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# *A Comparative Study of Functionalized Nano-Hydroxyapatite*

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***Abstract*—In an attempt to obtain appropriate components for complex transfection systems hydroxyapatite nanoparticles (nHAp) were synthesized by means of wet chemical precipitation method, under atmospheric pressure, in the absence or presence of different low-molecular or polymeric cationic dispersants, i.e. arginine, linear or branched polyethylenimine (LPEI, bPEI). The effect of added compounds' type and concentration on nHAp characteristics (surface chemistry, size, morphology, crystallinity, dispersibility) was comparatively studied. The chemical composition and surface modification of the synthesized nano-HAp was investigated using Fourier Transformed Infrared Spectroscopy (FTIR), Energy Dispersive X-Ray (EDX) analysis, Zeta potential measurements (DLS- dynamic light scattering) and UV–vis spectrophotometry. The crystal morphology and particle size were characterized by means of transmission electron microscopy (TEM), scanning electron microscopy (SEM) and X-Ray Diffraction technique (XRD). The DNA binding ability of the functionalized nHAp was tested by agarose gel electrophoresis assay.***

***Keywords*— hydroxyapatite, nanoparticles, gene delivery, arginine, polyethyleneimine***

## I. INTRODUCTION

Hydroxyapatite (HAp) is a bioceramic used on a large scale in the technical (catalysis, particulate emulsifier) and biomedical fields (tissue engineering, drug/gene delivery, dentistry - reparative materials, biocompatible coatings etc.) due to its stability at temperature, pH and chemical composition of the physiological fluid coupled with non-immunogenicity and non-oncogenicity [1, 2]. The increasing demand for this biomaterial gave rise to the development of a high number of synthesis approaches, including dry or wet methods, such as solid state reaction**,** emulsion technique**,** hydrothermal method**,** sonochemical precipitation**,** biomimetic method, sol-gel method, electrochemical deposition, microwave processing and wet chemical precipitation [1-3]. Different applications of HAp require specific properties which are dependent on morphology, surface area, particle size and particle size distribution [1-3]. Thus it is important to choose the right synthesis method that allows control over the properties specific to the envisaged application. In this work, HAp nanoparticles (nHAp) were synthesized using the wet precipitation method in the presence of cationic compounds, i.e. arginine and polyethyleneimine, as the end goal was the product use as a component in a complex gene transfection system [4]. The effect of the cationic compounds on the nHAp characteristics was comparatively investigated using XRD, FTIR, EDAX, TEM and DLS.

## II. EXPERIMENTAL

### *A. Materials*

Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$), sodium ammonium phosphate ($NH_4NaHPO_4.4H_2O$), L-arginine and branched polyethylenimine (bPEI) were purchased from Sigma-Aldrich. The linear polyethylenimine (LPEI) used for functionalization was obtained through hydrolysis of amine terminated poly(2-ethyl-2-oxazoline), with a medium degree of polymerization of ~40, according literature data [5, 6]. The other reagents and solvents were of analytical purity. Bidistilled water was used in all experiments.

### *B. nHAp preparation*

5 samples were prepared by dropwise adding aqueous $Ca(NO_3)_2\ 4H_2O$ (6.16g in 50 ml water) to $NH_4NaHPO_4$ solution (2.25g/50 ml water), under stirring, over 1h, in the presence or absence of the additives (arginine, bPEI, LPEI) on ice bath. The used additives' concentrations were calculated relative to expected final HAp amount (10 wt%/g solid product for arginine and bPEI, 5 and 10 wt%/g for LPEI, corresponding to samples code $HAp_A$, $HAp_{bPEI}$, $HAp_{LPEI-I}$ and $HAp_{LPEI-II,}$ respectively). The mixture was kept for 24 h at room temperature (under stirring) and another 24 h (without stirring) in an oven at 60°C, for aging. A pH of 9 was maintained by adding ammonia $(NH_4)OH$.

### *C. Characterization*

The amount of PEI adsorbed on the nHAps was determined through the spectrophotometric analysis of the supernatant resulted from the separation of nHAp. The analysis is based on the formation of a copperammonia complex [7] in the presence of $Cu^{2+}$/potassium acetate and was done with a UV-6300PC device (VWR).

The DLS data were acquired with a Laser Shimadzu SALD 700 device. The FT-IR spectra were obtained on a Bruker