Purification of RNA from recombinant lysates using biocompatible amino-acid-based ionic liquids

Ana F. Pereira¹, Augusto Q. Pedro¹, Leonor S. Castro¹, Maria J. Quental¹, Ana P. M. Tavares¹, Luís C. Branco², João A. P. Coutinho¹, Fani Sousa³, Mara G. Freire¹

¹CICECO – Instituto de Materiais de Aveiro, Departamento de Química, Universidade de Aveiro, Portugal

²LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal

³CICS-UBI – Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, Portugal

Keywords:

Biopharmaceuticals, Aqueous biphasic systems, Amino acid ionic liquid, Purification;

RNA-based biopharmaceuticals are being envisioned as a powerful tool for the development of innovative medicines for prevalent diseases. Despite their undeniable relevance, the ubiquitous unstable nature of RNA coupled with the laborious and costly methods required for its extraction and purification still challenge the widespread application of this biopolymer.

On the basis of the high affinity between amino-acids and RNA and the favorable nucleic acids-stabilization properties exhibited by amino-acid-based ILs (AA-ILs), a set of ILs is herein studied both as preservation media and components of aqueous biphasic systems (ABS). The global aim of this work is to develop alternative cost-effective and sustainable purification-preservation platforms for RNA with the ultimate goal of purifying RNA from a complex recombinant lysate, taking advantage of the "designer solvent" character of ILs. AA-ILs comprising cholinium, L-arginine, L-lysine and L-histidine as cations and combined with chloride, DL-aspartate, L-tyrosine or L-phenylalanine were synthesized, characterized, and their ability to form two phases with distinct salts and polymers investigated. All the AA-ILs in study were able to form ABS with polypropylene glycol with a molecular weight of 400 g.mol⁻¹ (PPG 400), being subsequently investigated as extraction and preservation platforms for RNA. It was demonstrated that RNA was successfully extracted to the IL-rich phase while ensuring that its integrity and stability

are preserved. Ongoing work is focusing the application of the most promising AA-ILbased ABS for the separation of RNA and genomic DNA from complex recombinant lysates.

Acknowledgements:

This work was developed within the scope of the projects: CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC); EIC-Pathfinder YSCRIPT project with reference 101047214, supported by the budgets of the Horizon Europe Program; and mVACCIL (EXPL/BII-BTI/0731/2021), financially supported by national funds (OE), through FCT/MCTES. Ana Pereira, Leonor Castro, Augusto Pedro and Ana Tavares acknowledge FCT, respectively, for the PhD grants 2022/13247/BD and 2020/05090/BD and the research contracts CEECIND/2020/02599 and CEECIND/2020/01867 under the Scientific Stimulus – Individual Call.