BIOCOMPATIBLE LIPOSOMES CO-ENCAPSULATING TRIPLE ANTIRETROVIRAL COMBINATIONS AND TLR AGONISTS AS NANOPLATFORMS TO TARGET HIV RESERVOIRS

M. Heredia [a], F.J. Ostos [a], J.A. Lebrón [a], M. López-López [b], M.L. Moyá [a], E. Bernal [a], Rut Fernández-Torres [c], H. D. T. Mertens [d], M.J. Almazán [a], L. F. López-Cortés [e], E. Ruiz-Mateos [e], M. Rafii-El-Idrissi [e,f], P. López-Cornejo [a]

[a] Department of Physical Chemistry, Faculty of Chemistry, University of Seville, Seville, Spain, [b] Engineering, Physical Chemistry and Materials Science, University of Huelva, Huelva, Spain, [c] Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, Seville, Spain, [d] European Molecular Biology Laboratory (EMBL), DESY, Hamburg, Germany, [e] Institute of Biomedicine of Seville, IBiS/Virgen del Rocío University Hospital/CSIC/University of Seville, Clinical Unit of Infectious Diseases and Microbiology, Seville, Spain, [f] Department of Medical Biochemistry, Molecular Biology, and Immunology, University of Seville, Seville, Spain.

mheredia3@us.es

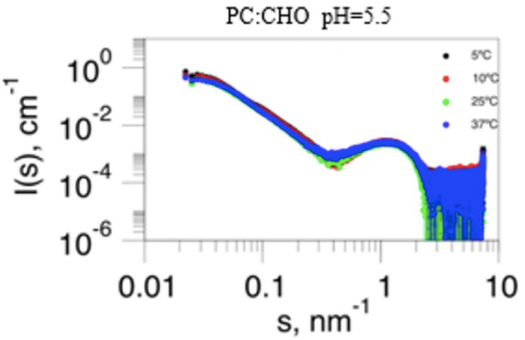
Persistent cellular and anatomical HIV-1 reservoirs prevent durable remission after antiretroviral therapy (ART) interruption. We developed biocompatible phosphatidylcholine/cholesterol (PC:CHO) liposomes, with or without a cationic Ru(II)-based metallolipid (RuC19C19), to co-deliver triple antiretroviral combinations and Toll-like receptor (TLR) agonists as a dual pharmacological and immunomodulatory strategy.

Two regimens (tenofovir/emtricitabine/nevirapine and tenofovir/emtricitabine/bictegravir) together with the TLR9 agonist (CpG ODN 2216) were encapsulated in the aqueous core, while TLR4 (LPS) and TLR7 (GS-9620) agonists were incorporated in the lipid bilayer. Liposomes were prepared by thin-film method to obtain unilamellar nanostructures that were characterised by dynamic light scattering, ζ-potential and small-angle X-ray scattering measurements. Particle sizes do not shown changes neither the temperature nor the pH. High antiretroviral encapsulation efficiencies were obtained in all the working conditions studied. Drug release at 310 K followed apparent first-order kinetics, with slower release from Ru-based liposomes and faster release for nevirapine- versus bictegravir-based antiretroviral combinations.

In vitro assays in several immunological cell lines and primary human Peripheral Blood Mononuclear Cells (PBMCs) indicated low cytotoxicity for PC:CHO liposomes over the tested concentration range. However, Ru-liposomes required low-to-intermediate concentrations to maintain cell viability. PC:CHO liposomes were non-haemolytic (≤5%), while Ru-liposomes showed increased haemolysis only at the highest doses. Ru-liposome uptake by PBMCs reached ~75% after 6 h at 37 °C.

Ongoing studies quantify TLR-driven dendritic and monocyte activation by multiparametric flow cytometry. Results support PC:CHO (with and without RuC19C19) liposomes are promising nanocarriers to enhance long-acting ART delivery while enabling innate immune activation, warranting further ex vivo evaluation in reservoir-relevant models.

Keywords:
Nanocarriers, biocompatible liposomes, metalloliposomes, HIV reservoirs, antiretrovirals, TLR agonists.



Small-Angle Scattering Intensity versus wave vector (SAXS data) collected for PC:CHO liposomes at pH 7.4 at different temperatures.

Acknowledgements:
Ministry of Science, Innovation and Universities of the Spanish Government (PID2023-151642OB-I00); Consejería de Salud y Consumo, Junta de Andalucía (PI-0170-2024); EU/ Ministry of Finance and Public Service/European Funds/Regional Government of Andalusia/Department of Universities, Research and Innovation (PPIT2024-31730); Ayudas SGUS (CITIUS) 2025/00000399 and 2025/00000418.