IOSR Journal of Multidisciplinary Research (IOSR -JMR)
ISSN: 3048-7439 Volume 2, Issue 4, Series 1, (Jul. – Aug 2025), PP 65-74
www.iosrjournals.org

Understanding Phase Behavior in IL-Based Aqueous Two-Phase Systems for Sustainable Separation Processes

Dr. Udai Pratap Singh
Assistant Professor,
Department of Chemistry, D.A-V. College, Kanpur

Dr. Sunil Kumar Mishra
(Corresponding author)
Assistant Professor,
Department of Chemistry D. A-V. College, Kanpur

Abstract: *Aqueous two-phase systems (ATPS) incorporating ionic liquids (ILs) have gained considerable attention as a promising, environmentally benign platform for various separation processes. A fundamental comprehension of their phase behavior is paramount for the effective design and optimization of these systems, particularly in the context of achieving sustainable separation processes. This paper comprehensively reviews the recent advancements in understanding how different components, notably the structural characteristics of ionic liquids and the nature of salting-out agents, critically influence the formation and properties of IL-based ATPS. By detailing these complex interactions, this work aims to provide essential insights that will guide future research and foster the broader application of these green and efficient separation technologies.*

Keywords: *Aqueous Two-Phase Systems (ATPS), Ionic Liquids (ILs), Phase Behavior, Phase Equilibrium, Sustainable Separation, Salting-out Agent*

I. Introduction

Liquid-liquid extraction stands as a pivotal technique across various chemical and biochemical industries for the separation and purification of diverse compounds. Among the evolving approaches, aqueous two-phase systems (ATPS) have emerged as a particularly attractive and environmentally conscious alternative to conventional organic solvent-based methods. First introduced by Albertsson, ATPS are uniquely characterized by their two immiscible liquid phases, both predominantly composed of water. Initially, these systems were typically formed by mixing two incompatible polymers, or a polymer and an inorganic salt, which would spontaneously demix into two distinct phases above certain concentrations due to repulsive molecular interactions [1-4]

A significant advantage of ATPS lies in the high-water content of both phases, which provides a mild and biocompatible environment. This characteristic is crucial for preserving the activity and structural integrity of sensitive biomolecules, while simultaneously mitigating the environmental hazards and toxicity associated with volatile organic solvents [5]. Consequently, ATPS has found widespread application in the separation and purification of a broad spectrum of substances, including biological macromolecules, metal ions, natural products, and advanced carbon nanomaterials [6]. Despite these merits, a key limitation of traditional ATPS has been their relatively narrow polarity range, which can restrict their broader applicability in diverse separation scenarios.

The advent of ionic liquids (ILs) has provided a transformative solution to overcome these challenges. ILs are remarkable salts that remain in a liquid state at or near room temperature, distinguishing them from traditional electrolytes. Their unique suite of properties – including negligible vapor pressure, non-flammability, exceptional thermal and chemical stability, a highly tunable chemical structure, and potent solubilization capabilities – makes them ideal candidates for enhancing separation processes [7-9]. While hydrophobic ILs have been traditionally employed in simple IL/water biphasic systems, their inherent high viscosity could potentially compromise the integrity of sensitive biomacromolecules during extraction.

Recognizing the synergistic potential, the integration of hydrophilic ILs into ATPS marked a significant advancement. In 2003, Rogers and co-workers pioneered this field by reporting the first IL-based ATPS formed from 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl) and potassium phosphate (K_3PO_4) [10]. This breakthrough demonstrated that IL-based ATPS could effectively circumvent the limitations observed in both simple IL/water systems and conventional polymer-based ATPS, such as improved extraction efficiency and broader polarity windows. Since this foundational work, the field of IL-based ATPS has experienced rapid growth, with studies demonstrating their formation with various components including inorganic salts, polymers, and surfactants [11-13]. The ability to precisely tailor the cationic and anionic structures of ILs further allows for fine-tuning the properties of these ATPS, leading to enhanced phase separation kinetics and clearer interfacial boundaries compared to traditional polymer-based systems.

However, despite considerable progress, several critical aspects require further systematic investigation to fully realize the potential of IL-based ATPS for sustainable applications. The long-term stability of ILs under diverse process conditions, as well as the development of efficient and cost-effective strategies for their recovery and recycling after use, remain crucial challenges. Addressing these issues is paramount for truly establishing IL-based ATPS as a cornerstone of green chemistry [14-15]. This paper aims to provide a comprehensive review of the recent advancements in understanding the fundamental phase behavior of IL-based ATPS, particularly focusing on the intricate influence of ionic liquid structures and salting-out agents. By synthesizing existing knowledge on their properties, phase separation mechanisms, and equilibrium behavior, this work seeks to offer valuable insights that will stimulate further research and accelerate the practical implementation of these highly promising and sustainable separation technologies.

II. Properties of IL-Based ATPSs

The successful design and subsequent scaling-up of any separation process critically rely on a comprehensive understanding of the physical properties of the aqueous two-phase systems (ATPS) under various concentrations and temperatures. In the context of ionic liquid (IL)-based ATPS, these properties are profoundly influenced by the specific structural characteristics of the constituent ILs. Among the diverse array of ILs, hydrophilic variants are particularly favored and widely utilized in ATPS due to their advantageous attributes, which include a broad selection of compatible anions, lower cost, and generally reduced toxicity compared to their hydrophobic counterparts. Figure 1 illustrates the common chemical structures of ILs frequently employed in ATPS [16].

Within IL-based ATPS, the nature of the ionic liquid plays a pivotal role in dictating phase behavior. Specifically, chaotropic ILs tend to promote "salting-in" phenomena, meaning they increase the solubility of a substance, while anti-chaotropic ILs exhibit the opposite effect. This inherent ability of ILs to modulate phase polarities more effectively than traditional ATPS components is a key advantage [17]. Such intriguing and beneficial properties of IL-based ATPS have significantly spurred extensive research into their thermodynamic characteristics, predictive modeling, and diverse extraction applications.

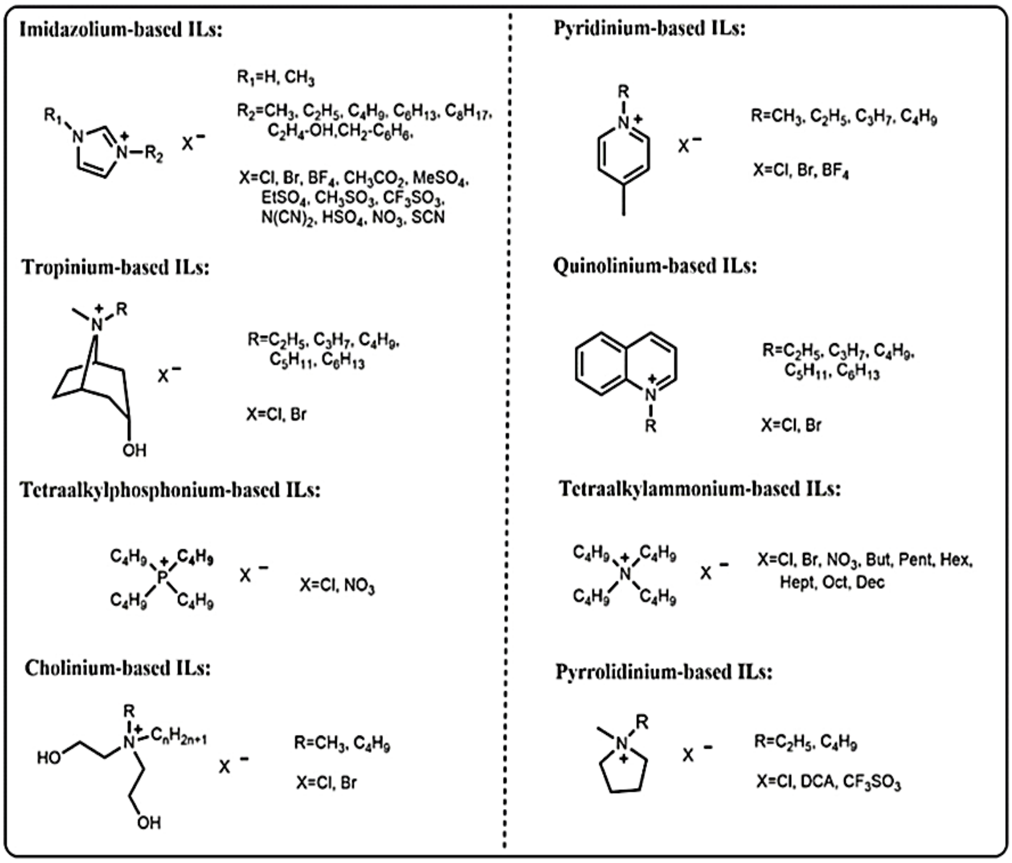

Figure1: Chemical Structure of the ILs Commonly used for ATPSs

2.1. Effects of ILs Cations

The cationic moiety of ionic liquids (ILs is highly amenable to structural modification, typically comprising a parent nucleus and various side chains. A significant portion of current research focuses on designing ILs with chaotropic cations, which are effectively "salted-out" by kosmotropic salts. This selective salting-out phenomenon is a crucial factor in the formation of aqueous two-phase systems (ATPS).

Studies have extensively investigated the impact of diverse cation structures on the phase behavior of IL-based ATPS. For instance, Bridges et al. [18] explored systems involving various chloride-based ILs—including 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl), 1-butyl-2,3-dimethylimidazolium chloride ([C_4mmim]Cl), N-butylpyridinium chloride ([C_4py]Cl), tetrabutylammonium chloride ([N_{4444}]Cl), and tetrabutylphosphonium chloride ([P_{4444}]Cl)—combined with different inorganic salts (e.g., K_3PO_4, K_2HPO_4, K_2CO_3, KOH, and $(NH_4)_2SO_4$). Their findings revealed a distinct order of decreasing chaotropicity among the ILs: [P_{4444}]Cl > [N_{4444}]Cl >> [C_4py]Cl >> [C_4mmim]Cl ≈ [C_4mim]Cl. This observed trend is directly linked to the inherent chemical differences in the cations, which dictate their chaotropic nature.

Specifically, quaternary onium salts, such as the tetrabutylammonium and tetrabutylphosphonium cations, possess highly shielded charges localized primarily on the heteroatom (nitrogen or phosphorus) surrounded by four bulky butyl chains. This structural feature renders them highly susceptible to salting-out by inorganic salts, thus promoting phase separation. In contrast, the pyridinium cation, while still possessing a localized charge on its nitrogen atom, exhibits less charge shielding compared to the quaternary onium salts. Imidazolium-based ILs, on the other hand, are characterized by a diffused charge distribution across the two nitrogen atoms and the C_2 carbon of the imidazolium ring. This delocalized charge facilitates multiple interactions with water molecules, making these ILs more challenging to salt out and contributing to their lower melting points.

Further investigations into imidazolium-based ILs, like those forming ATPS with K_3PO_4, including [C_4mim]Cl, 1-butyl-2,3-dimethylimidazolium chloride ([C_4mmim]Cl), and 1-hexyl-3-methylimidazolium chloride ([C_6mim]Cl) [19], indicated that the phase separating ability of [C_4mmim]Cl fell between that of [C_4mim]Cl and [C_6mim]Cl. This suggests that the hydrogen bonding capabilities of the C_2 hydrogen atom on the imidazolium cation have a comparatively minor influence on the overall phase behavior.

Beyond the specific nature of the heteroatom and charge distribution, the molar volume of the IL cation significantly impacts phase formation. Ventura et al. [20], while controlling pH with K_2HPO_4-KH_2PO_4, compared the phase separation ability of four substituted alkyl ILs with identical chain lengths. Their study showed the following sequence for phase formation ability: [C_4C_1pyr]Cl ≈ [C_4mim]Cl < [C_4C_1pip]Cl < [C_4C_1py]Cl. This demonstrates that cations with larger molar volumes or greater steric hindrance, such as those found in six-membered heterocycles (e.g., pyridinium and piperidinium), more readily disrupt the surrounding water's hydrogen bond network. Consequently, these ILs are generally easier to salt out compared to those with five-membered heterocycles like imidazolium and pyrrolidinium.

Furthermore, studies have reported on the physical properties of the formed phases. While density values between IL-based ATPS and typical polymer-based systems show no significant differences, phases containing phosphonium- or imidazolium-based ILs generally exhibit considerably lower viscosity compared to traditional polymer-rich phases, even at similar mass fraction compositions [21, 22]. This reduced viscosity can be a significant advantage in practical separation applications, facilitating faster mass transfer and easier handling.

The hydrophobicity of ILs is another critical factor governing ATPS formation. Generally, an increase in the alkyl chain length on the cation enhances the IL's hydrophobicity, leading to a stronger phase separation ability. This effect has been observed in ATPS based on series of n-alkyl-tropinium bromide ([C_nTr]Br, n = 2–5) and n-alkyl-quinolinium bromide ([C_nQ_n]Br, n = 2–6) with various salts, as illustrated by their binodal curves (Figure 2A). While increased hydrophobicity often correlates with enhanced phase formation for cationic side chain alkyl groups up to six carbons ($n \leq 6$) [23, 24], an excessively hydrophilic IL is detrimental to ATPS formation. Conversely, for longer alkyl chains ($n \geq 8$), anomalous behavior, possibly due to IL self-aggregation, has been reported [25]. Lastly, the substitution of different functional groups on the IL side chain also plays a role; hydroxyl and allyl groups, due to their higher water affinity, can significantly diminish the phase separation ability of ILs, whereas benzyl and n-heptyl groups have less pronounced effect.

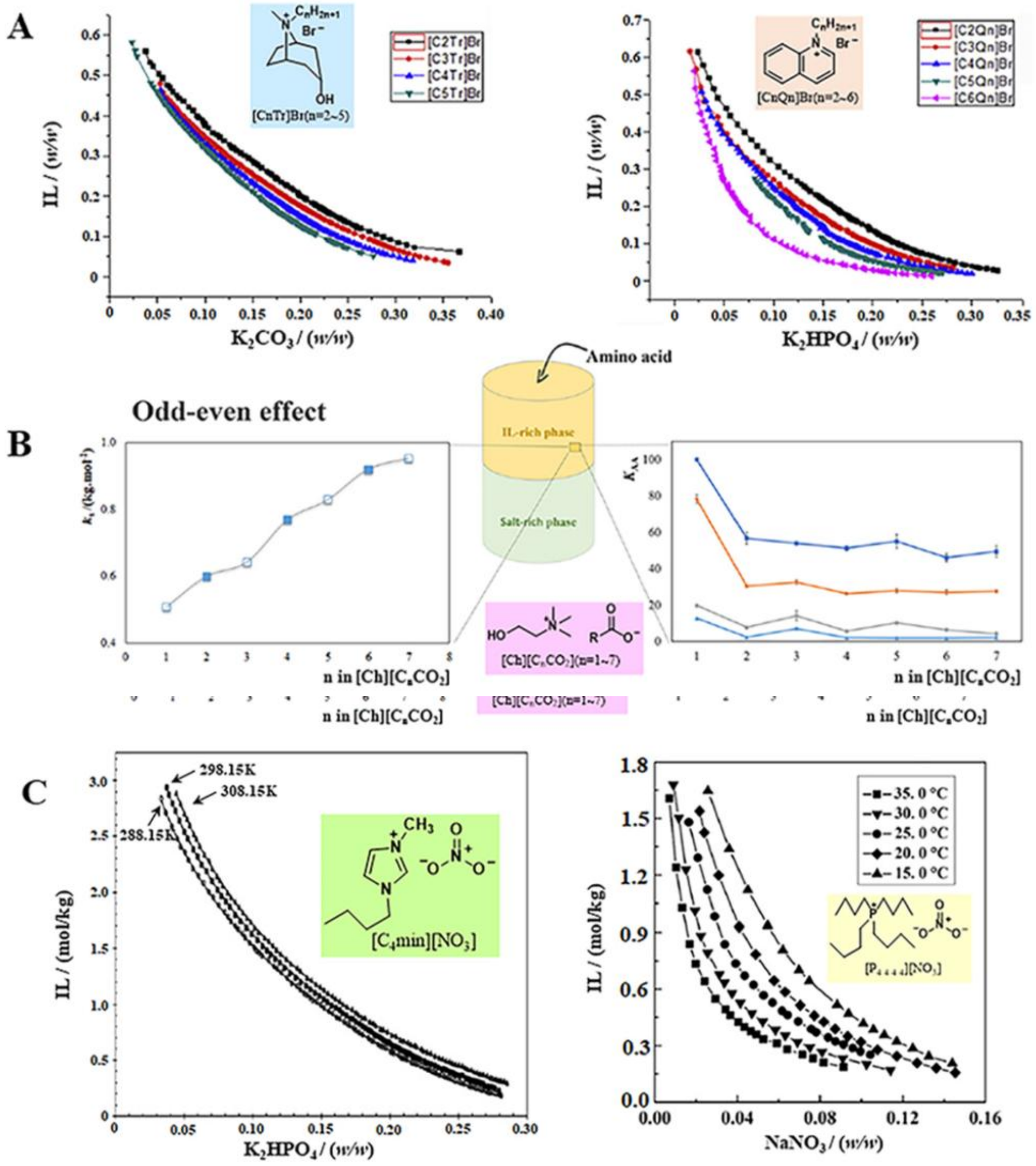

Figure 2. (A) Effect of ILs cations on the binodal curves [23]; (B) Odd-Even effect of the ATPS based on [Ch][C_nCO_2], comprising anions with odd and even alkyl chain lengths [24]; (C) Effect of temperature on the phase formation ability of different IL-based ATPS [25]

2.2. Effects of ILs Anions

While the cationic structure of ionic liquids (ILs has been widely explored for its influence on ATPS formation, the impact of IL anions on phase separation ability has received comparatively less systematic investigation, primarily due to the vast diversity in their chemical structures, which makes establishing a unified standard for comparison challenging.

Early insights into the role of anions emerged from the work of Pei and co-workers in 2007 [49], who first compared the phase separation capabilities of halide anions (Br^- andCl^-) Theyobservedthatfor1−butyl−3−methylimidazolium$([C_4mim])^+$and1−hexyl−3−methylimidazolium$([C_6mim])^+$ cations,thebromideanionconsistentlyyieldedsuperiorphaseformationcomparedtothechlorideanion.Thisphenomenonwasattributedtodifferencesintheirgibbsenergiesofhydration($\Delta_{hyd}G$) for Cl^-(−340 kJ/mol) compared to Br^- (−315 kJ/mol) signifies a stronger hydration tendency for chloride. Consequently, ILs with the Cl^- anion bind more water molecules, hindering phase separation upon salt addition.

Subsequent research expanded to a wider range of IL anions to gain further mechanistic understanding of their role in ATPS formation. Studies using 1-ethyl-3-methylimidazolium and 1-butyl-3-methylimidazolium cations evaluated anions such as chloride, bromide, acetate, hydrogensulfate, methanesulfonate, methylsulfate,

ethylsulfate, trifluoromethanesulfonate, trifluoroacetate, and dicyanamide. These investigations revealed consistent trends in phase formation ability, for instance: $[C_2mim][CF_3SO_3] > [C_2mim][C_2SO_4] > [C_2mim][MeSO_4] > [C_2mim][Br] > [C_2mim]Cl \approx [C_2mim][CH_3CO_2] > [C_2mim][CH_3SO_3]$, and $[C_4mim][CF_3SO_3] > [C_4mim][N(CN)_2] > [C_4mim][HSO_4] > [C_4mim][TFA] > [C_4mim]Br > [C_4mim]Cl \approx [C_4mim][CH_3CO_2] \approx [C_4mim][CH_3SO_3]$ [26]. A general conclusion drawn from these studies is that as the hydrogen bond accepting capacity (or hydrogen-bond alkalinity) of the anion increases, the ability of the IL to form an ATPS with the same cation tends to decrease [20]. This is likely because stronger hydrogen bond acceptors promote greater hydration of the IL, weakening its phase separation propensity. Given that most anions are relatively small and lack long hydrophobic alkyl chains, hydrogen bonding interactions are considered the predominant factor influencing their role in phase separation. Conversely, a weak hydrogen-bond alkalinity can sometimes negatively impact solute distribution and extraction efficiency, thereby limiting the application of certain IL-based ATPSs in separation contexts [27].

The influence of alkyl chain length within the anion has also been investigated. Deive et al. [28] explored ethyl-methylimidazolium alkylsulfate ILs, $[C_2mim][C_nSO_4]$ (where n = 2, 4, 6, or 8), forming ATPS with high charge-density inorganic salts. Their findings indicated that the phase separation ability of these ILs improved with increasing alkyl chain length in the anion. Similarly, Basaiahgari et al. [29] synthesized a series of tetrabutylammonium carboxylate ILs to form ATPS with K_3PO_4, observing a trend in phase formation ability: [TBA][But] < [TBA][Pent] ≈ [TBA]Br < [TBA][Hex] < [TBA][Hept] ≈ [TBA][Dec] < [TBA][Oct]. These results strongly suggest that the hydrophobicity of the anion plays a dominant role in driving phase formation, with longer alkyl chains leading to increased hydrophobicity and, consequently, enhanced phase splitting.

In systems where a random co-polymer like UCON was used as a phase-forming component alongside imidazolium-based ILs with various anions (chloride, bromide, acetate, or dicyanamide), Jimenez et al. [30] observed a significant effect of the anion on ATPS immiscibility. The heterogeneous region area followed the order: $OAc^- > Cl^- > Br^- > SCN^-$ which aligns with the absolute values of the Gibbs energies of hydration for these anions [31].

Amino acid ionic liquids (AAILs) represent another important class, offering tunable hydrophobicity, acid/base behavior, low toxicity, and biodegradability, making them highly suitable for ATPS applications. Korchak et al. [32] compared the phase separation ability of various AAILs (e.g., L-Leucine, L-Valine, L-Lysine anions) with inorganic salts. The ability to form two phases increased in the sequence: [Lys] > [Val] > [Leu] > Cl > Br. This indicates that AAILs can facilitate wider heterogeneous regions compared to halide ILs. Interestingly, an "odd-even effect" related to alkyl chain length was noted in ATPS formed with cholinium carboxylate ILs ($[Ch][C_nCO_2]$, where n = 1–7) [23]. As depicted in Figure 2B, ILs with even-numbered alkyl chains in their carboxylate anions exhibited slightly higher Setschenow salting-out coefficients (ks), indicating a greater propensity for phase separation. However, this trend was reversed for the extraction performance of amino acids, where odd-numbered alkyl chain anions led to slightly higher partition coefficients, likely due to enhanced dispersive interactions between the hydrophobic amino acids and the IL anion's aliphatic moieties.

In essence, the overall phase separation efficiency and extraction performance of IL-based ATPS are governed by a complex interplay of microscopic factors, including hydrophobicity, hydrogen bond accepting capacity, and dispersive interactions. Research into these anionic influences provides valuable strategies for optimizing and enhancing the phase separation capabilities of ILs.

2.3. Effects of Temperature

Temperature is a critical parameter influencing the formation and characteristics of IL-based ATPS. Generally, a decrease in temperature leads to an expansion of the heterogeneous (two-phase) region, indicating enhanced phase-forming ability. This is often observed because lower temperatures reduce IL solubility, promoting demixing. Studies using ILs like $[C_4mim]Br$ with tri-potassium citrate [33] or $[C_4mim][BF_4]$ with $(NH_4)_2SO_4$ [34] consistently show this trend, where lower temperatures result in larger two-phase areas and improved phase separation efficiency. This is particularly beneficial for extracting temperature-sensitive compounds like proteins.

However, the influence of temperature is complex and not universally consistent. A few IL-inorganic salt systems, notably those involving tetrabutylphosphonate cations (e.g., $[P_{4444}][NO_3]$ with $NaNO_3$), demonstrate the opposite behavior, where the immiscibility region expands with increasing temperature (Figure 2C). For IL-polymer ATPS (e.g., with PEG or PPG), temperature effects are further complicated by the disruption of hydrogen-bonding interactions, potentially leading to lower critical solution temperature (LCST) or upper critical solution temperature (UCST) behaviors depending on IL concentration [35]. In summary, temperature's impact on phase separation is a multifaceted phenomenon, governed by a delicate balance of hydrogen bonding, IL concentration, and the specific nature of all coexisting components.

III. Mechanism of Phase Separation

The primary driving force behind the phase separation in IL-based ATPSs, particularly those involving ILs and salts, is the salting-out effect. This mechanism relies on a competition for water molecules between the ionic liquid and the added salt. Initially, ILs are stably dispersed in water. When a salt aqueous solution is introduced, the differing abilities of the IL and the salt to attract and bind water molecules become critical.

Specifically, the formation of an IL-based ATPS occurs when kosmotropic ions (e.g., CH_3COO^-,$SO_4{}^{2-}$, $HPO_4{}^{2-}$, Mg^{2+},Ca^{2+},Li^+,H^+,OH^-, etc) from the added salt effectively "salt-out" the IL. These kosmotropic ions strongly structure water molecules around them, leading to a more ordered arrangement and lower free energy for water in their vicinity. This strong interaction reduces the amount of "free" water available to hydrate the IL, pushing the IL out of the homogeneous solution and into its own phase.

Both the hydrophobicity of the IL and the nature of the salt significantly influence this process. More hydrophobic ILs tend to be more easily salted out, thus promoting ATPS formation [3, 36]. Similarly, the stronger the kosmotropic nature of the salt ions, the greater their ability to induce phase separation. Essentially, the tendency for two-phase separation is directly linked to the increased structuring of water molecules by the kosmotropic ions, driving the ILs to demix.

IV. Phase Equilibrium of the IL-Based ATPSs

The phase equilibrium of IL-based aqueous two-phase systems (ATPS) is crucial for their design and application in separation. These systems typically form an IL-rich top phase and a salting-out agent-rich bottom phase, both primarily aqueous. Phase behavior is visually represented by phase diagrams, which use a binodal curve to delineate homogeneous from heterogeneous regions. A larger two-phase area indicates stronger phase separation. Tie lines connect the equilibrium compositions of the two phases, with their length reflecting compositional differences until they converge at the critical point, where phases merge. Many factors, including IL structure and coexisting components (salts, polymers, etc.), significantly influence these phase diagrams.

4.1. Salts

Salts are widely used as co-components in IL-based ATPS, driving phase separation primarily through the salting-out effect. Various inorganic and organic salts, as detailed in Table 1, are employed to induce ATPS formation. Studies show that the salting-out strength varies significantly among these salts; for instance, K_2HPO_4 often demonstrates superior phase-separation ability compared to K_2CO_3. This effect is frequently consistent with the Hofmeister series and directly correlates with the Gibbs energy of hydration ($\Delta_{hyd}G$) of the ions involved.

Emerging as a green alternative, choline amino acid ILs ([Ch][AA]) form stable ATPS with salts, proving effective for environmentally friendly extractions like those for food samples due to their low toxicity and biodegradability.

More significantly, Magnetic Ionic Liquids (MILs) offer a novel approach. These functionalized ILs, like imidazolium-based [C_4mim][$FeCl_4$] or cholinium-based MILs, enable ATPS formation with specific salts. For example, K_2HPO_4 and K_2CO_3 are found to be better phase-formers than Na_2CO_3 in these systems. A key advantage of MIL-based ATPS is the ability to achieve faster phase assembly and separation under an external magnetic field, representing a significant improvement over conventional IL-based ATPS. The salting-out ability of salt anions and cations in MIL systems generally follows orders consistent with their hydration properties, suggesting entropy as a driving force for two-phase formation. In summary, kosmotropic salts facilitate ATPS formation by strongly interacting with water molecules, while chaotropic ions are less effective.

Table 1. Some salts commonly used in the IL-based ATPSs.

Salt Name	Chemical Formula	Common Ion(s)	Type (Inorganic/Organic)	References
Potassium Phosphate	K_3PO_4	K^+, PO_4^{3-}	Inorganic	[18]
Dipotassium Hydrogen Phosphate	K_2HPO_4	K^+,HPO_4^{2-}	Inorganic	[20]
Potassium Carbonate	K_2CO_3	K^+, CO_3^{2-}	Inorganic	[23]
Ammonium Sulfate	$(NH_4)_2SO_4$	NH_4^+, SO_4^{2-}	Inorganic	[28]
Sodium Carbonate	Na_2CO_3	Na^+, CO_3^{2-}	Inorganic	[32]
Sodium Citrate	$Na_3C_6H_5O_7$	Na^+, $C_6H_5O_7^{3-}$	Organic	[37]
Sodium Chloride	NaCl	Na^+, Cl^-	Inorganic	[38]

Sodium Dihydrogen Phosphate	NaH_2PO_4	Na^+, $H_2PO_4^-$	Inorganic	[39]

4.2. Polymers

IL-polymer-based ATPS represent a distinct class, commonly utilizing neutral polymers such as polyethylene glycol (PEG) and polypropylene glycol (PPG), some of which are detailed in Table 2. In these systems, unlike IL-salt combinations, the phase separation is often driven by a "washing-out" phenomenon rather than direct salting-out. This occurs when water molecules preferentially hydrate the IL anion, disrupting the hydrogen bonds between the IL and the polymer, thereby inducing demixing.

Studies on PEG-based ATPS show that while long alkyl chains in IL cations can initially promote PEG solubilization at low concentrations, higher concentrations might lead to precipitation due to the dual hydrophilic/hydrophobic character of ILs. Crucially, the molecular weight of the polymer significantly impacts phase formation: higher molecular weight PEGs (e.g., PEG-3400, PEG-8000) enhance phase separation ability, necessitating lower concentrations of both IL and polymer for two-phase formation (as exemplified in Figure 5A and B from the original paper). This is primarily attributed to increased hydrophobicity with ascending molecular weight, which in turn diminishes the polymer's water solubility.

For PPG, which is inherently more hydrophobic than PEG, an increase in IL hydrophobicity can paradoxically hinder phase separation due to stronger IL-PPG interactions. However, similar to PEG systems, a general trend of higher molecular weight PPGs (e.g., EO10PO90 > PPG-1000 > PPG-400) correlating with enhanced phase-forming ability is observed. Some IL-PPG systems can also display thermoresponsive behaviors, such as Lower Critical Solution Temperature (LCST) or Upper Critical Solution Temperature (UCST), enabling temperature-controlled phase separation and facilitating the recovery of components like PPG-400 simply by adjusting temperature.

Overall, polymer-IL-based ATPS expand the accessible hydrophilic and hydrophobic ranges of the coexisting phases, which is beneficial for achieving more selective separations. This unique property, coupled with the potential for both polymers and ILs to act as salting-out agents in varying contexts, presents new opportunities for designing highly tailored ATPS. Common polymers employed in these systems are summarized in Table 2.

Table 2. Some polymers commonly used in the IL-based ATPSs.

Polymer Name	**Molecular Weight (Approx./Range)**	**Commonly Paired ILs (Examples)**	**References**
Poly(ethyleneglycol) (PEG)	Various (e.g., PEG-600, PEG-1000, PEG-2000, PEG-3400, PEG-3500, PEG-4000, PEG-6000, PEG-8000)	$[C_8NH_3][OAc]$, $[C_nmim]Cl$ (n=2–10), $[C_4mim]Cl$, $[C_6mim][C_{12}SO_3]$, $[C_2mim][BF_4]$, $[C_4mim][BF_4]$, $[C_2mim]Br$, $[C_4mim]Br$	[35], [39]
Poly(propylene glycol) (PPG)	Various (e.g., PPG-400, PPG-700, PPG-1000)	$[C_4mim][C_2H_5SO_4]$,$[C_4mim][CF_3SO_3]$, $[C_4mim][N(CN)_2]$, $[C_4mim][SCN]$, $[C_5mim]Cl$, $[C_6mim]Cl$, $[C_7mim]Cl$, DIMCARB, DPCARB, DACARB, DBCARB, Cholinium glycolate, Cholinium propionate, Cholinium lactate, Choline chloride, [Ch][BES]	[34], [37],
Poly(ethylene glycol) dimethyl ether (PEGDME)	PEGDME-250	Ch[L-Ala]	[15]
UCON (Ethylene Oxide–Propylene Oxide Copolymer)	Not specified	$[C_2mim]Cl$, $[C_2mim]Br$, $[C_2mim][SCN]$, $[C_2mim][OAc]$	[30]
EO–PO Copolymer ($EO_{10}PO_{90}$)	$EO_{10}PO_{90}$	Cholinium glycolate, Cholinium propionate, Cholinium lactate, Choline chloride	[38]

4.3. Surfactants

Research on IL-surfactant ATPS was limited until 2011, when Wei et al. first reported systems formed by $[C_4mim][BF_4]$ and sodium dodecyl benzene sulfonate (SDBS) [40]. Phase separation in these systems is often attributed to the formation of large micelle aggregates. Evidence suggests that cation-π interactions between the IL cation and the surfactant's benzene ring can increase micelle size, promoting demixing. Inorganic salts, particularly kosmotropic ones like K2HPO4, play a crucial role in maintaining ATPS stability by strengthening water-ion interactions.

Further studies with anionic surfactants, such as sodium dodecyl sulfonate (SDS) with 1-ethylpiperazinium tetrafluoroborate ($[C_2pi][BF_4]$), highlighted that ILs can significantly reduce the critical micelle concentration (CMC) of surfactants. This is due to the IL cation acting as a cosurfactant, influencing the micelle's electric double layer and promoting a "packing effect" that enhances micelle formation and efficiency [41].

Nonionic surfactants (e.g., Triton X, Tween family) also form ATPS with ILs, often exhibiting unique "island-type" phase diagrams where immiscibility occurs only in the ternary region, expanding with increasing temperature [42]. In these systems, the IL can act as a salting-out agent for the nonionic surfactant. The surfactant's hydrophobicity, quantifiable by its HLB (Hydrophilic-Lipophilic Balance) value, plays a key role; lower hydrophilicity (lower HLB) often correlates with larger biphasic regions [43]. Some common surfactants used in IL-based ATPS are summarized in Table 3.

Table 3. Some surfactants/saccharides commonly used in the IL-based ATPSs.

Name	Type (Saccharide/Surfactant)	Commonly Paired ILs (Examples)	References
Glucose	Saccharide	$[C_3mim][BF_4]$, $[C_4mim][BF_4]$, $[C_nmim][BF_4]$ (n = 3–10), $[C_2C_1py][C_4F_9SO_3]$, $[N_{1112}OH][C_4F_9SO_3]$, $[C_2mim][C_4F_9SO_3]$	[34]
Fructose	Saccharide	$[C_4mim][BF_4]$, $[C_2C_1py][C_4F_9SO_3]$, $[N_{1112}OH][C_4F_9SO_3]$, $[C_2mim][C_4F_9SO_3]$	[44]
Sucrose	Saccharide	$[C_nmim][BF_4]$ (n = 3–10), $[C_2C_1py][C_4F_9SO_3]$	[35]
Maltose	Saccharide	$[C_nmim][BF_4]$ (n = 3–10), $[C_2C_1py][C_4F_9SO_3]$	[44]
Xylose	Saccharide	$[C_nmim][BF_4]$ (n = 3–10), $[C_2C_1py][C_4F_9SO_3]$	[44]
D-sorbitol	Saccharide (Sugar Alcohol)	$[C_2C_1py][C_4F_9SO_3]$, $[N_{1112}OH][C_4F_9SO_3]$, $[C_2mim][C_4F_9SO_3]$	[43]
SDBS	Surfactant	$[C_4mim][BF_4]$, $[C_2pi][BF_4]$, $[Phpi][BF_4]$	[41]
SDS	Surfactant	$[C_4mim]Cl$, $[C_6mim]Cl$, $[C_8mim]Cl$, $[C_2pi][BF_4]$, $[Phpi][BF_4]$	[43]
Triton X-100	Surfactant	$[C_2mim][C_2SO_4]$, [Ch]Cl, $[C_2mim]Cl$	[42]
Tween 20	Surfactant	[Ch]Cl, $[N_{1112}OH][C_4H_5O_6]$	[43]

4.4. Amino Acids

Amino acids, as small organic compounds with low charge density, can also serve as salting agents to form ATPS with ILs. Zhang et al. [45] first reported ATPS formation between hydrophilic $[C_4mim][BF_4]$ and amino acids (glycine, L-serine, L-proline). Unlike typical IL-salt systems, the amino acid-rich phase was on top, and lower temperatures favored ATPS formation. The ability to form ATPS with $[C_4mim][BF_4]$ followed the order: glycine > L-serine > L-proline, indicating that not all hydrophilic ILs are suitable.

Further studies by Domínguez-Pérez et al. [46] showed that for $[C_4mim]^+$ ILs, the anion's influence on ATPS formation with amino acids followed: $[BF_4]^- > [CF_3SO_3]^- > [N(CN)_2]^-$ This behavior is akin to salting-out salts and is explained by the competition for water hydration, strongly linked to the IL anion's hydrogen-bond basicity (β) [47]. Lower β values (weaker hydrogen-bond acceptors) enhance ATPS formation. Amino acids' phase formation ability increased with their water solubility, as more soluble amino acids more readily form hydrates, precipitating the IL.

Compared to inorganic salts, amino acids have a weaker salting-out ability. They facilitate phase separation by forming water-amino acid complexes, which in turn reduce solution ionic strength and can lessen ion exchange between phases, aiding IL recycling. The balance of water-amino acid, IL-amino acid, and water-IL interactions, determined by side chain affinities, dictates salting-in/salting-out phenomena, emphasizing that stronger amino acid hydrophilicity leads to stronger phase separation. Similar to IL-inorganic salt ATPS, the immiscibility region generally decreases with increasing temperature, favoring lower temperatures for ATPS formation. Currently, only imidazolium-based ILs have been widely observed to form ATPS with amino acids, highlighting an area for further research.

4.5. Saccharides

Low molecular weight saccharides can also induce ATPS formation with ILs. Initially, $[C_4mim][BF_4]$ was shown to form ATPS with fructose, with lower temperatures favoring separation. More systematic studies revealed that while some imidazolium-based ILs (e.g., $[C_nmim]Cl$, $[C_nmim]Br$) failed to form ATPS with carbohydrates, fluorinated ILs like $[C_4mim][BF_4]$ and $[C_3mim][BF_4]$ successfully did [34]. The size of the alkyl chain on the imidazolium ring and the hydrophobicity of the IL (e.g., longer fluorinated alkyl chains in anions) both enhance phase separation. The phase-separation ability of carbohydrates generally follows: glucose > maltose > sucrose > xylose. Interestingly, adjusting glucose amounts can even reverse the IL-rich and glucose-rich phases.

The ability of carbohydrates to induce demixing is primarily linked to their extensive hydration, particularly through hydrogen bonding of their numerous hydroxyl groups with water. More hydroxyl groups generally lead to stronger phase separation [48]. For example, polyols like maltitol with more -OH groups show higher phase-forming ability. Even with the same number of hydroxyls, structural differences (e.g., pyranose vs. furanose rings in disaccharides) can influence salting-out strength.

The underlying mechanism involves a competition for water molecules between the ILs and saccharides. Saccharides, having a higher innate affinity for water, essentially "win" this competition, leading to the dehydration of IL ions and their precipitation from the aqueous solution. This results in the formation of two distinct microphases of water. As the concentration of either IL or saccharide increases, the stability of the solution is disrupted, leading to coagulation and phase separation. In these systems, saccharides primarily act as salting-out agents. Some saccharides commonly used in IL-based ATPS are listed in Table 3.

V. Conclusions

ATPS incorporating ionic liquids represent a significant advancement over traditional liquid-liquid extraction methods. These systems combine the inherent benefits of ILs—such as non-volatility, non-flammability, high thermal stability, and structural tunability—with the advantages of ATPS, including simplicity, high efficiency, rapid phase separation, and a biocompatible environment. This synergy positions IL-based ATPS as a highly competitive and environmentally favorable alternative for separation processes in an era of increasing environmental awareness.

Despite their promising outlook, IL-based ATPS still face notable challenges for widespread industrial adoption, including high viscosity, elevated costs, and the crucial need for efficient IL recovery and reuse. While temperature adjustment can reduce viscosity, it might compromise ATPS properties or suitability for heat-sensitive substances. Future research must prioritize economic aspects, such as reducing raw material costs and optimizing processes, alongside developing robust recycling strategies for ILs.

Key knowledge gaps that urgently need addressing for practical application include a deeper understanding of the physicochemical data, microstructure, and fundamental phase separation mechanisms of IL-based ATPS. Furthermore, establishing clear guidelines for IL selection tailored to specific analytes is essential. It's also critical to acknowledge that ILs, despite their "green" reputation, are not entirely pollution-free and often resist biodegradation. Therefore, ongoing efforts in IL-based ATPS are strongly geared towards developing even more environmentally benign approaches, aligning with the principles of green analytical chemistry, with the expectation of fostering increasingly sustainable separation systems.

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