

Ionic-liquid-based aqueous biphasic systems for improved detection of bisphenol A in human fluids†‡

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Received 25th May 2012, Accepted 12th July 2012

DOI: 10.1039/c2ay25536g

Bisphenol A is a human endocrine disruptor. Its normally low levels in biological fluids make it difficult to detect via conventional techniques. This work demonstrates the complete extraction of bisphenol A, and its possible concentration up to 100-fold in a single-step procedure, using ionic-liquid-based aqueous biphasic systems.

Bisphenol A (BPA, 4,4'-(propane-2,2-diyl)diphenol, CAS no. 80-05-7) is a ubiquitous organic compound in human life. BPA is a key monomer in the production of epoxy resins and the most common form of polycarbonate plastics (Fig. 1). These two applications account for 95% of worldwide BPA consumption (over 3.8 million tons per year) for the fabrication of regular daily products, e.g. baby

bottles, food and beverage containers, plastic tableware, toys, eyeglass lenses, sports equipment, medical devices, household electronics, industrial floorings, adhesives, automotive primers, and printed circuit boards.¹

Products using BPA-based plastics have been in commercial use since the 1950s. Nevertheless, the leaching of this compound, particularly from food storing items, became a matter of concern for governmental agencies after its recognition as an endocrine disruptor. In humans, it exerts hormone-like properties leading to altered immune functions, imbalanced hormone ratios, decreased semen quality, obesity, diabetes, heart disease, and behavioural alterations in children.² The detection of BPA in 92.6% of human urine samples, collected among the US population between 2003 and 2004, played a strong role in raising awareness of its hazards amongst the popular press.³ Since then, it has been identified in air, water, sediments, soil and house dust, food items, and other human biological fluids (including serum, plasma, placenta, semen and breast milk).⁴ These findings pointed out the need to establish regulations regarding the production and market placement of BPA. Canada has classified BPA as a toxic substance and has established a provisional tolerable daily intake (TDI) of 25 µg BPA per kg body weight per day, whereas in Europe the TDI is 50 µg BPA per kg body weight per day.⁵ This higher TDI value is actually recommended by the US Environmental Protection Agency.⁵ Yet, some criticism has been raised regarding the difficulties in detecting and quantifying BPA.⁶ The low content of BPA in biological samples is the major obstacle towards its identification and accurate quantification. Usually, liquid–liquid or solid–liquid extractions are used to increase BPA concentrations from food and biological samples;¹ yet, they are time-consuming and require large quantities of volatile organic solvents.

Aqueous biphasic systems (ABS) are currently recognized as efficient pre-treatment techniques for concentrating metabolites from one aqueous phase to another.⁷ ABS are composed of two aqueous phases formed by the concomitant addition of two structurally different polymers, a polymer and a salt, or two salts and/or surfactants. In the last decade, ionic liquids have been also studied as novel alternatives for the polymers or inorganic salts commonly used.⁸ Since these systems are mainly made up of water, the use of organic and harmful volatile solvents is totally avoided, and the result is, thus, “greener”. Moreover, the use of ionic liquids in ABS has proven to allow for the tailoring of the polarities of the coexisting phases, and hence it offers a controlled partitioning of the most diverse (bio)

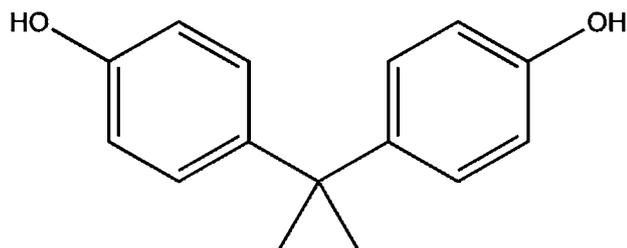


Fig. 1 Molecular structure of bisphenol A.

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† This work was financed by national funding from FCT-Fundação para a Ciência e a Tecnologia, through the project PTDC/QUI-QUI/121520/2010. ACAS, MRP and MGF also acknowledge FCT for the postdoctoral grants SFRH/BPD/65884/2009, SFRH/BPD/26689/2006 and SFRH/BPD/41781/2007, respectively.

‡ Electronic supplementary information (ESI) available: Materials, experimental procedure, ternary phase diagrams, tie-lines and tie-line lengths, additional results at different compositions, and detailed extraction efficiencies. See DOI: 10.1039/c2ay25536g

molecules.^{8,9} In this context, ionic-liquid-based ABS may represent a new vehicle for extracting BPA from human fluids. Although one report regarding BPA extraction using ionic liquids has been found in the literature,¹⁰ this study made use of expensive, toxic, and non-water-stable fluorinated ionic liquids.¹¹

In this work, we tested the ability of several hydrophilic ionic liquids combined with K_3PO_4 (a strong salting-out species) as constituents of ABS for extracting BPA. Both model aqueous systems and more complex matrices, such as human urine-type samples, were investigated. To this endeavour, the extraction efficiencies of BPA for the ionic-liquid-rich phase were measured at 298 K. The percentage extraction efficiencies of BPA (EE%) are defined as the percentage ratio between the amount of BPA in the ionic-liquid-rich phase and that in the total mixture. The quantification of BPA in both aqueous phases was carried out by UV spectroscopy, and the mass balance of the solute was always confirmed. Blank control samples were used to eliminate any interference of the salts, ionic liquids, or urea towards the BPA quantification. At least 3 independent extractions were performed for each ABS, and the respective standard deviations were determined. The usual concentration of BPA at the aqueous phase to form each ABS was $4.3 \times 10^{-4} \text{ mol dm}^{-3}$. The composition of the artificial human urine is described in the ESI.† Details regarding the mapping of the ternary phase diagrams, the composition of the phases, equilibration conditions, purification and purity control of all compounds, and general experimental procedure are provided in the ESI.‡

While maintaining the K_3PO_4 inorganic salt as the common salting-out agent, several chloride-based ionic liquids were tested. A list including the definition of their acronyms is provided as a footnote.§ The extraction efficiencies of BPA in the ABS formed by several chloride-based salts, K_3PO_4 , and water are depicted in Fig. 2. The detailed extraction efficiencies, associated standard deviations, mixture compositions, and the pH values of the coexisting phases are presented in the ESI.‡

Remarkably, extraction efficiencies of BPA higher than 98.5% were attained amongst all the investigated systems. This feature

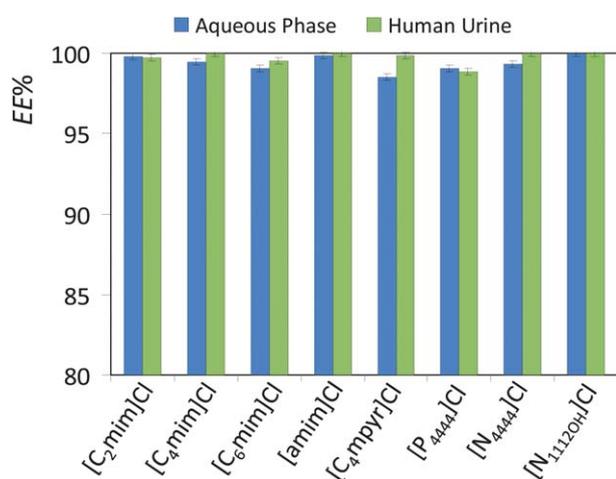


Fig. 2 Percentage extraction efficiencies of BPA, EE%, in the different ABS at 298 K. All ABS are composed of 15 wt% of K_3PO_4 + 25 wt% of chloride-based ionic liquid + 60 wt% of aqueous phase/human urine, except for the $[N_{1112OH}]Cl$ -based system with a concentration of 15 wt% of K_3PO_4 + 40 wt% of $[N_{1112OH}]Cl$ + 45 wt% of aqueous phase/human urine.

mirrors that of the low affinity of BPA for water and preferential partitioning for organic-rich phases. Indeed, the reported $\log K_{ow}$ value (octanol–water partition coefficient) of BPA varies from 3.32 to 3.82.¹² Nevertheless, the high extraction efficiencies obtained are also a direct result of the strong salting-out ability of K_3PO_4 (high-charge density anion with an improved ability to create hydration complexes) and which leads to the “exclusion” of BPA from the inorganic-salt-rich phase to the more “organic” ionic-liquid-rich phase. Albeit BPA is in a charged form ($pK_a = 9.59\text{--}11.30$)¹³ due to the alkaline medium used for extraction, it seems that the electrostatic interactions between the salt cation (K^+) and the BPA negative ion are of low importance, with the endocrine disruptor migrating preferentially for the ionic-liquid-rich phase. On the other hand, the counterions of the chloride-based salts play a role in the partitioning of BPA between the coexisting phases and which could indicate the presence of electrostatic interactions between each ionic liquid cation and the charged BPA. Taking into account the “neutral” molecular structure of BPA (two large phenyl groups, as well as two electron-rich hydroxyl groups and two methyl groups) and the range of ionic liquids employed, the following interactions can also be expected: (i) hydrogen-bonding interactions; (ii) $\pi \cdots \pi$ interactions between the aromatic groups; and (iii) dispersive-type interactions between the aliphatic groups. The results show that an increase in the cation side alkyl chain length of the 1-alkyl-3-methylimidazolium-based ionic liquids leads to a decrease in the extraction efficiencies of BPA. Moreover, the use of quaternary ammonium- and phosphonium-based ionic liquids also decreases the extraction efficiencies when compared with the imidazolium-based fluids. This effect of the imidazolium alkyl chain length and the four butyl chains at $[P_{4444}]Cl$ and $[N_{4444}]Cl$ seems to indicate that although there is a need to accommodate the non-polar parts of BPA, the dispersive-type interactions are non-favourable for its enhanced extraction. In fact, it seems that $\pi \cdots \pi$ interactions and hydrogen-bonding interactions are vital requirements for the complete extraction of BPA. Examples of such factors at work can be noticed with $[C_4mim]Cl$ and $[C_4mpyr]Cl$ (aromatic and non-aromatic five-sided rings) and with $[amim]Cl$ (enhanced hydrogen-bonding capability due to the allyl group). Even with choline chloride this tendency is confirmed since the hydroxyl group at the longest aliphatic chain favours the hydrogen-bonding between the choline cation and BPA.

Fig. 2 compares ABS made up of different ionic liquids. Nevertheless, in order to create more economic processes, the amount of ionic liquid added could be optimized without losing the high extraction efficiencies of BPA. Therefore, while maintaining the inorganic salt concentration, we studied the BPA extraction efficiencies in a range of concentrations of $[C_2mim]Cl$ and $[N_{1112OH}]Cl$ – two of the best candidates for the extraction of BPA. Fig. 3 shows the effect of the ionic liquid concentration in the extraction of BPA.

In general, an increase in the ionic liquid concentration, *i.e.* an increase in the tie-line length, leads to improved extraction efficiencies. However, to achieve complete extractions of BPA, the minimum concentrations of 15 wt% of K_3PO_4 + 25 wt% of $[C_2mim]Cl$ and 22 wt% of K_3PO_4 + 28 wt% of $[N_{1112OH}]Cl$ are required (or mixture compositions with higher amounts of inorganic salt and less ionic liquid and which fit within the same tie-line).

To guarantee that the concentration of BPA in the aqueous phase neither provides erroneous results nor leads to the saturation of the phases, the extraction efficiencies of BPA at initial concentrations of 100, 50, 5 and $1 \mu\text{g g}^{-1}$ in the aqueous phase were determined in the

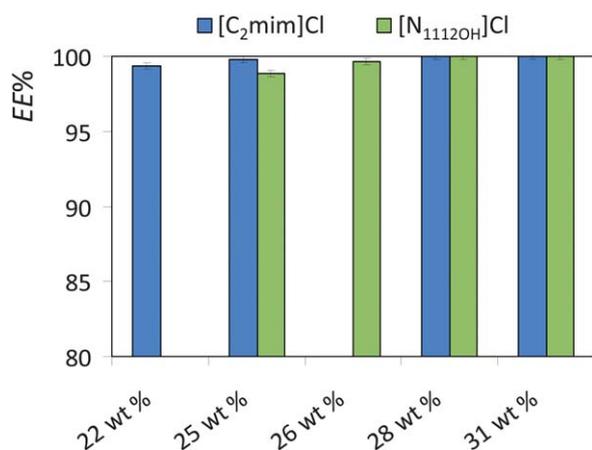


Fig. 3 Percentage extraction efficiencies of BPA, EE%, in the ABS composed of 15 wt% of K₃PO₄ + [C₂mim]Cl at different concentrations and 22 wt% of K₃PO₄ + [N₁₁₁₂OH]Cl at different concentrations, at 298 K.

two systems composed of 15 wt% of K₃PO₄ + 25 wt% of [C₂mim]Cl and 15 wt% of K₃PO₄ + 40 wt% of [N₁₁₁₂OH]Cl. In all of these studies, the complete extraction of BPA for the ionic-liquid-rich phase was observed and the mass balance was always confirmed. It is well known that BPA is poorly water soluble. If the saturation of the phases is reached, erroneous results could appear in clinical trials providing lower contents than the real ones. From our data it is safe to admit that concentrations up to 100 μg g⁻¹ of BPA can be analyzed, which supports the applicability of the proposed systems to real samples since this value is well above those recently found in human biological fluids.¹⁴

As pointed out before, one of the major concerns related to the BPA analysis is its very low concentration in body fluids. In order to explore the maximum concentration of BPA achievable, several extractions were carried out at different compositions in the same tie-line. The main goal is to reduce the volume of the ionic-liquid-rich phase up to a minimum capable of concentrating the BPA that is actually present in a larger volume of an aqueous medium, for instance, in biological fluids. The various initial compositions are along the same tie-line, yet different initial concentrations lead to a different volume ratio. The results obtained are depicted in Fig. 4. Detailed information and the representation of the composition mixtures at the phase diagrams are provided in the ESI.†

The results shown in Fig. 4 indicate that along the same tie-line it is possible to control the volume ratio of the aqueous phases, aiming at decreasing the volume of the ionic-liquid-rich phase, while keeping the complete extraction of BPA in a single-step. In Fig. 4, the volume ratio (ionic-liquid-rich/salt-rich phase) ranges between 4 and 0.5 (as determined by the lever-arm rule). Therefore, different mixture compositions along these tie-lines always lead to the complete extraction of BPA. In this context, the concentration of BPA can be increased at least up to 100-fold by the reduction of the total volume of the extractive phase (making use, for instance, of the following mixture compositions: 2.5 wt% of [N₁₁₁₂OH]Cl + 45 wt% of K₃PO₄ or 2.7 wt% of [C₂mim]Cl + 37 wt% of K₃PO₄).

After fine-tuning the ionic liquids and respective compositions with model systems composed of water, the direct extraction of BPA from artificial human urine was further evaluated to ascertain the applicability of these systems as novel extractive techniques from human

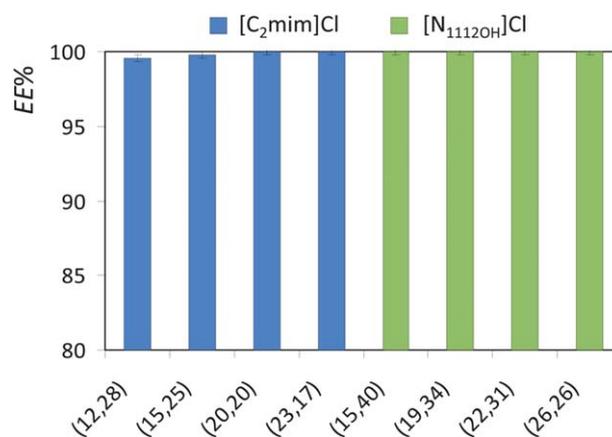


Fig. 4 Extraction efficiency percentages of BPA, EE%, in [C₂mim]Cl + K₃PO₄ and in [N₁₁₁₂OH]Cl + K₃PO₄ ABS at 298 K and at different mixture compositions (wt%) along the same TL (*x*,*y*) at the *xx* axis represent the percentage weight fractions of the salt and ionic liquid, respectively).

biological fluids. The urine sample results are shown in Fig. 2. In general, the presence of a more complex matrix, now including NaCl and urea, favours the partitioning of BPA for the ionic-liquid-rich phase. Indeed, for most systems, 100% of extraction was attained.

To the best of our knowledge, we report here for the first time the remarkable ability of ionic-liquid-based ABS to extract BPA from aqueous biological samples in a single-step procedure. We show that by obtaining a complete extraction and possible concentration up to 100-fold, human fluid samples can be effortlessly checked for their BPA content.

The pre-concentration of BPA and related metabolites from biological fluids is traditionally carried out by eminent solid-phase extraction (SPE) techniques and involve, according to well-known adopted protocols, the use of organic solvents (*e.g.* methanol, ethyl acetate, or MTBE).¹⁵ Besides the requirement on the use of organic and toxic molecular solvents, there is also the need of SPE cartridges which are usually of high cost. Therefore, the alternative process presented here is, in comparison to the latter, greener, safer, time saving and (arguably one of the most important characteristics in modern science) more economical. Besides the replacement of the conventional volatile organic solvents, the systems herein proposed, especially those composed of 1-ethyl-3-methylimidazolium chloride and choline chloride, require small amounts of ionic liquids of low toxicity and low cost.¹⁶

In conclusion, small kits containing the optimized ionic liquids and K₃PO₄ in fixed amounts, to which the human biological fluids could be simply added, can be conceptualized as a new and commercial complement to analytical/clinical strategies where the identification/quantification of BPA is required. It should be stressed that the most used analytical method for the determination of BPA is high-performance liquid chromatography (HPLC), either combined with mass spectrometry (MS) using electrospray ionization (ESI) interface or with an electrochemical detector (ED).¹⁷ There is already evidence that, for instance, the direct quantification of opium alkaloids, testosterone and epitestosterone in ionic-liquid-rich phases using high performance liquid chromatography (HPLC) is possible, and no interferences with the phase forming components of ABS were found.¹⁸

Notes and references

§ Chloride (Cl^-) counterions: 1-alkyl-3-methylimidazolium ($[\text{C}_n\text{mim}]^+$ with $n = 2, 4$ and 6), 1-allyl-3-methylimidazolium ($[\text{amim}]^+$), 1-butyl-1-methylpyrrolidinium ($[\text{C}_4\text{mpyr}]^+$), tetrabutylphosphonium ($[\text{P}_{4444}]^+$), tetrabutylammonium ($[\text{N}_{4444}]^+$), and (2-hydroxyethyl)trimethylammonium, choline ($[\text{N}_{1120\text{H}}]^+$).

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