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Using thermoreversible aqueous biphasic systems and ionic liquids to improve the manufacturing of mRNA nanomedicines

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Abstract:

If there is one thing the COVID-19 pandemic has taught us is the enormous potential of messenger RNA (mRNA) vaccines as an effective tool to contain infectious disease outbreaks. Over conventional vaccines, there are several advantages to mRNA vaccines, namely improved safety and efficacy, and the possibility of repeatedly administration [1]. However, mRNA nanomedicine production is still a complex and expensive process that requires improved technologies to produce more stable and widely accessible products, meeting a timely and sufficient manufacturing capacity.

Ionic liquids (ILs) are molten salts comprising organic cations, with a remarkable structural diversity and with promising applications as solvents and catalysts. If properly engineered, ILs can improve the stability of RNA [2] and contribute to the achievement of highly selective purification processes when applied as components of aqueous biphasic systems (ABS) [3]. Therefore, this work aims to integrate the production and clarification steps of mRNA nanomedicine manufacturing processes by using thermoreversible ABS comprising ILs, simplifying subsequent purification steps. Initially, the production of mRNA by *in vitro* transcription using a T7 polymerase-based cell free system was implemented. Several quality control methods (UV spectroscopy, electrophoresis, fluorescence-based mRNA quantification, PCR, dot-blot, circular dichroism, etc) were designed to evaluate the integrity and purity of mRNA. 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 ionic liquids (ILs) as adjuvants were deeply characterized. These systems generally display an upper critical solution temperature (UCST) behavior, which renders them promising candidates for the development of the integrated process. Preliminary mRNA extraction experiments using the developed ABS indicate that the mRNA is preferentially partitioned toward the DEX-rich phase, being recovered with high yield and with high integrity. Ongoing work, having into consideration the most promising ILs able to maintain the stability and integrity of mRNA, is focused on the selection of the best integrated production-clarification platform resorting to thermoreversible IL-based ABS, to be achieved by a careful selection of the ABS components and mixture points.

In conclusion, the development of a new integrated production-clarification platform, resorting to thermoreversible ABS comprising ILs, can be used to overcome the challenges of mRNA nanomedicine production, namely by lowering costs and environmental impact of current manufacturing processes while improving mRNA stability, yield, and speed of production.

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