One step towards the quantification of p53-minicircle DNA

Ana I. Valente<sup>1</sup>, Ana P. M. Tavares<sup>1</sup>, Fani Sousa<sup>2</sup>, Mara G. Freire<sup>1</sup>

 <sup>1</sup>CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
 <sup>2</sup>CICS-UBI – Health Sciences Research Center, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

## Abstract

p53-minicircle DNA (mcDNA) is a biopharmaceutical with potential use in the genetic therapy of cancer. Despite the therapeutic relevance of DNA-based products, current clarification and purification steps are complex, resulting in high-cost biopharmaceuticals [1]. The existing strategies for mcDNA isolation are based on chromatographic techniques, such as size-exclusion, hydrophobic interaction, and affinity chromatography. Despite the selectivity achieved with the affinity strategy, it depends on the genetic manipulation of the vector to include specific sequences and/or in the use of enzymes to eliminate impurities [2,3]. Thus, up to date, one of the best strategies resorts to size exclusion chromatography, allowing the recovery of 66% of mcDNA with a purity of 98% [2].

Due to the lack of an efficient purification strategy, a quantification method for mcDNA is non-existent. Considering this, an analytical method based on ion exchange chromatography for mcDNA quantification is under development. Firstly, the p53-mcDNA is produced through the culture of transformed *Escherichia coli*. Then, the mcDNA is extracted with a commercial kit. The samples obtained after the extraction are applied in developing the new analytic method. Also, an extraction method using ionic liquid (IL)-based aqueous biphasic systems (ABS) is under evaluation for the replacement of the commercial kit used for the purpose. For that, the cytotoxicity of several analogues of glycine-betaine ILs is being also assessed.

## Keywords

Biopharmaceutical, Minicircle DNA, Extraction, Quantification, Ionic Liquids, Citotoxicity

## References

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## Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC) and CICS-UBI projects UIDB/00709/2020 & UIDP/00709/2020 financed by national funds through the FCT/MCTES. This work was developed within the scope of the project PureDNA (2022.03394.PTDC, Development of cost-effective platforms based on ionic liquids for the purification of p53-minicircle DNA biopharmaceuticals with application in oncology), financially supported by national funds (OE), through FCT/MCTES. Ana P.M. Tavares acknowledges the FCT for the research contract CEECIND/2020/01867 and Ana I. Valente acknowledges the FCT PhD grant (SFRH/BD/08352/2021).