European Commission

Surface engineering of fluorescent silica nanoparticles for biolabelling applications

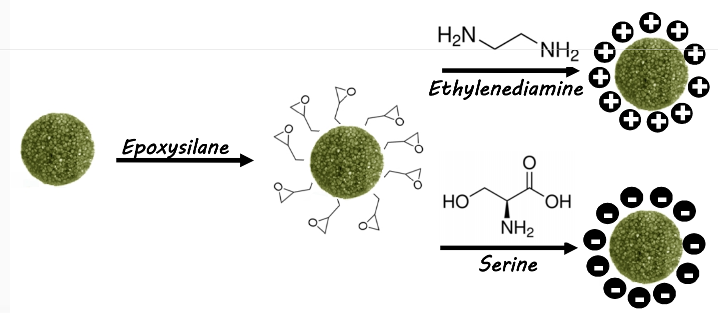
Silica nanoparticles (SiO_2 NPs) are a versatile class of mesoporous materials based on the base-catalyzed hydrolysis of alkyl silicates and subsequent condensation of silicic acid. Due to their outstanding properties, SiO_2 NPs are rapidly becoming a part of our daily life as they are produced on an industrial scale as additives to cosmetics, drugs, printer toners and foods.

Recently, they have also been easily co-synthesized with a variety of fluorophores such as $Ru(bpy)_3$, fluorescein, rhodamine or Quantum Dots, in order to produce robust and biocompatible NPs. Fluorescent labeling of SiO_2 NPs offers the possibility of quantitative measurement and visualization of particle uptake, also providing more insights into the uptake mechanism and survival strategy *in vitro*.

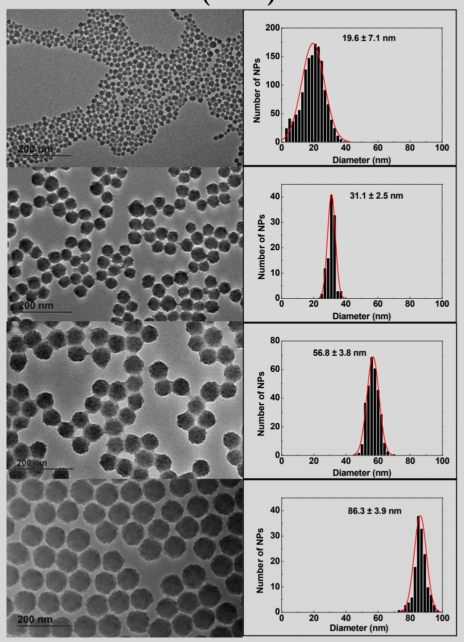
Functionalized SiO_2 NPs are being applied in biotechnology and biomedicine as drug delivery systems, in cancer therapy, for gene transfection and in biosensing and imaging applications. In their way towards real applications in the field of biomedicine, surface engineering for modulating NP biodistribution and performance under physiological conditions remains still a challenging issue.

In this context, the present study deals with a simple and biofriendly procedure to control the size of NPs, by using a regrowth procedure in water, and to exploit the epoxysilane chemistry as a convenient and versatile route for activation of the silica surface.

Surface modification of SiO_2 nanoparticles



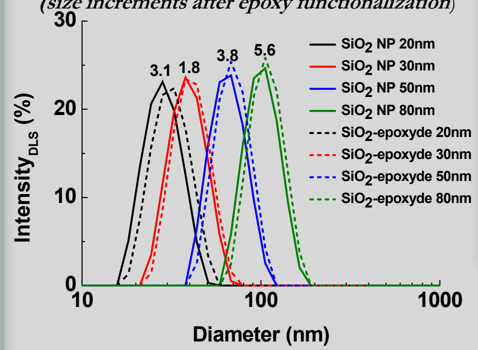
Morphology by Transmission Electron Microscopy (TEM)



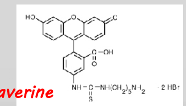
- $Ru(bpy)_3$ can be added to the reaction mixture to produce fluorescent NPs, by absorbance only 0.1 % remains free.
- Scaled up to 250 mL solutions of ~ 2 mg/mL.

Dynamic Light Scattering (DLS)

(size increments after epoxy functionalization)

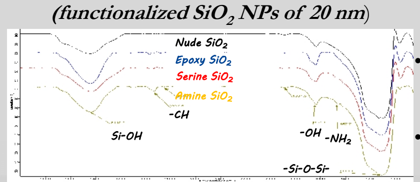


- Estimated a total number of 165 available epoxy groups per NP, by derivatization with a dye-molecule.



Fourier Transformed-Infrared spectra

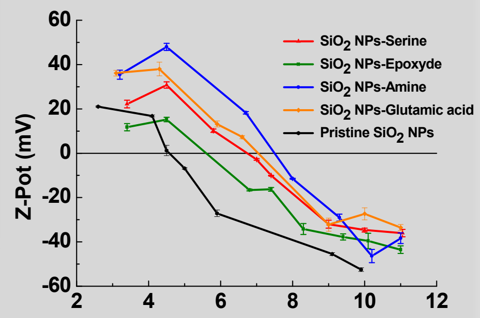
(functionalized SiO_2 NPs of 20 nm)



- C-H stretching vibration at 2927-2944 cm^{-1} (epoxy)
- C-H bending vibration at 1457 cm^{-1} amine

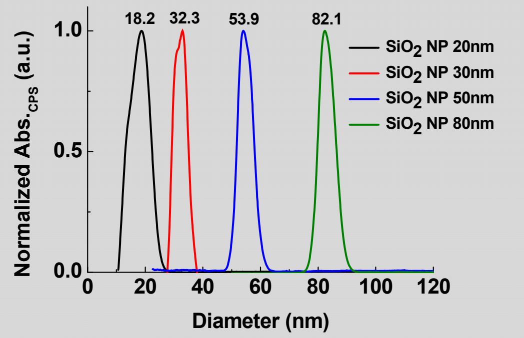
Surface charge vs pH

(functionalized SiO_2 NPs of 20 nm)



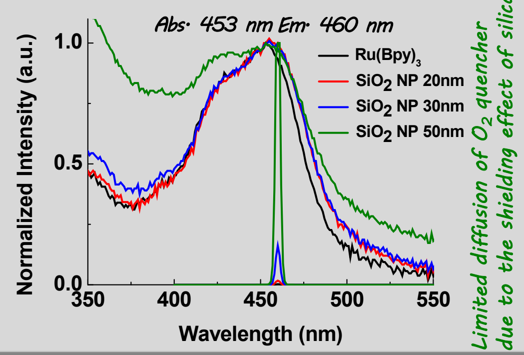
- Stability compromised near the Zero Point Charge (ZPC)

Size distribution by centrifugal sedimentation (CPS)



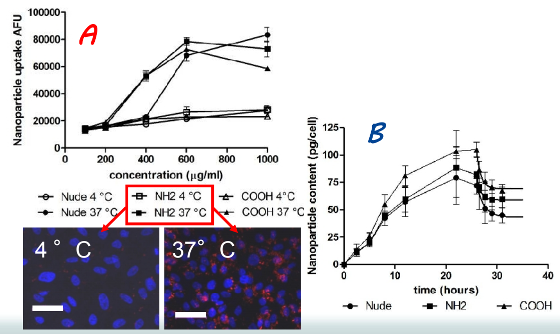
Absorption/Fluorescence

(effect on the fluorescence of encapsulated dye molecules)



In vitro evaluation nanoparticle uptake in lung epithelial cells (A549)

(functionalized SiO_2 NPs of 20 nm)



A: Saturation type concentration-dependent uptake at 5 hr in cCCM

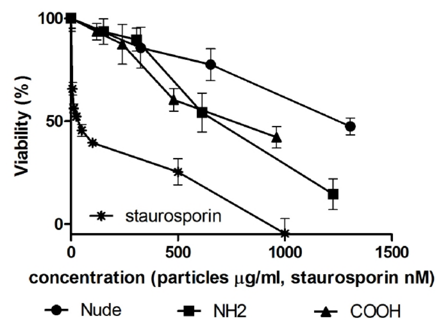
B: Total uptake (75-100 pg/cell) and efflux (after 26 hr)

Stability in CCM

(functionalized SiO_2 NPs of 20 nm)

Sample	Medium	Functionalization	Hydrodynamic Diameter (nm) by intensity	PdI	Z-Potential (mV) at pH 7.4
SiO_2 NPs	H_2O	nude	22.48	0.197	- 27.6
		amine	22.71	0.302	11.3
		carboxylic acid	23.20	0.321	-21.3
	CCM	nude	24.09	0.389	2.05
		amine	28.54	0.373	-7.35
		carboxylic acid	26.39	0.337	6.69
SiO_2 $Ru(bpy)_3$ NPs	H_2O	nude	28.16	0.164	-21.1
		amine	25.31	0.145	9.17
		carboxylic acid	27.61	0.143	-36.2
	CCM	nude	27.75	0.210	-19.9
		amine	26.21	0.127	-18.4
		carboxylic acid	24.43	0.210	-24.9

Cytotoxic effect in A549



Ratio of living cells by propidium-iodide test

No significant cytotoxic effect detected at 72 hr below 250 µg/mL

Conclusions and Outlooks

- The present study demonstrates the successful functionalization of SiO_2 nanoparticles with a variety of ligands *via* a simple, fast and biofriendly process while preserving the main outstanding properties of the particles, i.e. size distribution, morphology and stability.
- Silica encapsulation of dye molecules has a dramatic effect on the fluorescence as a result of the shielding effect of the silica nanomatrix, which prevented the dye molecules from being quenched by the surrounding media.
- The versatility of the epoxy functionalization will be soon evaluated with different relevant biomolecules.

References

1. J. S. Nowak, D. Mehn, P. Nativo, C. Pascual-García, S. Gioria, I. Ojea-Jiménez, D. Gilliland, F. Rossi, Silica nanoparticle uptake induces survival mechanism in A549 cells by the activation of autophagy but not apoptosis, Toxicology Letters, 2013, 224 (1), 84-92.
2. S. Liang, K. Shephard, D. T. Pierce, J. X. Zhao, Effects of a nanoscale silica matrix on the fluorescence quantum yield of encapsulated dye molecules, Nanoscale, 2013, 5, 9365-9373.

www.jrc.ec.europa.eu

The work leading to these results has received funding from the European Union Seventh Framework Programme (FP7 / 2007-2013) under grant agreement n° NMP4-LA-2013-310451

Joint Research Centre

Contact Isaac Ojea-Jiménez, Matteo Pedroni, Dora Mehn, Sabrina Gioria, Douglas Gilliland, François Rossi
European Commission • Joint Research Centre • Institute for Health and Consumer Protection (IHCP)
21027, Ispra (VA), Italy.
Tel. +39 (0)332786688• Email: Isaac.OJEA-JIMENEZ@ec.europa.eu