Stabilization and purification of RNA using biocompatible ionic liquids

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The widespread use of RNA for the development of novel therapies has been hindered due to issues associated with its inherent chemical instability and fast degradation by ribonucleases[1]. In addition, the traditional extraction and purification techniques of this biomolecule are time-consuming and require highly toxic reagents, demanding for improved technologies able to provide RNA with high integrity, purity and biological activity.

To overcome the described bottlenecks, ionic-liquid-based aqueous biphasic systems (IL-based ABS), which are mainly composed of water[2], are herein investigated as more sustainable and efficient techniques for RNA purification. Due to the high affinity between amino-acids and RNA and the favourable nucleic acids-stabilization properties exhibited by amino-acid-based ILs (AA-ILs)[1], they were selected as components of ABS formulations. After identifying the most promising systems able to protect RNA from ribonuclease-mediated degradation, the ultimate goal of this work is to purify RNA from a complex recombinant lysate, envisaging the development of integrated purification-preservation platforms.

AA-ILs comprising L-arginine, L-tryptophan and L-lysine as cations combined with chloride or DL-aspartate ([Arg]Cl, [Trp]Cl, [Lys]Cl, [Arg][Asp], [Trp][Asp], and [Lys][Asp]) were synthesized and characterized, and their ability to form two phases with distinct salts

and polymers investigated. With exception of [Trp]Cl and [Lys][Asp] that are unable to form ABS with the compounds in study, all the remaining AA-ILs form ABS with polypropylene glycol 400 (PPG 400). Initial studies on the stability and integrity of RNA have been performed in aqueous solutions of the studied ILs to assess its potential to act as preservation media. Several promising IL-based ABS were found and are currently under investigation as integrated purification-preservation platforms for RNA envisaging its use as biotherapeutics.

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Keywords: RNA, Aqueous biphasic systems, Amino acid ionic liquid, Purification

References:

(1) Quental, M. V.; Pedro, A. Q.; Pereira, P.; Sharma, M.; Queiroz, J. A.; Coutinho, J. A. P.; Sousa, F.; Freire, M. G. Integrated Extraction-Preservation Strategies for RNA Using Biobased Ionic Liquids. *ACS Sustain. Chem. Eng.* **2019**, *7* (10), 9439–9448. https://doi.org/10.1021/acssuschemeng.9b00688.

(2) Pereira, J. F. B.; Freire, M. G.; Coutinho, J. A. P. Aqueous Two-Phase Systems: Towards Novel and More Disruptive Applications. *Fluid Phase Equilib.* **2020**, *505*, 112341. https://doi.org/10.1016/j.fluid.2019.112341.