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Production and purification of non-viral vectors for gene therapy applications

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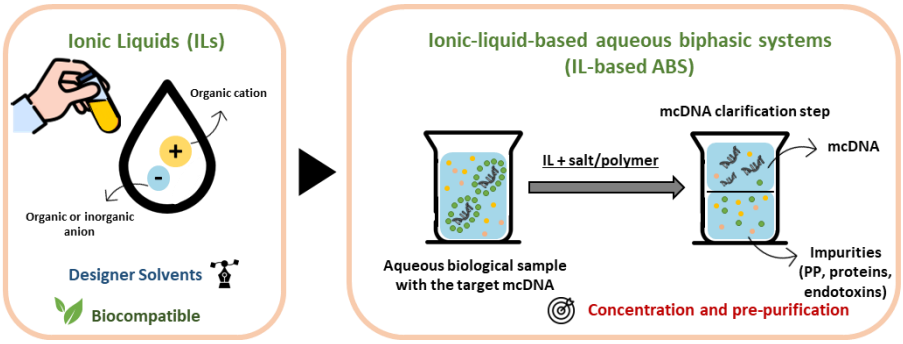
Cancer is the second cause of death worldwide with relevant societal and economic burdens. Approximately 19.3 million new cases and 10 million cancer deaths were registered in 2020, being projected an increase in cancer incidence in the coming years.[1] To counteract this trend and mitigate consequences, multiple innovations have been achieved in oncology, from which the manufacturing of biopharmaceuticals as effective therapeutic agents stands out. Nowadays, there are more than 540 biopharmaceuticals approved in the EU/USA, and nucleic acids are gaining momentum both in the prophylaxis of infections and as therapeutic agents in gene therapy.[2] Gene therapy is based on the transfection of eukaryotic cells with gene-based products to correct the target malfunction. Among these, non-viral vectors including plasmid DNA and minicircle DNA (mcDNA) attracted increased importance.[3] Despite their clinical relevance, current manufacturing strategies are still complex and involve multi-step purification processes, ultimately increasing their cost.[3] To overcome this obstacle, this work investigates the application of ionic-liquid-based aqueous biphasic systems (IL-ABS) as a primary capture strategy of p53-mcDNA biopharmaceuticals with broad therapeutic efficiency to multiple cancer types (scheme 1). Considering the biological medium complexity in which the target biopharmaceutical is produced, a clarification and concentration step, to be achieved by IL-ABS, is key before moving to high-resolution chromatographic purification techniques. p53-mcDNA was produced using recombinant *Escherichia coli* cells in shake flasks and using a suitable culture medium. To promote cell growth and parental plasmid (PP) bioproduction, cells were incubated at optimized conditions. Afterward, recombination was successfully induced using L-arabinose, yielding the p53-mcDNA. The fraction containing the PP and p53-mcDNA was subsequently isolated using a commercial kit, and their partitioning behavior in ABS comprising bromide-based ILs and citrate potassium salt was investigated. Ongoing studies are focused on optimizing the separation of PP and p53-mcDNA using the designed IL-ABS, after which a sample of increased complexity without pretreatment will be applied.



Scheme 1: Proposed application of IL-ABS as a capture strategy for mcDNA biopharmaceuticals.

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