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TITLE: Application of ionic-liquid-based aqueous biphasic systems for the extraction, purification and concentration of phenolic contaminants from biological tissues

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AIM: This project aims to use liquid-liquid extraction based on more efficient, environmental benign and economically viable systems, such as aqueous biphasic systems (ABS) composed of ionic liquids (ILs), as an extraction and purification technique in the detection of phenolic contaminants, namely triclosan, in biological tissues.

PROCEDURE: In line with previous results obtained by Dr. Nguyen Minh Tue during his staying in University of Aveiro in 2018, the ability of aqueous biphasic systems (ABS) composed of commercial ionic liquids (ILs) to extract and purify triclosan in chicken blood samples was evaluated. ABS were prepared by mixing the commercial available ILs tetrabutylammonium bromide ($[N_{4444}]Br$) and tetrabutylphosphonium bromide ($[P_{4444}]Br$) with a citrate buffer solution ($K_3C_6H_5O_7$:$C_6H_8O_7$) at pH 7.1 in which chicken blood sample was diluted. Preliminary tests on the effect of IL concentration and the addition of salts, namely $NaN(CN)_2$ and NaSCN, in the blood matrix precipitation rate and triclosan extraction were also carried-out. Solvent extraction by using hydrophobic eutectic solvents composed of mixtures of trioctylphosphine oxide (TOPO) with decanoic acid and thymol and menthol, mixed with citrate buffer solution were also evaluated. All systems were spiked with triclosan at known concentrations. Chicken blood was commercially acquired at a local supermarket.

RESULTS: It was observed by Dr. Nguyen Minh Tue during his staying in University of Aveiro in 2018, that the glycine-betaine ILs have a good ability to induce the precipitation of blood matrix, allowing the detection of phenolic compounds without matrix influence. However, this type of ILs is not commercially available and requires a syntheses procedure before their application. Thus, in the current year, it was our goal to evaluate the ability of commercially available ILs to extract and purify triclosan. The obtained results are summarized in the Table 1. It was previously reported that $[P_{4444}]Br$-based ABS was able to induce the complete precipitation and/or denaturation of bovine

serum albumin.[1] Taking into account these results, ABS composed of both [N_{4444}]Br and [P_{4444}]Br were here evaluated. Unfortunately, none of these commercial ILs were able to induce the complete precipitation of chicken blood matrix and some constituents were also extracted to the IL-rich phase (top phase) – *cf.* Figure 1A.

Table 1. Summary of the obtained results with IL-based ABS and solvent extraction by HES.

Commercial IL	IL (wt %)	Buffer (wt %)	Blood Sample (wt %)	Results
[N_{4444}]Br	24	29	9	Partial precipitation; blood matrix was also extracted to IL-rich phase.
[P_{4444}]Br	26	27	8	
HES	**Aqueous:Organic (v/v)**		**Blood Sample (vol %)**	**Results**
TOPO:Decanoic Acid	1:1		10	Almost complete/complete blood matrix precipitation
Menthol:Thymol	1:2		10	

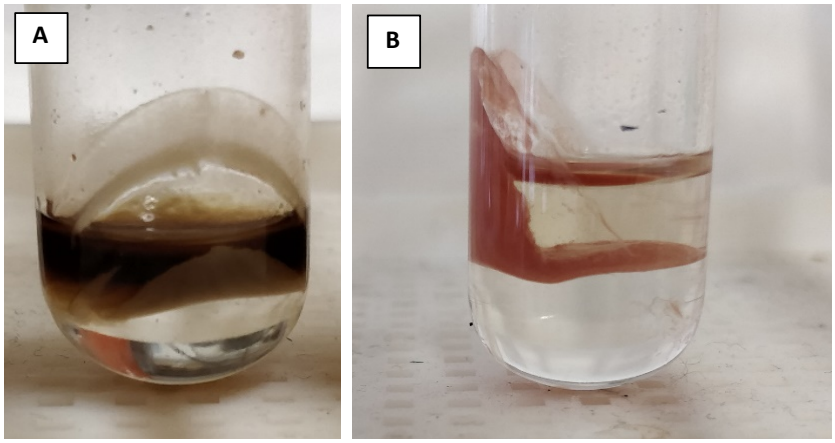

Figure 1. Macroscopic aspect of chicken blood matrix precipitation by: **A** - IL-based ABS; **B** - solvent extraction with HES.

Taking into account the good results obtained by Dr. Nguyen Minh Tue with the application of glycine-betaine-based ILs composed of dicyanamide ($N(CN)_2$) anions, the addition of $NaN(CN)_2$ to the ABS composed of [P_{4444}]Br was evaluated here. However, no changes on the precipitation rate were observed. The same happens when NaSCN was added to the system. Thus, to better understand what is inducing the almost complete precipitation of blood matrix in the presence of glycine-betaine-based ILs (observed by Dr. Nguyen Minh Tue) it is necessary to do more experimental work.

Solvent extraction by using two different HES composed of TOPO:decanoic acid (1:1) and menthol:thymol (1:1) was also carried-out, and the obtained results are summarized in Table 1. Remarkably both systems were able to induce the precipitation of most blood matrix – *cf.* Figure 1B. In fact, in 2019, Schaeffer et al.[2] demonstrated the ability of HES composed of menthol:thymol to purify violacein, extracted from bio-engineered

Yarrowia lipolytica yeast, by the complete precipitation of contaminant proteins at the solvent interface. Thus, the results obtained here are in good agreement with data previously reported in literature and demonstrate the potential of these systems composed of HES to be used in the extraction and purification of phenolic compounds from biological tissues.

PERSPECTIVES IN FUTURE: The results obtained by Dr. Nguyen Minh Tue during his staying in University of Aveiro in 2018 and those reported here demonstrate the potentiality of liquid-liquid extraction based on aqueous biphasic systems composed of glycine-betaine based ILs, and HES to be used in the pre-treatment of blood samples for a more accurate detection of phenolic contaminants, such as triclosan. To the good development of this methodology, several parameters will be evaluated aiming to attain high triclosan extraction efficiency and blood matrix precipitation: (i) the influence of the concentration of the IL and buffer when ABS are used; (ii) the influence of the addition of additives in IL-based ABS; (iii) the use of other types of HES composed of bio-derived compounds of low cost and easy acquisition; (iv) optimization of the ratio HES:buffer solution to decrease the amount of HES used and increase the concentration of triclosan in organic phase for an easier detection.

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