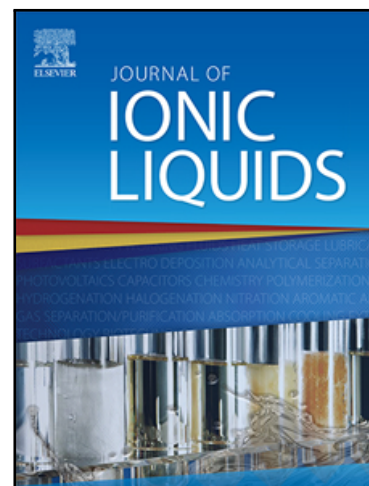


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Aqueous Biphasic Systems Composed of Ionic Liquids and Dextran: From the Understanding of Phase Formation to the Potential of Exopolysaccharide Recovery

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Abstract

Aqueous biphasic systems (ABS) are effective strategies for separating and purifying (bio)molecules. Ionic liquid (IL)-based ABS are particularly attractive, as combining ILs with salts, saccharides, amino acids, or polymers produces systems with tuneable phase polarities suited for customised liquid–liquid extraction. Although dextran is widely used in polymer–polymer ABS, its combination with ILs remains underexplored. This work shows, for the first time, that IL-based ABS can be formed with dextran. New ternary phase diagrams for systems composed of water, imidazolium-based ILs and dextran polymers were determined at 298 K. The effects of dextran molecular weight and IL cation/anion structure were assessed and related to the ABS-forming ability. Higher molecular weight dextran polymers, containing more hydroxyl groups, interact more strongly with water and promote a stronger sugaring-out effect, thereby enhancing phase separation. ILs with weaker cation/anion–water interactions showed expanded biphasic regions, with ABS formation correlating with IL anion’s hydrogen-bond basicity. Increasing dextran concentration decreases the influence of the IL chemical structure, leading to overlapping binodal curves and demonstrating that phase behaviour is primarily governed by the polymer’s sugaring-out capacity and its competition with IL–water interactions. Owing to dextran’s high hydrophilicity relative to polyethers typically used in IL/polymer ABS, IL–dextran interactions exert minimal impact on phase diagram behaviour. Finally, by selecting an appropriate IL, the concentration of exopolysaccharides (EPS) such as dextran in one ABS coexisting phase can be maximised. This strategy enables ABS formation directly in EPS-rich matrices (e.g., fermentation broths), supporting *in situ* EPS recovery. This possibility was demonstrated using a [C₄mim][C₁SO₄]/Dextran 40 kDa ABS and a simulated broth containing 20 wt% of sucrose, achieving high concentration factors for sucrose in the IL-rich (top) phase ($K_{\text{sucrose}} \approx 32$) and for dextran in the EPS-rich (bottom) phase ($K_{\text{dextran}} \approx 0.04$).

Keywords: Aqueous Biphasic System; Ionic Liquid; Dextran; Separation; Exopolysaccharides.

1. Introduction

The demand for more benign and sustainable liquid-liquid extraction (LLE) processes has driven the development of new biphasic systems capable of recovering target solutes with enhanced efficiency and selectivity, particularly from bio-based, water-rich matrices. The main challenge has been to achieve a balance between extraction efficiency and compatibility with the physicochemical characteristics of both solvents and solutes, especially by avoiding the use of harmful chemicals while maintaining suitable system properties. Viewed as a greener alternative to conventional aqueous/organic systems, Aqueous Biphasic Systems (ABS) (also known as Aqueous Two-Phase Systems, ATPS) have emerged as an eco-friendly and biocompatible system to operate LLE processes [1].

ABS consist of two aqueous-rich phases formed by mixing in water at least two water-soluble components above a critical concentration, enabling the extraction of target biomolecules by manipulating their affinity for each phase. As both phases contain large amounts of water, these systems preserve the native conformation and biological activity of biomolecules in solution [2]. These features have led to the widespread exploration of ABS for the extraction and purification of a variety of solutes, including proteins, natural dyes and pigments [3], metals [4] and industrial solutes [5].

A landmark contribution in ABS research was made by Rogers' research group, who pioneered the studies on the formation of ABS using hydrophilic ionic liquids (ILs) and concentrated aqueous solutions of inorganic salts [6]. This development was a *game-changer* in the field of ABS, particularly influential to our research group, as it inspired us to explore the use of ILs in ABS to separate a wide range of high-value compounds while also addressing the molecular-level mechanisms ruling phase separation. It opened doors and paved the way of collaborative efforts between the two research groups, leading to new studies that combined ILs and polymers, and their application to a wide range of separation and purification processes [7–11].

The tunable nature of ILs, achieved through the appropriate selection of their constituent ions, enables the formation of systems spanning a wider hydrophilicity–hydrophobicity spectrum [12]. This versatility allows the design of biphasic systems with tailored properties that enhance phase separation and selectivity towards specific solutes [13]. Moreover, the increasing focus on environmentally benign extraction techniques encourages the use of ILs instead of volatile organic solvents that may present some negative impact due to their toxic and denaturing characteristics [14]. ILs have thus

gained attention as alternative solvents for diverse separations and reactions. Combining the properties of conventional salts with those of organic liquids, ILs exhibit low to negligible vapour pressures, do not evaporate under atmospheric conditions, and remain thermally stable up to high temperatures [15,16]. They are also liquid across wide temperature ranges, possess high thermal conductivity, and have broad electrochemical windows [17,18]. Owing to these properties, ILs have been applied in catalysis [19,20], organic synthesis [21], chemical and enzymatic reactions [22], multiphase bioprocess operations [23], mass spectrometry [24], biofuel production [25], among other relevant examples.

Although ILs can be costly, their potential for recovering and reuse may turn their application more feasible and cost-effective [26]. IL-based ABS present notable advantages over conventional systems, including minimal emulsion formation, low viscosity, rapid phase separation, and high extraction efficiency, features that can be tuned through appropriate ion selection [27,28]. Such systems have been successfully employed in the separation, concentration, and purification of proteins [29,30], heavy metal ions [31,32], small organic molecules [33,34], and antibiotics [35,36]. Recently, more environmentally benign systems have been reported, using amino acids or carbohydrates instead of inorganic salts to form IL-based ABS [37]. Carbohydrates, including mono- and disaccharides, exhibit high water affinity and can salt out ILs from aqueous media, giving rise to ABS [38–40]. Dextran, a polysaccharide of glucose units produced by *Leuconostoc* or *Streptococcus* species [41], is a well-known carbohydrate polymer used in polymer–polymer ABS [42]. Despite its potential as a two-phase promoter, no studies have yet explored the use of dextran for forming IL-based ABS.

To address this research gap, this study investigates novel IL + dextran + water systems. The ternary phase diagrams for 20 different IL/dextran ABS were experimentally determined at 298 K. The effects of dextran molecular weight, IL cation/anion alkyl chain length, IL anion nature, and polymer concentration on ABS formation were systematically evaluated. Furthermore, the main phase separation mechanisms governing these systems are discussed, providing new insights into their thermodynamic behaviour and highlighting the potential of IL/dextran ABS for the enrichment of exopolysaccharides (EPS) from fermentation broths.

2. Experimental Section

2.1 Materials

The ionic liquids (ILs) investigated in this work were: 1-ethyl-3-methylimidazolium trifluoromethanesulfonate, $[\text{C}_2\text{mim}][\text{CF}_3\text{SO}_3]$; 1-butyl-3-methylimidazolium dicyanamide, $[\text{C}_4\text{mim}][\text{N}(\text{CN})_2]$; 1-butyl-3-methylimidazolium ethylsulfate, $[\text{C}_4\text{mim}][\text{C}_2\text{SO}_4]$; 1-butyl-3-methylimidazolium methylsulfate, $[\text{C}_4\text{mim}][\text{C}_1\text{SO}_4]$; 1-butyl-3-methylimidazolium octylsulfate, $[\text{C}_4\text{mim}][\text{C}_8\text{SO}_4]$; 1-butyl-3-methylimidazolium tetrafluoroborate, $[\text{C}_4\text{mim}][\text{BF}_4]$; 1-methyl-3-methylimidazolium tosylate, $[\text{C}_4\text{mim}][\text{TOS}]$; 1-butyl-3-methylimidazolium trifluoromethanesulfonate, $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ and 1-hexyl-3-methylimidazolium dicyanamide, $[\text{C}_6\text{mim}][\text{N}(\text{CN})_2]$. The chemical structure of the dextran polymer is presented in **Figure S1** in the **Supplementary Information (SI)**.

All ILs were supplied by Iolitec with a purity higher than 98 wt%. The dextrans, obtained from *Leuconostoc spp.*, were purchased from Sigma-Aldrich. Three dextrans of different molecular weight were studied: 6 kDa, 40 kDa, and 100 kDa.

3,5-dinitrosalicylic acid (DNS) 98% pure and sucrose >99.5 wt% pure were purchased at Panreac Sintesis and Himedia, respectively.

The water used in all experiments was doubly distilled, passed through a reverse osmosis system, and further purified using a Milli-Q Plus 185 water purification apparatus.

2.2 Determination of Ternary Phase Diagrams

For the determination of ternary phase diagrams, aqueous solutions of Dextran (6 kDa, 40 kDa, and 100 kDa) at 40 wt% were prepared. The ILs that are liquid at room temperature were used as received (100 wt%), while those solid at room temperature were prepared as aqueous solutions at 80 wt%.

The binodal (solubility) curves were determined at room temperature (298 ± 1) K and atmospheric pressure using the cloud point titration method, with temperature controlled by a thermostatic bath [28]. Briefly, this method involves the dropwise addition of the IL (either pure or as an aqueous solution) to the dextran aqueous solution under continuous stirring until the cloud point is reached (identified by the appearance of turbidity), indicating ABS formation. Subsequently, water is added dropwise until the solution becomes clear and homogeneous again, marking the return to the monophasic region. Additional IL is then introduced to the clear solution to induce a new phase separation, and this cycle is repeated until no further turbidity is observed.

The mass fraction compositions were determined gravimetrically (to within 10^{-4} g) at each cloud and clear point using an analytical balance (Mettler TOLEDO Excellence, XS205 Dual Range).

2.3 EPS Partitioning

To demonstrate the potential of IL/Dextran ABS for the concentration of EPS from fermentation broths, a model system simulating microbial dextran production was prepared using a sucrose aqueous solution with the same concentration as that typically found in fermentation broths (20 wt%) [43]. The ABS was composed of 43.5 wt% of $[C_4mim][C_1SO_4]$, 20 wt% of Dextran 40 kDa, and 36.5 wt% of sucrose aqueous solution. The sucrose solution was prepared by dissolving 10 g of sucrose in 40 g of water. Subsequently, 2.55 g of this solution were mixed with 3.05 g of IL and 1.40 g of Dextran 40 kDa, resulting in a total extraction system mass of 7 g. The biphasic mixture was vigorously vortexed, centrifuged, and the phases were carefully separated using a syringe and weighed. Approximately 3 mL of each phase was obtained after separation, and 2 g of each being used for subsequent dextran and sucrose quantification.

The biphasic mixture was vigorously vortexed, centrifuged, and the phases were carefully separated using a syringe and weighed. Dextran precipitation was carried out by adding absolute ethanol (1:1) to each phase. The solutions were vortexed again and centrifuged at 3500 rpm at room temperature for 30 min. The supernatant was collected for sucrose quantification, while the precipitate was dried in a 378 K oven for gravimetric determination of dextran content, up to constant weight.

Sucrose present in the supernatant of both phases was hydrolysed by adding undiluted HCl (340 K, 5 min), followed by pH neutralisation with NaOH. A sucrose calibration curve (**Figure S2** in the **Supplementary Information (SI)**) was constructed using the reaction with 3,5-dinitrosalicylic acid (DNS) [44]. Absorbance measurements were performed with a Pharma-Spec Spectrometer (SHIMADZU UV-1700) at 540 nm.

The concentration values of dextran and sucrose determined in each phase were then used to calculate the partition coefficients of sucrose and dextran, expressed as the ratio of their concentrations in the top (IL-rich) phase to those in the bottom (dextran-rich) phase.

3. Results and Discussion

3.1 Phase Diagrams

As a pioneering study on the use of dextran as an ABS-inducing polymer, novel ternary phase diagrams were determined for IL + dextran + water systems at room temperature (298 K) and atmospheric pressure. In total, 20 phase diagrams were successfully determined from the 21 possible combinations, with only the Dextran 6 kDa + [C₄mim][C₁SO₄] + water system failing to form a biphasic system.

This extensive dataset enabled a comprehensive evaluation of the effects of dextran molecular weight, the alkyl chain length of IL cations and anions, and the intrinsic nature of the IL anion on the phase separation mechanisms governing ABS formation. All binodal curves are expressed in molality units (mol of IL or dextran *per* kg of solvent), allowing the direct comparison of the phase-forming ability of ILs and dextran polymers while avoiding inconsistencies arising from differences in their molecular weights. The experimental molality data are compiled in **Tables S1–S7**, while the corresponding weight percentage (wt%) data are presented in **Tables S8–S14** of the **Supplementary Information (SI)**. **Table 1** presents the experimental data obtained for the [C₄mim][CF₃SO₃]/Dextran 40 kDa ABS as a representative example.

Table 1. Experimental molality and weight percentage data of the [C₄mim][CF₃SO₃] (IL)/Dextran 40 kDa ABS. The data of the remaining phase diagrams is presented as **SI**. All experimental data were determined at 298 K and 1 atm with an uncertainty of $\pm 10^{-4}$ g.

Molality (mol.kg ⁻¹)				wt%			
IL	Dextran 40 kDa	IL	Dextran 40 kDa	IL	Dextran 40 kDa	IL	Dextran 40 kDa
0.36748	0.01418	1.07232	0.00260	36.19	9.58	9.42	23.61
0.39816	0.01235	1.07844	0.00256	33.07	10.30	9.29	23.72
0.42733	0.01087	1.08625	0.00253	30.31	10.97	9.18	23.85
0.46891	0.01017	1.08242	0.00247	28.93	11.91	8.98	23.78
0.49651	0.00931	1.10445	0.00242	27.13	12.52	8.84	24.15
0.53100	0.00878	1.10172	0.00237	25.98	13.28	8.66	24.11
0.54854	0.00810	1.10607	0.00234	24.47	13.65	8.54	24.18
0.58253	0.00774	1.11073	0.00230	23.64	14.38	8.43	24.25
0.59521	0.00718	1.11759	0.00227	22.31	14.65	8.33	24.37
0.62419	0.00691	1.12312	0.00224	21.64	15.25	8.23	24.46
0.63177	0.00669	1.12712	0.00221	21.11	15.41	8.13	24.52
0.65739	0.00646	1.13460	0.00218	20.54	15.93	8.03	24.65
0.67846	0.00619	1.14140	0.00216	19.84	16.36	7.95	24.76
0.70722	0.00596	1.15005	0.00213	19.26	16.94	7.86	24.90
0.71350	0.00560	1.15684	0.00211	18.31	17.06	7.77	25.01
0.72302	0.00544	1.16170	0.00208	17.87	17.25	7.69	25.09
0.74015	0.00525	1.16142	0.00205	17.35	17.59	7.58	25.08
0.75884	0.00509			16.92	17.95		
0.77407	0.00493			16.48	18.24		
0.79435	0.00479			16.09	18.63		
0.81120	0.00466			15.70	18.95		
0.82590	0.00452			15.33	19.23		
0.83973	0.00440			14.97	19.49		
0.85773	0.00429			14.64	19.83		
0.86140	0.00412			14.15	19.89		
0.87426	0.00402			13.86	20.13		
0.88778	0.00393			13.58	20.38		
0.90088	0.00385			13.34	20.62		
0.91343	0.00377			13.11	20.84		
0.91176	0.00365			12.73	20.81		
0.92286	0.00358			12.51	21.01		
0.93578	0.00350			12.29	21.25		
0.94614	0.00344			12.09	21.43		
0.95562	0.00337			11.88	21.60		
0.96956	0.00331			11.69	21.85		
0.96794	0.00321			11.38	21.82		
0.97703	0.00316			11.21	21.98		
0.98598	0.00310			11.04	22.13		
0.99694	0.00305			10.88	22.32		
1.00643	0.00300			10.72	22.49		
1.01535	0.00295			10.57	22.64		
1.02182	0.00291			10.42	22.75		
1.03055	0.00286			10.27	22.90		
1.03589	0.00281			10.11	23.00		
1.04425	0.00277			9.98	23.14		
1.05366	0.00273			9.83	23.30		
1.06245	0.00268			9.69	23.45		
1.06586	0.00264			9.56	23.50		

3.1.1 Effect of Dextran Molecular Weight

Given the large number of ABS formed, an understanding of the factors governing phase separation could be achieved through systematic analysis of the phase diagrams and the corresponding ABS formation trends. The first parameter examined was dextran molecular weight. To this end, the phase diagrams of $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ -based systems containing dextran of 6 kDa, 40 kDa, and 100 kDa were compared, as shown in **Figure 1**.

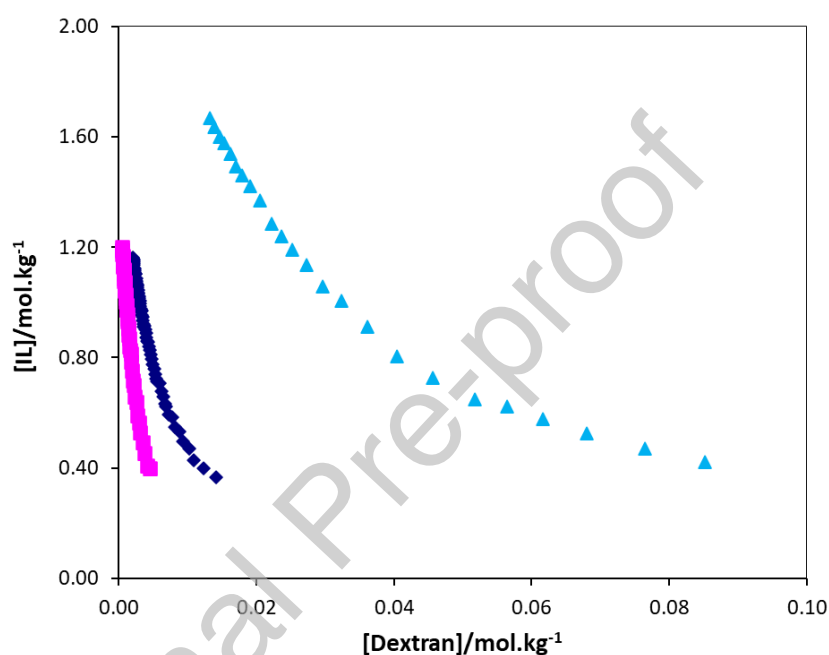


Figure 1. Phase diagrams for the ternary systems composed of $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ + Dextran + water at 298 K: (\blacktriangle) Dextran 6 kDa; (\blacklozenge) Dextran 40 kDa; (\blacksquare) Dextran 100 kDa.

Analysis of **Figure 1** clearly shows that increasing dextran molecular weight leads to a substantial expansion of the biphasic region, following the trend 6 kDa < 40 kDa < 100 kDa. This behaviour is attributed to the higher number of hydroxyl groups and enhanced hydration capacity of higher-molecular-weight dextran polymers, which interact strongly with water molecules, reducing free water, and effectively inducing a “sugaring-out” effect on the IL from the polymer’s vicinity [39,40].

These results are in good agreement with observations for IL-based ABS composed of mono- and disaccharides or polyols, in which the carbohydrate exerts a sugaring-out effect over the IL [39,40]. Furthermore, higher concentrations of these smaller carbohydrates are required to induce phase separation compared with high-molecular-weight polysaccharides such as dextran. This difference stems primarily from the lower

number of hydroxyl groups in the structures of simpler sugars [38–40]. Moreover, the high hydrophilicity of dextran and its high-water affinity minimise IL-polymer dispersive interactions that typically arise involving more hydrophobic ILs, an effect often observed in other IL/polymer ABS [11,45,46].

The effect of increasing polymer molecular weight in enhancing ABS formation observed here is analogous to that seen in IL/polyether ABS, such as those involving polyethylene glycol (PEG) and polypropylene glycol (PPG), although the underlying mechanisms differ due to the intrinsic relative hydrophobicity of polyether polymers [45,47]. In PEG and PPG polymers, hydroxyl groups are located only at the chain ends; therefore, increasing molecular weight primarily extends the aliphatic backbone and enhances the overall hydrophobic character of the polymer. Consequently, higher-molecular-weight PEG or PPG polymers exhibit weaker polymer–water interactions and enhanced phase separation [45–48].

In contrast, for IL/dextran ABS, the main mechanisms responsible for enlarging the biphasic region are clearly the enhanced hydrophilicity of the dextran and the intensified sugaring-out effect of dextran over the IL. Meanwhile, in polyether-based ABS, IL–polymer interactions play a crucial role in determining phase behaviour. Strong dispersive interactions between IL ions and the hydrophobic moieties of PEG or PPG can increase mutual miscibility [11,45], thereby narrowing the biphasic region, particularly in systems containing more hydrophobic ILs and in regions with lower water content (*i.e.*, close to the binary polymer-IL region [8]). In certain cases, such strong interactions may even lead to the formation of Type 0 phase diagrams, where no phase separation in the binary mixtures occurs, but is achieved on the ternary system [49].

3.1.2 Effect of the IL Cation and Anion Alkyl Chain Length

After establishing the effect of dextran molecular weight on ABS formation, the influence of the ion's alkyl chain length was investigated. **Figure 2** presents the ternary phase diagrams of ABS composed of ILs containing the dicyanamide ($[\text{N}(\text{CN})_2]^-$) anion with $[\text{C}_4\text{mim}]^+$ and $[\text{C}_6\text{mim}]^+$ cations, as well as systems with ILs containing the trifluoromethanesulfonate ($[\text{CF}_3\text{SO}_3]^-$) anion with $[\text{C}_2\text{mim}]^+$ and $[\text{C}_4\text{mim}]^+$, and the $[\text{C}_4\text{mim}]^+$ cation with $[\text{C}_1\text{SO}_4]^-$ and $[\text{C}_2\text{SO}_4]^-$ anions. For consistency, dextran 40 kDa was used as the polymer phase-forming component in all systems.

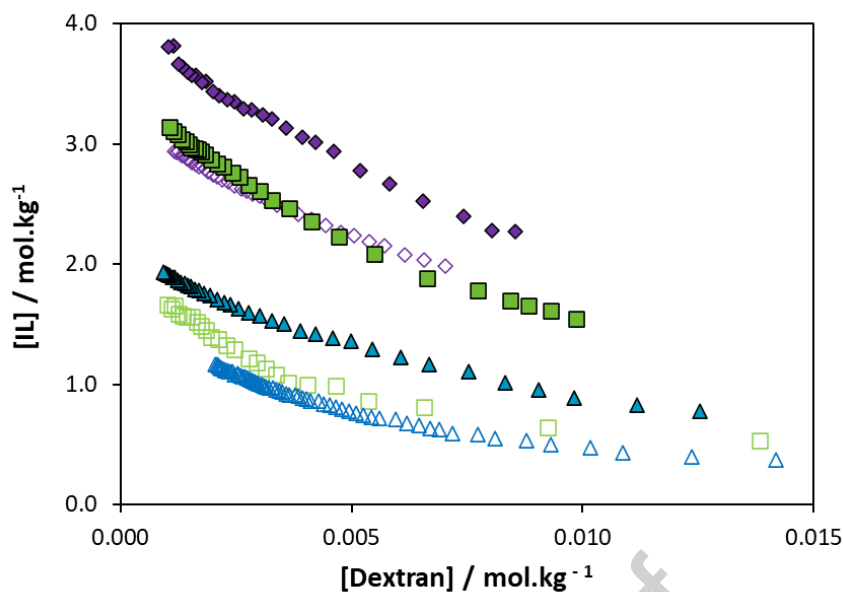


Figure 2. Phase diagrams for the ternary systems composed by IL + Dextran 40 kDa + water at 298 K: (■) $[C_4mim][N(CN)_2]$; (□) $[C_6mim][N(CN)_2]$; (▲) $[C_2mim][CF_3SO_3]$; (Δ) $[C_4mim][CF_3SO_3]$; (◆) $[C_4mim][C_1SO_4]$ and (◇) $[C_4mim][C_2SO_4]$.

From the results shown in **Figure 2**, it is clear that for the $[N(CN)_2]^-$ -based systems, the $[C_4mim]^+$ -based ATPS exhibits a larger biphasic region than the corresponding $[C_2mim]^+$ -based system. Similarly, for the $[CF_3SO_3]^-$ -based ATPS, the $[C_6mim]$ -based system presents a larger biphasic region than the $[C_4mim]^+$ -based one. Taken together, these observations indicate that, irrespective of the anion nature ($[N(CN)_2]^-$ or $[CF_3SO_3]^-$), increasing the alkyl chain length of the IL cation promotes the expansion of the biphasic region, following the trend: $[C_6mim]^+ > [C_4mim]^+ > [C_2mim]^+$. This behaviour with the increased relative hydrophobicity of the IL cations bearing longer cationic alkyl chains, which reduces their affinity for water molecules [1,50]. As a result, the sugaring-out effect induced by dextran is enhanced, decreasing the concentrations of the phase-forming components required for phase separation. Therefore, stronger dextran–water interactions, combined with weaker IL cation–water interactions, favour phase segregation and promote ABS formation.

This trend is commonly observed in IL/salt and IL/sugar systems, where phase separation is generally driven by the salts' salting-out/sugars' sugaring-out ability and the relative hydrophobicity of the IL [1,39,40,51]. In contrast, for IL/polymer ABS containing polyethers, as previously discussed, mutual solubility between the IL and the polymer becomes an additional factor influencing phase demixing. In these systems, hydrophobic interactions between the IL and the polymer play a crucial role in promoting liquid–liquid

separation, representing an extra governing force beyond the typical polymer–water and IL–water interactions [7,45].

From an application perspective, the negligible IL–polymer interactions observed here allow for a more predictive and controlled design of IL/dextran ABS tailored for specific solutes. The strong sugaring-out ability of dextran reduces the amount of carbohydrate required for phase separation and allows finer control over phase composition (e.g., tie-line lengths). Since IL/dextran ABS typically exhibit broader biphasic regions than IL/sugar systems, these systems represent a promising and tuneable platform for selective and efficient extraction in (bio)technological applications.

The influence of the IL anion's alkyl chain length on ABS formation was investigated by comparing systems containing dextran 40 kDa, water, and [C₄mim][C₁SO₄] or [C₄mim][C₂SO₄]. The respective ternary phase diagrams presented in **Figure 2** show that the formation of larger biphasic regions correlates positively with increasing IL anion alkyl chain length, following the trend [C₄mim][C₂SO₄] > [C₄mim][C₁SO₄]. This observation confirms the enhanced relative anion hydrophobicity of the longer-chain anion in [C₄mim][C₂SO₄], which weakens anion–water interactions and facilitates its sugaring-out by dextran. As a result, increasing the anion's alkyl chain length enhances the overall hydrophobicity of the IL and decreases its affinity for water, thereby promoting demixing and expanding the biphasic region, which is behaviour similar to that observed by increasing the IL cation alkyl chain length[39,40].

3.1.3 Effect of the IL Anion Nature

Given the large number of ternary phase diagrams and ILs examined, it was possible to analyse the influence of the anion nature on the formation of IL/dextran ABS, as well as its dependence on polymer concentration. The latter analysis was achieved through visual inspection of the binodal curve patterns across increasing dextran concentrations along the x-axis. The ternary systems investigated ([C₄mim]-based IL + Dextran + water) are presented in **Figure 3** corresponding to dextran polymer with molecular weights of 6 kDa, 40 kDa, and 100 kDa, respectively.

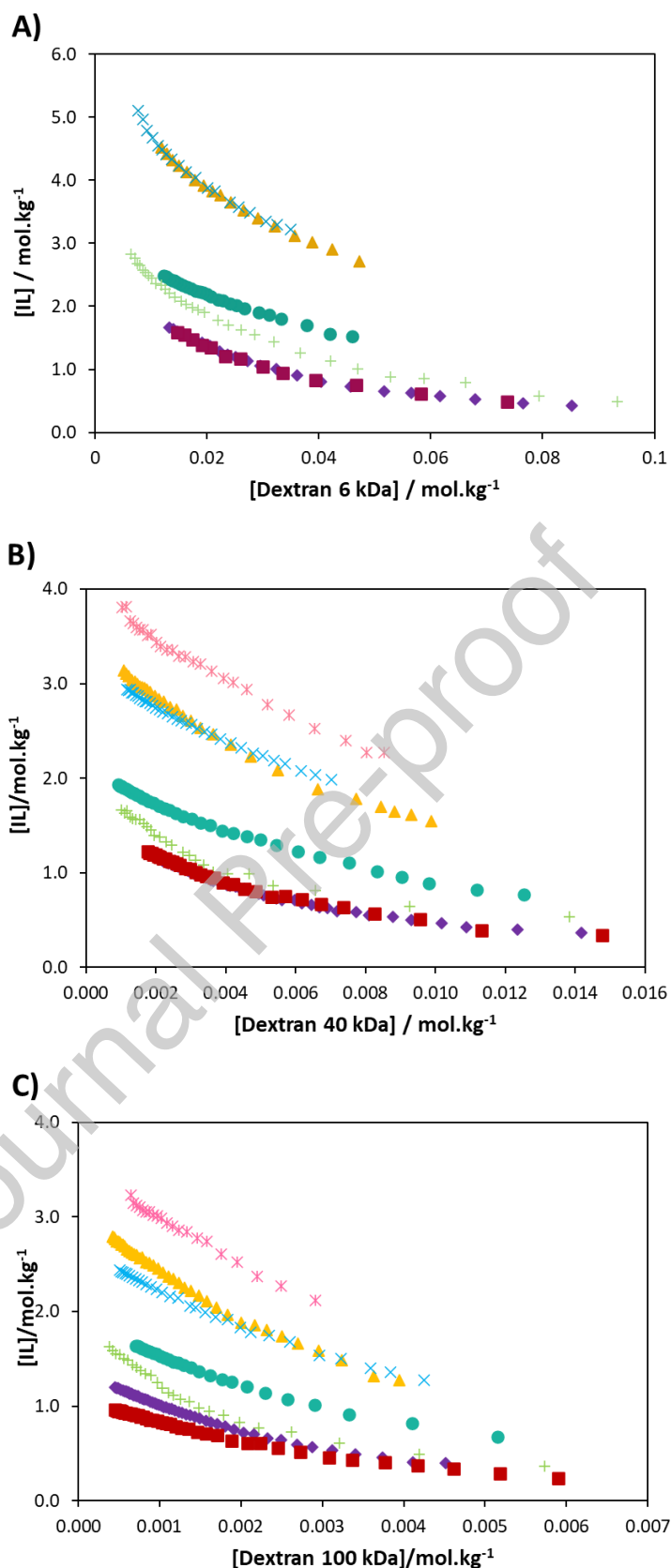
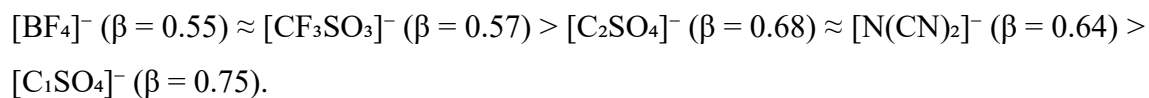


Figure 3. Phase diagrams for all ternary systems composed of IL + Dextran + water at 298 K. The ILs are denoted as follows: [C₄mim][C₁SO₄] (*); [C₄mim][N(CN)₂] (▲); [C₄mim][C₂SO₄] (×); [C₂mim][CF₃SO₃] (●); [C₆mim][N(CN)₂] (+); [C₄mim][CF₃SO₃] (◆); and [C₄mim][BF₄] (■).

As shown in **Figure 3**, the anion nature exerts a consistent influence on the expansion of the biphasic region across all systems containing [C₄mim]-based ILs, regardless of the molecular weight of the dextran. The observed trend in ABS formation correlates strongly with the anions' hydrogen-bond basicity (β) [52], following the order:



Anions with higher β values form stronger hydrogen bonds with water and are therefore more hydrophilic, which diminishes their ability to promote ABS formation in IL/Dextran aqueous mixtures. Consequently, the decrease in the anion's capacity to interact with water through hydrogen bonding appears to be one of the main driving forces for phase separation, making these ILs more prone to be sugared out by the different dextran polymers.

This behaviour closely resembles that observed for IL/sugar ABS [38–40], where increasing anion hydrophobicity and weaker anion–water interactions expand the biphasic region and enhance the tendency for ABS formation. In contrast, IL/polymer ABS composed of PEG or PPG [7,45] frequently exhibit the opposite trend: stronger IL–polymer dispersive interactions associated with more hydrophobic anions can increase miscibility and suppress phase separation. Furthermore, the incorporation of dextran enables ILs with higher β values to form ABS, unlike systems induced by small sugars [38–40], which require ILs with significantly lower β values to promote phase separation. This widens the range of accessible biphasic systems and permits the design of more versatile formulations that can be tailored to specific liquid–liquid extraction processes and applications.

To provide a clearer visual interpretation of the discussed relationship, **Figure 4** presents the correlation between the minimal equal weight percentages of IL and the different dextrans required to form ABS ($[\text{IL}] = [\text{Dextran}]$, wt%) and the corresponding β values of the anions. The established correlation enables the evaluation of how the ability of each [C₄mim]-based IL to form an ABS relates to the intrinsic β value of its respective anion. Because ILs and dextran differ greatly in molecular weight, their molar concentrations required to induce phase separation vary by several orders of magnitude. Therefore, phase diagrams expressed in weight percentage were used to determine the minimal equal amounts of both components. The intersection points of the binodal curves with the line $[\text{IL}] = [\text{Dextran}]$ represent these minimal equal concentrations used for analysis.

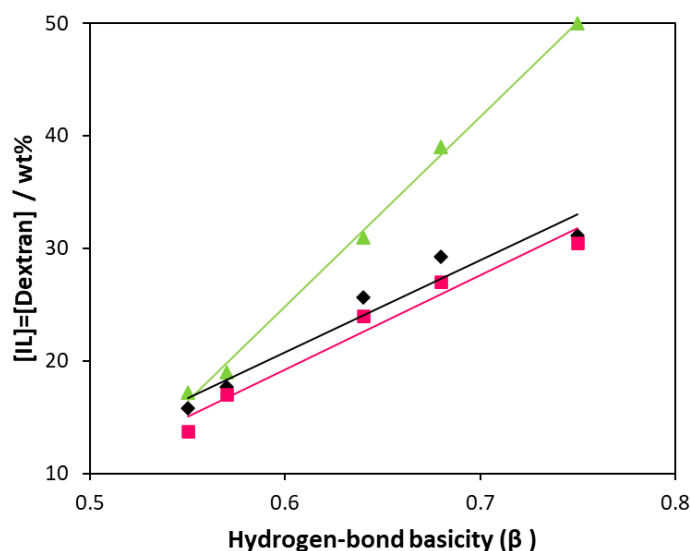


Figure 4. Correlation between the minimal equal mass fractions (wt%) of IL and Dextran required to induce phase separation and the hydrogen-bond basicity (β) of the anions in the tested $[\text{C}_4\text{mim}]^+$ -based ILs. The Dextrans are denoted as follows: (\blacktriangle) Dextran 6 kDa; (\blacklozenge) Dextran 40 kDa; (\blacksquare) Dextran 100 kDa. Linear equations and respective correlation coefficients are displayed in the SI (Table S15).

A linear relationship between the minimal equal mass fractions of IL and dextran required for phase separation and the anion β values is evident in **Figure 4**. ILs containing anions with higher β values require larger quantities of both IL and polymer to induce phase separation, resulting in narrower immiscibility regions. This confirms that the dextran's sugaring-out ability plays a key role in ABS formation and that weaker IL–water interactions favour broader biphasic regions. Moreover, the correlation with anion hydrogen-bond basicity demonstrates that β is a useful predictor of ABS formation for ILs sharing the same cation.

For systems containing the same polymer, larger biphasic regions consistently occur with ILs bearing anions with weaker water affinity, in line with decreasing β values. The similar linear regressions obtained for Dextran 40 kDa and 100 kDa (**Figure 4**) indicate that the high molecular weight of these polymers, and their correspondingly strong sugaring-out effect, significantly reduces the influence of IL structure on phase separation. In contrast, the weaker sugaring-out ability of Dextran 6 kDa allows IL properties to play a more pronounced role in defining the immiscibility region of IL/Dextran ABS.

This effect is particularly evident in systems with the $[\text{C}_1\text{SO}_4]^-$ anion, which exhibits the highest β value and strongest hydrogen-bonding affinity for water. When combined with the Dextran 6 kDa, which contains fewer hydroxyl groups, no phase separation occurs, as the polymer's sugaring-out effect is insufficient to overcome strong IL–water interactions (**Figure 3A**). Increasing the dextran molecular weight to 40 kDa enhances polymer–water interactions and enables $[\text{C}_4\text{mim}][\text{C}_1\text{SO}_4]$ to form a biphasic system (**Figure 3B**), with phase separation becoming even more pronounced with dextran 100 kDa (**Figure 3C**). These observations confirm that ABS formation depends on a balance between the polymer's sugaring-out effect and the anion-water interactions of the IL.

Further insights into the mechanisms governing phase behaviour can be obtained by examining the binodal curve patterns in **Figure 3**. At lower dextran concentrations, clear differences in phase separation behaviour are observed among ILs, reflecting variations in ion-water affinity and their relative hydrophobicity. As dextran concentration increases, however, the binodal curves progressively converge, indicating a diminishing influence of IL structure. This convergence is particularly evident in **Figure 3B**, where distinct phase behaviours are observed up to Dextran 40 kDa concentration of $0.005 \text{ mol}\cdot\text{kg}^{-1}$. Above this threshold, the phase diagrams become increasingly similar, despite substantial differences in IL composition, specially for systems containing $[\text{C}_6\text{mim}][\text{N}(\text{CN})_2]$, $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$, and $[\text{C}_4\text{mim}][\text{BF}_4]$.

These results indicate that the structure and nature of the IL exert a stronger influence at lower polymer concentrations, where variations in IL hydrophobicity and ion–water affinity more significantly affect the size and position of the biphasic region. At higher polymer concentrations, however, enhanced polymer–water interactions and the stronger sugaring-out effect of dextran overshadow these differences, rendering the polymer's hydrophilicity the dominant factor governing phase behaviour.

An even more pronounced convergence is observed for Dextran 100 kDa (**Figure 3C**), where binodal curves become nearly indistinguishable above dextran concentrations of $0.0025 \text{ mol}\cdot\text{kg}^{-1}$. Below this concentration, phase separation is governed by the balance in between IL–water and dextran–water interactions; above it, the strong sugaring-out effect of the polymer predominates, leading to similar phase behaviour regardless of IL water affinity.

These findings highlight dextran's high hydrophilicity as the primary driver of phase separation in IL/dextran systems. Although reduced anion–water affinity can enhance IL–

polymer interactions in some polymer-based ABS [7,45], the abundance of hydroxyl groups in dextran promotes dominant polymer–water interactions that suppress such effects. Consequently, phase separation in IL/dextran systems is primarily driven by polymer–water and IL–water interactions rather than by direct IL–polymer affinity.

Overall, phase separation in IL/dextran systems is dictated by the interplay between polymer–water and IL–water interactions. The strong sugaring-out effect of dextran, particularly at molecular weights of 40 kDa and 100 kDa, reduces the availability of free water molecules around IL ions and promotes phase demixing. Owing to dextran’s high hydrophilicity and weak IL–polymer interactions, the influence of the IL is largely determined by ion–water affinity, with weaker interactions producing broader biphasic regions. This behaviour distinguishes dextran from more hydrophobic polymers such as PEG or PPG [45,47], leading to fundamentally different demixing mechanisms.

From a thermodynamic perspective, dextran-based IL/polymer ABS differ markedly from IL/polyether systems, exhibiting behaviour more similar to that of IL/sugar systems. In IL/polyether systems, increasing the polymer molecular weight generally promotes phase demixing due to increasingly unfavourable polymer–water interactions; however, this trend persists only up to a certain molecular weight threshold. Beyond this point, stronger IL–polymer interactions may enhance their mutual solubility, introducing an additional interaction regime that significantly influences phase separation behaviour and ABS formation [7,45].

In contrast, in IL/sugar ABS, increasing the molecular weight of the carbohydrate enhances its hydration capacity, thereby intensifying the sugaring-out effect. Consequently, phase separation in IL/dextran systems is governed by a less complex balance of intermolecular interactions than that observed in IL/polyether systems, exhibiting demixing mechanisms more closely related to those reported for IL/sugar ABS [38–40].

The insights gained here provide a foundation for the rational design of dextran-based IL/polymer ABS and support their potential application in sustainable, biocompatible, and environmentally benign liquid–liquid extraction processes, particularly for the enrichment of exopolysaccharides from complex fermentation broths (see next section).

3.2 Exopolysaccharides Enrichment via IL-based ABS

Having proved that dextran is an effective polymer for inducing ABS formation in IL-containing systems, and given that exopolysaccharides (EPS) are polymers typically produced extracellularly and present at high concentrations in fermentation broths, developing integrated enrichment processes based on dextran-rich media combined with IL addition represents a promising biotechnological application of this new class of ABS.

As illustrated in **Figure 5**, the extracellular polysaccharides present in fermentation broths, such as dextrans, can be separated *in situ* by adding an IL to promote phase separation. This produces two immiscible aqueous phases: an IL-rich (top) phase and an EPS-rich (bottom) phase. Such an integrated approach concentrates the majority of EPS molecules into a single phase, providing a more straightforward and efficient route for polysaccharide recovery from fermented broths.

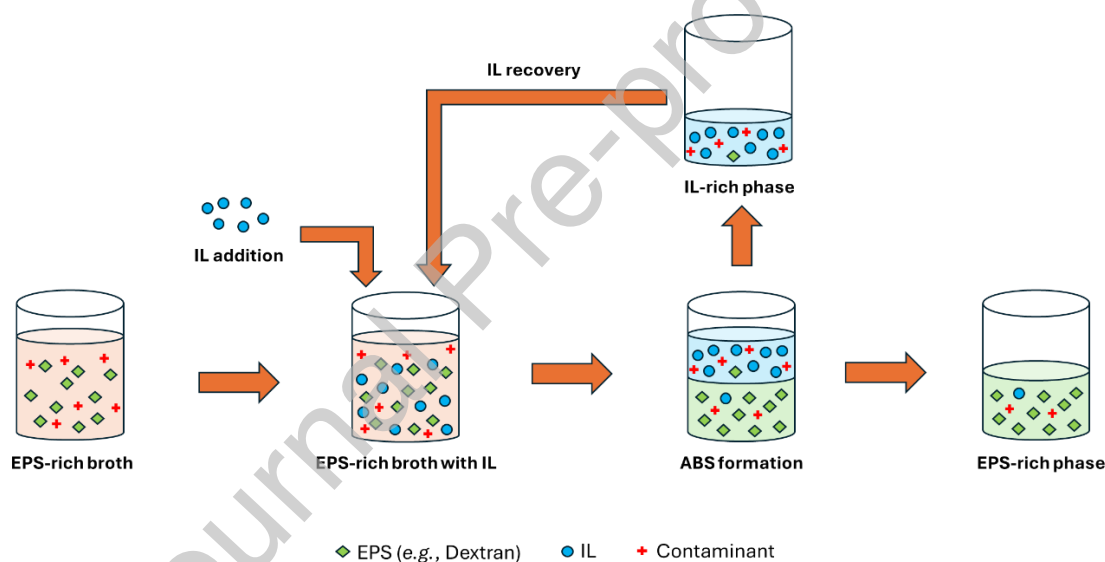


Figure 5. Schematic representation of an integrated IL-based ABS process for EPS enrichment from fermented broths.

Compared with conventional extraction techniques, often time-consuming, energy-intensive, reliant on large amounts of organic solvents, and potentially degrading polysaccharides under harsh processing conditions [53], the IL/dextran-based process offers a more sustainable and controllable alternative. By fine-tuning phase composition and manipulating tie-line lengths to control the degree of EPS enrichment, IL-based ABS can significantly streamline recovery. Overall, this strategy could provide a cost-effective and technologically viable solution to the downstream processing challenges typically associated with EPS production and purification.

To validate the proposed EPS enrichment approach, a simulated fermentation broth containing 20 wt% sucrose in water [43] was used to form an ABS composed of [C₄mim][C₁SO₄] and Dextran 40 kDa. The final mixture consisted of 43.5 wt% IL, 20 wt% Dextran 40 kDa and 36.5 wt% of the aqueous sucrose solution. Partition coefficients for sucrose (K_{sucrose}) and dextran (K_{Dextran}) were then determined. This system was selected as a worst-case scenario—previously shown to have limited ability to induce phase separation (**Figure 3B**)—to demonstrate the viability of the approach as a proof of concept for the integrated process illustrated in **Figure 5**. The results obtained are summarised in **Table 1**.

Table 1. Concentrations and partition coefficients of sucrose and dextran in an EPS enrichment process using an ABS composed of [C₄mim][C₁SO₄], Dextran 40 kDa, and a simulated fermentation broth containing 20 wt% sucrose.

IL/Dextran ABS-based EPS enrichment	Sucrose concentration (g/L)	Sucrose partition coefficient (K_{sucrose})	Dextran concentration (g/L)	Dextran partition coefficient (K_{Dextran})
[IL]-rich phase	25.99 ± 0.27	31.73 ± 1.40	19.41	0.043
[Dex 40kDa]-rich phase	0.82 ± 0.04		456.25	

The results provided in **Table 1** clearly demonstrate that the high K_{sucrose} and low K_{Dextran} values reflect strong ability of this ABS to concentrate sucrose in the IL-rich (top) phase while retaining dextran in the bottom phase. Thus, IL/Dextran ABS provide a promising platform for concentrating EPS from fermented broths that contain substantial amounts of sucrose as a major contaminant. This approach not only yields an EPS-rich phase while but also allows the IL-rich phase to be recovered and reused in closed-loop processes.

However, it is important to recognise that employing ABS for EPS recovery does not guarantee that the EPS-rich phase (*i.e.*, the product phase) will be free from contaminants. Fermented broths may contain residual media components (e.g., small saccharides, salts) and substances released by microorganisms, such as lipids and proteins from some partial cell lysis. The extent to which these compounds partition into the EPS-rich or IL-rich phase depends on their chemical structure and physicochemical properties. Consequently,

some contaminants may accumulate in the EPS-rich phase, reducing its purity and possibly necessitating further purification depending on end-use requirements.

Cross-phase contamination is also likely: trace amounts of IL may remain in the EPS-rich phase, while some EPS may be retained in the IL-rich phase. Residual IL could limit the applicability of the recovered EPS in processes requiring high-purity, whereas EPS retained in the IL-rich phase may hinder successive IL reuse. Over repeated cycles, the accumulation of such impurities may alter the composition of the IL-rich phase, affecting process performance and posing a key challenge for techno-economic feasibility. Enhancing IL phase reuse is therefore essential, as efficient recovery would reduce operational costs, minimise fresh IL consumption, and improve the economic and environmental sustainability of the process.

From a green chemistry perspective, the use of ILs for EPS recovery is highly attractive when compared to conventional organic solvents. Their negligible vapour pressure, high chemical and thermal stability, low volatility, and tuneable physicochemical properties can enhance extraction efficiency and selectivity. Consequently, ILs may represent more sustainable alternatives to the most part of petrochemical-based solvents commonly used in LLE processes [54]. This can reduce atmospheric emissions and minimise health and safety risks associated with the handling of volatile organic compounds (VOCs). Nevertheless, ILs are not inherently benign, and their potential toxicity and environmental impact must be carefully considered. Therefore, the selection of environmentally friendly and sustainable ILs is essential to ensure compliance with green chemistry principles [54].

In addition, the high ionic strength of IL-based systems may increase the risk of equipment corrosion, making the selection of suitable construction materials important for ensuring long-term operational stability [28,54]. From an economic perspective, ILs are generally more expensive than conventional organic solvents, making efficient IL recovery and reuse essential for process viability and sustainability. In this context, the process proposed in **Figure 5** addresses this challenge by enabling the recycling of the IL-rich phase while minimising IL consumption. Specifically, the simultaneous concentration of EPS in the bottom phase and IL in the top phase facilitates the direct reuse of the IL-rich phase in subsequent extraction cycles, reducing the need for complex regeneration steps, minimising IL contamination in the EPS-rich phase, and improving the overall techno-economic feasibility of the proposed process.

The recovery and recycling of ILs must nevertheless be supported by suitable purification strategies selected according to the intrinsic properties of the ILs, such as hydrophobicity, viscosity, density, and water solubility [55]. Common regeneration approaches for IL-based systems include membrane technologies, adsorption, back-extraction, and distillation, each presenting specific advantages and limitations. Although some of these methods are already implemented at industrial scale and can achieve high IL recovery efficiencies, important challenges remain, including thermal degradation at high temperatures, energy-intensive operation, limited mass transfer caused by high viscosity, the potential release of volatile compounds, and the environmental impact of regeneration agents[55].

For the IL-based systems investigated in this work, membrane-based technologies appear particularly promising due to their operational simplicity, scalability, and cost-effectiveness. Their main limitation is the high viscosity of ATPS coexisting phases, which may hinder mass transfer. However, in the proposed process, IL regeneration would only be required after multiple extraction cycles to remove sucrose and other contaminants accumulated in the IL-rich phase. In this case, viscosity could be reduced by adding water to the IL-rich phase, facilitating IL recovery and reuse. Overall, this strategy supports the development of a more circular process, which should be further explored and experimentally validated in future studies.

Overall, the formation of IL/dextran ABS and their underlying mechanisms, driven primarily by the sugaring-out effect, provide strong evidence that IL-based ABS could offer an effective, more sustainable alternative to conventional downstream processing methods for EPS enrichment. Nonetheless, further research is required to address existing current challenges, including contamination control, phase recovery and reuse, and hydrodynamic optimisation, to fully realise their potential as an industrial biotechnology platform.

4. Conclusions

In this work, IL-based aqueous biphasic systems (ABS) composed of imidazolium-based ionic liquids (ILs), dextran, and water were reported for the first time. The effects of dextran molecular weight, IL cation/anion structure, and polymer concentration on phase behaviour were systematically investigated through the determination of ternary phase diagrams.

Higher-molecular-weight dextran promoted phase separation due to its stronger hydration ability and enhanced sugaring-out effect, leading to larger biphasic regions. Conversely, ILs with weaker ion–water interactions, typically associated with longer alkyl chains and lower hydrogen-bond basicity, favoured ABS formation by reducing their affinity for water. Polymer concentration also played a significant role in phase behaviour, particularly for ILs with weaker IL–water interactions, where the balance between dextran hydration and IL ion–water interactions strongly influenced the shape of the binodal curves.

Overall, the phase behaviour of IL/dextran ABS differs markedly from that of conventional IL/polymer systems and more closely resembles that of IL/sugar ABS, with phase separation being mainly governed by polymer–water and ion–water interactions. These systems also showed promising potential for EPS enrichment from fermentation broths, enabling the simultaneous concentration of sucrose in the IL-rich phase and dextran in the polymer-rich phase. Therefore, IL/dextran ABS emerge as promising platforms for sustainable downstream processing and biomolecule separation applications.

CRedit authorship contribution statement

Sara F. Carvalho: Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Alexandre M. S. Jorge:** Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **Isabel Boal-Palheiros:** Writing – review & editing, Validation, Formal analysis. **João A. P. Coutinho:** Conceptualization, Writing – review & editing, Resources, Visualization, Supervision, Project administration, Funding acquisition. **Jorge F. B. Pereira:** Validation, Writing – review & editing, Resources, Visualization, Supervision, Project administration, Funding acquisition. **Mara G. Freire:** Conceptualization, Methodology, Validation, Writing – review & editing, Resources, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of generative AI in scientific writing

During the preparation of this study, ChatGPT was used to improve the readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as required and took full responsibility for the publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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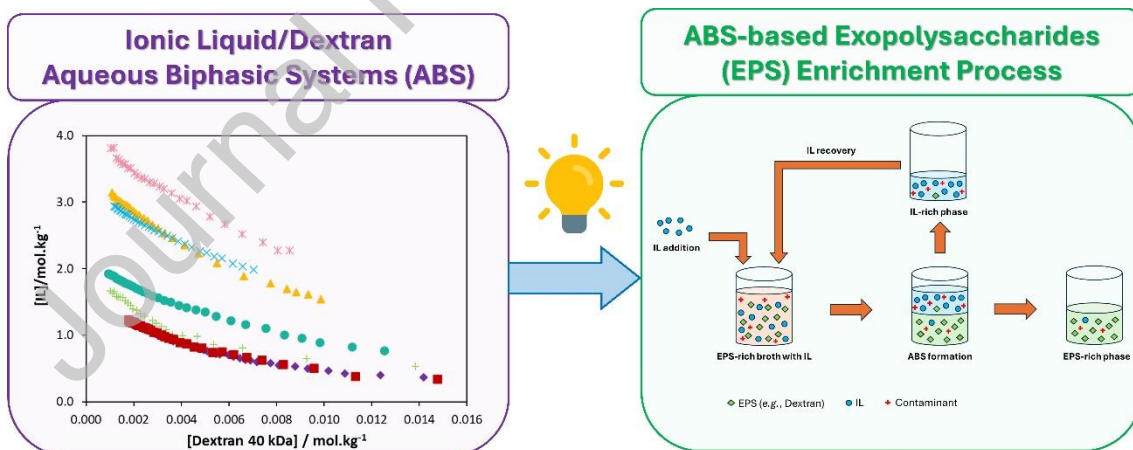
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Graphical abstract



Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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