Improving the manufacturing of mRNA nanomedicines using thermoreversible aqueous biphasic systems and ionic liquids

***Maria I. Sousa*[1(*)], Luís C. V. Silva[1], Andreia Correia[1], Mara G. Freire[1], Francisca A. e Silva[1], Augusto Q. Pedro[1]**

[1] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal*

[(*)] *minessousa@ua.pt*

Keywords: mRNA nanomedicines; ionic liquids; thermoreversible aqueous biphasic systems; *in vitro* transcription.

ABSTRACT

It is undeniable the potential of messenger RNA (mRNA) vaccines as effective tools to manage infectious disease outbreaks. However, mRNA nanomedicines production is complex and expensive, requiring improved technologies to produce stable and widely accessible products, whilst meeting required production times and fulfilling market demands.

Ionic liquids (ILs) are composed of organic cations and organic or inorganic anions. Given their remarkable structural diversity, ILs can be properly designed to improve RNA stability. Moreover, as phase-forming agents of aqueous biphasic systems (ABS), ILs contribute to develop highly selective purification processes. This work aims to integrate production-clarification steps of mRNA vaccines, using thermoreversible IL-based ABS, simplifying subsequent purification steps. Several quality control methods were designed to evaluate integrity and purity of mRNA manufactured resorting to *in vitro* transcription. ABS formed by dextran from Leuconostoc spp. with an average molecular weight of 450.000-650.000 g/mol (Dex 500) and polyethylene glycol (PEG) 3350 g/mol, containing ILs as adjuvants were characterized regarding their thermoreversible nature.

Preliminary mRNA partition experiments using IL-based ABS indicate that mRNA is preferentially partitioned toward the IL-PEG-Rich phase. Promising systems for mRNA purification have been identified. Ongoing work focuses on selecting the most promising IL-based ABS for initial mRNA clarification. In the future, mRNA production-clarification will be integrated into the best performing system to overcome current challenges in mRNA nanomedicine manufacturing, such as sustainability and production speed, whilst improving mRNA stability and yield.

Acknowledgements:
This work was developed within the scope of: CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through FCT/MCTES (PIDDAC); and mVACCIL (EXPL/BII-BTI/0731/2021), financially supported by national funds (OE), through FCT/MCTES. Augusto Q. Pedro and Francisca A. e Silva acknowledge FCT respectively, for the research contracts CEEC-IND/02599/2020 and CEEC-IND/03076/2018 under the Scientific Stimulus–Individual Call. Maria I. Sousa acknowledges FCT for the PhD grant 2022.11476.BD.