



A Comparison between Biocompatibilities of Nanocomposites of Silica Doped in HA/collagen and those Doped in HA/gelatin

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ABSTRACT

In this study, biocompatibility of nano composites of silica doped in HA/collagen was compared against those doped in HA/gelatin. For this purpose, hydrothermal samples were synthesized before being investigated by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). Size and morphology of the samples were further characterized by scanning and transmission electron microscopy (SEM). Moreover, energy-dispersive X-ray spectroscopy (EDS) was used to undertake an elemental analysis on the prepared composites. FTIR spectras confirmed chemical structure of the nano composites and EDS patterns showed that, even though the nano composites of silica doped in HA/collagen had equivalent Ca/P ratios to the theoretical value as repeated for HA chemical structure of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, corresponding Ca/P ratios to the nano composites of silica doped in HA/gelatin were just close to the theoretical value. A comparison on the obtained XRD patterns showed that, the nano composites of silica doped in HA/collagen had their crystallite size smaller than that of the nano composites of silica doped in HA/gelatin. FESEM images indicated smaller particle sizes for the nano composites of silica doped in HA/collagen, as compared to those doped in HA/gelatin. Reduced crystallite size and decreased particle size are known to be associated with larger contact surface in chemical reactions, leading to better pharmacological efficacy in terms of bone repair.

Keywords: hydroxyapatite, gelatin, collagen, silica, nanocomposite.

INTRODUCTION

Improving biomaterials for enhanced osseointegration and tissue engineering (for bone remodeling) represents an international research target. Some of the most significant approaches to such a target have been reviewed in a special issue of *Science*¹. During the last decade, a considerable deal of attention has been directed towards the use of bioactive materials, where bioactivity is defined as interfacial bonding of an implant or a bioactive scaffold to tissue by forming a biologically active hydroxyapatite (HA) layer on the bioactive material surface². Among all calcium phosphate bioceramics, HA (with the chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is the most extensively used, biocompatible ceramic material for bone tissue engineering, as it has its chemical composition well-similar to those of the bone mineral phase³. A recently established materials concept of biomimetic composites based on silica, collagen, and calcium phosphates was adapted for the preparation of porous scaffolds suitable for tissue engineering applications⁴⁻⁶. Synthetic biodegradable scaffolds are becoming highly promising materials for bone substitution and regeneration⁷. They can provide an adequate structural support and a 3-D system on which cells can grow and migrate while the tissue regenerates, acting as a temporary extracellular matrix inducing the natural process of tissue regeneration and development⁸. The use of bioactive HA coatings on metal implant surfaces have been reported to improve fixation in orthopedic implants, especially during early stages of healing^{9,10,11}. The requirement for biomaterials to assist in many medical applications replacing original musculoskeletal tissues and improve quality of life

is rapidly increasing. HA has been widely used, as a bone graft material, in a range of medical and dental applications. However, pure HA suffers from low reactivity with existing bone^{12,13}. HA and calcium-deficient HA in nano-particulate form are beginning to play significant roles in bone-related therapies owing to their unique functional properties including high surface area-to-volume ratio and ultrafine structure, which are similar to those of biological apatite. This similarity is of importance in cell-biomaterial interactions¹⁴⁻¹⁹.

EXPERIMENTAL

Synthesis of nanocomposites of silica doped in HA/collagen

In situ synthesis of HA rods was carried out in a collagen matrix. In order to prepare modified HA, 2.8 g of collagen was mixed with 70 ml of distilled water while continuously applying a magnetic stirrer at its maximum speed at around 70°C. After stirring for 2 h, 80 ml of 0.1 M calcium chloride solution was gradually added to the prepared biopolymer solution. The solution was subsequently stirred for 1 h before introducing 48 ml of 0.1 M sodium phosphate in a drop wise fashion while stirred at a pH value of 10. The solution was placed in an oven at 100°C and kept for half an hour before filtering the formed precipitate. Once finished with preparing collagen-based HA nanocomposite, 30 cc of double-distilled water along with 7.0 g of Na_2SiO_3 were mixed in a beaker and then introduced into the solution while stirred for half an hour at 36.5°C. The samples were incubated at 36.5°C for 72 h after which time they were filtered and washed with double-distilled water, and then were dried at room temperature.

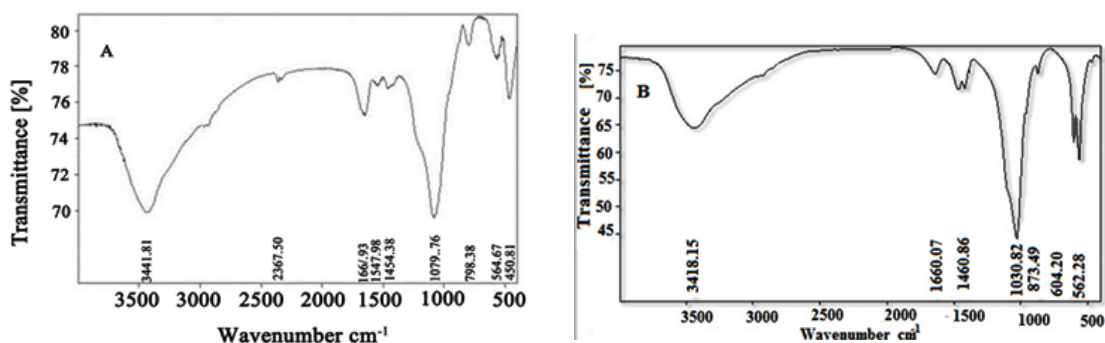


Fig. 1: FT-IR spectra for nanocomposites of silica doped in: A) HA/collagen, and B) HA/gelatin

Synthesis of nanocomposites of silica doped in HA/gelatin

All synthesis steps explained in Section 2.1 were repeated with gelatin instead of collagen.

Table 1: Important Infrared Peaks Assigned To The Synthesized Nanocomposites

Nanocomposites	The wavelength cm^{-1}
Crystal structure of HA	464, 605, 566
P-O Stretching	1036
O-H Stretching	3851
N-H stretching	3443
C=O vibration	1651

Table 2: A Comparison Between Particle Sizes of the two Nanocomposite Samples

Sample	Particle size
HA +Si+ gelatin	54.07 nm
HA +Si+ collagen	46.85 nm

Table 4: EDS Quantitative Results For Silica Nanoparticles Doped In HA/Gelatin

Elt	W%	A%
C	12.11	18.82
O	49.38	57.62
Na	7.69	6.69
Si	8.36	5.55
P	8.67	4.90
Ca	13.79	6.42
	100.0	100.0

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy (FT-IR)

Fig. 1 provides a comparison between FT-IR spectrum captured for synthesized nanocomposites of silica doped in HA/collagen (Fig. 1 A) and that of HA/gelatin (Fig. 1 B).

The peaks at of 562 cm^{-1} at 603 cm^{-1} specify crystal structure of HA, while the peak at 1617 cm^{-1} specifies N-H, C- H stretching in collagen. The peak within $3200\text{-}3500 \text{ cm}^{-1}$ attributes to the absorption of H_2O on HA, with the one at 1010 cm^{-1} representing P-O stretching. The peak at 875 cm^{-1} specifies C-O stretching related to CO_2 content of the air. The peak

Table 3: A Comparison Between Crystallite Sizes Of The Nanocomposite Samples

Samples	Crystallite size [\AA]	Crystallite size [nm]
(HA+ Gelatin +Si)	1073	10.73nm
(HA+ Collagen +Si)	419	4.19nm

Table 5: EDS Quantitative Results For Silica Nanoparticles Doped In HA/Collagen

Elt	W%	A%
C	10.36	16.68
O	48.24	58.27
Na	3.67	4.79
Si	8.70	5.99
P	10.85	5.54
Ca	18.13	8.74
	100.0	100.0

Table 6: A Comparison Between Ca/P Ratios of The Two Nanocomposite Samples

Samples	Ca/P ratio	Comments
HA + Si + gelatin	1.59	Close to the theoretical value
HA + Si + collagen	1.67	Equivalent to the theoretical value

at 1175 cm^{-1} (C-H, N-H) refers to stretching amine (II), confirming the presence of HA/collagen. Non-presence of peaks related to the vibration absorption of Si-H (2100 cm^{-1}) and Si-O bonds (1100 cm^{-1}) in the spectrums proves the silica sample to be doped.

Table 1 reports several important infrared peaks assigned to the synthesized nanocomposites, confirming the formation of both products.

Field emission scanning electron microscopy (FESEM)

Fig. 2 comes with FESEM spectra for the synthesized silica nanocomposites doped in HA/gelatin (A) and HA/collagen (B). Table 2 indicates particle sizes for synthesized silica nanoparticles doped in HA/gelatin (54.07 nm) and HA/collagen (46.85 nm).

X-ray diffraction (XRD)

Fig. 3 shows XRD patterns for silica nanoparticles doped in HA/collagen (A) and HA/gelatin (B). The XRD peaks confirm crystalline planes of HA crystal system²⁰. On the other hand, the presence of silica dope in nanocomposites cannot change the HA crystal system.

Table 3 shows crystallite sizes of the synthesized silica nanoparticles doped in HA/gelatin (10.73 nm) and HA/collagen (4.19 nm).

Energy-dispersive X-ray spectroscopy (EDS)

Elemental analysis were undertaken via energy-dispersive X-ray spectroscopy (EDS), so as to confirm nanocomposite preparation. Fig. 4 and Table 4 present the results obtained for the

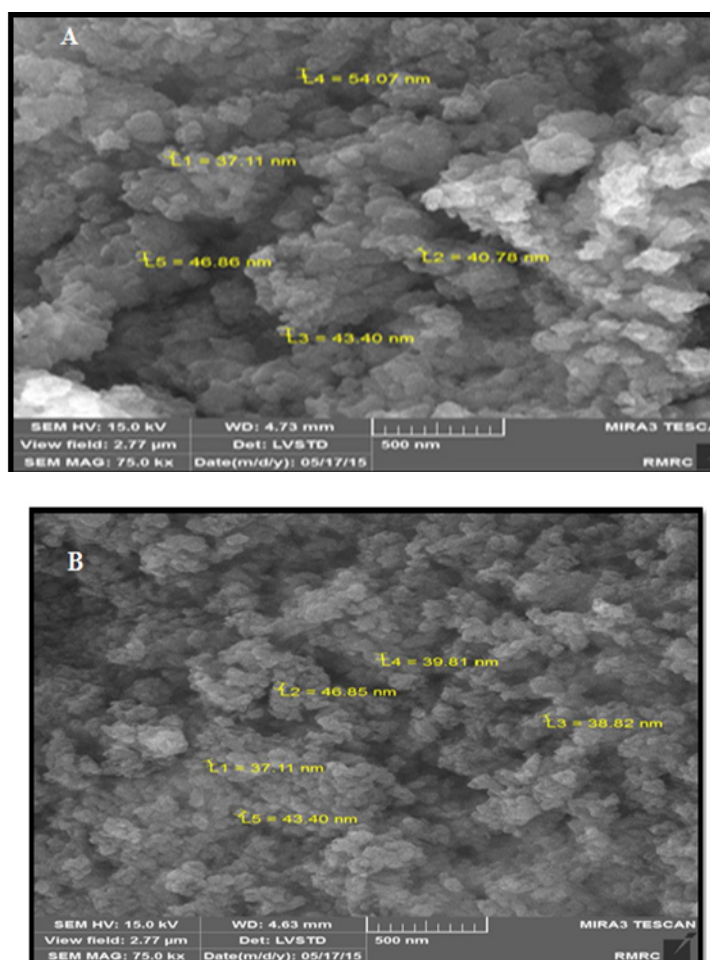


Fig. 2: FESEM images of silica nanoparticles doped in: A) HA/gelatin, and B) HA/collagen

synthesized silica nanocomposites doped in HA/gelatin, while Fig. 5 and Table 5 come with the corresponding results to silica nanoparticles doped in HA/collagen. The presence of silica in Figs. 4 and 5 and Tables 4 and 5 confirms the doping of silica

in synthesized nanocomposites. Continuing with the investigations, Ca/P ratios were calculated using the amounts of calcium and phosphorus. Accordingly, Ca/P ratios of 1.59 (close to theoretical value) and 1.67 (equivalent to the theoretical value) were

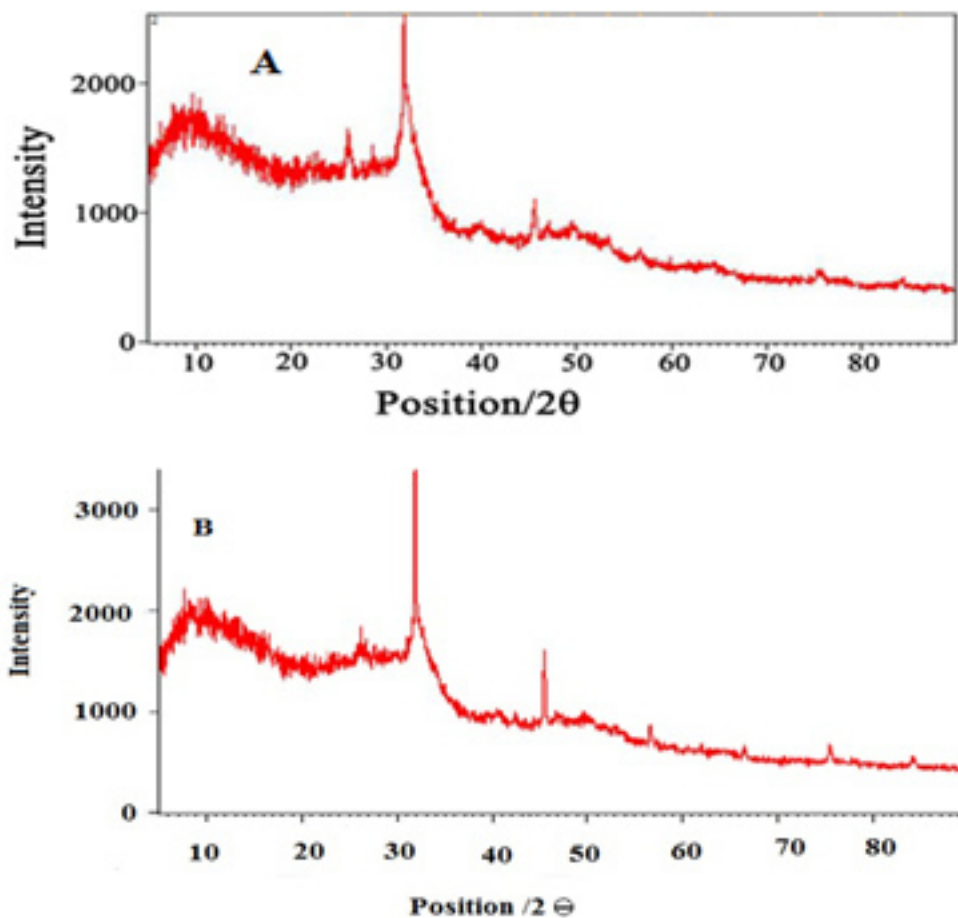


Fig. 3: XRD patterns of silica nanocomposites doped in: A) HA/collagen, and B) HA/gelatin

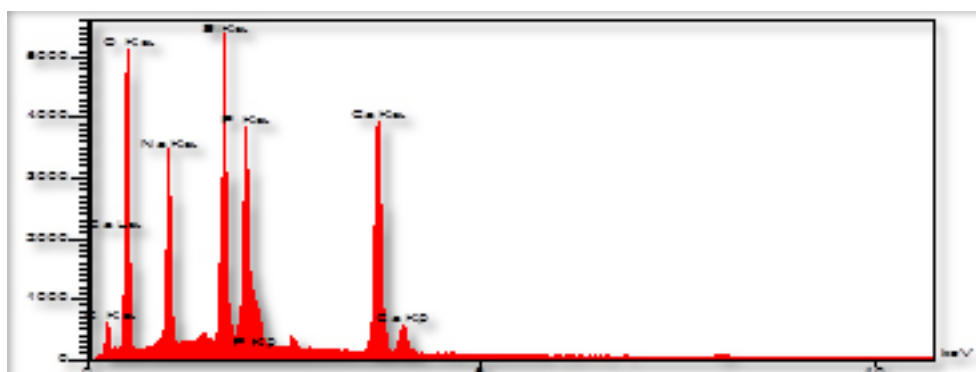


Fig. 4: EDS pattern for silica nanoparticles doped in HA/gelatin

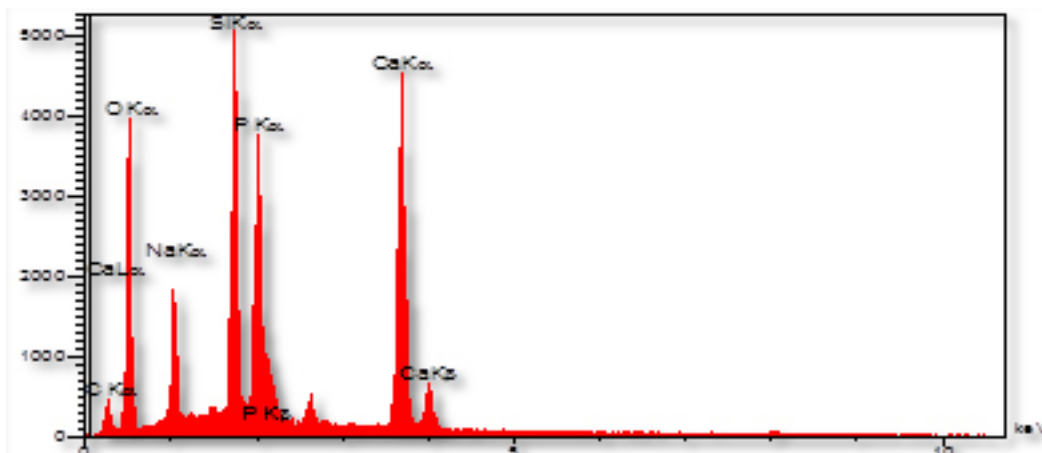


Fig. 5: EDS pattern for silica nanoparticles doped in HA/collagen

obtained for silica nanoparticles doped in HA/gelatin and in HA/collagen, respectively.

CONCLUSION

In this study, two hydrothermal HA nanocomposites were synthesized with their biocompatibilities investigated. According to the FTIR results, the synthesized HA nanocomposites had their chemical structures confirmed. Furthermore, based on the results of EDS method in terms of calcium-to-phosphorus ratios, the nanocomposites of silica doped in HA/collagen were observed to

exhibit a Ca/P ratio of equivalent to the theoretical value as repeated for HA chemical structure of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Moreover, the results of spectroscopic XRD and SEM showed that the silica nanocomposites doped in HA/collagen were composed of smaller crystallite and particles than those of the silica nanocomposites doped in HA/gelatin. As reducing crystallite size and particle size increase contact surface, the silica nanocomposites doped in HA/collagen are expected to exhibit higher levels of reactivity in chemical reactions, making them of better pharmacological efficacy when it comes to bone repair.

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