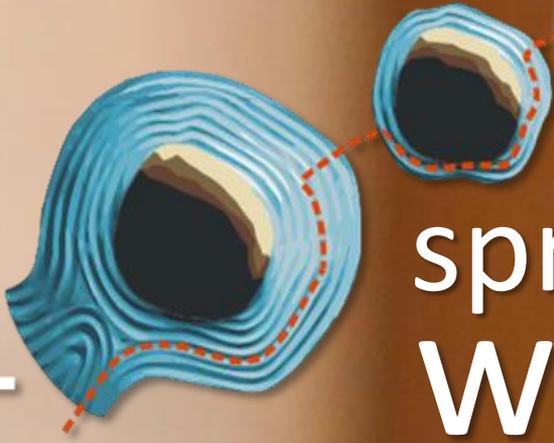


path



spring workshop



May 9th 2016

Montebelo Hotel
Vista Alegre - Ílhavo

PATH Spring Workshop

May 9th 2016

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BOOK OF ABSTRACTS

Organizing Committee:

João A.P. Coutinho

Mara G. Freire

Sónia P. M. Ventura

Scientific Program

09:00 – Opening session

09:10 – Switchable systems

Thermoreversible ionic-liquid-based aqueous biphasic systems (Helena Passos)

Effect of ionic liquids into the development of thermosensitive aqueous micellar two-phase systems (Filipa A. Vicente)

pH-driven reversible aqueous biphasic systems composed of ionic liquids (Ana M. Ferreira)

10:00 – Multiphasic systems

Ionic liquids as phase-forming components of aqueous multiphasic systems (Helena Passos)

Switchable nature of aqueous multiphasic systems consisting of polymers and salts (João Henrique P. M. Santos)

10:30-11:00 – Coffee Break

11:00 – Polarity changes by combining PEGs and ILs

Ionic liquids as a tool to control the phase behaviour and extraction capability of aqueous two-phase systems (Álvaro Silva Lima)

Addition of ionic liquids to tune the phases' polarities of polymer-salt aqueous biphasic systems (Mafalda R. Almeida)

11:30 – Characterization of the relative polarities of ABS coexisting phases

Evaluation of alkaloids partitioning as effective probes to determine the relative hydrophobicity of aqueous biphasic systems (Jorge F. B. Pereira)

Abraham's linear free-energy relationships for characterizing ionic-liquid-based aqueous biphasic systems (Pedro P. Madeira)

New insights into the physicochemical behaviour of ionic-liquid-based aqueous biphasic systems (Helena Passos)

12:00 – New evidences on ILs vs Salts characteristics

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Temperature influence on polymer aqueous biphasic systems: a mechanism to reveal the nature of salts (Jorge F. B. Pereira)

12:30-13:30 – Lunch

13:30 – Lecture

Millions of new ionic liquids are hiding in plain sight: understanding the nature of the bonding in double salt ionic liquids (aka ionic liquid mixtures) (*Robin D. Rogers*)

14:30 – Alternative IL-based ABS

Mixtures of ionic liquids as an alternative approach to tailor the phase's separation ability and extraction performance of aqueous biphasic systems (*Teresa B. V. Dinis*)

Selective separation of amino acids using aqueous biphasic systems composed of ionic liquids (*Emanuel V. Capela*)

Ionic-liquid-based aqueous biphasic systems for the fractionation of carbohydrates: case studies with glucose/fructose and dextrans of different molecular weights (*Pankaj Bharmoria*)

Aqueous biphasic systems comprising two ionic liquids aiming at the development of improved separation techniques (*Maria V. Quental*)

Novel aqueous biphasic systems composed of ILs and copolymers (*Francisca A. e Silva*)

15:45-16:15 – Coffee Break

16:15 – Hydrotropic- and micelle-mediated effects of ILs in biomass processing

Ionic liquids aqueous solutions in the extraction and purification of biocompounds (*João A.P. Coutinho*)

Micelle-mediated extraction of phycobiliproteins (*Filipa A. Vicente*)

Extraction and recovery of cynaropicrin from *Cynara cardunculus L.* using ionic liquids aqueous solutions (*Emanuelle L. P. de Faria*)

16:45 – Extraction and purification of proteins using ILs

Extraction and purification of IgG from serum using liquid-liquid systems comprising ionic liquids (*Dibyendu Mondal*)

Purification of IgY using polymer-based aqueous biphasic systems combined with centrifugal partitioning chromatography (*Mafalda R. Almeida*)

Development of alternative early-stage cancer diagnosis methods by the application of ionic liquids (*Matheus M. Pereira*)

17:30 – Closing

Thermoreversible Ionic-Liquid-based Aqueous Biphasic Systems

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The ability to induce reversible phase transitions between aqueous homogeneous solutions and biphasic liquid-liquid systems, at pre-defined and suitable operating temperatures, is of outstanding relevance in the design of separation and purification processes for valued-added proteins. It was already demonstrated that phase transitions in mixtures involving hydrophobic ionic liquids (ILs) and other solvents can be induced by changes in temperature or by reversible reactions with CO₂ forming salts or ILs. However, most of the binary liquid-liquid systems comprising ILs typically occur at temperatures far from room temperature and are confined to mixture compositions imposed by the upper or lower critical points of the phase diagrams. Moreover, these systems are composed of an IL-rich phase (typically with hydrophobic characteristics) and a molecular-solvent-rich phase.

In this work, we show that aqueous biphasic systems (ABS), i.e., ternary systems where the major solvent is water, may have their reversible behavior triggered by small changes in temperature (as low as 1 °C). The applicability of the temperature-induced phase switching is further demonstrated with the complete extraction for the IL-rich phase of two valued-added proteins, namely cytochrome c and azocasein, achieved in a single-step. It is shown that the temperature induced phase switching provided by IL-based ABS is significantly more versatile than classical liquid-liquid systems which are constrained by their specific critical temperatures. Instead, this approach allows to combine and to work in a wide range of temperatures and compositions which can be tailored to fit the requirements of a given separation process.

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Effect of Ionic Liquids into the Development of Thermosensitive Aqueous Micellar Two-Phase Systems

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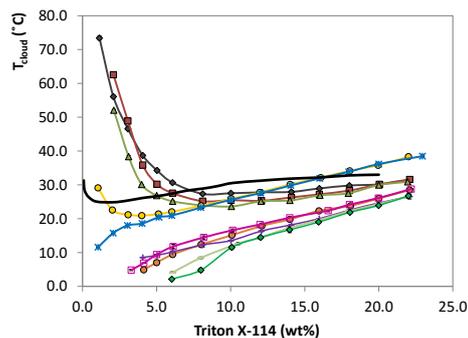
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Aqueous micellar two-phase systems (AMTPS) using surfactants, are advantageous thermosensitive extraction systems, since they are mostly composed of water, emerging as an appellative type of liquid-liquid extraction (LLE). AMTPS composed of two surfactants enhanced the extraction of biomolecules, hence exploiting the advantage of introducing ionic liquids (ILs) as a new class of co-surfactants came as an attractive alternative due to their unique properties. Though, it is necessary to firstly design and understand the phase diagrams of such systems. Therefore, the binodal curves of conventional and novel AMTPS were carried out to evaluate the effect of distinct tensioactives ILs, belonging to the imidazolium and phosphonium families, upon the systems' cloud points (T_{cloud}). Herein, several ILs' structural features were taken into consideration, namely the cation and anion effect, the cation alkyl side chain and symmetry influence, as well as the IL concentration. Additionally, the binodal curves were determined for various nonionic surfactants, such as Triton X-114 (Figure 1) and surfactants belonging to the Tergitol and Pluronic families. The effect of the hydrophobic/hydrophilic nature of the different ILs as co-surfactants was addressed, as well as the influence of the surfactant nature.

Figure 1. Binodal curves for Triton X-114 based-AMTPS with different ILs tested as co-surfactants at 0.3 wt%, pH 7: —, without IL; ♦, [C₁₀mim]Cl; ■, [C₁₂mim]Cl; ▲, [C₁₄mim]Cl; ●, [P_{6,6,6,14}]Cl; *, [P_{6,6,6,14}]Br; ●, [P_{6,6,6,14}][Dec]; +, [P_{6,6,6,14}][N(CN)₂]; □, [P_{6,6,6,14}][TMPP]; —, [P_{8,8,8,8}]Br; ◆, [N_{8,8,8,8}]Br. The effect of ILs' structural features is provided separately in the insets to facilitate the analysis.



Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors thank financial support from Fundação para a Ciência e a Tecnologia (FCT) through the doctoral and post-doctoral grants SFRH/BD/94901/2013 and SFRH/BPD/79263/2011 of F. A. e Silva and S.P.M. Ventura, respectively. Santander Scholarship granted to Filipa A. Vicente and the financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Process N° 2011/20521-0) given to Luciana P. Malpiedi. The authors also thank the financial support from FAPESP through the project FAPESP 2012/12022-6. This project was also afford by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) from Brazil.

pH-Driven Reversible Aqueous Biphasic Systems Composed of Ionic Liquids

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Recently, dynamic and reversible biphasic systems constituted by ionic liquids (ILs) revealed to be potential extraction strategies.¹ It was previously demonstrated that mixtures involving ILs and other solvents can be switched between the homogeneous regime and a two-phase system either by a temperature-driven phenomenon or by adding CO₂/N₂.¹ These systems have shown to be remarkable in the selective separation of several value-added compounds, such as proteins.¹ Furthermore, the research in liquid–liquid extractions using ILs focuses on two main approaches: the direct use of non-water miscible hydrophobic ILs and the use of aqueous biphasic systems (ABS) composed of ILs and organic/inorganic salts. Between the two types of systems, ABS constitute a “greener” and more biocompatible option since they are mainly composed of water (up to 70 wt%).² Furthermore, these systems can lead to the complete extraction of a wide variety of compounds and to high concentration factors.² The major goal of this work consists on the exploitation of switchable IL-based ABS triggered by a pH-dependent phenomenon or addition CO₂/N₂.

First, a large array of ABS, obtained by the combination of potassium citrate with different ILs, was initially investigated by the determination of their ternary phase diagrams at different pH values. The first set of reversible IL-based ABS was ascertained by the addition of citric acid or potassium hydroxide *i.e.*, by the manipulation of the speciation of the organic salt used. Their reversibility behaviour was demonstrated for at least 3 times. In addition to IL-salt mixtures, polymer IL-based ABS were also investigated while reaching their switchable behaviour through the IL anion speciation. Second, it was study reversible ABS formed by aqueous solutions of polymers and ethanolamine-based compounds able to form salts by a reversible reaction with CO₂/N₂. The dynamic change between a homogeneous solution and a two phase system is a key advantage in the separation field. This reversible picture occurs at room temperature and is achieved with low cost compounds.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. A. M. Ferreira acknowledges FCT for the PhD grant SFRH/BD/92200/2013.

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Ionic Liquids as Phase-Forming Components of Aqueous Multiphasic Systems

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Aqueous multiphasic systems (AMS) were introduced by Mace et al.¹ in 2012, by the combination of a large number of polymers and surfactants mixtures in aqueous solutions. AMS share all the benign characteristics of the well-studied aqueous biphasic systems (ABS); yet, selective and improved separations of high-value compounds are better achieved by the presence of more aqueous phases.² For instance, the successful separation of nanoparticles by rate-zonal centrifugation was already reported using AMS.³ However, and to the best of our knowledge, AMS formed by ionic liquids (ILs) and their possible applications were not explored up to date.

In this work, novel AMS formed by quaternary mixtures composed of cholinium ([N₁₁₁₂OH])⁺-based ILs, polymers, inorganic salts and H₂O are presented. The respective phase diagrams which define the monophasic and biphasic regions, as well as the corresponding tie-lines, were determined. The ability of these new systems for selectively extracting a mixture of three dyes was also evaluated. The outstanding performance of IL-based AMS will be clearly demonstrated as a result of the tailoring features afforded by ILs.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors also acknowledge FCT for the doctoral grant SRH/BD/85248/2012 of H. Passos. M. G. Freire acknowledges the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013) / ERC grant agreement n° 337753.

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Switchable Nature of Aqueous Multiphasic Systems Consisting of Polymers and Salts

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Aqueous multiphasic systems are structured and more complex systems, in this case, composed of mixtures of aqueous solutions of polymers, using water as the main component. These systems have been largely ignored regarding their characterization but mainly their possible application, however their potential is undoubtedly promising. Multiphasic systems formed by propylene glycol (PPG 400) and sodium polyacrylate (NaPA 8000) and small contents of sodium chloride (NaCl) used in this work as an electrolyte were investigated. Studies of different contents on electrolyte and temperature effect were done. The three aqueous phases were characterized by ATR-FTIR and chloride titration. The relative hydrophobicity of the phases was visually described by the use of three dyes of very different hydrophobic/hydrophilic characteristics, namely sudan III, ultramarine blue pigment (PB29) and tartrazine (E102). The results demonstrated the switchable behavior of these multiphasic systems, triggered by a temperature and salt content dependent phenomenon.

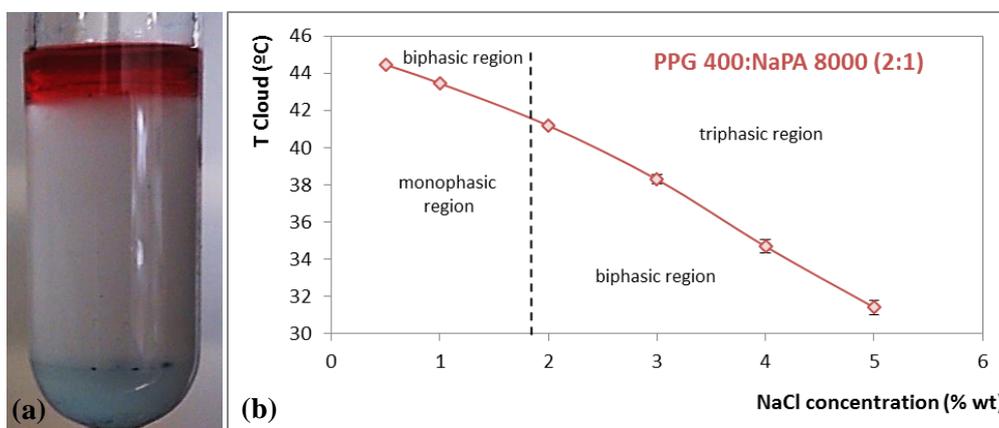


Figure 1. (a) Visual aspect of the multiphasic systems under study. (b) Impact of temperature and electrolyte (NaCl) concentration (% wt) in the aqueous-phase equilibria for systems based in PPG 400:NaPA 8000, in a mass ratio of (2:1).

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors are thankful to FCT for the financial support on the ambit of the doctoral grant SFRH/BD/102915/2014 of João H. P. M. Santos and post-doctoral grant SFRH/BPD/79263/2011 of Sónia P.M. Ventura.

Ionic Liquids as a Tool to Control the Phase Behaviour and Extraction Capability of Aqueous Two-Phase Systems

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Aqueous two-phase systems (ATPS) can be formed by mixture of a pairs of phase-forming constituents above a critical thermodynamic point. Traditionally, they are composed by polymer-polymer or polymer-salt system. In this context, polyethylene glycol (PEG) has been applied together salts (phosphate, citrate and carbonate) to form ATPS. However, the hydrophilic nature of PEG and restrict range of polarities of the coexisting phases limit its application. To overcome this limitation, the literature has been seeking some alternatives such as addition of electrolytes like sodium chloride to increase the hydrophobicity difference between the phases; the PEG functionalization to enhance the affinity between target molecule and PEG; ionic liquid (IL) as a constituent of system, allowing the manipulation of physicochemical properties of system; and, the addition of small amount of IL, which act as an adjuvant in ATPS. In several cases, the system using IL as an adjuvant display a better phase separation than PEG- or IL-salt systems, suggesting that mixtures of IL-PEG may be more hydrophobic than their pure components and are more easily salted-out by salts in aqueous media. The effect of ILs on binodal curves follows the trend imposed by the ILs' hydrophobic character, which leads to reduce miscibility and increase the phase separation. The trend is similar those found for ATPS based on IL-salts. Moreover, the addition of IL in small amount makes the system cheaper than those ATPS based on IL-salt. For ATPS based on PEG-salt, ILs migrate preferentially for the PEG-rich phase and can drag the target molecule or contaminants due to interaction $\pi \cdots \pi$ with it. IL drags the L-tryptophan to PEG-rich phase in PEG-600 + Na₂SO₄ system. In other case, ILs takes the contaminant protein to PEG-rich phase while the lipase migrate to salt-rich phase, improving the purification factor (PF) of enzyme. PEG-salt and IL-salt systems have lower PFs than system using adjuvants (Table 1). Moreover, IL is more efficient than NaCl as adjuvant in lipase purification (PF = 245 fold).

Table 1. Purification Factor of Lipase using different aqueous two-phase systems

ATPS Constituents			ATPS Constitution (wt %)			PF (fold)
W ₁	W ₂	W ₃	W ₁	W ₂	W ₃	
PEG 1500	K ₂ HPO ₄ /KH ₂ PO ₄	[C ₆ mim]Cl	15	15	5	245.0
PEG 8000	K ₂ HPO ₄ /KH ₂ PO ₄	NaCl	20	18	6	201.5
PEG 8000	K ₂ HPO ₄ /KH ₂ PO ₄	-	20	18	-	123.9
[C ₈ mim]Cl	K ₂ HPO ₄ /KH ₂ PO ₄	-	25	30	-	51.0

Addition of Ionic Liquids to Tune the Phases' Polarities of Polymer-Salt Aqueous Biphasic Systems

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Aqueous biphasic systems (ABS) have been successfully applied in the purification of the most diverse biological materials, such as cells, lipids, nucleic acids and proteins. Despite the several advantages of conventional ABS composed of polymer-polymer or polymer-salt combinations, these systems still exhibit a restricted polarity difference among the coexisting phases which has been preventing their successful application in the extraction/purification of some added-value biomolecules. However, the introduction of ionic liquids (ILs) can lead to a tailoring of the phases' polarities allowing thus an improved performance in extraction and purification approaches.

In this work, the phase diagrams of ABS composed of water, poly(ethylene glycol) and phosphate buffer were determined, as well as those where 1-butyl-3-methylimidazolium chloride was introduced. The IL concentration in the ABS formation ability was evaluated. Furthermore, aiming at exploring the “untypical” behavior observed, where small amounts of IL largely favor the formation of a two-phase system when compared with IL-salt and polymer-salt ABS, the Kamlet–Taft parameters, such as hydrogen-bond donating ability (α), hydrogen-bond accepting ability (β), and dipolarity/polarizability (π) were also determined. Finally, the relative hydrophobicity of the phases was evaluated with extraction and purification results regarding different biomolecules, with different characteristics and properties.

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Evaluation of Alkaloids Partitioning as Effective Probes to Determine the Relative Hydrophobicity of Aqueous Biphasic Systems

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Recently, several Aqueous Biphasic Systems (ABS) have been studied, in particular, those that have a biocompatible and environmental character. Although a large interest in this type of systems as improved extraction and purification platforms appeared in the past few years, the characterization of their relative polarity and of the coexisting aqueous phases has been lagged behind. In this context, this work addressed the characterization of the relative hydrophobicity of polymer-salt ABS by determination of the partition of three alkaloids (caffeine, theobromine and theophylline) used here as relative hydrophobicity probes. For that purpose, a series of ABS composed of polyethylene glycol (PEG, with an average molecular weight of 600 g.mol⁻¹) and cholinium-based compounds (salts and ILs) were prepared, and the experimental results of the alkaloids' partitioning demonstrated that in all the systems alkaloids migrate preferentially for the low water content PEG-rich phase. The experimental results have shown a linear dependence between the logarithmic function of the partition coefficients and the water content ratio between the two phases in equilibrium. These results were supported by COSMO-RS (Conductor-like Screening Model for Real Solvents), namely, to compute the screening charge distributions of the salts/IL components of ABS and alkaloids, excess enthalpy, and activity coefficients at infinite dilution. In summary, this work demonstrates that the determination of the partition coefficients of alkaloids in ABS can be used to evaluate the relative hydrophobicity of the coexisting phases, and consequently to support the development of predictive models to be used for developing effective ABS at an industrial scale for the separation and recovery of added-value biomolecules.

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Abraham's Linear Free-Energy Relationships for Characterizing Ionic-Liquid-based Aqueous Biphasic Systems

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Ionic-Liquid-based Aqueous Biphasic Systems (IL-based ABS) have received attention towards the development of novel and more efficient separation processes. While our understanding of the properties of IL-based ABS has increased considerably in recent years, we are not yet to the point of being able to “tailor make” them having specific physical and chemical properties.

One of the most widely used tools for characterizing the contribution of intermolecular interactions to separation processes is the solvation equation approach that Abraham has developed over the last 25 years:

$$\log P = c + eE + sS + aA + bB + vV + (j^+ \cdot J^+ + j^- \cdot J^-) \quad (1)$$

The solute descriptors in Eq. (1) were defined for neutral compounds as follows: E is the solute excess molar refractivity in units of $(\text{cm}^3\text{mol}^{-1})/10$, S is the solute dipolarity/polarizability, A and B are the overall or summation hydrogen bond acidity and basicity, and V is the McGowan volume, V_x , in units of $(\text{cm}^3\text{mol}^{-1})/100$. The coefficients in Eq. (1) are not just fitting constants but reflect the chemistry of the system in question. In particular, the a-coefficient will reflect the hydrogen bond basicity of the system (because a hydrogen bond solute will interact with a system that is a hydrogen bond base) and the b-coefficient will reflect the hydrogen bond acidity of the system. Thus the coefficients in Eq. (1) serve to characterize particular systems. For the partition of ions and ionic species Eq. (1) will be used with an extra descriptor for cations, J^+ , and an extra descriptor for anions, J^- .

We are engaged in the application of Abraham's approach for the study of IL-based ABS. A couple of examples of its applicability will be briefly shown in this communication. We will also present the results obtained thus far and discuss the ongoing/future work.

New Insights into the Physicochemical Behaviour of Ionic-Liquid-based Aqueous Biphasic Systems

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Aqueous biphasic systems (ABS) have appeared as more benign liquid-liquid separation processes since they consist in two immiscible aqueous-rich phases and can be created by the mixture of two polymers, a polymer with a salt or two salts. However, and despite these more conventional ABS, in 2003, it was demonstrated that ABS can be also created by the mixture of ionic liquids (ILs) and inorganic salts. These IL-based ABS present additional and outstanding advantages when compared to the more traditional polymer-based systems, such as low viscosity, quick phase separation and high extraction efficiencies for the most diverse biomolecules.¹ All these features contribute to the development of cost-effective industrial processes.

A large amount of research studies have already demonstrated the improved potential of IL-based ABS for the extraction of some value-added compounds.¹ However, their actual use at an industrial level was not attempted up to date since a deeper understanding on the mechanisms which rule the partitioning of the biomolecules among the coexisting phases is still missing. Thus, in the present work, our most recent results in the determination of the relative hydrophobicity as well as the identification of new probes and approaches to assess the solvatochromic parameters (solvent dipolarity/polarizability, π , solvent hydrogen-bond donor acidity, α , and solvent hydrogen-bond acceptor basicity, β) of the coexisting phases of ABS composed of ILs and inorganic salts will be presented. The ILs investigated were selected to primarily determine the IL anion impact through the relative hydrophobicities and solvatochromic properties displayed in IL-based ABS.

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Narrowing the Gap Between Ionic Liquids and Higher Melting Salts Using Aqueous Biphasic Systems

Francisca A. e Silva¹, Jorge F. B. Pereira², Kiki A. Kurnia¹, Sónia P. M. Ventura¹, Artur M. S. Silva³, Robin D. Rogers⁴, João A. P. Coutinho¹ and Mara G. Freire¹

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Ionic liquids (ILs) are currently defined as salts with melting temperatures below 100 °C. Opposing to higher melting salts, ionic liquids are formed by low-charge density ions that allow a rich variety of non-coulombic interactions to dramatically influence their properties. Although being useful, this melting temperature-based definition is yet simplistic, once it undervalues the complexity and richness of interactions occurring in ILs. In this work, a new definition for ionic liquids considering not only their melting points, but also their phase-forming abilities with polyethylene glycol (PEG) in aqueous biphasic systems (ABS) is proposed. It is known that the formation ability of IL-polymer-based ABS is influenced by temperature: while for imidazolium-based ILs + PEG combinations in aqueous media the phase separation ability is hampered by an increase in temperature, the opposite pattern is verified for cholinium-based ILs-PEG-based systems. Based on this scenario, the ternary phase diagrams of a large set of quaternary ammonium chlorides, covering compounds that are unquestionably higher melting temperature salts (*e.g.* tetramethylammonium chloride, [N₁₁₁₁]Cl and cholinium chloride, [N_{1112(OH)}]Cl) or ILs (*e.g.* tetrabutylammonium chloride, [N₄₄₄₄]Cl) + PEG 2000 + water were determined at distinct temperatures. The results obtained indicate that these compounds can display either higher melting salt-like or ionic-liquid-like behaviour. The molecular-level mechanisms controlling these opposite behaviours were ascertained by means of ¹H NMR spectroscopy and COnductor-like Screening Model for Real Solvents (COSMO-RS). At the end, the restrictive nature of the currently accepted IL definition is highlighted, suggesting the crucial need for its reformulation.

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Temperature Influence on Polymer Aqueous Biphasic Systems: *a* Mechanism to Reveal the Nature of Salts

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The common definition of ionic liquids (ILs) are based on their melting temperatures, where they are generally defined as salts with melting temperatures below 100°C. This is a direct result of their low charge density and symmetry between the ions pair, as well cation-anion intermolecular forces. Although, the large assumption of this definition, it is quite restrictive and it dismisses part of complexity and large array of interactions of ILs. On the other hand, recently, the investigation of several series of aqueous biphasic systems (ABS) have been allowing to gauge some particular features of the phase forming agents, where their high or low capability to induce a biphasic region is a direct result of type and physical state of the water-soluble compounds used on the ABS formation. Thus, herein, an approach based on the study of temperature effect on the solubility curves of ABS composed of polyethylene glycol (PEG, with an average molecular weight of 200 g.mol⁻¹) and N-alkyl-, dialkyl, and trialkyl-ammonium salts of acetate was proposed. The results obtained demonstrated that the liquid-liquid demixing temperature dependency is quite dependent with the salts/ILs melting temperatures. The results show that occurs a reduction or enlargement of two-phase region with the increase of temperature, if a longer (such as, hexylammonium acetate) or shorter (such as, ammonium or propylammonium acetate) alkyl side chain lengths were used. These patterns are in agreement with IL-polymer-like or salt-polymer-like behavior in ABS, respectively. The inversion of pattern observed for the longer alkyl side chains ammonium salts reveals their IL nature. On the other hand, although propylammonium acetate have a melting point below 100°C, their aptitude to induce the ABS formation is similar than other common inorganic salts. Therefore, these results support the restrictive nature of the currently accept ILs' classification, and the crucial need for its reformulation, where the study of complex systems can allow to have further insights about the ionic species nature.

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Millions of New Ionic Liquids are Hiding in Plain Sight: Understanding the Nature of the Bonding in Double Salt Ionic Liquids (aka Ionic Liquid Mixtures)

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Ionic liquids (ILs) certainly have some interesting properties and this has led to tremendous growth in interest, knowledge, and applications of these materials in a diverse array of science and technology. However, the field is still dominated by study of the simplest binary salts and an over reliance on ‘magic’ solvent properties which currently limits their creative use for new technologies. Lately, the scientific community has been attracted towards “IL/IL mixtures” prepared by mixing two or more ILs, and (as was the case with the early studies of the binary salts), a focus on specific physicochemical properties. Interestingly, crystalline salts dissolved in ILs are seen as a different class of liquids (e.g., high ionicity ILs). We have found that the physical, chemical, and biological (i.e., the materials) properties of IL/IL mixtures (or Double Salt Ionic Liquids, DSILs, as we like to call them) seem to be mainly controlled by the intrinsic ion interactions which are influenced by the exact ion composition. Perhaps, a new way of thinking about these multi-ion fluids is needed in order to direct research toward an understanding of the complex and varied types of interionic forces possible in a liquid. We have proposed the DSIL concept to help guide our work towards obtaining this deeper understanding. In this nomenclature, any homogeneous liquid containing three or more ions (no matter how they are prepared) are described as DSILs and are defined by the specific ion combinations present.

In this presentation, these concepts will be illustrated by discussion of the chemical, physical, and biological properties of several DSIL systems chosen to explore different interactions between the ions (i.e., Coulombic, hydrogen bonding, dispersion, etc.). While we will present physical properties as a function of composition, we will focus more on chemical properties illustrated by the solubilities of pharmaceuticals and on biological properties illustrated by changes in membrane transport, both as a function of ion composition. Spectroscopic studies will be presented to provide insight into specific ion-ion interactions. For example, the solubility of active pharmaceutical ingredients with different acidity and lipophilicity are finely tunable by varying the ratio of the ions (Figure 1). Such studies support the concept that each DSIL is a unique ionic fluid that is able to provide tunable property sets derived from the ions present and that these properties can be controlled by choosing the nature and the abundance of the ions.

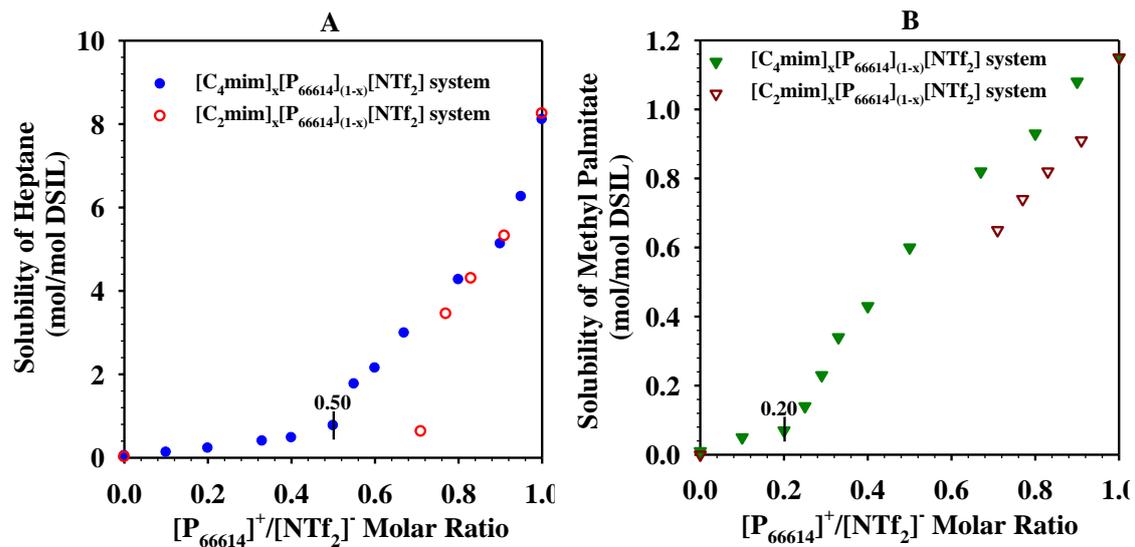


Figure 1. Comparison of the solubilities of heptane (A; circle) and methyl palmitate (B; triangle) in [C₄mim]_x[P₆₆₆₁₄]_(1-x)[NTf₂] (solid symbols) with those in the possible compositions of [C₂mim]_x[P₆₆₆₁₄]_(1-x)[NTf₂] (open symbols).

Mixtures of Ionic Liquids as an Alternative Approach to Tailor the Phase's Separation Ability and Extraction Performance of Aqueous Biphasic Systems

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In the past decade, ionic liquids (ILs) were introduced as phase-forming components and as promising replacements of polymers in ABS.¹ Due to the wide variety of their chemical structures, the introduction of ILs in ABS allows to overcome the narrow hydrophilic-hydrophobic range of polymer-based ABS. As a result, IL-based ABS have shown to provide higher extractions performance and increased selectivity for a wide plethora of compounds.² Phase diagrams and possible applications of ABS composed of ILs and salts were widely investigated in the past decade³ and it is now accepted that the phases' demixing ability and polarities of the coexisting phases are largely dependent on the IL anion hydrogen-bond basicity.³

In this work, mixtures of two ILs with a common cation and different anions were investigated aiming a more controlled manipulation of the phases' polarities and ABS ability to undergo liquid-liquid demixing. Novel ABS phase diagrams were determined and characterized for systems composed of K₂CO₃, water and mixtures of [C₄C₁im][CF₃SO₃] and [C₄C₁im]Cl in different mole fractions. The relative polarity of the coexisting phases was additionally evaluated by partitioning experiments of a series of dinitrophenylated (DNP) amino acids. In general, the formation capability of the systems investigated decreases and the partitioning extent for the IL-rich phase increases with the content of [C₄C₁im]Cl. It will be shown that ABS composed of mixtures of ILs are a promising strategy for developing more selective separation platforms.

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Selective Separation of Amino Acids Using Aqueous Biphasic Systems Composed of Ionic Liquids

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Aromatic amino acids, such as L-tryptophan, L-phenylalanine and L-tyrosine, can be produced by bacterial fermentation.¹ However, these are difficult to obtain at a high production yield and purity level, and several approaches have been attempted, which include their continuous removal from the fermentative medium by liquid-liquid extraction.¹ To this goal, the application of ionic-liquid-based aqueous biphasic systems (IL-based ABS) could be seen as a valuable alternative to guarantee a continuous extraction and purification of aromatic amino acids during fermentation. In the field of IL-based ABS, those formed with inorganic/organic salts are the most studied.² However, recently it has been demonstrated that aliphatic amino acids can behave as salting-out agents and form IL-based ABS.³ Based on this possibility, in the current work, we investigated the ability of ABS formation combining ammonium- and phosphonium-based ILs with aliphatic amino acids, namely L-lysine, L-lysine·HCl and L-proline, and we then evaluated the potential of these systems to separate aromatic amino acids (L-phenylalanine, L-tyrosine and L-tryptophan).

The ABS phase diagrams were firstly determined at 25°C and then used for the design of aqueous two-phase extraction routes envisaging the selective separation of aliphatic and aromatic amino acids. According to the gathered results, [P₄₄₄₄]Br + Lysine reveals to be the better system to selectively separate aromatic and aliphatic amino acids, attaining extraction efficiencies up to 85 % for opposite phases in a single-step. The obtained results reveal that IL-based ABS are promising approaches for the separation of mixtures of amino acids, being their selectivity highly dependent on the IL employed.

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Ionic-Liquid-based Aqueous Biphasic Systems for the Fractionation of Carbohydrates: Case Studies with Glucose/Fructose and Dextrans of Different Molecular Weights

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The commercial potential of carbohydrates in food and pharmaceutical industries is a factual figure and has been rising exponentially.^{1,2} Yet, challenges still persist on their cost-effective production in terms of separation and purification to meet quality demands. Recently, the use of ionic-liquid-based aqueous biphasic systems (IL-ABS) to purify a variety of biomolecules *via* liquid-liquid extraction has shown large potential.³ Herein, the application of IL-ABS for the fractionation of carbohydrates with a special focus on mixtures of glucose/fructose and dextrans of different molecular weights was investigated. In particular, the application of IL-ABS for the separation of glucose/fructose mixtures will be discussed in light of (1) production of “high fructose corn syrup (HFCS)” directly from sugarcane juice; and (2) extraction of individual glucose and fructose from underutilized sources of “date palm fruit”. On the other hand, the separation and purification of dextrans of different molecular weights from fermentation broths will be discussed. To these aims, the respective ternary phase diagrams composed of carbohydrates and ILs have been determined and the effect of the ILs molecular structure in light of their ability to form IL-ABS has been investigated. After, the selective separation of carbohydrates mixtures was studied with model systems. Finally the most promising systems were applied to real samples of sugarcane juice, date palm and fermentation broths are produced to selectively separate glucose from fructose and to fractionate dextrans of different molecular weights.

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Aqueous Biphasic Systems Comprising Two Ionic Liquids Aiming at the Development of Improved Separation Techniques

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In the last decade, ionic-liquid-based aqueous biphasic systems (IL-ABS) have been deeply investigated due to the possibility of tuning the polarities and affinities of the coexisting phases by employing ILs with different chemical structures.^{1,2} However, this tailoring ability is mainly attained at the IL-rich phase since the opposite phase is enriched in the other phase-forming component, *e.g.*, polymers or salts. In this work, the expansion of the tailoring ability in both phases is demonstrated by the formation of ABS composed of two ILs. A wide range of ILs combinations was explored and the respective liquid-liquid phase diagrams were determined at 25°C. Given that these ABS are formed by two ILs and water, the extension of the ionic exchange between the coexisting phases was also ascertained. Finally, the enhancement in the extraction performance and selectivity of IL-IL ABS, attained by tailoring the polarities of both phases, was explored with a series of alkaloids of different hydrophobicity. Outstanding extraction efficiencies and more selective extractions were obtained with IL-IL ABS when compared with ABS formed by the same ILs and salts or polymers.

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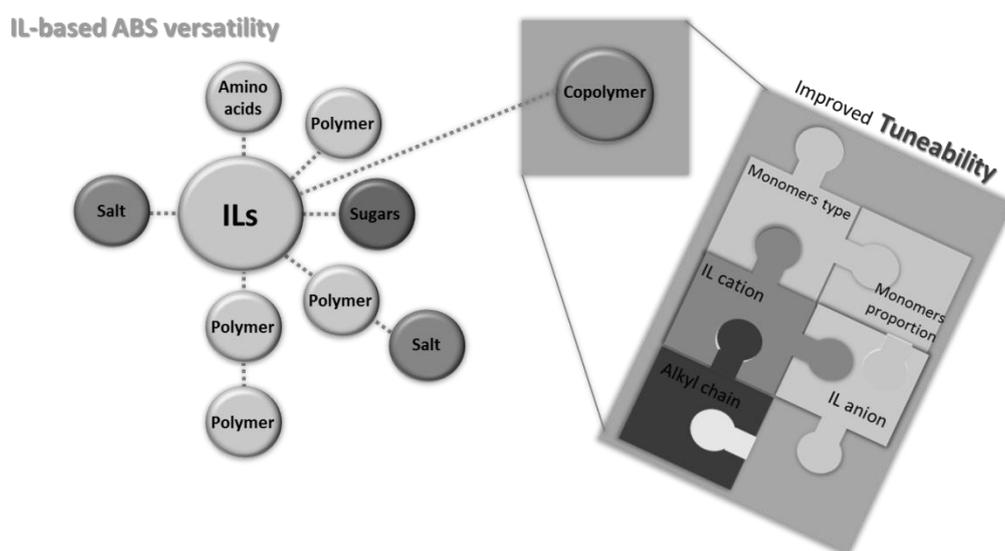
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Novel Aqueous Biphasic Systems Composed of ILs and Copolymers

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Aqueous biphasic systems (ABS) composed of ionic liquids (ILs) have been recognized as enhanced routes for purification processes as a way of overcoming the low specificity afforded by the conventional techniques. This recent trend results from three main aspects described as the i) IL designer solvent status, ii) the variety of compounds (e.g. salts, polymers, amino-acids and carbohydrates) with which these can be combined with to create ABS and iii) the extended hydrophobic/hydrophilic range that these can offer. Despite the remarkable aptitude to extract several molecules of interest IL-based ABS still exhibit drawbacks in the selective extraction of structurally similar compounds. Under this scenario, copolymers, i.e. tunable compounds formed by the polymerization of two (or more) distinct monomeric units, can offer unique opportunities, providing an extra degree of tuneability in the domain of IL-based ABS. This work focuses on understanding the physical-chemistry of novel ABS composed of cholinium-based ILs and copolymers and on studying their ability to selectively extract similar biomolecules



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Ionic Liquids Aqueous Solutions in the Extraction and Purification of Biocompounds

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Amongst the many solvents that ionic liquids may be combined with, water is the most interesting given its green solvent character and its unique properties, which can be further modulated by ionic liquids. While the extraction of biocompounds from natural sources is usually carried out with non-polar or mildly polar organic solvents, due to their low solubility in water, it will be shown in this communication that ionic liquids possess an hydrotropic nature that allows the solubilization of poorly soluble compounds, making of aqueous solutions of ionic liquids excellent solvents to extract compounds from biomass. The effects of the IL chemical structures, their concentration and the effect of temperature on the solubility of various biocompounds were evaluated and compared with the performance of conventional hydrotropes. Using Dynamic Light Scattering, NMR and molecular dynamics (MD) simulations, the molecular level mechanisms that enhance the solubility of biomolecules in aqueous solutions were disclosed and shown to be related to the formation of IL-biomolecule aggregates. It will be also discussed how the hydrotropy can be used to recover solutes from aqueous media by using water as anti-solvent.

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Micelle-Mediated Extraction of Phycobiliproteins

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Aqueous micellar two-phase systems (AMTPS) are advantageous thermosensitive extraction systems, since they are mostly composed of water, emerging thus as an appellative type of liquid-liquid extraction (LLE). AMTPS composed of two surfactants, while introducing ionic liquids (ILs) as a new class of co-surfactants, is an attractive alternative which may enhance these systems extraction performance. Taking into account the excellent results of selectivity achieved with AMTPS using ILs as co-surfactants,¹ their application in the purification of phycobiliproteins is now being considered. AMTPS formed by Triton X-114 and Tergitol 15-S-7 as main surfactants and several imidazolium ($[C_n\text{mim}]\text{Cl}$, with $n = 10, 12$ and 14) and phosphonium ($[P_{6,6,6,14}]\text{X}$, with $\text{X} = \text{Cl}, \text{Br}, \text{Decanoate}$ and trihexyltetradecylphosphonium bis (2,4,4-trimethylpentyl)phosphinate, $[P_{4,4,4,14}]\text{Cl}$ and $[P_{8,8,8,8}]\text{Br}$) ILs as co-surfactants are being investigated. The main goal is to extract and purify phycobiliproteins from other contaminant proteins and, if possible, to isolate R-phycoerythrin from the remaining phycobiliproteins existent in algae biomass. Furthermore, different process conditions are being optimized, namely the surfactant concentration, the time of extraction as well as the phycobiliproteins concentration. The search for the most selective system and conditions taking into account the purification of phycobiliproteins is being carried out.

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Extraction and Recovery of Cynaropicrin from *Cynara cardunculus L.* Using Ionic Liquids Aqueous Solutions

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The production of chemicals (including those with therapeutic properties), materials and energy is highly dependent on non-renewable fossil fuels and, in the past decades, large efforts have been carried out on the search of alternative raw materials - biomass. On the other hand, currently, there is a great demand for natural ingredients with therapeutic purposes. In this context, and within the scope of pharmaceutical industries, alternative, more environmentally-friendly and cost-efficient solvents have been investigated in the past years aiming at extracting and recovering natural products. In particular, a large interest has been observed on the exploitation of ionic liquids (ILs) aqueous solutions as alternative solvents.¹

Cynaropicrin, one of the main compounds of *Cynara cardunculus L.*, displays a wide range of biological activities, including anti-inflammatory, antispasmodic, antitrypanosomal, and proapoptotic properties. Currently, the cost of cynaropicrin is *circa* 20,000 €/g due to difficulties in extraction and purification steps. In this work, aqueous solutions of ILs were investigated as alternative solvents for the extraction of cynaropicrin from *Cynara cardunculus L.* The effects of the IL chemical structure, IL concentration, time of extraction, solid-liquid ratio and temperature on the extraction yield of cynaropicrin were evaluated. The extraction yields obtained with ILs aqueous solutions were also compared with those obtained with conventional organic volatile solvents, demonstrating the high potential of ILs to extract value-added compounds from biomass. Finally, it was demonstrated that water can be used as an anti-solvent to recover cynaropicrin from ILs aqueous solutions.

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Extraction and Purification of IgG From Serum Using Liquid-Liquid Systems Comprising Ionic Liquids

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Specific needs of new affordable biopharmaceuticals to treat age- and prosperity-related diseases will considerably increase in the following years. Biopharmaceuticals, and in particular antibodies from mammals, namely immunoglobulin G, have largely improved the treatment of many diseases, and in some cases are the only approved therapies available for specific human disorders. These biologic-based products have application in several medicinal areas, such as in vaccination, immunization, oncology, and in autoimmune, cardiovascular, inflammatory and neurological diseases. Continuous discoveries in molecular biology and genetics, combined with advances in media and feed development, have significantly increased the production titres of biopharmaceuticals. However, their current large cost is a main result of a lack of cost-effective purification techniques.

Envisaging a widespread use of biopharmaceuticals, in this work, we investigated the potential of aqueous biphasic systems composed of ionic liquids (IL-based ABS) for the extraction and purification of IgG. Within the search of more environmentally friendly and biocompatible separation processes, two types of ABS were investigated: (i) ABS composed of polymers and salts using ILs as adjuvants; and (ii) ABS composed of polymers and bio-based ILs. Remarkably, the complete extraction of IgG in a single-step was accomplished, with ca. 46% and 100% recovery yields using ABS formed by ILs as adjuvants and ABS composed of bio-based ILs, respectively. The optimized systems were also applied to the extraction and purification of IgG directly from rabbit serum samples. The stability of IgG before and after the extraction was also evaluated by SE-HPLC, SDS-PAGE and FTIR analysis to evaluate the appropriate systems capable of maintaining the structural stability of IgG. In most cases, IgG was found to retain its native structure, without degradation or denaturation effects, which further suggest the usefulness of IL-based ABS for the purification of high-cost biopharmaceuticals.

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Purification of IgY Using Polymer-based Aqueous Biphasic Systems Combined with Centrifugal Partitioning Chromatography

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Biopharmaceuticals, such as antibodies, have greatly improved the treatment and diagnosis of some diseases. Antibodies are key glycoproteins which act as organism specific responses to foreign substances; they are produced when an antigen receptor binds specifically to a B-cell, triggering a mechanism which involves activation, cell division and differentiation. Because of their versatility, antibody-based therapies can be developed against any existing pathogen, and with high specificity. Currently, mainly mammalian monoclonal or polyclonal antibodies (immunoglobulins G, IgG) are used in diagnosis and therapeutic purposes. These antibodies have however a weak immunogenic response and are produced in low amounts. These disadvantages can be overcome by egg yolk antibodies (immunoglobulin Y, IgY). Nevertheless, the widespread use of IgY is nowadays restricted by the lack of effective isolation and purification techniques. Therefore, to ensure the IgY application at a large scale, as alternative antibodies for use in passive immunotherapy, the existing methods should be optimized and/or new techniques should be developed. In this work, different polymer-salt aqueous biphasic systems (ABS) were investigated to purify IgY from the complex egg yolk matrix. Partition coefficients, extraction efficiencies, recovery yields and purification factors were determined for different systems and at different compositions. ABS combined with ultrafiltration were also investigated. Finally, the most promising ABS were applied in centrifugal partitioning chromatography (CPC) envisaging the technology scale-up.

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Development of Alternative Early-Stage Cancer Diagnosis Methods by the Application of Ionic Liquids

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Worldwide, prostate cancer (CaP) and colorectal cancer (CC) are the most common diagnosed male cancers with more than 8.2 million and 500,000 deaths *per* year, respectively.¹ Thus, investments in the treatment and on the development of techniques for cancer early-stage diagnoses are a main priority in order to increase the lifespan of patients and to decrease costs associated to treatment. The prostate specific antigen (PSA) and lactate dehydrogenase (LDH) are proteins commonly found in cancer patients fluids (urine and serum), and which have received increased attention as prognostic tumor biomarkers and in the monitoring of cancer treatment.² However, due to their low concentration and complexity of body fluids, their identification and quantification are usually inaccurate leading therefore to false-positives and false-negatives. In order to develop more efficient and accurate cancer diagnosis methods, while also allowing the use of more versatile equipment such as HPLC instead of the commonly used immunoassays, a series of aqueous biphasic systems (ABS) composed of organic salts and phosphonium-based ionic liquids (ILs) were investigated for the extraction, purification and concentration of PSA and LDH from human fluids. The effects of the IL chemical structure were deeply investigated aiming at reaching the complete extraction of PSA and LDH for the IL-rich phase in a single-step, as well as their concentration (up to 250 times). Moreover, their purification was also accomplished by the one-step precipitation of main metabolites in body fluids using appropriate ILs. According to the obtained results, IL-based ABS allow the one-step extraction, purification and concentration of tumor biomarkers from human fluids, and can thus be considered as adequate strategies for early-stage cancer diagnosis.

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