[28] and/or the competition of the charged species for the water molecules are the driving forces behind the phase separation.[27] Meanwhile, when ionic liquids are used as electrolytes[31] the interactions are far more complex. Even though there are still different visions on the molecular-level phenomenon ruling the phase separation, these systems have shown some interesting results regarding the purification of biomolecules.[28] In general, in PEG-NaPA systems, the manipulation of the electrolyte nature affects the biomolecule partition and their selectivity to one of the phases, which are a result of specific interactions occurring between the phase-forming components, the electrolyte, and the target biomolecules or their contaminants.[21,26,30] Biomolecules of higher complexity and molecular weight, like cytochrome c,[31] hemoglobin,[32] lysozyme[32] and protease,[29] and simpler biomolecules, such as clavulanic[30] and chloranilic acids,[31] were studied using PEG-NaPA-based ABS with different electrolytes. In these studies, the authors paid attention to the maintenance of the biomolecules' biological activity and stability during the purification process.

However, one of the major drawbacks associated with the use of polymer-based ABS is the difficulty to transpose the high yields of extraction and the purity levels obtained in the lab, to continuous processes at a larger scale, culminating in an industrial process. To this end, centrifugal partition chromatography (CPC) can enhance the resolution of the separations and convert liquid–liquid extractions based on ABS into processes scalable to large flow rates.[33] This chromatographic downstream technology operates with liquid stationary and mobile phases, which, in this work, will correspond to the two phases of the polymer-based ABS. The stationary phase is immobilized by a strong centrifugal force while the mobile phase is pumped through the stationary phase,[34] allowing a multistage separation process by the continuous partition of the biomolecules between the two phases (Fig. S1†). CPC does not need an expensive solid stationary phase, and both the quantity and quality requirements regarding the solvents/phases to be used could be much more similar in terms of their properties (*e.g.* viscosity and density) than those applied in more standard liquid chromatographic techniques, such as counter current chromatography (CCC).[34] Compared to traditional chromatographic techniques which employ stationary phases, CPC reduces the sample losses which could occur by irreversible adsorption, imposing no restrictions regarding the flow related to solids or the adsorbent porosity, and no changes of the analyte structure.[35] Due to the different affinity of the target compounds and contaminants for each phase, all components of a sample mixture injected at the beginning of the multistage cascade will elute at different times. CPC has been applied to fractionate various biomolecules derived from biomass, showing separations with high selectivity and extraction efficiency values. Phenolic compounds from plant extracts were previously successfully purified by CPC using other immiscible phases, *e.g.* heptane/ethyl acetate/methanol/water (1 : 5 : 1 : 5; v/v) and hexane/ethyl acetate/ethanol/water (4 : 5 : 3 : 3; v/v).[36–38] Recent trends aim for the valorisation of wastes and by-products of the (bio)chemical and agro-food industries in the framework of a circular economy. Moreover, the Green Chemistry principles recommend that new processes should minimise their solvent usage and raw material consumption, and increase their waste processing. This should lead to improvements in the environmental benignity and economic viability of these processes. The downstream processes, representing more than half of the cost of the final products, are following the same trend. New strategies to minimise the consumption of solvents and energy, as well as the production of wastes, are being followed aiming for an enhanced sustainability.[39–41] Therefore, it is important to provide green metrics for these processes to evaluate their sustainability and identify opportunities for decreasing their environmental impact.[42,43]

In this work, PEG 8000 + NaPA 8000-based ABS, using ionic liquids or inorganic salts as electrolytes, were studied in the separation of five model phenolic compounds: three phenolic acids (caffeic, ferulic and protocatechuic acids) and two aldehydes (vanillin and syringaldehyde), derived from lignocellulosic depolymerisation. The selection of the best ABS and its optimization was performed, followed by its application in CPC to reinforce the technique scale-up. After the development and characterization of the integrated process to fractionate the mixture of phenolic compounds, an environmental evaluation was done considering the carbon footprint as the main parameter/output.

Experimental

Materials

The polymer-based ABS studied are formed by two polymers, namely polyethylene glycol (PEG 8000 g mol^{-1}; purum) and sodium polyacrylate (NaPA 8000 g mol^{-1}; 45 wt% in water), both from Sigma-Aldrich.

Inorganic salts used as electrolytes were sodium chloride (NaCl) and sodium sulphate (Na_2SO_4), both purchased from Sigma-Aldrich, with a purity ≥99 wt%.

Ionic liquids used as electrolytes were 1-ethyl-3-methylimidazolium chloride ([C_2mim]Cl), 1-ethyl-3-methylimidazolium triflate [C_2mim][CF_3SO_3], 1-ethyl-3-methylimidazolium methanesulfonate [C_2mim][CH_3SO_3], 1-ethyl-3-methylimidazolium tosylate [C_2mim][TOS], and 1-ethyl-3-methylimidazolium dicyanamide [C_2mim][$N(CN)_2$]. The ionic liquids were purchased from Iolitec, with a purity >97 wt% (Fig. 1). The investigated phenolic compounds (Fig. 1), namely caffeic (CA), ferulic (FA) and protocatechuic acids (PA), and the phenolic aldehydes, vanillin (VN) and syringaldehyde (SA), were acquired from Sigma-Aldrich (purity > 98 wt%).

Preparation of polymer-based ABS using inorganic salts or ionic liquids as electrolytes to fractionate phenolic compounds

The ABS used for the partition studies of phenolic compounds were prepared using graduated centrifuge tubes by weighing the appropriate amount of each phase component and each phenolic aqueous solution. The extraction point adopted to study the partition of phenolic compounds was 15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 5 wt% of each electrolyte + 75.5 wt% of an aqueous solution containing each phenolic compound, namely CA (28 μg mL^{-1}), FA (20 μg mL^{-1}), PA (20 μg mL^{-1}), VN (30 μg mL^{-1}) and SA (30 μg mL^{-1}). This mixture point falls within the biphasic region and it was chosen based on the phase diagrams reported elsewhere.[31] The different phenolic concentrations were selected to ensure sufficient accuracy within the analytical technique employed for their quantification. After the complete dissolution of all components by stirring, all mixtures were left to equilibrate for 12 hours in an air oven, at (298 ± 1) K, to achieve the complete partition of each phenolic compound between the two aqueous phases. The phases were then carefully separated and the phenolic compounds were quantified at both top and bottom phases by UV-spectroscopy, using a Synergy HT spectrometer microplate reader, at the wavelengths of 287, 277, 256, 279 and 306 nm, for CA, FA, PA, VN and SA, respectively. Calibration curves for each phenolic compound were established at the respective maximum absorption wavelengths. Three independent assays were prepared for each mixture, and the quantification of each phenolic compound was performed in triplicate, where the final absorbance results are reported as the average of the triplicates accompanied by the respective standard deviation. Possible interferences of the phase-forming electrolytes were considered using blank controls (represented by the same mixture points, but without the presence of the phenolic compound under study). Different parameters were determined to evaluate the partition performance of each phenolic compound (PC), namely their partition coefficients (K_{PC}) those representing K_{CA}, K_{FA}, K_{PA}, K_{VN} and K_{SA}, their recovery in the top phase ($Rec_{Top}PC\%$), which corresponds to the PEG-rich phase, and the selectivity data (S), as represented by eqn (1) to (3), respectively.

$$K_{PC} = \frac{[PC]_{Top}}{[PC]_{Bot}} \quad (1)$$

$$Rec_{Top}PC(\%) = \frac{100}{1 + \left(\frac{1}{K_{PC} \times R_v}\right)} \quad (2)$$

$$S = \frac{K_{PC1}}{K_{PC2}} \quad (3)$$

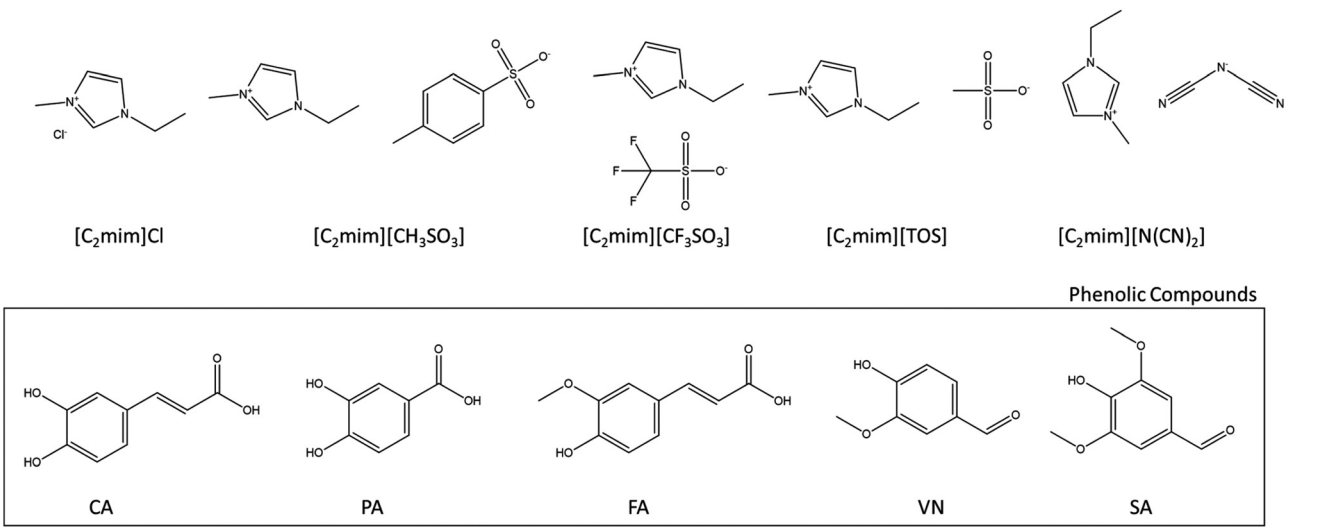

Fig. 1 Chemical structure of ionic liquids and phenolic compounds studied in this work.

where $[PC]_{Top}$ and $[PC]_{Bot}$ represent the phenolic compound concentration at the top and bottom phases, respectively. Specific abbreviations representative of the partition coefficients for each phenolic compound will be adopted. R_v represents the volume ratio between the top and bottom phase volumes, and K_{PC1} and K_{PC2} represent the partition coefficients of two different phenolic compounds, in which $K_{PC1} \geq K_{PC2}$ represents $S \geq 1$. The detailed recovery data are presented in Table S1 in the ESI.†

Centrifugal partition chromatography to separate phenolic compounds

A Fast Centrifugal Partition Chromatography (FCPC)® system, model FCPC-C, from Kromaton Rousselet-Robatel (Annonay, France), was used for the separation of phenolic compounds. The equipment design comprises a pattern of cells interconnected by ducts and dug in a stainless steel disk. The cell design, also called twin cells, contains a restriction in the middle ducts of the canal creating two superimposed chambers. The rotor consists of 13 associated disks, each one containing 64 twin cells, making a total of 832 twin cells. The total cell volume is 50 mL, with 10 mL or 20% of the column volume corresponding to the connecting ducts. The maximum theoretical liquid stationary phase retention factor ($S_f = V_S/V_C$) is 80%, since 20% of the connecting duct volume can only contain the mobile phase. The maximum rotor rotation is 3000 rpm generating a maximum centrifugal field of ~1500*g*. Two rotating seals are displayed at the rotor entrance and exit (also called "head" and "tail", respectively), which allow a maximum pressure of 7 MPa. The CPC system is connected to an ECOM ECB2004 gradient box with a degasser, an ECOM ECP2010 analytical HPLC pump, an ECOM Flash 14 DAD detector (four wavelengths were simultaneously analysed with 280 nm selected as the wavelength with no significant interferences), and to a continuous scan (ECOM spol. S.r.o., Czech Republic). Several fractions are collected with an ADVANTEC® Super Fraction Collector CHF122SC (Advantec Toyo Kaisha, Ltd, Tokyo, Japan). Each sample was injected manually using a Rheodyne valve model 3055-023 through a 10 mL sample loop. Analogical detector signals were processed using the ECOMAC software (ECOM spol. S.r.o., Czech Republic).

The CPC separations were carried out using a system composed of 15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 75.5 wt% of water + 5 wt% of NaCl. This system was set to work in the ascending mode. The rotor was entirely filled with the NaPA-(bottom)-rich phase at 600 rpm to achieve homogeneous solvent re-equilibration on the rotor. Then, the rotation was set up at 2000 rpm for an appropriate stationary phase retention. After the working rotational speed was set up, the PEG 8000-rich-(top) phase was pumped through the stationary phase to reach the equilibrium, *i.e.* when only the mobile phase came out of the column and the signal baseline is stabilized. The mobile phase flow rate was studied to increase the stationary phase retention ratio and, simultaneously, to decrease the purification time, and the best flow rate found was 1.5 mL min^{-1}. The stationary phase retention, S_f, was calculated by the ratio of the stationary phase volume (V_S) and the column volume (V_C): $S_f = V_S/V_C$. In this case, values of 37% and 20% of S_f for 1.5 and 1.0 mL min^{-1} of flow rate, respectively, were achieved.

For the separation of phenolic acids, the sample loop was filled with 5 mL of the ABS composed of 15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 75.5 wt% of water + 5 wt% of NaCl containing the CA, FA and PA at higher concentrations (0.4 mg mL^{-1}) than those used in the lab-scale ABS experiments. After 55 min of elution with the top PEG-rich phase to extract the CA, the mobile phase was changed to the NaPA-rich (bottom) phase to elute separately the PA and FA, by applying an elution–extrusion process.[44]

To prove the success of the CPC performance in the separation of phenolic compounds from the depolymerisation of lignin, a more complex mixture was also tested. This is composed of the three acids previously mentioned and two aldehyde-derived phenolic compounds. In this case, the same experimental procedure was performed, and the same operational conditions (flow rate, stationary phase volume, rotation and injection volume) were maintained. Each phenolic compound was injected at a concentration of 0.4 mg mL^{-1}, for a total volume of 5 mL. However, in this purification step, the change of phase elution from the PEG 8000 to NaPA 8000-rich-phase was performed at 65 min, instead of 55 min.

Isolation of phenolic compounds from aqueous polymeric phases

Phenolic compounds were isolated from the PEG 8000- and NaPA 8000-rich fractions through dialysis. A Spectra/Por membrane (cut-off: 1 kDa; diameter 24 mm) was used, against a volume of 10 mL of ultra-pure water, at room temperature. The amount of polymer solution was *ca.* 37.5 wt% of PEG 8000 and 40 wt% of NaPA 8000. The permeate was evaluated by UV-Vis spectroscopy to quantify the amount of each phenolic compound present. The phenolic aldehydes were recovered together by dialysis from the PEG 8000-rich fractions. In the specific case of CA, when the equilibrium was reached, a new volume of water (10 mL) was needed to recover the remaining phenols, with the PEG 8000 fraction being retained in the membrane and CA quantified also by UV-Vis spectroscopy.

The recovery/purity of the recovered PA and FA was evaluated by ^{1}H NMR. The ^{1}H NMR results were just performed for PA and FA, because their quantification was masked by the presence of NaPA 8000. The ^{1}H NMR measurements were performed on a Bruker Avance 300 spectrometer operating at 300.13 MHz. For the remaining phenolic acids, their quantification and recovery yield determination were assessed by UV-Vis spectroscopy.

To prove the purity of the polymeric phase formers, ATR-FTIR was also used to demonstrate the elimination of the polymers after the polishing step (dialysis), still proving the absence of any phenolic compound. Attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy (Tensor 27 FTIR spectrometer, Bruker Co., USA) was used to characterize the phases after the polishing step and to quantify

the polymers (PEG and NaPA 8000) before and after the dialysis. The experiments were carried out with a wavenumber ranging from 350 to 4000 cm^{-1}, with a resolution of 4 cm^{-1}, in a scan number of 256, and referenced against distilled water. The ATR-FTIR results are depicted in Fig. S8 in the ESI.†

Environmental assessment

The environmental assessment of the integrated system proposed for scale-up using the CPC followed by an isolation step was performed by calculating the carbon footprint, *i.e.* the sum of greenhouse gas (GHG) emissions expressed as carbon dioxide equivalent ($CO_{2\ eq}$) from a life cycle perspective. From the industrial point of view, ultrafiltration was used in this analysis, although the experimental results were obtained by dialysis. Thus, besides the CPC and ultrafiltration processes, this assessment includes also the production of PEG 8000, NaPA 8000, NaCl, water, and the electricity consumed. Two scenarios were considered, namely with and without the reuse of PEG 8000- and NaPA 8000-rich phases, to better understand the environmental gains of reusing these phases.

Data on the amounts of PEG 8000, NaPA 8000, NaCl, water and electricity consumed in CPC were obtained during and after the CPC experiment. For the scenario with the reuse of PEG 8000- and NaPA 8000-rich phases, the same recovery rates of chemicals determined by ATR-FTIR for dialysis were assumed. Data on electricity consumption in the ultrafiltration and reuse flow were taken from the literature[45] and pump catalogues, respectively (Table 1). Data on GHG emissions from the production of PEG 8000, NaPA 8000, NaCl and electricity were sourced from Ecoinvent database version 3.4,[46] while GHG emissions from the production of water were taken from the literature[47] (Table S2†). All data refer to 1000 g of ABS used in CPC.

Results and discussion

Evaluation of PEG-NaPA-based ABS to separate phenolic compounds

The phase diagrams of the systems investigated in this work, as well as the electrolytes' preferential partition (see Table S3†), were reported in a previous study.[31] Aiming at the better understanding of the separation of these phenolic compounds, the pH of the phases was also determined, as presented in Table S4 in the ESI.† Table S4† also presents the partition coefficient data for the five phenolic compounds between the two phases. Despite the absence of a buffering agent to maintain the pH, the pH of the studied systems is *ca.* 7 (6.7 < pH top phase < 7.5 and 6.6 < pH bottom phase < 7.4). From these results, it seems that this condition (pH) is independent of the electrolyte nature and type, probably because these are present at low concentrations. At these pH values, the phenolic acids are negatively charged in all the partition experiments, and the phenolic aldehydes are either neutral or negatively charged (*cf.* their dissociation curves in Fig. S2–S6 in the ESI†).

In this work, two types of electrolytes were investigated,[31] five imidazolium-based ionic liquids and two inorganic salts. The partition tests were performed using systems composed of 15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 75.5 wt% of water + 5 wt% of the electrolyte. The electrolyte concentration of 5 wt% was selected in this work because not only it corresponds to the biphasic region according to the binodal curves previously reported and to the high selectivity and good performance on the separation of molecules,[31] but also it allows the appropriate conditions of the phase volume ratio required for the CPC operation. It should be highlighted that all the [C_2mim]-based ILs used as electrolytes are predominately partitioned towards the NaPA 8000-rich phase, due to the electrostatic interaction between the large IL cation and the negatively charged polymer[31] (the K_{IL} data are depicted in the ESI as Table S3†).

Fig. 2 depicts the partition coefficients and recovery results achieved, in which two profiles can be distinguished for the phenolic acid partition: (i) non-electrolyte dependent (FA, VN and SA) and (ii) electrolyte-dependent (PA and CA). The preferential partition of FA ($\log K_{ow}$ = 1.67 (ref. 48)) for the NaPA 8000-rich phase ($\log K_{FA}$ < 0; Fig. 2A) observed in this work follows the results previously reported,[21] similarly to the PA partition profile, which in general displays a higher affinity to the more hydrophobic phase (being the systems based in Na_2SO_4 and [C_2mim][CF_3SO_3] the exceptions). The partition of both PA and CA seems to be however dependent on the phenolic-acid–electrolyte interactions, which is obvious in their partition coefficients ($-0.32 < \log K_{CA} < 0.44$ and $-1.47 < \log K_{PA} < 0.29$; Fig. 2A) and recovery experimental data (Fig. 2B). In general, phenolic acids preferentially partition towards the NaPA 8000-rich phase ($\log K < 0$), the same partition tendency observed for some of the electrolytes used.[31] Exceptions to this behaviour are observed with the systems formed by [C_2mim]Cl and [C_2mim][$N(CN)_2$]. On the other hand, for ABS using the inorganic salts (NaCl and Na_2SO_4) and the ionic liquid [C_2mim][CF_3SO_3] with the lowest partition coefficient ($K_{IL} \ll 1$), it is observed that at least one of the phenolic acids partitions to the PEG 8000-rich phase. The PA is partitioned to the PEG 8000-rich phase in the ABS using Na_2SO_4 ($\log K_{PA}$ = 0.23 and $Rec_{Top}PA$ = 64.31 ± 0.10%) and [C_2mim][CF_3SO_3] ($\log K_{PA}$ = 0.29 and $Rec_{Top}PA$ = 85.5 ± 1.7%) as electrolytes. The CA also partitions to the PEG 8000-rich phase when NaCl ($\log K_{CA}$ =

Table 1 Inputs to the centrifugal partition chromatography (CPC) and ultrafiltration (UF) for the two scenarios considered, (i) without and (ii) with the reuse of PEG 8000- and NaPA 8000-rich phases. The amounts refer to 1000 g of ABS used in CPC

Input	Without reuse	With reuse
PEG 8000 (g)	150.00	16.03
NaPA 8000 (g)	45.00	15.00
NaCl (g)	50.00	2.00
Water (g)	755.00	6.97
Electricity in CPC (kWh)	1.19	1.19
Electricity in UF (kWh)	2.86×10^{-4}	1.05×10^{-3}

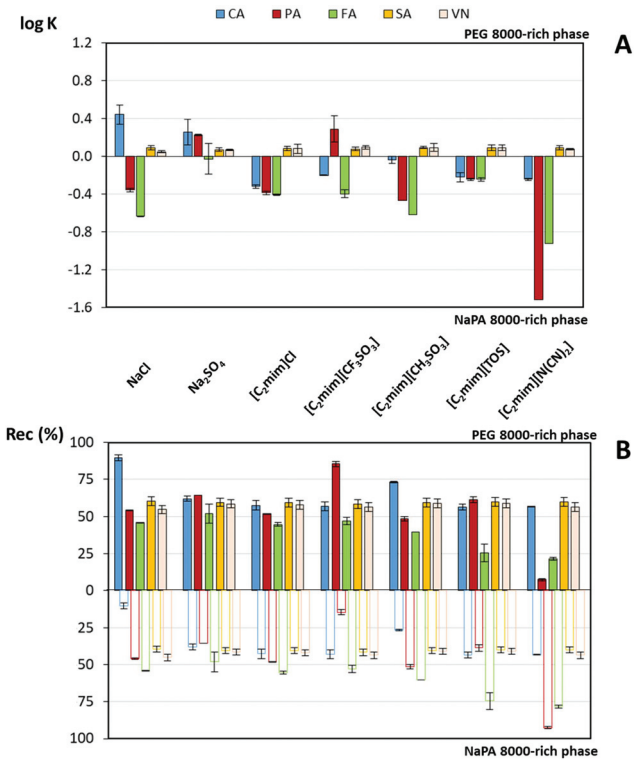

Fig. 2 (A) Partition coefficient in $\log_{10}$, ($\log_K$), and (B) recovery, Rec (%), of phenolic compounds between the PEG 8000-(top)- and NaPA 8000-(bottom)-rich phases in different polymer-based ABS.

0.44 and $Rec_{Top}CA$ = 89.6 ± 1.8%) and Na_2SO_4 ($\log K_{CA}$ = 0.26 and $Rec_{Top}CA$ = 62.0 ± 1.9%) are used as electrolytes. Regarding the phenolic aldehydes' partition profile, it can be observed that in all the studied ABS both VN and SA show a preference for the PEG 8000-rich phase. Thus, the negative charge of the phenolic aldehydes at the system pH, combined with the negative charge of NaPA species, leads to the partition of the VN and SA towards the PEG-rich phase ($\log K_{VN} > 0$ and $\log K_{SA} > 0$), through electrostatic interactions. Additionally, the electrolyte nature is a negligible factor for the phenolic aldehyde partition, with the NaPA 8000–VN and NaPA 8000–SA repulsion interactions being the main driving-forces for these biomolecules.

The selectivity was also determined (Table 2) to assess the capacity of the investigated ABS to separate the mixture of phenolic compounds. The capacity of each ABS to separate each phenolic compound is different and depends on the electrolyte used.

The best results of selectivity were achieved for the ABS formed when NaCl ($1.1 < S < 12.1$), $[C_2mim][CF_3SO_3]$ ($1.0 < S < 4.9$), $[C_2mim][CH_3SO_3]$ ($1.0 < S < 5.2$) and $[C_2mim][N(CN)_2]$ ($1.0 < S < 36.3$) were applied as electrolytes. Considering the results obtained in the optimization step, the most promising ABS to be used in CPC was the one using NaCl as the electrolyte. This ABS was selected due to its low cost, high selectivity and good separation performance, *i.e.* low partition coefficients, yet sufficiently different for the various phenolic compounds. Moreover, this was shown to be the system with the highest selectivity for the two phenolic aldehydes which are the most similar compounds in terms of partition profile. Although $[C_2mim][N(CN)_2]$ has, in general, a higher range of selectivity values, the partition coefficients of PA and FA are below the acceptable K values required to use CPC, and thus the NaCl-based system was the best option to be scaled-up.

Table 2 Selectivity values (S) obtained for ferulic (FA), protocatechuic (PA) and caffeic (CA) acids, vanillin (VN) and syringaldehyde (SA) using inorganic salts and ionic liquids as electrolytes in PEG/NaPA-based ABS

Selectivity among the same class of phenolic compounds						
Electrolyte	$S_{CA/SA}$	$S_{CA/VN}$	$S_{PA/SA}$	$S_{PA/VN}$	$S_{FA/SA}$	$S_{FA/VN}$
NaCl	2.3	2.5	2.8	2.5	5.3	4.9
Na_2SO_4	1.5	1.5	1.4	1.4	1.3	1.3
$[C_2mim]Cl$	2.5	2.5	3.0	3.0	3.1	3.1
$[C_2mim][CF_3SO_3]$	1.9	2.0	1.6	1.6	3.0	3.1
$[C_2mim][CH_3SO_3]$	1.3	1.3	3.6	3.6	5.2	5.2
$[C_2mim][TOS]$	2.1	2.1	2.2	2.2	2.2	2.2
$[C_2mim][N(CN)_2]$	2.2	2.1	36.1	35.1	10.3	9.9

Selectivity among different classes of phenolic compounds				
Electrolyte	$S_{FA/CA}$	$S_{CA/PA}$	$S_{FA/PA}$	$S_{VN/SA}$
NaCl	12.1	6.3	1.9	1.1
Na_2SO_4	2.0	1.1	1.9	1.0
$[C_2mim]Cl$	1.2	1.2	1.1	1.0
$[C_2mim][CF_3SO_3]$	1.6	3.1	4.9	1.0
$[C_2mim][CH_3SO_3]$	3.8	2.7	1.4	1.0
$[C_2mim][TOS]$	1.1	1.1	1.0	1.0
$[C_2mim][N(CN)_2]$	4.8	16.8	3.5	1.0

Separation of phenolic compounds using centrifugal partition chromatography (CPC)

To show the advantages of using CPC to promote the purification of complex mixtures derived from lignin depolymerisation, two scenarios were tested: scenario (A) in which three phenolic acids were purified and scenario (B) in which the separation of a more complex mixture of five phenolic compounds (three phenolic acids and two phenolic aldehydes) was investigated.

(A) Model mixture of phenolic acids (FA, PA and CA)

The best ABS identified before, composed of 15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 5 wt% of NaCl + 74.5 wt% of water, was used for the separation of the three phenolic compounds using CPC. As previously mentioned, this ABS presents partition coefficients (K) for the phenolic acids in an adequate range for use in CPC (K_{CA} = 2.78 ± 0.2, K_{PA} = 0.44 ± 0.04 and K_{FA} = 0.23 ± 0.01) and higher selectivity values. To select the optimum conditions to separate the three phenolic acids, preliminary studies were performed considering the combination of two different mobile phases, respectively the PEG- and NaPA 8000-rich phases; flow rates, respectively 1.0 and 1.5 mL min^{-1}; rotation speeds, respectively 1500 and 2000 rpm; and ascending or descending modes. After some preliminary assays, the flow rate of 1.5 mL min^{-1}, the rotation speed of

2000 rpm, and the ascending mode were selected to perform further studies. These conditions provide a stationary phase retention (*i.e.* the volume ratio between the stationary phase and the column volume) of 37% allowing one to perform a fast separation of the target acids under constant pressure (4.5 MPa). Fig. 3 presents the chromatogram resulting from the batch injection of a mixture (5 mL) of CA (1 mg), PA (1 mg), and FA (1 mg), performed in the ascending mode and with a mobile phase flow rate of 1.5 mL min^{-1}. The top PEG 8000-rich phase was used as the mobile phase during the initial stage of elution (from 0 to 55 min). Afterwards, and for the elution of CA, the mobile phase was changed to the NaPA 8000-rich phase, to separately elute the PA and FA, respectively. This change in the mobile phase was required to decrease the elution time of the two last phenolic acids, since both acids have a higher affinity to the stationary phase used during the initial stage of elution, the NaPA 8000-rich phase. No losses of the stationary phase were observed during the separation run, and the complete separation of the three compounds was achieved, with the CA eluted in fractions 5–8, the FA in fraction 25, and the PA in fraction 28. Since NaPA 8000 absorbs strongly in the UV region, it was not possible to observe the phenolic characteristic peaks of FA and PA since these are masked by the polymer. The identification of these two phenolic acids in the respective fractions was achieved after the removal of NaPA 8000 from the fractions collected using dialysis. The peak observed in the fractions 18–19 corresponds to the change of the mobile phase from PEG to NaPA aqueous solutions. In agreement with the partition coefficients determined for the PEG 8000 + NaPA 8000 + water + NaCl system (presented in Table 2), it is here confirmed that CA has the highest affinity to the PEG 8000-rich phase, the first compound being eluted. Both PA and FA have a low affinity to the PEG 8000-rich phase ($K < 1$), which justifies the application of an elution–extrusion process.[44] By changing the mobile phase from a PEG 8000- to a NaPA 8000-rich phase, the FA was first eluted (due to its smaller K value), followed by the PA. A higher separation resolution was achieved at a mobile phase flow rate of 1.5 mL min^{-1} (Fig. 4) than with the rate of 1.0 mL min^{-1} (Fig. S7 in the ESI†). This was attributed to the lower retention time of the phenolic acid elution and to the higher stationary phase retention (two times greater S_f), with the compounds being eluted at lower elution times. In addition, by increasing the flow rate from 1.0 mL min^{-1} to 1.5 mL min^{-1}, a better resolution of protocatechuic and ferulic acids was obtained. With a flow rate of 1.0 mL min^{-1}, the PA and FA were co-eluted.

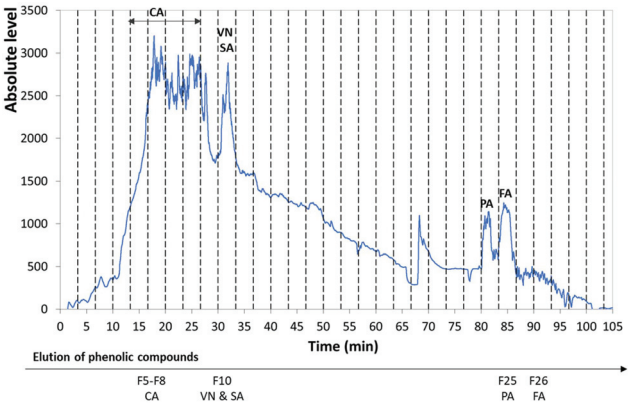

Fig. 4 Separation of phenolic compounds by CPC using the system composed of 15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 75.5 wt% of water + 5 wt% of NaCl. Experimental conditions: rotation speed of 2000 rpm min^{-1}; flow-rate of 1.5 mL min^{-1}; S_f = 37%; $P \approx 4.5$ MPa; detection wavelength: 280 nm, concentration of phenolic compounds: 0.4 mg mL^{-1}.

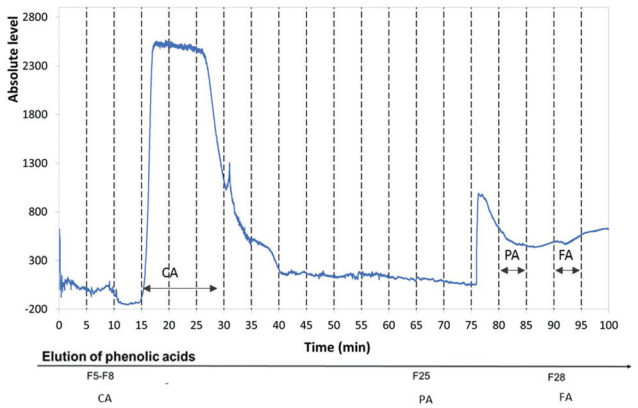

Fig. 3 Separation of phenolic acids by CPC using the system composed of 15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 75.5 wt% of water + 5 wt% of NaCl. Experimental conditions: rotation speed of 2000 rpm min^{-1}; flow-rate of 1.5 mL min^{-1}; S_f = 37%; $P \approx 4.5$ MPa; detection wavelength: 280 nm, concentration of phenolic compounds: 0.4 mg mL^{-1}.

Summing up, the experiments and results discussed before demonstrate the potential of polymer-based ABS as alternative and versatile platforms for the separation of phenolic acids using CPC. It was shown that any of the studied polymeric phases could be used as the stationary or the mobile phase in CPC. In spite of the viscosity of the NaPA 8000-rich phase, the design of the twin-cells of the CPC column enabled the use of high flow rates, while attaining a high stationary phase retention.[49]

(B) Model mixture of five phenolic compounds (phenolic acids and phenolic aldehydes)

To extend the range of phenolic compounds derived from the lignocellulosic depolymerisation process that could be fractionated using CPC, a more complex mixture with five compounds, namely the three phenolic acids (CA, FA and PA) previously studied and two phenolic aldehydes (VN and SA), was tested. Before the CPC purification, the partition profile of these two phenolic aldehydes was evaluated as depicted in Fig. 2 and Table S4 (ESI†). From the results obtained it was found that these compounds have more affinity to the PEG-rich phase, a tendency revealed independent of the electrolyte used. The partition of these compounds follows the order: $K_{CA} > K_{VN} \approx K_{SA} > K_{PA} > K_{FA}$. It is expected that in CPC,

both aldehydes elute at the same time, after the CA and before the PA and FA.

As previously justified, the same ABS (15 wt% of PEG 8000 + 4.5 wt% of NaPA 8000 + 5 wt% of NaCl + 74.5 wt% of water) was used for the fractionation of these phenolic compounds using CPC.

Fig. 4 shows the chromatogram resulting from the batch injection of a mixture (5 mL) of CA (2 mg), PA (2 mg), FA (2 mg), VN (2 mg) and SA (2 mg) performed in the ascending mode and with a mobile phase flow rate of 1.5 mL min^{-1}. The top PEG 8000-rich phase was used as the mobile phase during the initial stage of elution (from 0 to 55 min) to elute separately the CA from the phenolic aldehydes. Afterwards, and for the elution of these compounds, the mobile phase was changed to the NaPA 8000-rich phase, to separately elute the PA and FA, respectively. The complete separation of the two classes of phenolic compounds (acid and aldehydes) was achieved with no losses of stationary phase witnessed. In fact, and using CPC, it was possible to obtain four fractions rich in FA, CA, PA, and both aldehydes, respectively. Only the separation of SA and VN was not achieved due to their similar hydrophobicity and structure.

Due to the high relevance of fractionating different compounds from the lignin depolymerisation, some studies have been reported with this objective. Supercritical carbon dioxide extractions, ionic liquid (IL) extractions, and adsorption in specific polymeric resins are some of the main fractionation techniques already described for lignocellulosic products (*e.g.* vanillin, syringaldehyde and *p*-hydroxybenzaldehyde).[17] However, the high cost and difficult scale-up are the main disadvantages of these techniques. Meanwhile, some other fractionation processes using CPC were found in the literature. However, when comparing the main results from the literature with our data, it can be concluded that the use of organic solvents (*e.g.* *n*-heptane, ethyl acetate, and methanol) and water as biphasic solvents lead to inefficient separations[37,38] represented also by lower selectivity values, since the phenolic acids are co-eluted in the same fraction (*i.e.* gallo-catechin, sinapic acid and two phenolic acid amides).[37]

Integrated purification process

Isolation of phenolic compounds and ABS phase-forming component recycling. The isolation of the studied phenolic compounds, namely CA and phenolic aldehydes from the PEG 8000-rich fractions and FA and PA from the NaPA 8000-rich fractions, after their separation by CPC, was experimentally addressed through dialysis. The dialysis of both the PEG 8000-rich and NaPA 8000-rich phases was executed using a membrane with a MWCO = 1 kDa, allowing the polymer retention and the release of the phenolic compounds in the permeate.

Isolation of CA. Around 87% of the initial mass of CA added to the system was recovered after two washing steps with water (68% and 19% in the first and second steps of dialysis, respectively). It should be highlighted that after the dialysis step, not only the CA was removed, but also the PEG 8000-rich phase was purified.

Isolation of phenolic aldehydes both concentrated in the same fraction. The isolation of phenolic aldehydes from the PEG 8000-rich fraction was achieved with a yield of 82% of VN and SA (both concentrated in the same fraction).

Isolation of FA and PA. For the isolation of FA and PA from the NaPA 8000-rich phase, a dialysis step was performed. In this polishing step of FA and PA, 84% and 65% were recovered, respectively. After lyophilisation of both FA and PA, a ^{1}H NMR (depicted in Fig. S9 and S10 in the ESI†) analysis was carried out confirming the high purity of each phenolic acid isolated. To summarize, Table S5† presents the experimental data obtained for the recovery of each phenolic compound after the dialysis step.

Polymers recycling after the removal of phenolic compounds by dialysis. After the isolation of the phenolic compounds, the purity of the polymeric fractions obtained after the phenolic compound isolation was also tested by ATR-FTIR. The data obtained by ATR-FTIR confirmed the separation of both polymers, PEG 8000- and NaPA 8000, from the phenolic fractions (Fig. S8†).

After the phenolic compound separation, NaPA 8000 and PEG 8000 aqueous solutions could be directly reused in a new purification cycle, as previously described for similar systems.[50–52] Taking the previous experimental results into account and envisaging the industrial application of the technique developed here, an integrated process for the purification of phenolic compounds was designed as presented in Fig. 5.

Environmental assessment

When the industrial implementation of one purification process is envisaged, it is important not only to characterize all the steps involved but also to evaluate the environmental impact of the proposed process. In this context, the carbon footprint of the integrated system using CPC and ultrafiltration was studied for 1000 g of ABS (Fig. 6). At this stage, two scenarios were considered: (i) considering the reuse of the phase-forming components (PEG 8000 and NaPA 8000) and (ii) not considering the reuse of the polymers. Fig. 6 shows an estimation of 0.87 kg $CO_{2\ eq}$ when there is no reuse of PEG 8000- and NaPA 8000-rich phases after ultrafiltration. The carbon footprint decreases by 36% (0.55 kg $CO_{2\ eq}$) when these phases are reused as the consumption of new chemicals and water is considerably reduced. In this scenario, there is a slight increase of electricity consumption for pumping the reused phases, but its carbon footprint is almost negligible. The main contribution to the carbon footprint in both scenarios comes from the production of the electricity consumed, mainly in CPC. This contribution is particularly relevant in the reuse scenario, being almost 90% of the total carbon footprint. The lack of published data on the carbon footprint of similar or alternative integrated purification systems of phenolic acids prevents a comparison with the results of the carbon footprint obtained in this study.

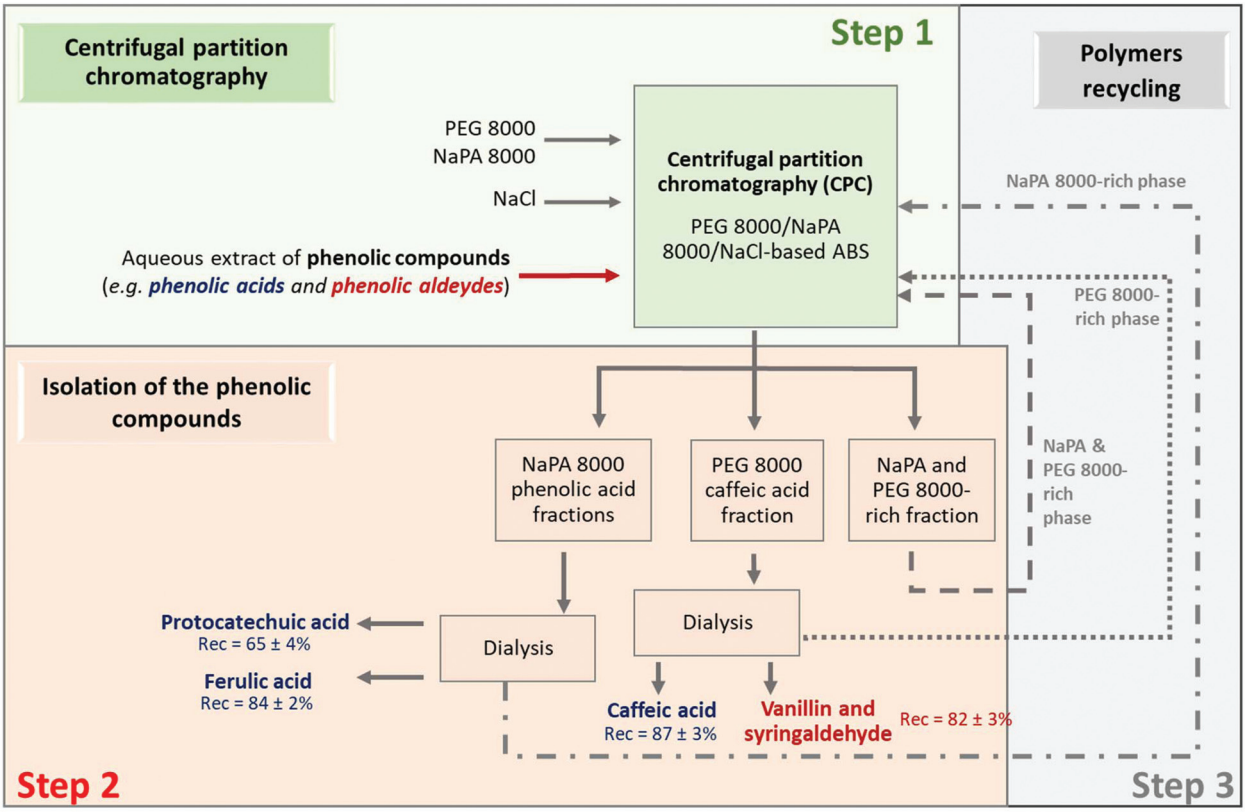

Fig. 5 Schematic representation of the envisioned scaled-up process of purification of five phenolic compounds by applying PEG 8000/NaPA 8000-based ABS using NaCl as the electrolyte and CPC. The isolation of the phenolic compounds from each aqueous phase and the respective phase-forming components' reuse are also presented. Steps 1–3 were experimentally carried out in this work (numerical results presented in Table S5 in the ESI†).

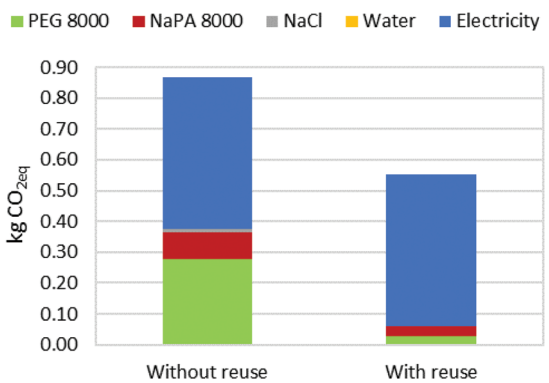

Fig. 6 Carbon footprint of the integrated system composed by CPC and ultrafiltration for 1000 g of ABS, for the scenarios without and with the reuse of PEG 8000- and NaPA 8000-rich phases after ultrafiltration.

Conclusions

PEG/NaPA-based ABS using inorganic salts or ionic liquids as electrolytes were investigated to separate phenolic compounds. A purification process using these polymeric-based ABS integrated with CPC was shown. The separation of three structurally similar natural compounds (phenolic acids) was firstly optimized, allowing one to prove the importance of the electrolyte nature, not only in terms of the phase diagram design (as discussed elsewhere[31]), but also mainly in terms of the different partition tendencies observed for each phenolic compound. Moreover, the study of a more complex mixture was also carried out, in which two different classes of compounds were tested, namely the three phenolic acids previously mentioned and two aldehydes (VA and SA). The PEG 8000/NaPA 8000/NaCl-based ABS was identified as the most efficient system for the separation of the most complex mixture, and was further used in CPC to support its scale-up. After the optimization of the operational conditions, successful results, *i.e.* the complete separation of the three phenolic acids and the separation of an aldehyde-rich fraction, were obtained with CPC. The recovery of the three phenolic acids was, respectively, 87%, 84% and 65% for CA, FA and PA, and 82% for the aldehyde-rich fraction. Based on these promising results, an integrated process implemented using CPC in a continuous regime was developed allowing the complete separation of monomeric phenolic lignin-based compounds. Finally, the environmental impact of the integrated process was evaluated. The reuse, or not, of the polymeric phases were the two scenarios under study. In this context, the carbon footprint of the

two processes (with and without the reuse of PEG 8000- and NaPA 8000-rich phases after ultrafiltration) was estimated. This green metric shows the importance of the reuse of these polymeric phases in terms of sustainability of the entire process, with the carbon footprint being decreased by 36%. Furthermore, novel approaches for the recovery of the phenolic compounds and the reuse of the phase-forming components have been developed. Based on the success of this work, its applicability for the processing of real matrices,[53] *e.g.* agro-industrial lignocellulosic residues, biomass wastes and forestry residues, is certainly an open topic for future investigations.

Conflicts of interest

There are no conflicts to declare.

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