X-ray absorption spectroscopy unveils highly stable doxorubicin-loaded iron oxide nanoparticles in tumor cells

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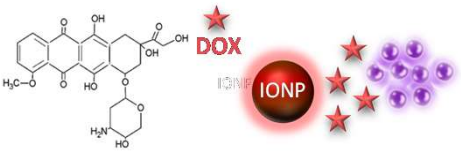
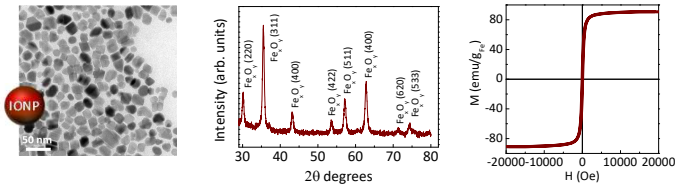
Introduction

Nanotechnology has brought new tools for the development of advanced materials for the diagnosis and treatment of diseases. Magnetic nanoparticles are a major class of nanomaterials due to their high potential in biomedicine, such as drug delivery, hyperthermia treatment and magnetic resonance imaging [1-3]. In this work, we report the synthesis of a multifunctional drug delivery system based on magnetic iron oxide nanoparticles (IONP) and doxorubicin (DOX) and their suitability for multimodal hyperthermia-based anticancer treatments. The stability of the DOX-loaded IONPs in the tumor environment is characterized by X-ray absorption spectroscopy (XAS).

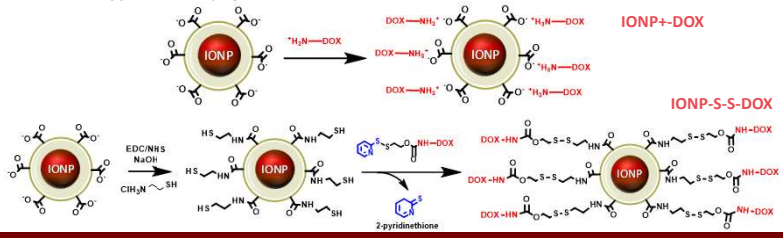


Synthesis and functionalization

15-nm core **superparamagnetic IONPs** composed of magnetite/maghemite

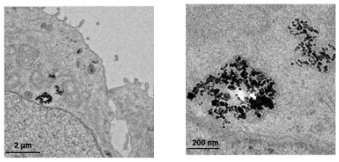


Two types of conjugation via a **disulfide linker** and **electrostatic interactions:**

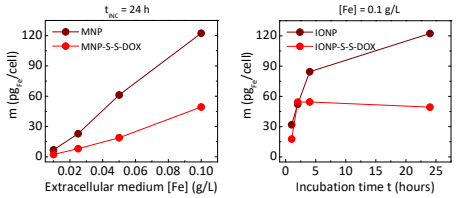


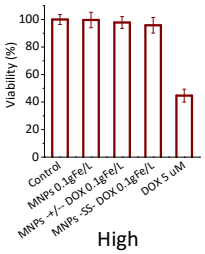
Cell uptake and viability

Incubation of DOX-loaded IONP in MCF-7 **tumour cells**



Iron cell uptake quantification by ICP

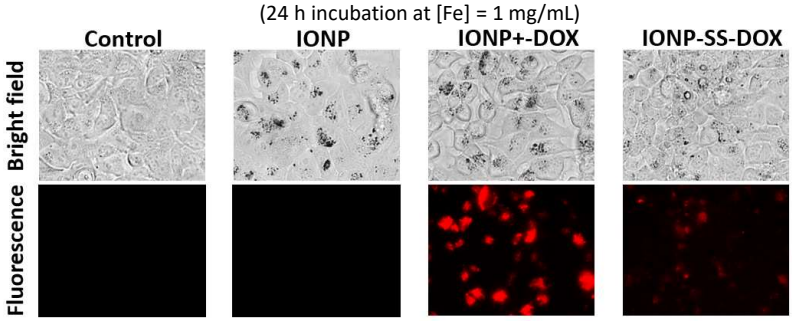




High **biocompatibility** & high internalization values up to **50-100 pg/cell**

Fluorescence microscopy

DOX-loaded IONP internalized in cancer cells (MCF-7)
(24 h incubation at [Fe] = 1 mg/mL)

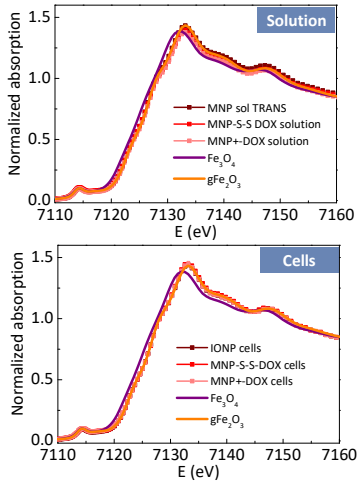


DOX is detected in the **green channel** (excitation at **488 nm**, emission at **561 nm**). Identification of DOX **inside the cells**.

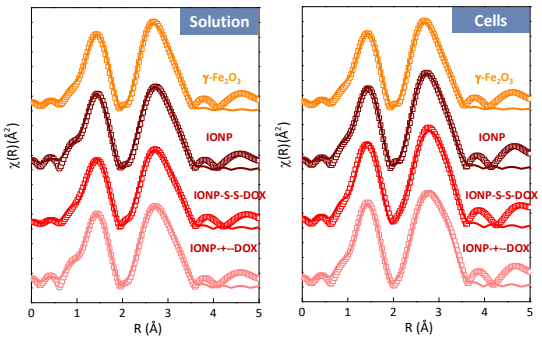
X-ray absorption spectroscopy

Fe K-edge

XANES



EXAFS



XANES spectra reveal that **Fe** is in its **+3 oxidation state** for all samples **both in solution and in cells. EXAFS spectra** are fitted to obtain the data on interatomic distance **(R)**, coordination number **(N)** and the Debye-Waller Factor **(σ^2)**. XAS measurements show that the structure of DOX-IONPs correspond to Υ-Fe_2O_3.

1st Shell Fe-O	N		R(Å)		σ^2(Å^2)	
Sample	Solution	Cells	Solution	Cells	Solution	Cells
γ-Fe_2O_3	5.25 ± 0.03		1.96 ± 3E-3		0.012 ± 5E-4	
IONP	5.05 ± 0.03	5.42 ± 0.02	1.95 ± 3E-3	1.96 ± 3E-3	0.011 ± 5E-4	0.011 ± 4E-4
IONP-S-S-DOX	4.38 ± 0.03	5.63 ± 0.06	1.96 ± 4E-3	1.96 ± 4E-3	0.011 ± 5E-4	0.011 ± 9E-4
IONP +- DOX	5.08 ± 0.03	5.60 ± 0.03	1.96 ± 2E-3	1.96 ± 3E-3	0.012 ± 4E-4	0.011 ± 5E-4

2nd Shell Fe-Fe	N		R(Å)		σ^2(Å^2)	
Sample	Solution	Cells	Solution	Cells	Solution	Cells
γ-Fe_2O_3	3.75 ± 0.04		3.00 ± 3E-3		0.013 ± 4E-4	
IONP	3.42 ± 0.04	4.10 ± 0.04	3.00 ± 4E-3	3.01 ± 3E-3	0.013 ± 4E-4	0.014 ± 4E-4
IONP-S-S-DOX	3.38 ± 0.03	4.64 ± 0.06	3.01 ± 4E-3	3.01 ± 5E-3	0.013 ± 5E-4	0.016 ± 1E-3
IONP +- DOX	3.61 ± 0.03	4.31 ± 0.03	3.00 ± 2E-3	3.01 ± 5E-3	0.014 ± 5E-4	0.015 ± 5E-4

3rd Shell Fe-Fe	N		R(Å)		σ^2(Å^2)	
Sample	Solution	Cells	Solution	Cells	Solution	Cells
γ-Fe_2O_3	8.25 ± 0.05		3.46 ± 3E-3		0.012 ± 5E-4	
IONP	8.16 ± 0.04	10.08 ± 0.04	3.46 ± 4E-3	3.47 ± 3E-3	0.012 ± 4E-4	0.012 ± 6E-4
IONP-S-S-DOX	7.39 ± 0.04	11.9 ± 0.1	3.47 ± 4E-3	3.48 ± 4E-3	0.011 ± 5E-4	0.012 ± 7E-4
IONP +- DOX	7.91 ± 0.04	11.8 ± 0.05	3.47 ± 3E-3	3.47 ± 3E-3	0.012 ± 4E-4	0.012 ± 4E-4

Conclusions

- ✓ The DOX-loaded magnetic nanoplatforms are composed of a core of maghemite that can act as nanothermal agents in either high frequency alternating magnetic fields or near-infrared light.
- ✓ IONP loaded with doxorubicin have a high cellular uptake and are highly biocompatible, i.e., the drug is not released until a stimulus is applied, making it an ideal system for drug delivery allowing bimodal treatments with hyperthermia.
- ✓ XANES spectra show that DOX-loaded IONP are composed of Υ-Fe_2O_3 both in solution and in cells. They are remain intact when internalized into cells, which is advantageous to fulfil their therapeutic mission in the cellular environment..
- ✓ The EXAFS data show an increase in coordination (N) in the cells but no large changes in disorder are observed. This indicates that the increase in N may be due to the aggregation that they suffer due to endosomal confinement.

References

[1] Espinosa, A., et al. (2018) **Adv. Funct. Mater.** 28 (11), 1803660.

[2] Lafuente-Gómez N. et al. (2021) **Chem. Comm**. 7, 13662–13677.

[3] Espinosa, A., et al. (2021) **Nano Letters.** 21 (1), 769-777.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (SEV-2016-0686, RED2018-102626-T, FPI SEV-2016-0686-20-1, FPU18/02323), Comunidad de Madrid(2017-T2/IND5395 Talento, 2018-T1 / IND-1005 Talento, 2018 / NMT-4321), AECC (project Ideas Semilla 2019) and H2020 European project (No 685795. NoCanTher). Special thanks to BM23 (ESRF) beamline staff for the support during the experiments.