Title

The use of thermoreversible ionic liquid-based aqueous biphasic systems as an integrated production-clarification platform in mRNA vaccines manufacturing

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Abstract

COVID-19 reinforced the potential of messenger RNA (mRNA) vaccines as effective platforms to control disease outbreaks, given their rapid development, safety and efficacy. Nevertheless, mRNA manufacturing can be optimized by combining production with initial clarification steps. This work aims to integrate mRNA production and initial clarification steps, using thermoreversible ionic liquid-based aqueous biphasic systems (IL-ABS), ultimately simplifying mRNA vaccine production.

Quality control methods (dot-bot, electrophoresis, among others) were designed to evaluate mRNA production by *in vitro* transcription, accessing its purity and integrity. Polymer-based ABS composed of 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, with and without ionic liquid as adjuvants (Tetrabutylphosphonium bromide; Tetrabutylammonium bromide; 1-butyl-3-methylimidazolium chloride; Tetrabutylphosphonium chloride) were studied. Polymer-ABS thermoreversability is achieved by lowering the monophasic system temperature from 37 to 25 degrees, enabling phase separation.

Preliminary extraction experiments with Dex-PEG ABS revealed mRNA can be recovered with high integrity in the Dex-rich phase as well as common contaminants such as double-stranded mRNA and T7 RNA Polymerase. However, the addition of ILs as adjuvants to the ABS changed the mRNA partition profile, being mainly found in the phase enriched in PEG and IL. Promising systems for mRNA purification have been identified.

Using the most promising IL-ABS previously identified, current work focusses of designing the integrated mRNA production-clarification process.

In conclusion, thermoreversible IL-ABS can be used as production-clarification platforms to diminish costs, ecological footprint and mRNA production time, while maintaining mRNA integrity and yield.

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Graphical Abstract