Thiol functionalised gold nanoparticles loaded with methotrexate for cancer treatment: synthesis, characterisation, and *in vitro* studies

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Gold nanoparticles (AuNPs) have been successfully prepared in the presence of two hydrophilic thiols in mixture: 3-mercapto-1-propanesulfonate (3MPS) and 2-(diethylamino)ethanethiol (DEA), following a single-phase wet chemical reduction method in water, where sodium borohydride is used as the reducing agent [1]. AuNPs with a controlled diameter lower than 10 nm and negative surface charge, suitable for their use as drug delivery carriers [2] were isolated and allowed to interact with methotrexate (MTX) to obtain the AuNPs-MTX nanoconjugate with a molar drug encapsulation efficiency optimised and found to be ≥ 70%.

The stability of the colloids upon interaction with the MTX drug was assessed monitoring the surface plasmon resonance (LSPR) band at 520 nm and the hydrodynamic diameter <2RH> = (9 ± 2) nm, with Dynamic Light Scattering (DLS) and Small-Angle X-Ray Scattering (SAXS). To gain information on the spatial arrangement of MTX drug onto the AuNPs, 1D, 2D Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared (FT-IR) spectroscopy, and X-ray Photoelectron Spectroscopy (XPS) experiments were compared, evidencing different non-covalent interactions.

The MTX loading slightly decreased the structural order of the system and increased the distance between the AuNPs as studied via solid state Grazing Incidence Small and Wide-Angle X-Ray Scattering (GISAXS, GIWAXS). The small size and stability of the AuNPs before and after the interaction with MTX was highlighted by microscopy observations with Field Emission Scanning Electron Microscopy (FE-SEM) and Transmission Electron Microscopy (TEM). The *in vitro* cytotoxicity of pristine and MTX-loaded AuNPs towards two different neuroblastoma cell lines, SJNKP and IMR5 with overexpressed n-Myc was studied and compared with free MTX drug. On both cell lines, free AuNPs showed no cytotoxicity whereas the nanoconjugate had a more potent effect compared with free MTX.

[1] Venditti I., Cartoni A., Cerra S., Fioravanti R., Salamone T. A., Sciubba, F., Tabocchini M. A., Dini V., Battocchio C., Iucci G., Carlini L., Faccini R., Collamati F., Mancini Terracciano C., Solfaroli Camillocci E., Morganti S., Giordano A., Scotognella T., Maccora D., Rotili D., Marchese C., Anastasiadou E., Trivedi P., Fratoddi I., Part. Part. Syst. Charact., **39**, 2100282, (2022).

[2] Beik, J.; Khateri, M.; Khosravi, Z.; Kamrava, S. K.; Kooranifar, S.; Ghaznavi, H.; Shakeri-Zadeh, A., Coord. Chem. Rev., **387**, 299-324 (2019).