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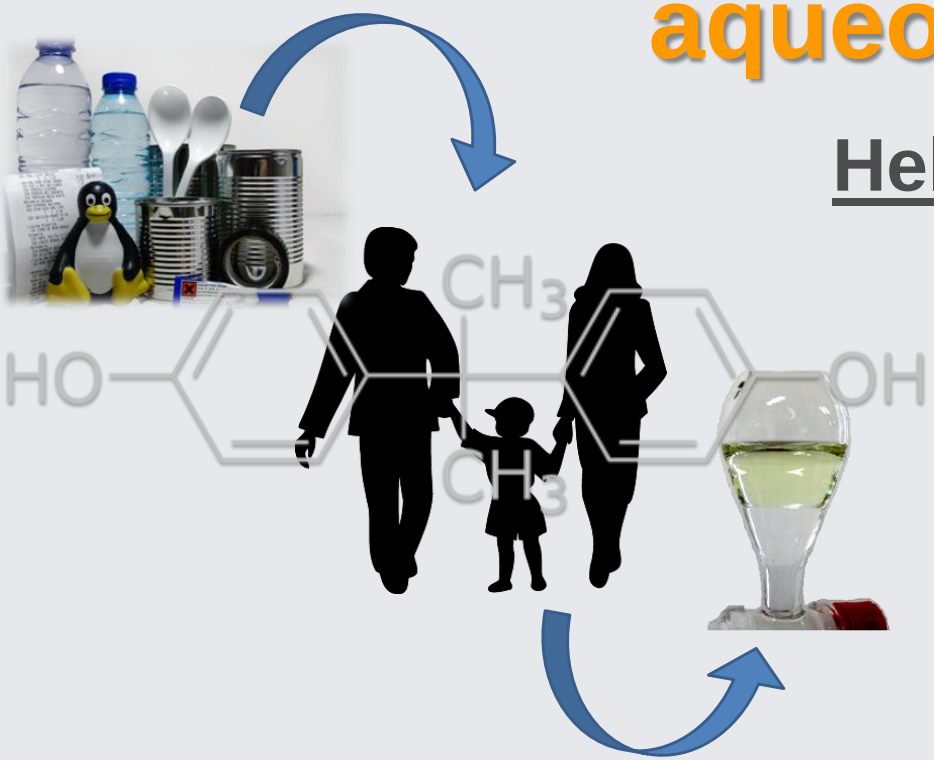
Extraction of bioactive compounds with ionic liquid aqueous solutions



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Dissertação/Projeto 5ºAno
Mestrado Integrado em Engenharia Química
20 de Julho de 2012

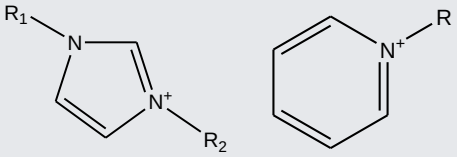
Summary

1. Introduction

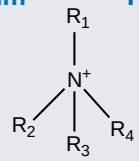
1.1. Ionic liquids (ILs)

ILs → are composed of

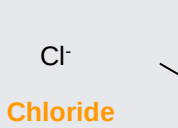
large organic **cations** + organic or inorganic **anions**



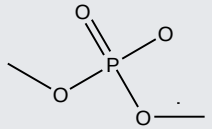
Imidazolium Pyridinium



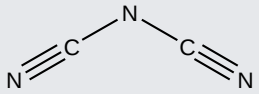
Ammonium



Chloride



Dimethylphosphate



Dicyanamide

ILs → exhibit unique properties

- ✓ negligible vapour pressure and flammability;
- ✓ high thermal and chemical stability;
- ✓ high solvation ability for several compounds;

} Volatile organic compounds substitutes

- ✓ improved selectivity and easy recycling;
- ✓ tunable properties. → Designer solvents

1.2. Aqueous two phase systems (ATPS)

Liquid-Liquid Extraction

ATPS

↓

two aqueous-rich phases

polymer	+	polymer
salt	+	polymer
salt	+	salt

IL-based ATPS

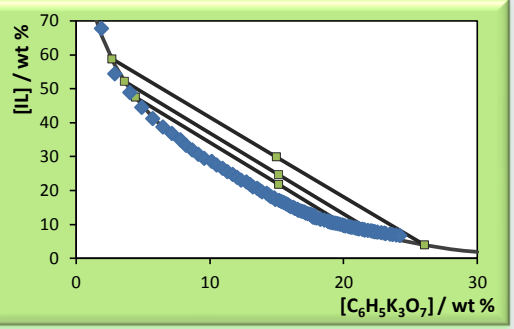
Additional advantages

- ✓ low viscosity;
- ✓ quick phase separation;
- ✓ high and tailored extraction efficiency.

ATPS composed of IL + Salt + H_2O.

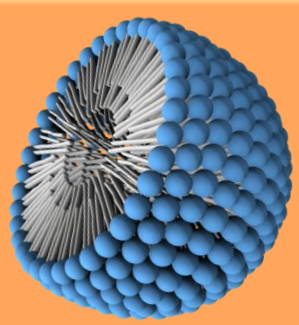
2. Objectives and scopes

✓ **To find more benign ATPS by substitution of the high charge density salts usually employed;**



✓ **To study the ability of novel ATPS for alkaloids extraction;**

✓ **To study the effect of the ILs self-aggregation and subsequent impact on the partition of different biomolecules;**



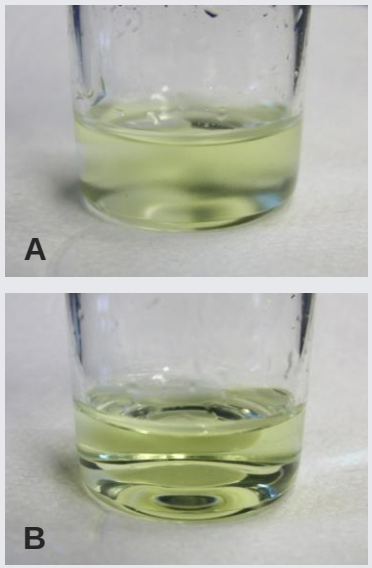
✓ **To demonstrated the potential of IL-based ATPS for the extraction of Bisphenol A (BPA).**

3. Experimental procedure

Determination of phase diagrams

↓

Cloud point titration method



Cloud point titration method.

Extraction of bioactive compounds

↓

1. ATPS preparation:
IL + Salt + Biomolecules

→ **2.** Phases separation

3. UV-spectroscopy quantification

4. Determination of:

ATPS composed of IL + Salt + H_2O.

✓ Partition Coefficient

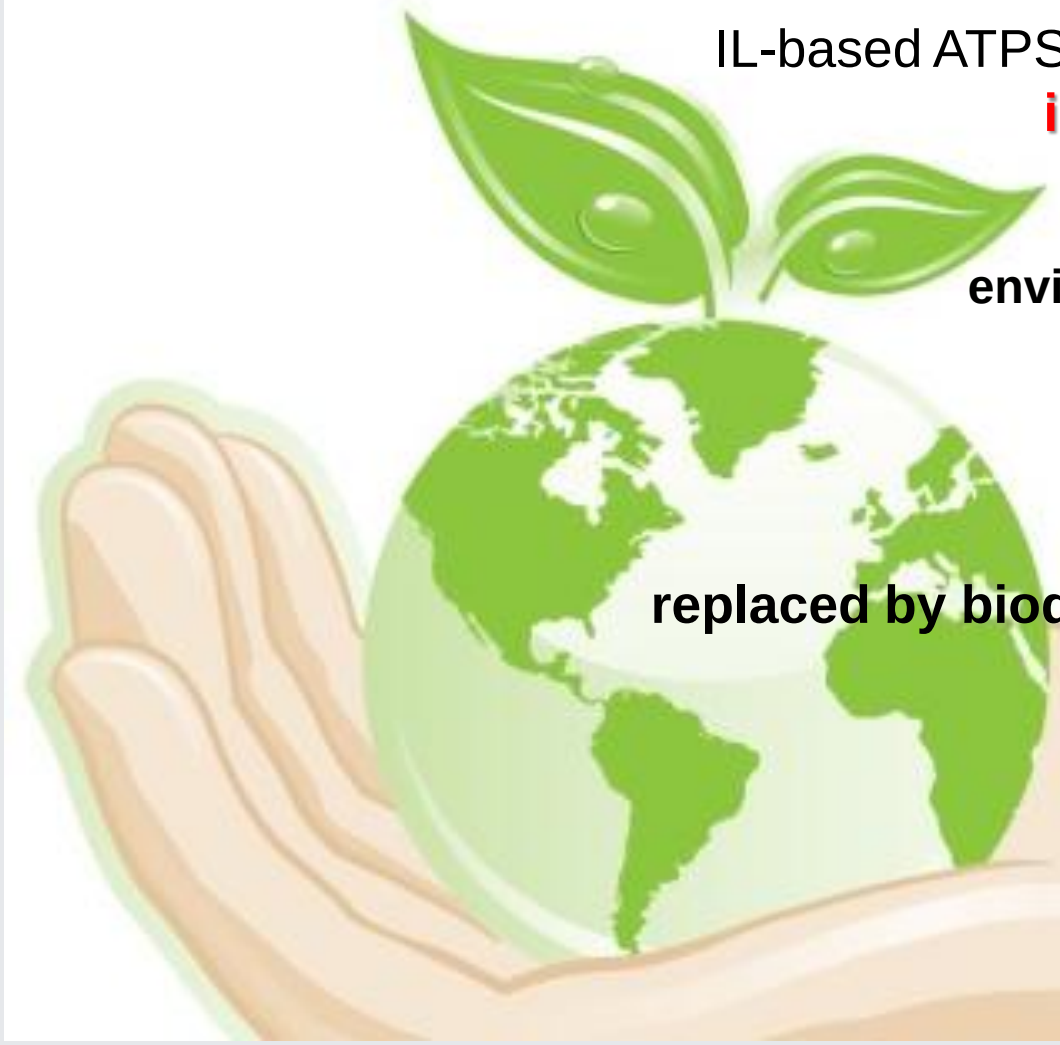
$$K_X = \frac{[X]_{IL}}{[X]_{Salt}}$$

✓ Extraction Efficiency

$$EE_X\,\% = \frac{m_X^{IL}}{m_X^{IL} + m_X^{Salt}} \times 100\%$$

4. Results and discussion

4.1. IL + H_2O + $C_6H_5K_3O_7$ ternary systems



IL-based ATPS are commonly composed by

inorganic salts

environmental concern

replaced by biodegradable and biocompatible

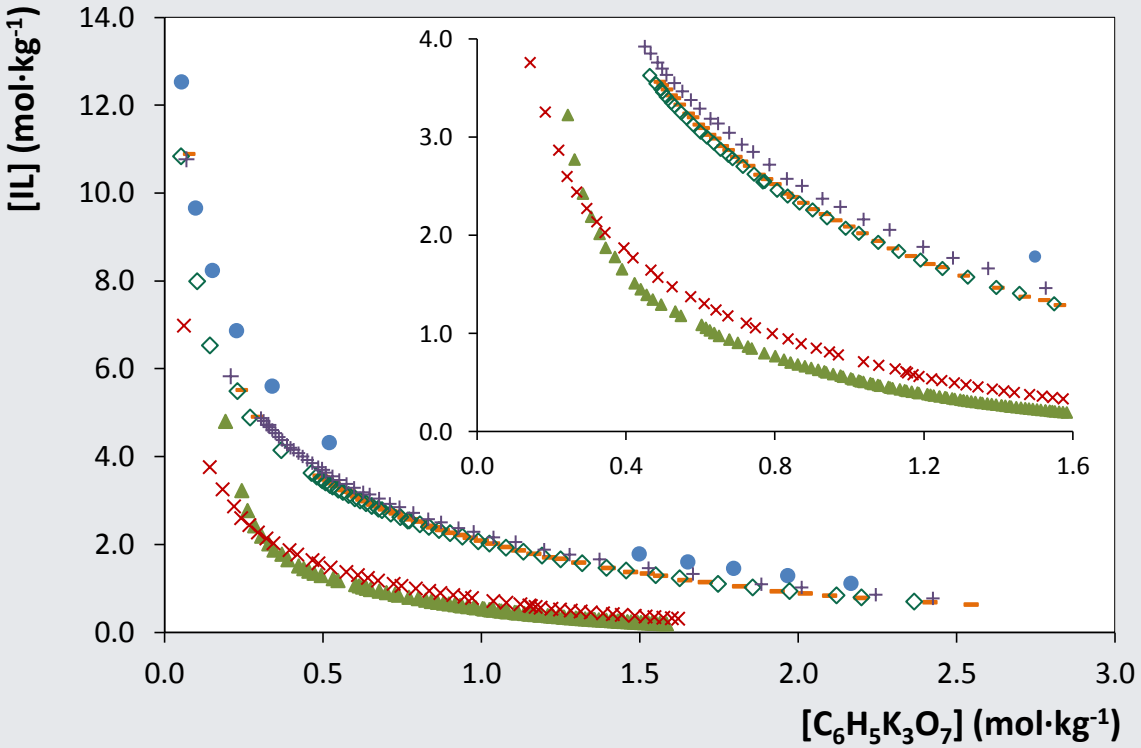
organic salts

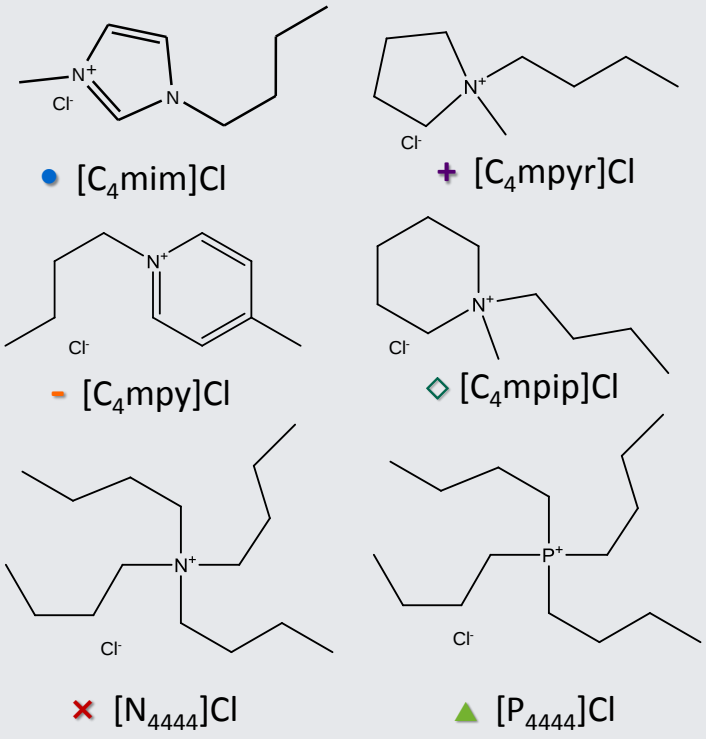
4. Results and discussion

4.1. IL + H_2O + $C_6H_5K_3O_7$ ternary systems

ATPS composed of IL + Potassium citrate + H_2O

1. Cation core effect





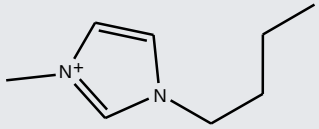
$[P_{4444}]^+ > [N_{4444}]^+ >> [C_4mpy]^+ \approx [C_4mpip]^+ > [C_4mpyr]^+ > [C_4mim]^+$

4. Results and discussion

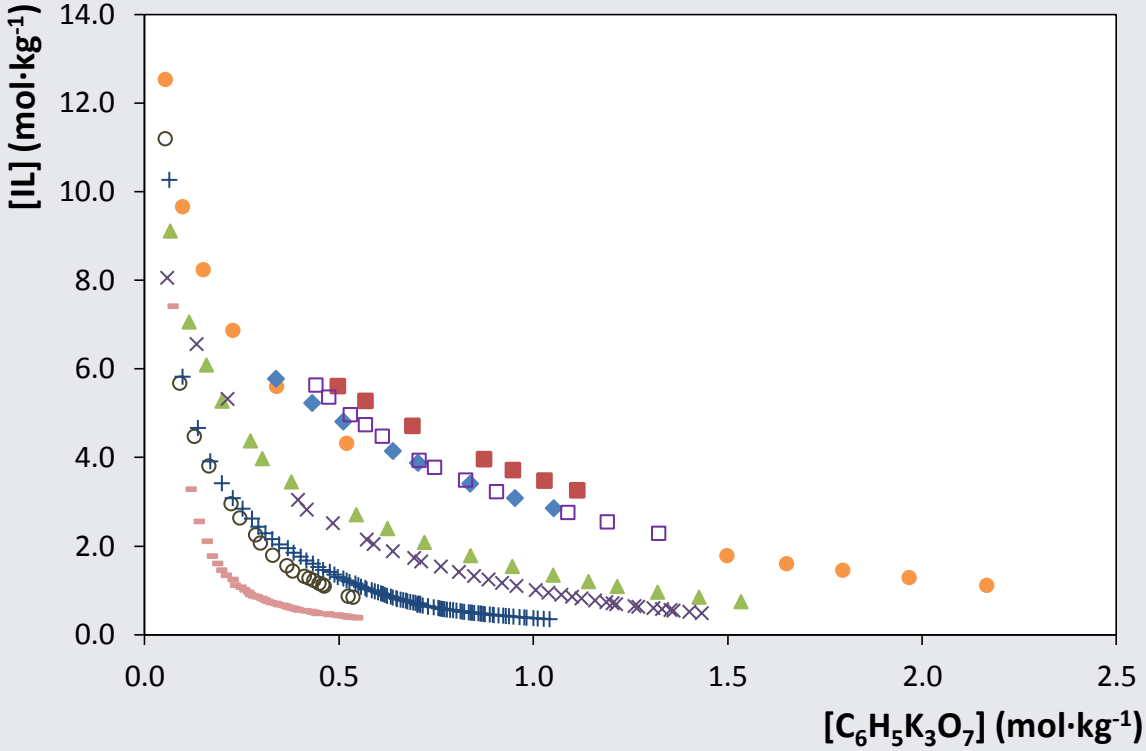
4.1. IL + H_2O + $C_6H_5K_3O_7$ ternary systems

ATPS composed of IL + Potassium citrate + H_2O

2. Anion effect



$[C_4mim]^+$ cation



- $[C_4mim][CH_3CO_2]$
- $[C_4mim][PO_4(CH_3)_2]$
- $[C_4mim][CH_3SO_3]$
- $[C_4mim]Cl$
- $[C_4mim]Br$
- $[C_4mim][CF_3CO_2]$
- $[C_4mim][N(CN)_2]$
- $[C_4mim][SCN]$
- $[C_4mim][CF_3SO_3]$

hydrogen bond basicity

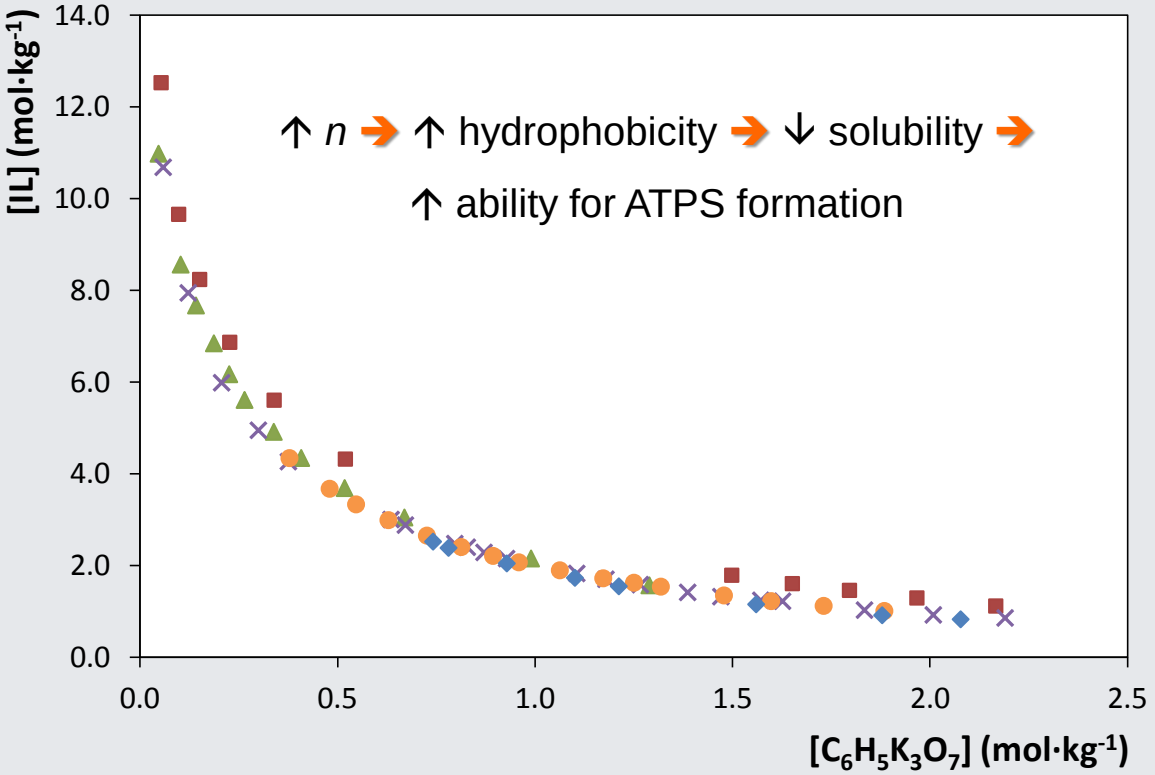
$[CH_3CO_2]^- < [PO_4(CH_3)_2]^- < [CH_3SO_3]^- < Cl^- << Br^- < [CF_3CO_2]^- << [N(CN)_2]^- < [SCN]^- < [CF_3SO_3]^-$

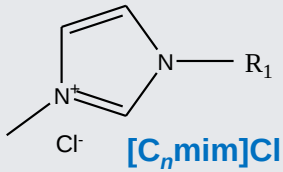
4. Results and discussion

4.1. ILs + H_2O + $C_6H_5K_3O_7$ ternary systems

ATPS composed of IL + Potassium citrate + H_2O

3. Cation alkyl side chain length effect





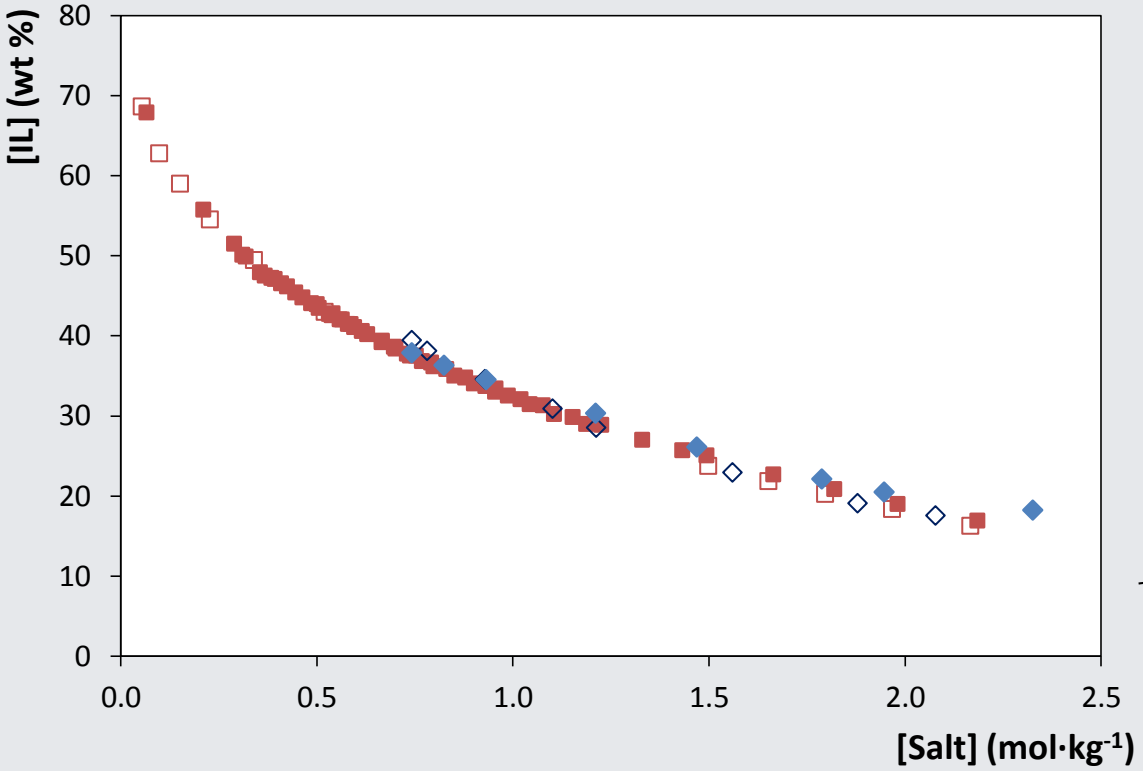
$[C_4mim]^- < [C_6mim]^- \approx [C_7mim]^- \approx [C_8mim]^- \approx [C_{10}mim]^-$

4. Results and discussion

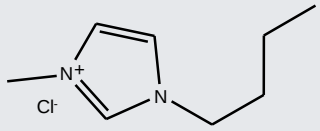
4.1. ILs + H_2O + $C_6H_5K_3O_7$ ternary systems

ATPS composed of IL + Potassium citrate + H_2O

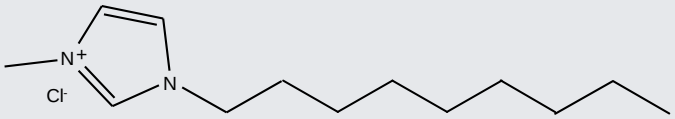
4. pH effect



pH 9
pH 7
[C_4mim]Cl



pH 9
pH 7
[C_{10}mim]Cl

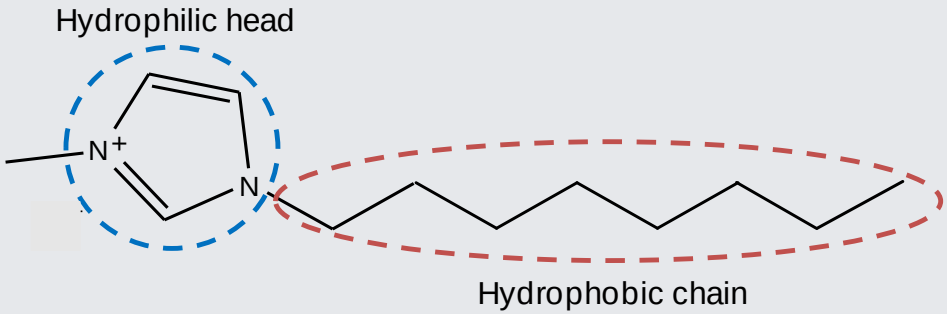


pH effect is negligible

4. Results and discussion

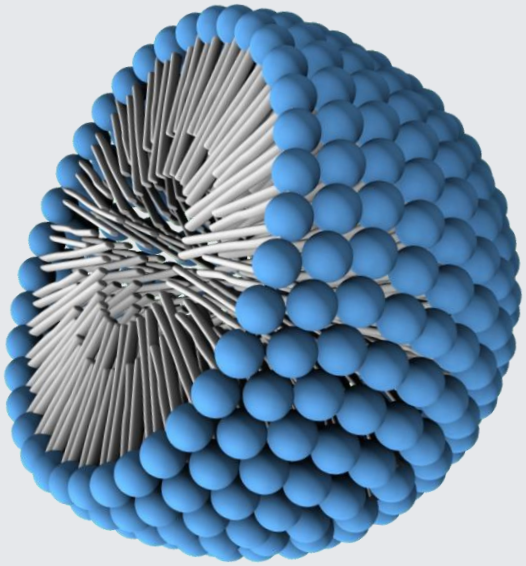
4.2. Self-aggregation in IL-based ATPS

$[C_n mim]^+$ are structurally similar to **ionic surfactants**



$[C_{10}mim]^-$ molecular structure.

ILs micelle formation in aqueous solution[1]



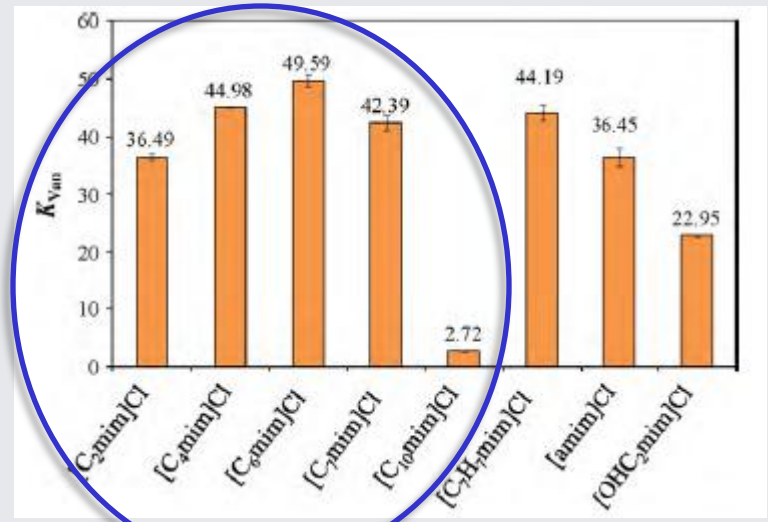
[1] C. Jungnickel et al., *Colloids and Surfaces A: Physicochem. Eng. Aspects* 316 (2008) 278–284.

4. Results and discussion

4.2. Self-aggregation in IL-based ATPS

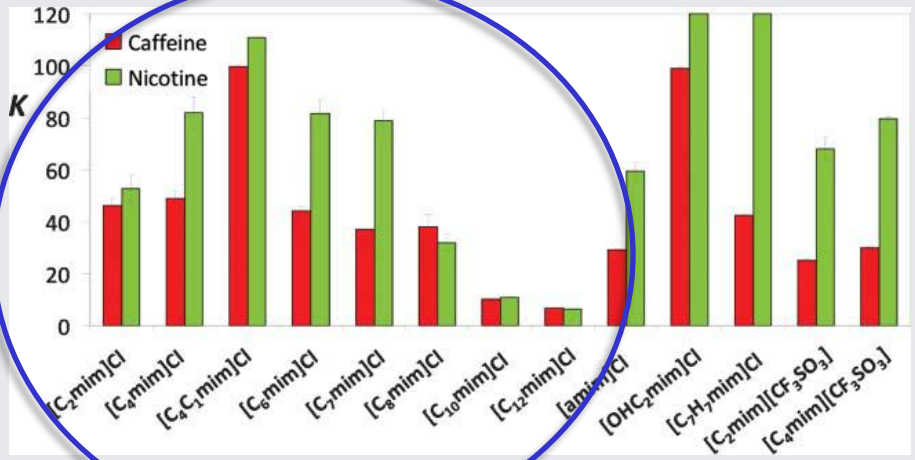
Micelle-mediated extraction can be used to increase or decrease the extraction efficiencies of a given molecule.

Extraction of vanillin using ionic-liquid-based aqueous two-phase systems



[3] A.F.M. Cláudio et al., *Separation and Purification Technology* 75 (2010) 39–47.

High-performance extraction of alkaloids using aqueous two-phase systems with ionic liquids

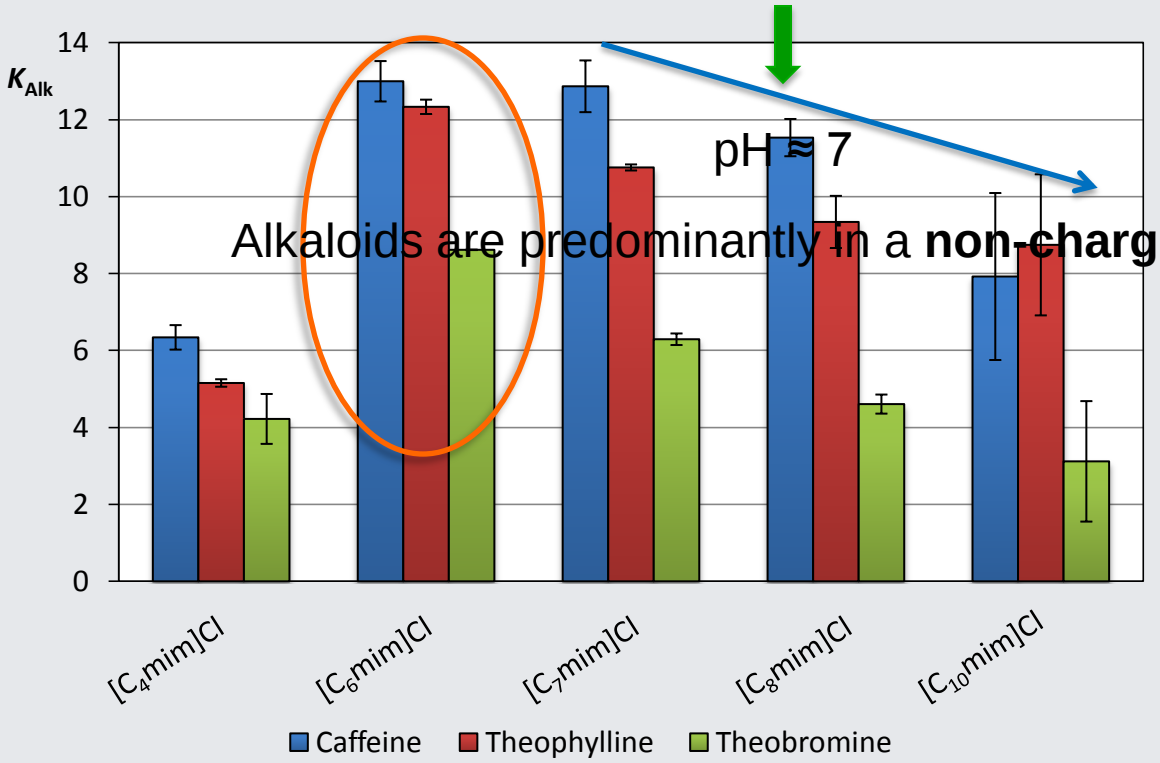


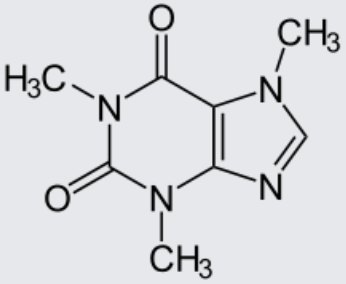
[2] M.G. Freire et al., *Green Chem.* 12 (2010) 1715–1718.

4. Results and discussion

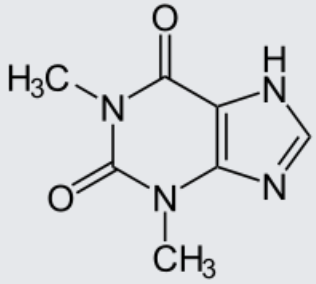
4.2. Self-aggregation in IL-based ATPS

Imidazolium-based IL + $C_6H_5K_3O_7/C_6H_8O_7$ + alkaloids

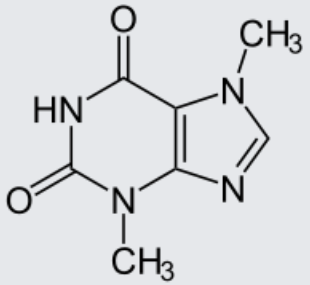




Caffeine

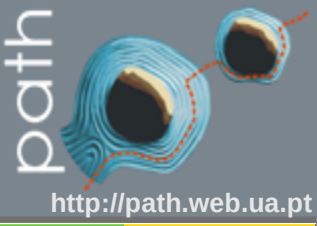


Theophylline



Theobromine

$n \geq 7$ → Maximum of extraction → [C_6mim]Cl → ↓ K_{Alk}

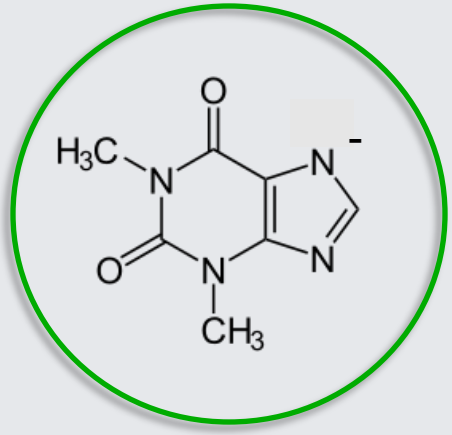


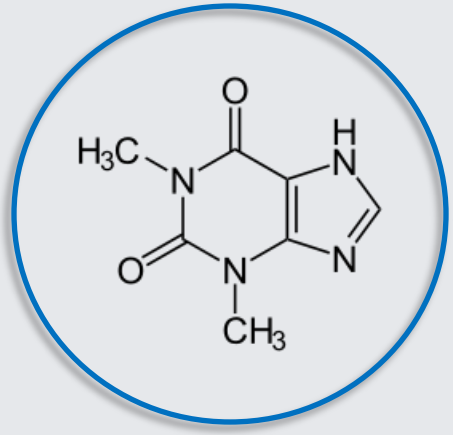
Imidazolium-based IL + $C_6H_5K_3O_7$ + alkaloids

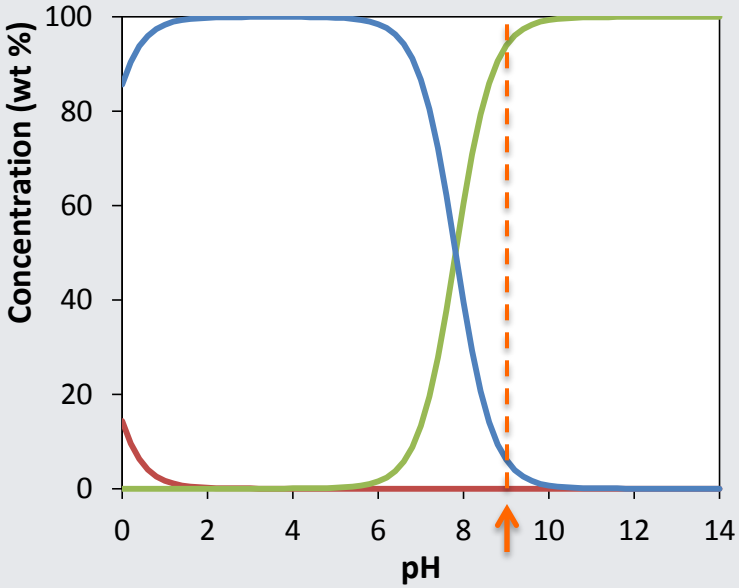
pH ≈ 9

Theophylline is predominantly in a **negative charged form**

Theophylline

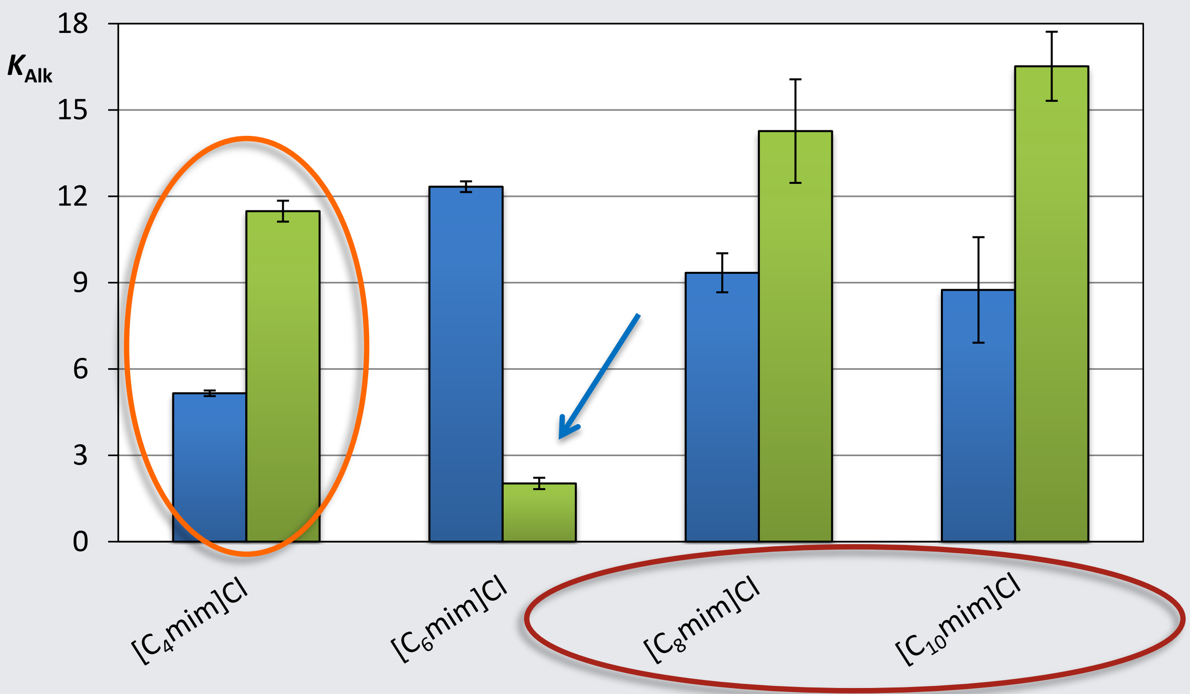




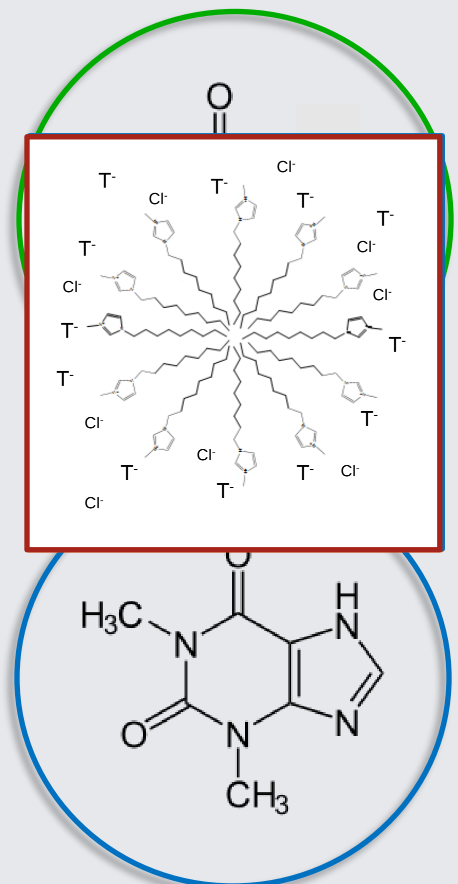


4.2. Self-aggregation in IL-based ATPS

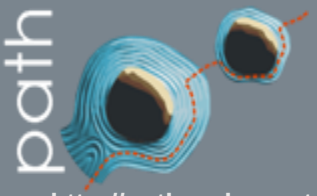
Imidazolium-based IL + $C_6H_5K_3O_7$ + alkaloids



Theophylline

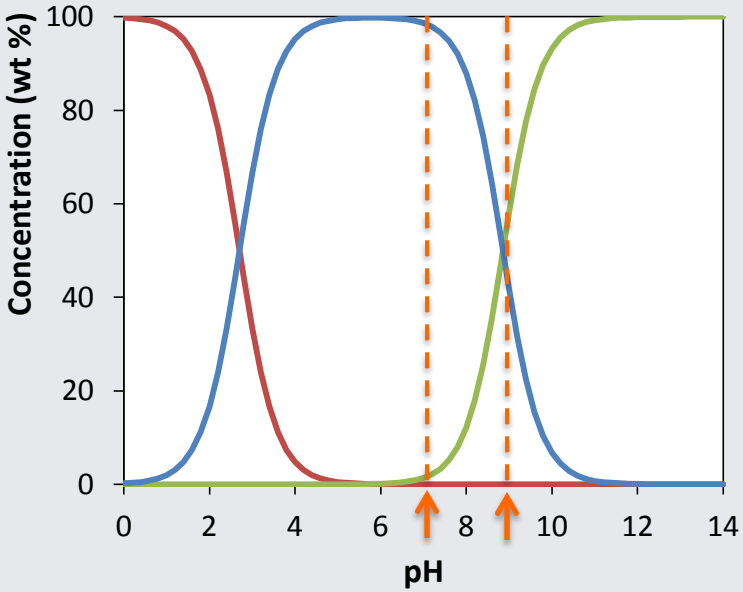


K_{Alk} ↑ for charged molecule → larger micelles → micelles formation → interaction opposite charges ↑ K_{Alk}

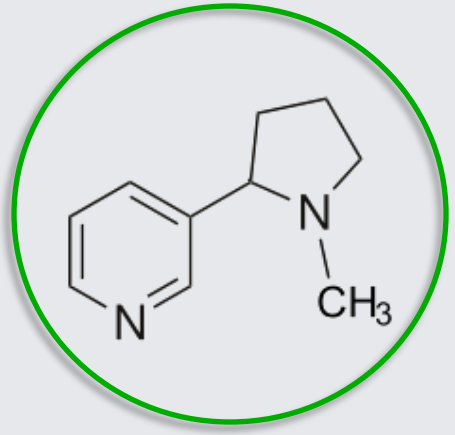


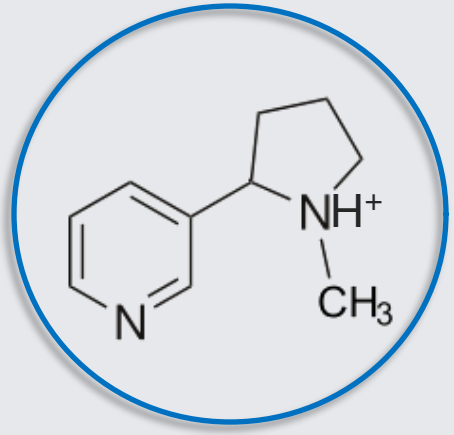
Imidazolium-based IL + $C_6H_5K_3O_7$ + alkaloids

- pH ≈ 7 ➔ Nicotine is predominantly in a **positive charged form**
- pH ≈ 9 ➔ Nicotine is preferentially in a **neutral form**
  (58 wt % of neutral molecule and 42 wt % of positively charged molecule)



Nicotine

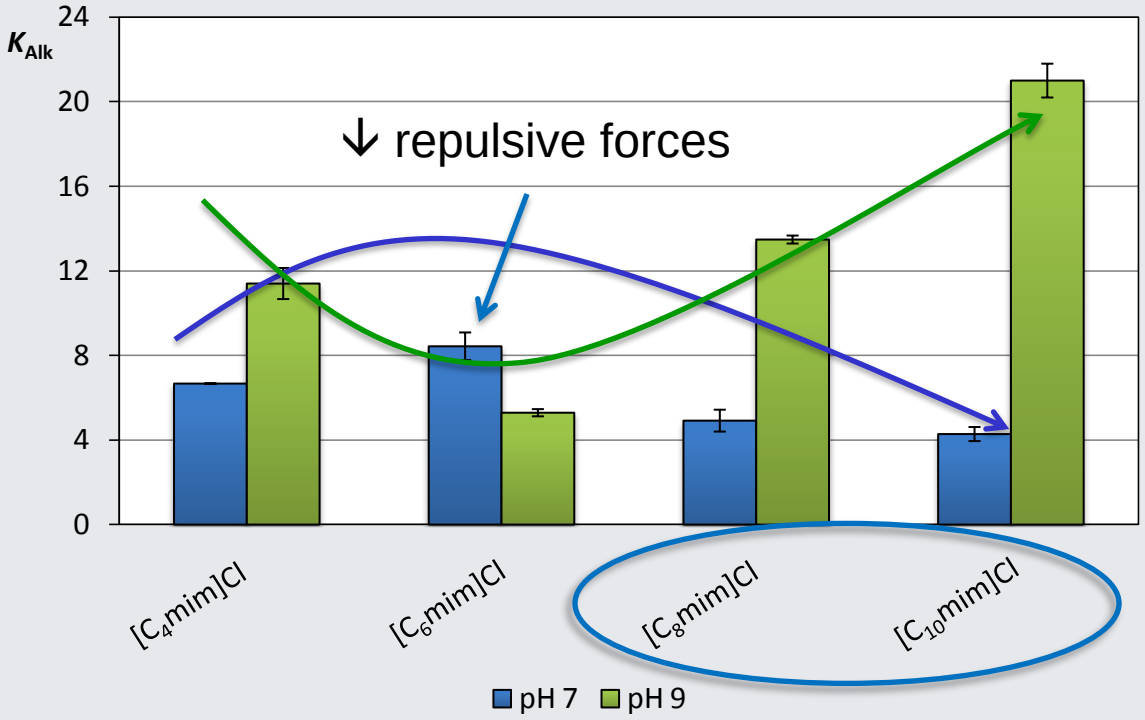




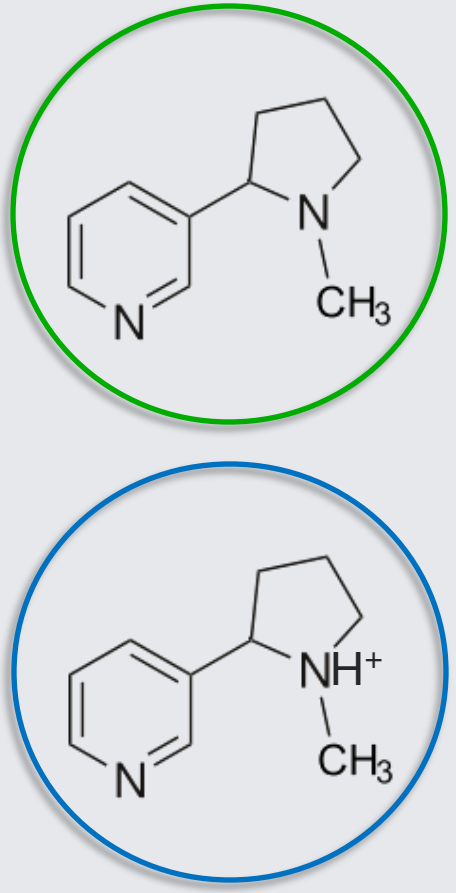
4. Results and discussion

4.2. Self-aggregation in IL-based ATPS

Imidazolium-based IL + $C_6H_5K_3O_7$ + alkaloids



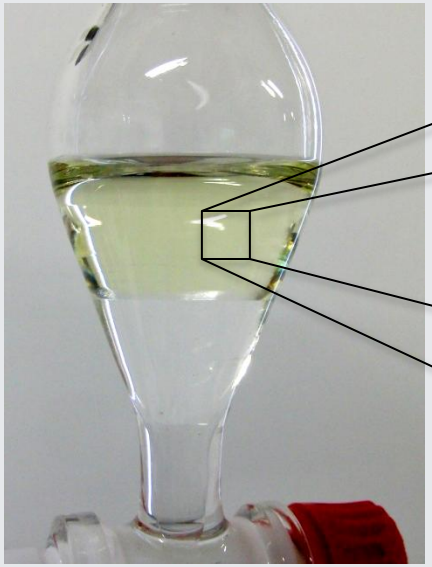
Nicotine



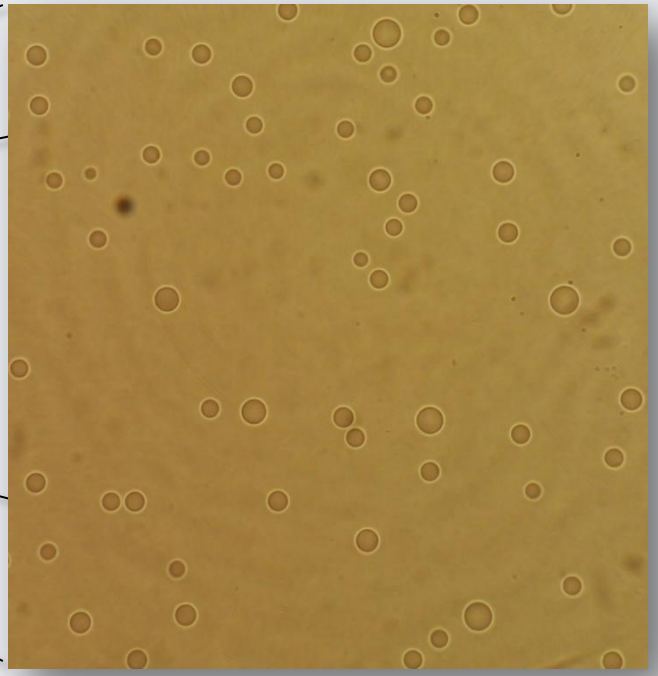
micelles formation → ↓ repulsive forces → ↓ K_{Nic}

Nicotine is the most hydrophobic compound studied → stay **inside of micelles**

Imidazolium-based IL + $C_6H_5K_3O_7$ ➔ Microscopy aplication

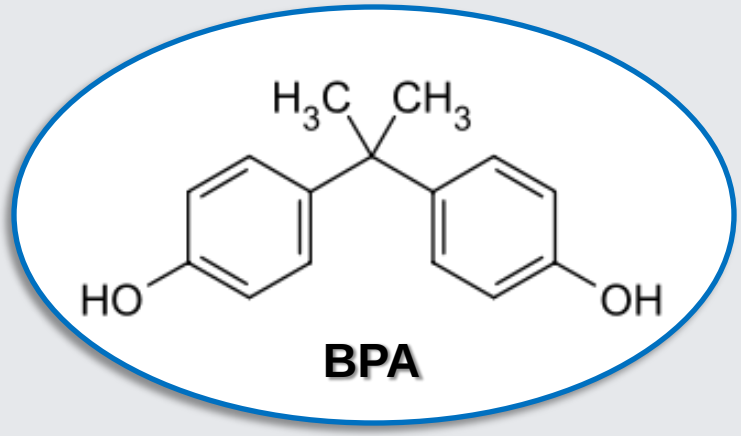


ATPS composed of IL + Salt + H_2O.



Microscope image of IL-rich phase
of ATPS composed of $[C_8mim]Cl + C_6H_5K_3O_7 + H_2O$.

Confirmed the presence of micelles in systems composed of
➔ **$[C_7mim]Cl$**, **$[C_8mim]Cl$** and **$[C_{10}mim]Cl$**



Applications of BPA

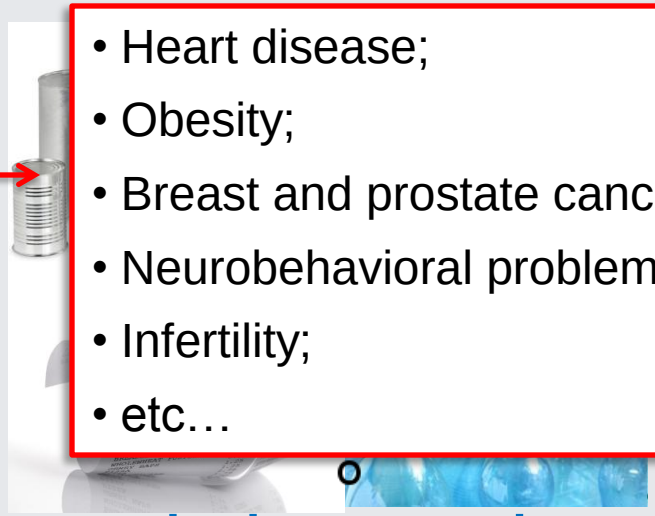
- **Plastic industry** as an intermediate in the production of epoxy resins and polycarbonate plastics;

- In the **manufacture of thermal paper**.

However...

BPA is an endocrine disruptor

- Production:**3 million tons/year**
- Release into the atmosphere: **100 tons/year**



- Heart disease;
- Obesity;
- Breast and prostate cancer;
- Neurobehavioral problems;
- Infertility;
- etc…

BPA is now an ubiquitous component in the atmosphere

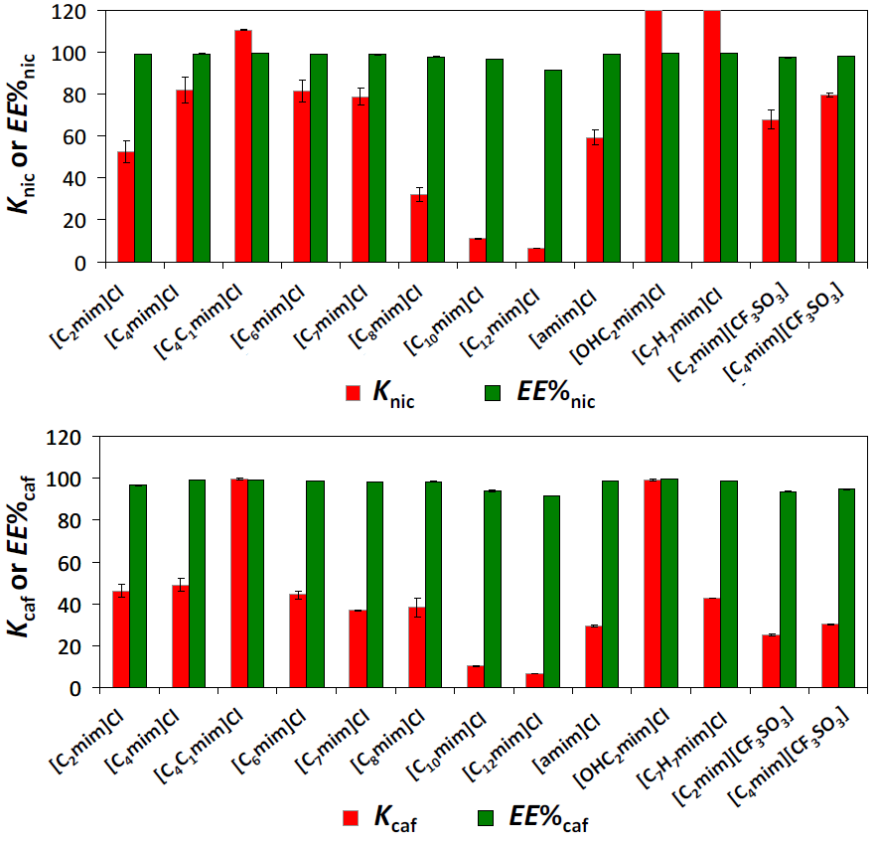
4. Results and discussion

4.3. Extraction of bisphenol A (BPA)

COMMUNICATION www.rsc.org/greenchem | Green Chemistry

High-performance extraction of alkaloids using aqueous two-phase systems with ionic liquids†

Mara G. Freire,*[a] Catarina M. S. S. Neves,[b] Isabel M. Marrucho,[a] José N. Canongia Lopes,[a] Luís Paulo N. Rebelo*[a] and João A. P. Coutinho[b]



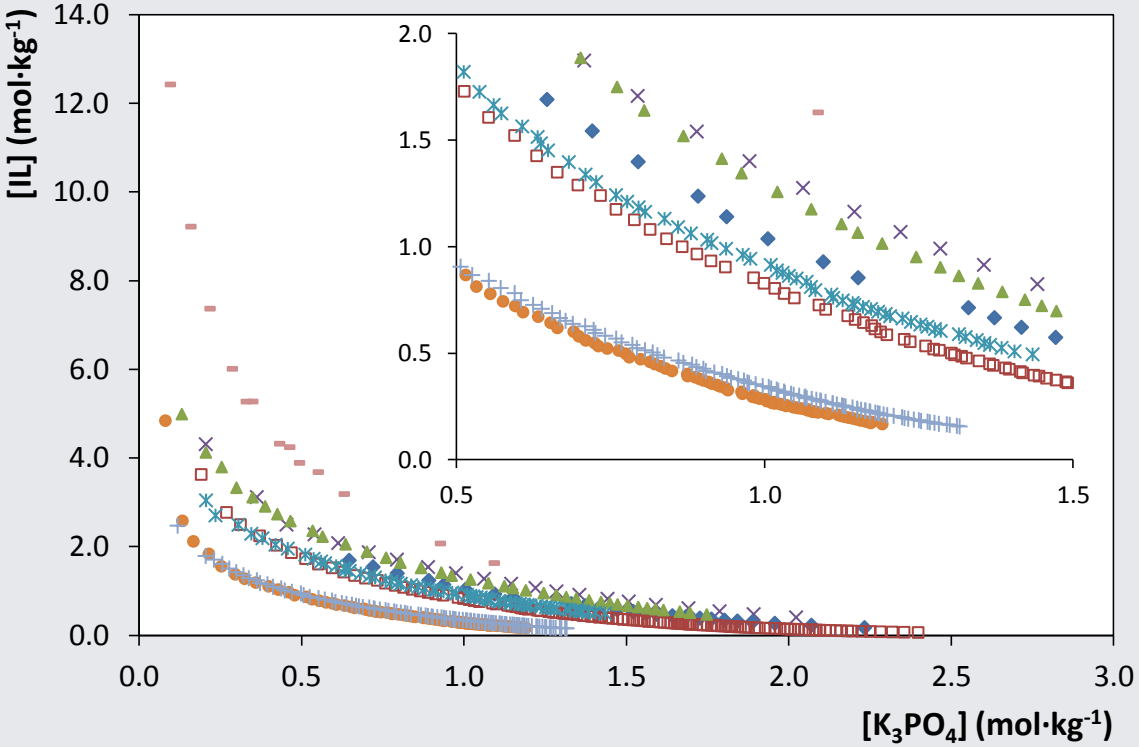
ATPS composed of
IL + K_3PO_4 + H_2O

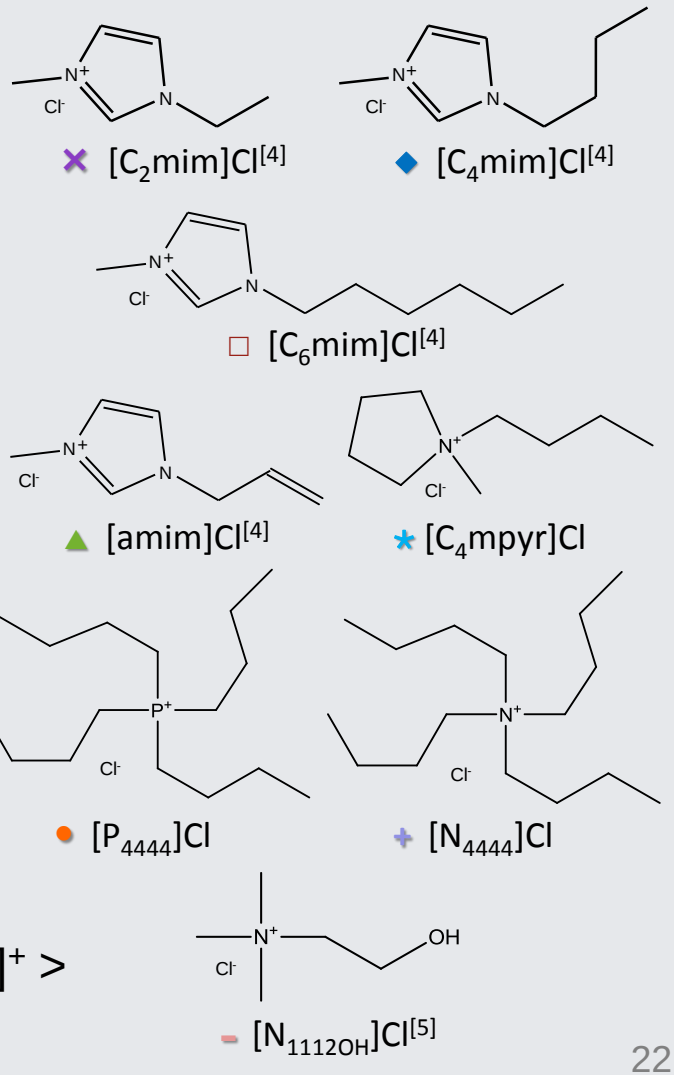
IL-based systems formed by the addition of **K_3PO_4** provide **high extraction efficiencies** due to the presence of the **strong salting-out salt**

4. Results and discussion

4.3. Extraction of bisphenol A (BPA)

ATPS composed of IL + K_3PO_4 + H_2O





$[P_{4444}]^+ > [N_{4444}]^+ >> [C_6mim]^+ > [C_4mpyr]^+ > [C_4mim]^+ > [amim]^+ > [C_2mim]^+ >> [N_{1112OH}]^+$

[4] Neves, C. M. S. S., et al, *J. Phys. Chem. B* 2009, 113, 5194–5199;
[5] Louros, C.L.S., MSc thesis, "Extraction of Biomolecules with Aqueous Two Phases Systems", University of Aveiro, Aveiro, Portugal (2009).

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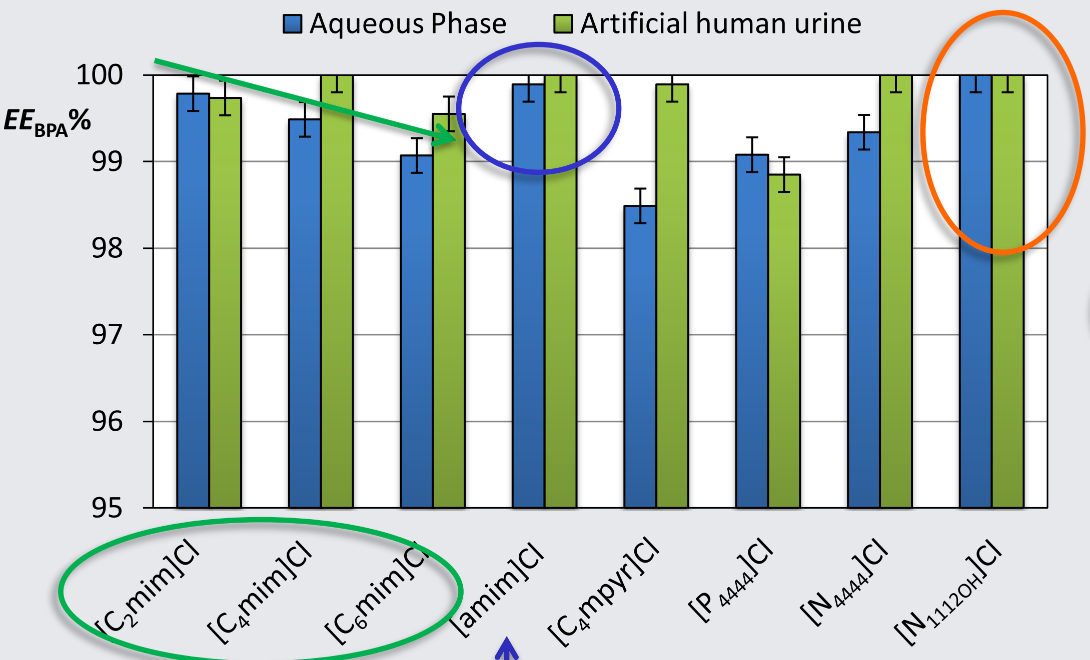

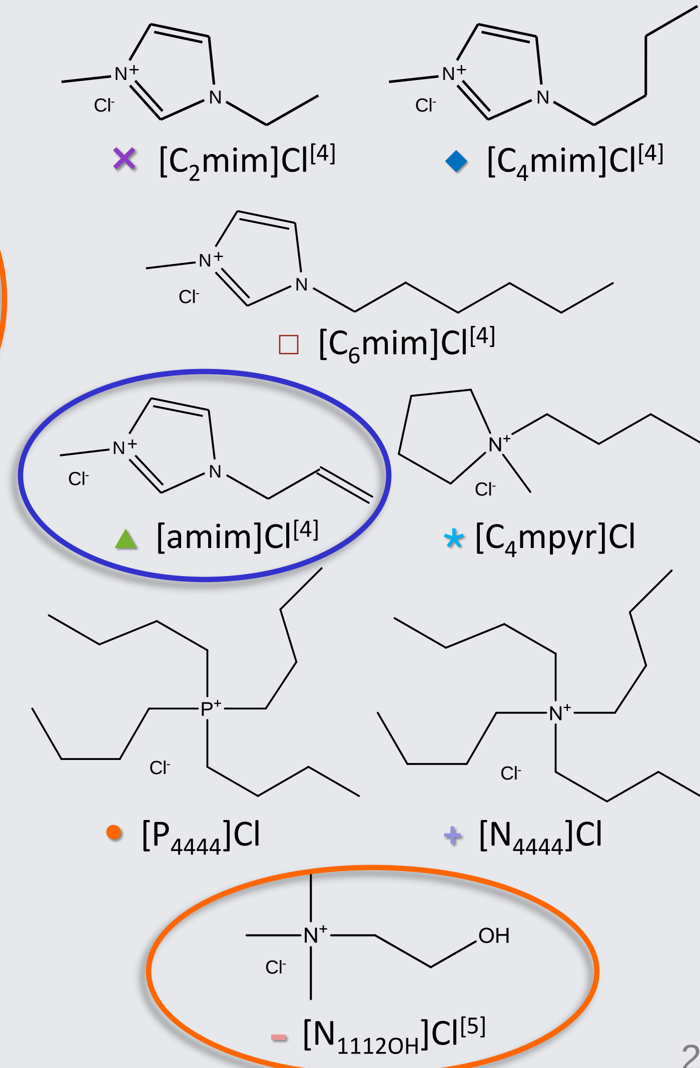

The presence of a **more complex matrix, favors the partitioning of BPA for the IL-rich phase**

↑ alkyl side chain length → ↑ $EE_{BPA}\%$

$[N_{1112OH}]Cl$ → ↑ $EE_{BPA}\%$

[4] Neves, C. M. S. S., et al, *J. Phys. Chem. B* 2009, 113, 5194–5199;
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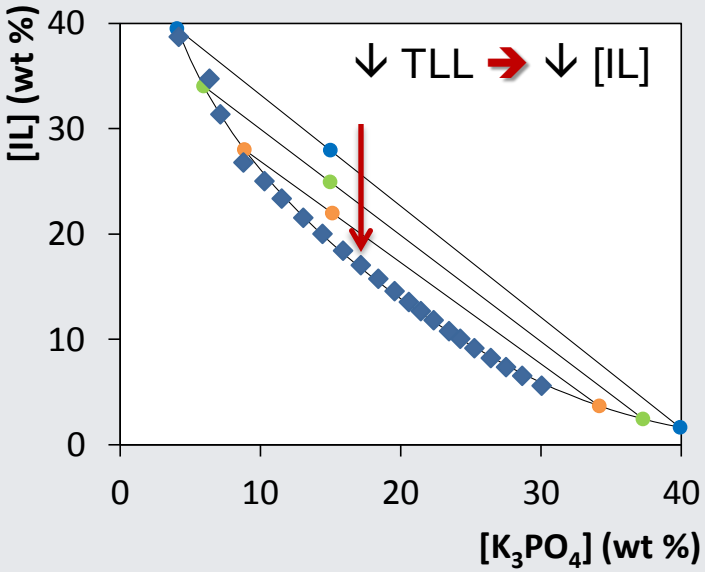
4. Results and discussion

4.3. Extraction of bisphenol A (BPA)

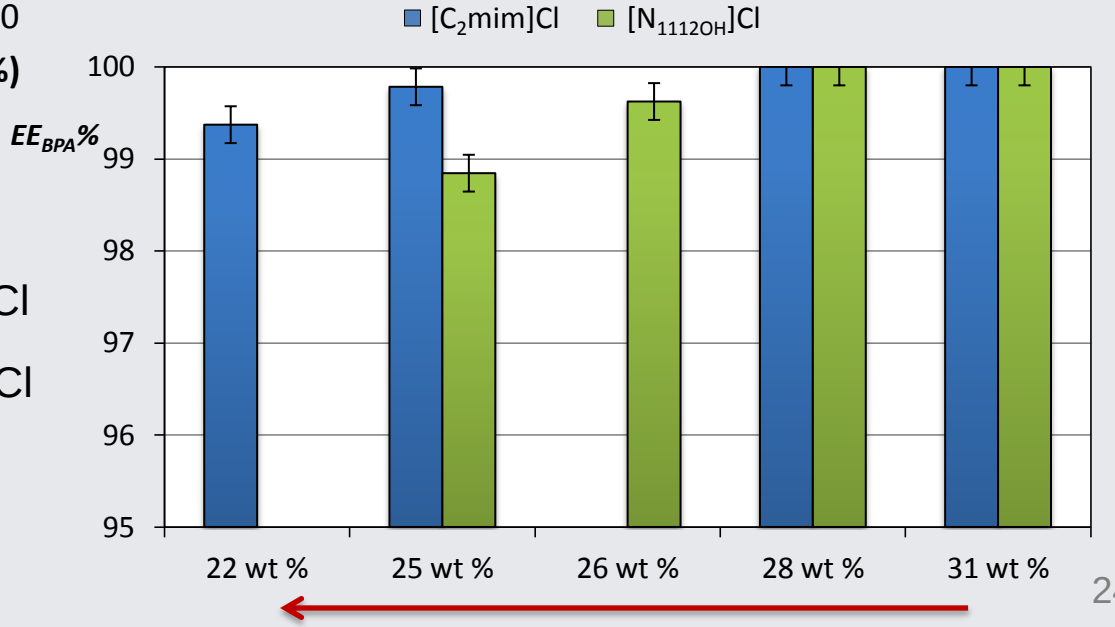


Optimization of **amount of IL** without losing the **high extraction efficiencies** of BPA

Minimum concentrations:

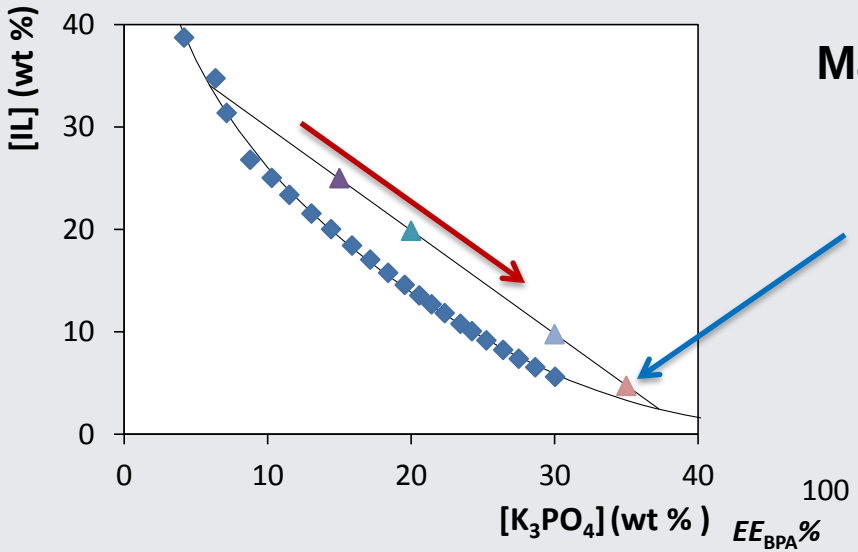
15 wt % K_3PO_4 + 25 wt % $[C_2mim]Cl$

22 wt % K_3PO_4 + 28 wt % $[N_{1112OH}]Cl$



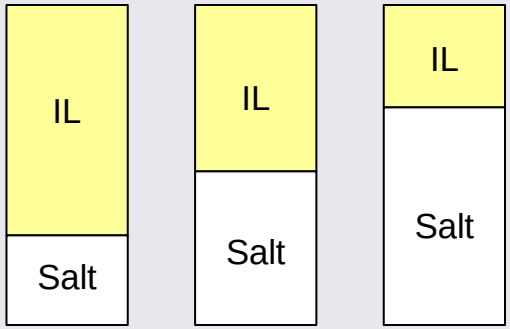
4. Results and discussion

4.3. Extraction of bisphenol A (BPA)

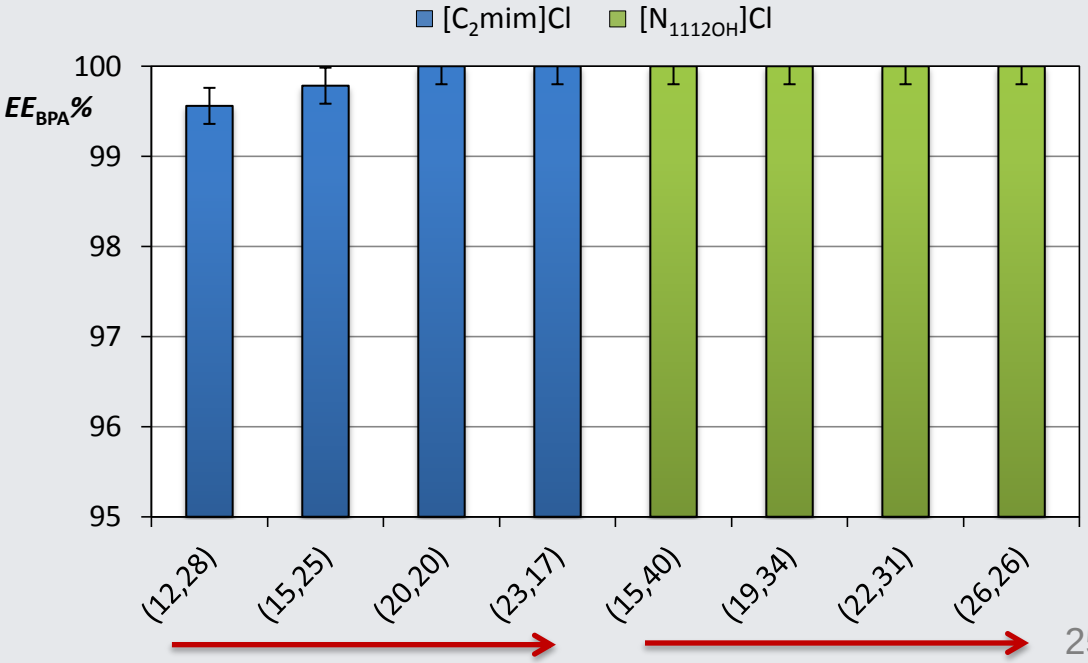


Maximum concentration of BPA achievable

The concentration of BPA can be increased at least up to **100-fold**.



↓ V_{IL} → ↑ [BPA] IL-rich phase



5. Conclusions

✓ The **organic salt** tri-potassium citrate showed to be a **good option for the substitution** of commonly used **inorganic salts**, in ATPS formation;

✓ For the first time:

- ✓ it was addressed the **effect of micelles formation** and their impact on the **extraction of (bio)molecules** (both charged and non-charged);

↓

Significant impact in extraction processes

✓ The application of IL-based ATPS shows to be an **improved technique for concentrating** the levels of BPA **from biological fluids**;

✓ For all investigated systems, **extraction efficiencies** of BPA are **higher than 98.5 %**.

6. Future work

✓ To **study novel ATPS** composed of **IL and other organic salts** and their **potential in the extraction** of different types of compounds;

✓ To understand better the **mechanisms underlying to the micelle formation** in these systems and **their effect in the extraction process;**

✓ To support the **finding of the micelle-mediated extraction** – application of transmission electron microscopy (TEM);

✓ Extraction of BPA with IL-based ATPS:

- ✓ To **work with real body fluid samples**, aiming at providing a general overview of **the levels of BPA in the Portuguese population** and its relation with **several types of diseases**.

Acknowledgements