3. Contents

TITLE: Aqueous biphasic systems composed of ionic liquids as a sample purification step in the detection and quantification of phenolic contaminants in biological tissues

MEMBERS:
Helena Passos, *University of Aveiro, CICECO, Chemistry Department*
Ana Catarina Sousa, *University of Évora, Department of Biology and Comprehensive Health Research Centre (CHRC)*
Mara G. Freire, *University of Aveiro, CICECO, Chemistry Department*
João A.P. Coutinho, *University of Aveiro, CICECO, Chemistry Department*
Nguyen Minh Tue, *Center for Marine Environmental Studies (CMES), Ehime University*
Tatsuya Kunisue, *Center for Marine Environmental Studies (CMES), Ehime University*

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) or solvent extraction with hydrophobic eutectic solvents, as extraction and purification techniques in the detection of phenolic contaminants in biological tissues.

PROCEDURE: In line with previous results, the ability of hydrophobic eutectic solvents (HES) to extract and purify bisphenol A (BPA) in chicken blood samples was evaluated. BPA was used as a model phenolic contaminant, since its identification and quantification can be easily attained by using UV-Vis spectroscopy allowing a faster characterization of the systems studied here. HES of trioctylphosphine oxide (TOPO):decanoic acid (1:1 mol:mol) and thymol:menthol (1:1), mixed with citrate buffer aqueous solutions at different volume ratios (1:2, 1:1, 2:1) were evaluated. All systems were spiked with BPA at known concentrations. Chicken blood was commercially acquired at a local supermarket.

RESULTS: The results obtained last year demonstrated the remarkable ability of HES to induce the precipitation of most blood matrix during solvent extraction step. These results agreed with previous data reported in the literature.[1] Thus, in the current year, a more detailed studied was carried-out by using these systems. HES composed of trioctylphosphine oxide (TOPO):decanoic acid (1:1 mol:mol) and thymol:menthol (1:1), mixed with citrate buffer aqueous solutions (pH 7) at different volume ratios (1:2, 1:1, 2:1) were evaluated (*cf.* Figure 1). In a first set of experiments, BPA extraction efficiency percentage (*EE*%) was determined in these systems in the absence of chicken blood. The *EE*% was calculated by Eq. 1 and the obtained results are presented in Figure 2.

$$EE\ \% = \frac{m_{BPA}^{Top}}{m_{BPA}^{Top} + m_{BPA}^{Bottom}}$$

(Eq. 1)

With m_{BPA} representing the weight of BPA determined on the HES phase (Top) and aqueous phase (Bottom).

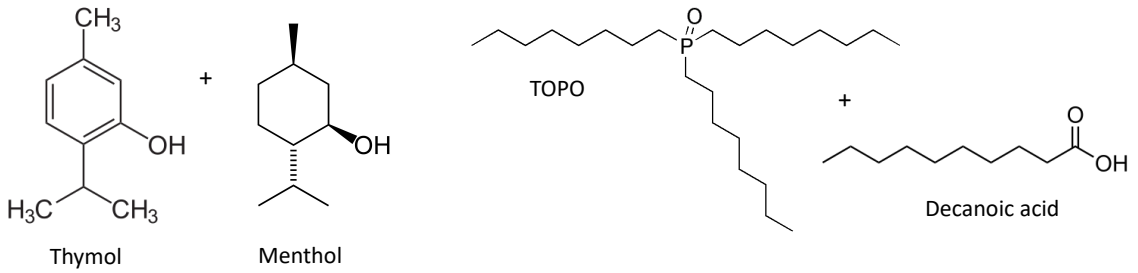

Figure 1. Chemical structure of HES components.

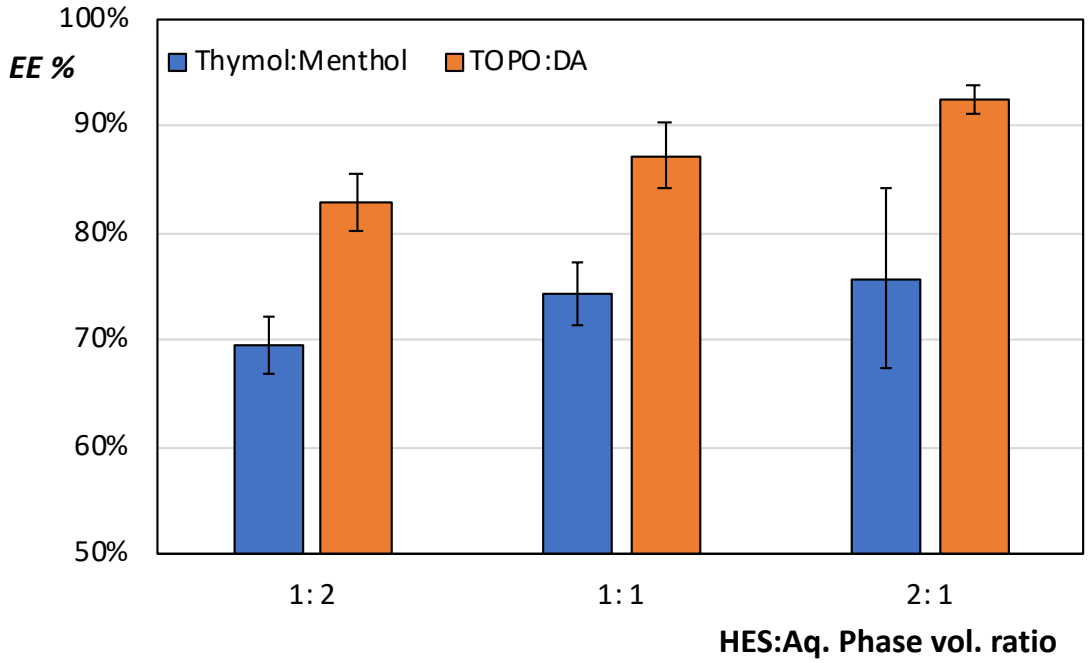

Figure 2. Extraction efficiency percentage (*EE* %) of BPA for HES phase by using different HES (thymol:menthol and TOPO:decanoic acid(DA)) and organic:aqueous phase volumetric ratios.

Results presented in Figure 2 demonstrated that both HES used in this work have good ability to extract BPA. The best results were attained with TOPO:Decanoic acid mixture, probably due to its more hydrophobic character which is favorable to the extraction of BPA. Furthermore the higher is the HES:Aqueous phase volumetric ratio, the highest is the extraction of BPA to the HES phase. Thus the best result was attained by using TOPO:Decanoic acid HES at HES:Aqueous phase 2:1 volumetric ratio: *EE* % = 92 % ± 1 %. Despite the remarkable results obtained, it is important to refer that HES composition was not optimized in this step, which may have a positive impact on BPA

extraction efficiency. This should be evaluated as future work.

Since previous results were obtained in the absence of chicken blood a simple test was carried-out to verify how the addition of chicken blood to the extraction system can affect the quantification of BPA on the aqueous phase through UV-Vis spectroscopy. Figure 3 discloses the UV-Vis spectrum of the blank aqueous phase, i.e. without BPA addition, in the presence and the absence of chicken blood to infer on blood matrix impact in the quantification technique. Since BPA is quantified at the wavelength of 277 nm, as it is possible to infer from Figure 3, chicken blood matrix will have an influence on this region of the spectra. Thus, it is important to take into account this influence and carry-out the previous experiments in the presence of chicken blood to see if BPA *EE* % is also affected. This will be evaluated as future work.

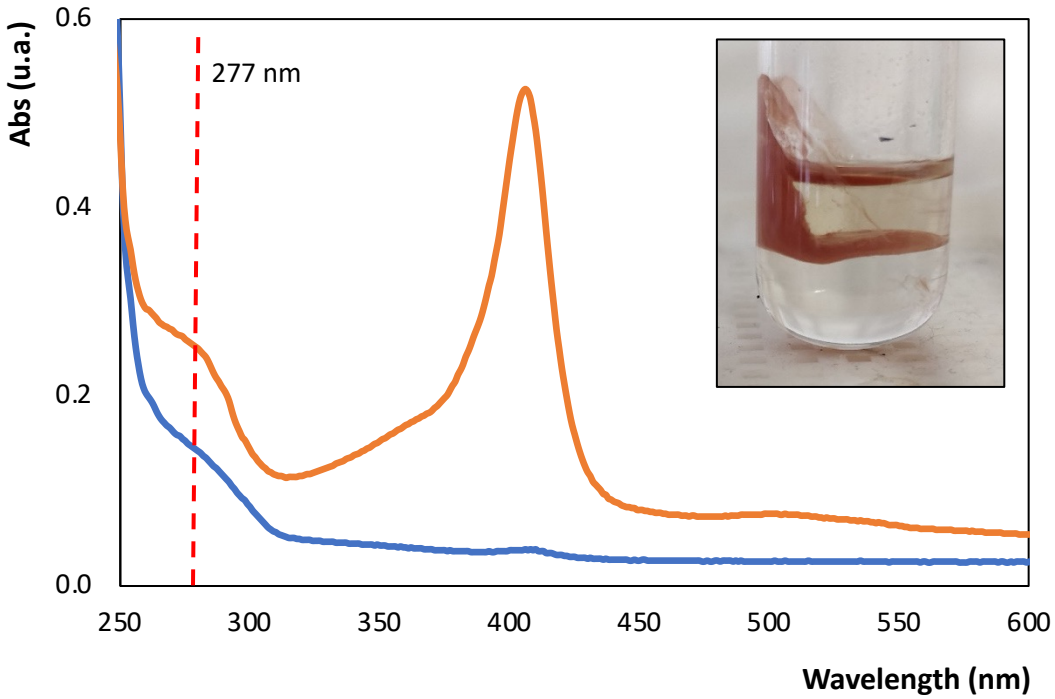

Figure 3. Blank aqueous phase UV-Vis spectrum in the presence (orange) or absence (blue) of blood chicken. Macroscopic aspect of chicken blood matrix precipitation by solvent extraction with HES.

PERSPECTIVES IN FUTURE: The results reported here demonstrate the potential of solvent extraction based on HES to be used in the pre-treatment of blood samples for a more accurate detection of phenolic contaminants. To the good development of this methodology, the future work should focus on the following aspects: (i) evaluation of HES composition effect on BPA extraction efficiency; (ii) evaluation of blood matrix impact on BPA extraction efficient; (iii) use of the optimized system for the extraction of more relevant phenolic contaminants, such as triclosan and 1-OH-pyrene; (iv) evaluation of HES phase composition analysis by GC-MS: direct analysis vs back-extraction.

REFERENCES:

[1] Schaeffer N, Kholany M, Veloso TLM, Pereira JL, Ventura SPM, Nicaud JM, Coutinho JAP (2019) Temperature-responsive extraction of violacein using a tuneable anionic surfactant-based system. Chemical Communications 55: 8643–8646.