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Article

Multifunctionality of Nanosized Calcium Apatite Dual-Doped with Li^+/Eu^{3+} Ions Related to Cell Culture Studies and Cytotoxicity Evaluation *In Vitro*

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Abstract: Li^+/Eu^{3+} dual-doped calcium apatite analogues were fabricated using a microwave stimulated hydrothermal technique. XRPD, FT-IR, micro-Raman spectroscopy, TEM, and SAED measurements indicated that obtained apatites are single phased, crystallize with a hexagonal structure, have similar morphology and nanometric size as well as show red luminescence. Lithium effectively modifies the local symmetry of optical active sites and thus affect the emission efficiency. Moreover, the hydrodynamic size and surface charge of the nanoparticles have been extensively studied. The protein adsorption (lysozyme, LSZ; bovine serum albumin, BSA) on the nanoparticle surface depended on the type of cationic dopant (Li^+, Eu^{3+}) and anionic group (OH^-, Cl^-, F^-) of the apatite matrix. Interaction with LSZ resulted in a positive zeta potential and the nanoparticles had the lowest hydrodynamic size in this protein medium. The cytotoxicity assessment was carried out on the human osteosarcoma cell line (U2OS), murine macrophages (J774.E), as well as human red blood cells (RBCs). The studied apatites were not cytotoxic to RBCs and J774.E cells, however, at higher concentrations of nanoparticles cytotoxicity was observed against U2OS cell line. No antimicrobial activity was detected against Gram-negative bacteria with one exception for *P. aeruginosa* treated with Li^+-doped fluorapatite.

Keywords: Nanoapatites; Eu^{3+} and Li^+ ions; Rare earth ions; Photoluminescence; Cytotoxicity; *In vitro* cell culture studies; Protein corona, Antibacterial evaluation; Theranostics

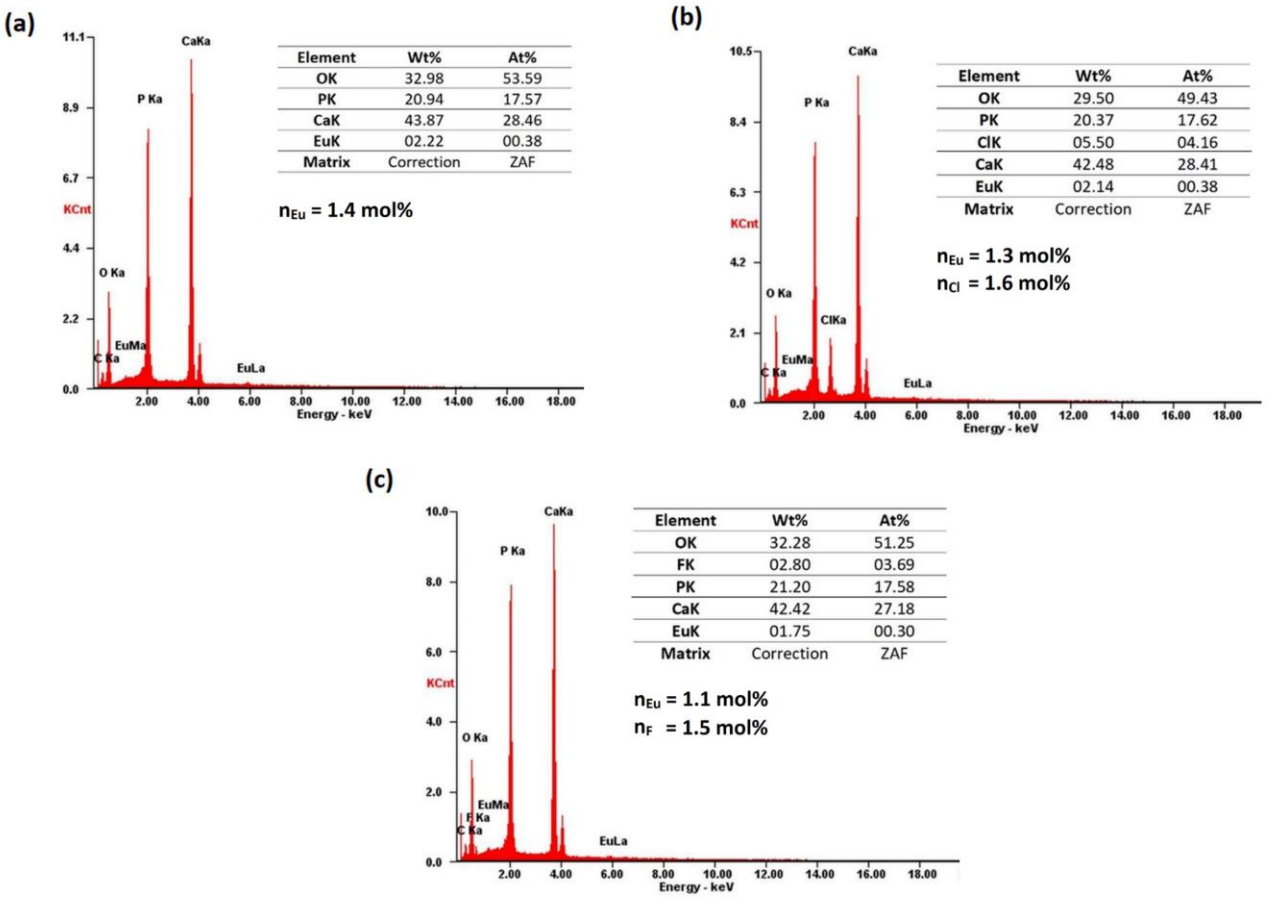

Figure S1: EDS spectra of the (**a**) $Ca_{10}(PO_4)_6(OH)_2$, (**b**) $Ca_{10}(PO_4)_6Cl_2$ and (**c**) $Ca_{10}(PO_4)_6F_2$ co-doped with 1 mol% Eu^{3+} and 2 mol% Li^+ ions, prepared at 500°C with the quantitative analysis of elements (inset).

Table S1. Lattice planes (hkl) assigned based on SAED images and ICSD data ($Ca_{10}(PO_4)_6(OH)_2$ (ICSD-26204), $Ca_{10}(PO_4)_6Cl_2$ (ICSD-24237) and $Ca_{10}(PO_4)_6F_2$ (ICSD-262707) of the $Ca_{10}(PO_4)_6(OH)_2$, $Ca_{10}(PO_4)_6Cl_2$ and $Ca_{10}(PO_4)_6F_2$ co-doped with 1 mol% Eu^{3+} and 2 mol% Li^+ ions, prepared at 500°C.

Sample	d (Å) SAED	(hkl)	d (Å) ICSD
$Ca_{10}(PO_4)_6(OH)_2$: 1 mol% Eu^{3+},2 mol% Li^+	3.4347	(002)	3.4395
	2.8253	(211)	2.8147
	2.2727	(310)	2.2636
	1.8690	(213)	1.8403
	1.3173	(431)	1.3169
$Ca_{10}(PO_4)_6Cl_2$: 1 mol% Eu^{3+},2 mol% Li^+	3.4231	(002)	3.4250
	2.7773	(112)	2.7801
	2.1901	(302)	2.1435
	1.8447	(213)	1.8418
$Ca_{10}(PO_4)_6F_2$: 1 mol% Eu^{3+},2 mol% Li^+	3.4270	(002)	3.4422
	2.7218	(300)	2.7041
	2.2419	(310)	2.2499
	1.8302	(213)	1.8372
	1.4555	(034)	1.4520