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Integrated extraction, purification and preservation of DNA with ionic liquid-based aqueous biphasic systems

Ana I. Valente[1], Teresa B.V. Dinis[2], Ana P. M. Tavares[1], Fani Sousa[3], Mara G. Freire[1]

[1] CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[2] LSRE-LCM - Laboratory of Separation and Reaction Engineering-Laboratory of Catalysis and Materials, Department of Chemical Engineering at Faculty of Engineering, University of Porto, Rua Dr Roberto Frias s/n, 4200-465 Porto, Portugal
[3] CICS-UBI – Health Sciences Research Center, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal
** E-mail: anaivalente@ua.pt*

The production of deoxyribonucleic acid (DNA) in large-scale for therapeutic purposes presents several challenges. An effective downstream process is highly demanding, as it should be capable of extracting, purifying, and preserving DNA integrity, by reducing its degradation by endonucleases.[1,2] A technique that allows the integration of several downstream steps is aqueous biphasic systems (ABS). Through the alignment of ABS with ionic liquids (ILs), IL-based ABS can be a possible platform to be included in DNA production when properly designed. Nonetheless, until our work[3], no attempt had been made to apply an IL-based ABS with DNA, particularly an ABS capable of separating endonucleases from nucleic acids. In this work, double-stranded DNA (dsDNA) was separated from deoxyribonuclease I (DNase I) endonuclease through the application of a three-phase partitioning system (TPP) formed by an ABS composed of biocompatible cholinium-based ILs. Taking advantage of the customized properties of ILs, dsDNA was completely extracted to the IL-rich phase, while DNase I was precipitated at the ABS interface. The system composed of [Ch][Gly] and PEG 400 demonstrated that an optimized ABS/TPP allows the dsDNA simultaneous extraction, purification, and preservation in the long term, paving the way for their application in the bioprocessing of DNA-based therapy products.

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