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Surface modification of Mg-doped fluoridated hydroxyapatite nanoparticles using bioactive amino acids as the coupling agent for biomedical applications

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Abstract

Hydroxyapatite (HA) has been extensively utilized in the field of biomaterials as a bioactive ceramic. Development of modified-HA by the substitution of Ca ions and OH⁻ groups not only makes its chemical composition similar to that of the natural bone tissue, but also improves the in vitro behavior of commercially synthesized HA. Accordingly, magnesium-fluoridated hydroxyapatite nanoparticles (Mg-FHA NPs) have been recently developed. However, due to the high surface energy of such NPs, they cannot be well dispersed in a biopolymer matrix to prepare a polymer/ceramic composite, which is usually demanded for tissue engineering applications. To overcome this shortcoming, the surface of Mg-FHA NPs was modified using a few well-known natural amino acids as the cost-effective and environment-friendly biomaterials in the present research. L-leucine, L-isoleucine, L-methionine, L-phenylalanine, L-tyrosine and L-valine amino acids were employed as the coupling agents and surface modification of Mg-FHA NPs was carried out by means of sonication technique. The results confirmed that using amino acid molecules led to the uniform dispersion of Mg-FHA NPs in the organic environment by making the surface of NPs hydrophobic, although the length and chemical reactivity of amino acid molecules affected the efficiency of NPs dispersion. The uniform distribution of Mg-FHA NPs could be regarded as a desired condition for polymer/ceramic composite preparation, with high applicability for biomedical purposes.

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1. Introduction

Hydroxyapatite (HA) bioactive ceramic with the chemical composition of Ca₁₀(PO₄)₆(OH)₂ has been extensively utilized for orthopedic, dental and maxillofacial applications due to its

similarity to the mineral phase of bone and tooth. Incorporation of fluorine into the apatite structure to form fluoridated hydroxyapatite [FHA:Ca₁₀(PO₄)₆OH_{2-x}F_x, where *x* represents the degree of fluoridation] improves the physical and biological properties of HA. At present, the HA partially substituted by fluorine has received considerable attention, particularly for clinical bone growth. Moreover, the presence of Mg ions instead of Ca ions in FHA structure (Mg-FHA) promotes its bioactivity and osteoconductivity characteristics. The Mg-FHA provides superior biocompatibility and biological properties compared to

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the unmodified FHA [1,2]. Besides, the biodegradability of the synthesized FA or FHA is controlled through the optimized substitution of Mg^{2+} into the FHA structure [3]. Recently, polymeric matrix composites have come into spotlight in bone tissue engineering applications owing to their superior biological and mechanical properties as compared to ceramics. In addition, the development of polymer/ceramic composites simulates the structure of natural bone since it is composed of HA in collagen matrix [1,4].

The bioactivity properties can be further improved using bioceramic nanoparticles (NPs) since the natural bone is also composed of nanostructured HA [1,5]. However, bioceramic NPs tend to be agglomerated in the polymeric matrix as a result of their high surface area and incompatible surface polarity with polymers. It is well-established that the surface compatibility of ceramics with the polymeric matrix significantly affects its biological properties such as protein adsorption and subsequent cellular attachment and proliferation on the structures [5,6].

In order to strengthen the interfacial bonding between the mentioned phases, understanding the surface and interfacial chemistry of nanobioceramic in a polymer matrix is very important. In this respect, the surface modifications of the NPs have been performed using the surface-active agent, the coupling agent, fatty acid and ethanol [5,7–11].

Amino acids are known as the cost-effective biomaterials with suitable biodegradability, biocompatibility, nontoxicity and eco-friendly properties, making them ideal candidates for tissue engineering applications. Moreover, the best solvent for amino acids is water as a nontoxic, natural, environmentally friendly and inexpensive solution [12–14].

Due to the presence of OH^- groups on the surface of Mg-FHA NPs, they considerably tend to be agglomerated in a polymeric matrix. In addition, these superficial OH^- groups cause the Mg-FHA NPs to be linked to amino acid molecules. As a result of the chemical bonding between superficial OH^- groups of Mg-FHA NPs and the amino acid molecules, the surface of Mg-FHA NPs is changed from a hydrophilic state to a hydrophobic one, leading to the uniform distribution of particles in an organic solution. Thus, the amino acids can be considered as appropriate choices for the surface modification of nanobioceramics, especially in a polymeric matrix [12–15].

In this study, the surface of Mg-FHA NPs was chemically modified by different natural bioactive amino acids to improve NPs dispersion in the polymeric matrix. The modified NPs were characterized by Fourier transmission infrared spectroscopy (FT-IR), dispersion stability, X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM) as well as thermogravimetry analysis (TGA).

2. Experimental method

2.1. Surface modification of Mg-FHA NPs

Mg-FHA NPs were prepared using the sol-gel technique according to a previous research by Biomaterials Research Group

lab [1]. Briefly, the starting materials were P_2O_5 (99.9%), $Mg(NO_3)_2 \cdot 6H_2O$ (99.9%) and $Ca(NO_3)_2 \cdot 4H_2O$ (99.9%). Appropriate amounts of the mentioned materials with the stoichiometric composition of $Ca_{9.5}Mg_{0.5}(PO_4)_6(OH)F$ were independently dissolved in absolute ethanol. These solutions were added drop wise to each other to obtain a solution with a (Ca, Mg)/P ratio of 1.67. The final mixture was continuously stirred for ~ 24 h at ambient temperature to form a gel. As-formed gel was aged for 24 h at ambient temperature and dried in an oven at $100^\circ C$ in air for another 24 h. The dried gel was sintered with a heating rate of $5^\circ C/min$ up to $650^\circ C$ for 1 h in a muffle furnace.

The natural amino acids, including L-leucine, L-isoleucine, L-methionine, L-phenylalanine, L-tyrosine and L-valine, were utilized without further purification. The aforementioned amino acids with the concentration of 10 wt% were dissolved in 20 mL of distilled water at room temperature. The prepared Mg-FHA NPs were dried at $120^\circ C$ for 24 h to remove the adsorbed water. Then, the Mg-FHA NPs (1.0 g) were added into the ethanol solution containing the amino acid, and stirred at room temperature for 24 h. The prepared mixture was placed into an ultrasonic bath for 30 min. Finally, the obtained suspension was filtered and washed by ethanol several times to eliminate unreacted amino acid molecules, and the remaining powder was subsequently dried at $60^\circ C$ for 24 h. The schematic diagram of the surface modification process for Mg-FHA NPs is presented in Fig. 1.

2.2. Characterization

The functional groups of the samples were identified by Fourier transform infrared spectroscopy (FT-IR: Bruker-Tensor 27, Jasco-680 spectrophotometer, Japan) in the range of $400\text{--}4000\text{ cm}^{-1}$. Dispersion stability of modified Mg-FHA NPs was determined by sedimentation experiments in two various mediums, including water and chloroform, according to the previous report [5]. Briefly, the NPs with the concentration of 1 g/L were ultrasonically dispersed in the mediums. The dispersion stability was estimated by measuring the time required for the sedimentation of all NPs wherein the solution became completely transparent.

The phase composition of the samples was analyzed by X-ray diffraction patterns (XRD, Philips XPert) with the voltage of 40 kV, and Cu $K\alpha$ radiation ($\lambda=0.15406\text{ nm}$). The crystallite size of the unmodified and modified Mg-FA NPs was calculated using the Debye-Scherrer equation (Eq. 1) [16]:

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where λ (nm) is the wavelength of X-ray used (0.15406 nm), β (rad) is the full width at half maximum (FWHM) of diffraction bands, θ (degree) is Bragg's angle and D is the average crystallite size. The XRD patterns were recorded in 2θ range of $20\text{--}60^\circ$ (step size of 0.02° and time per step of 1 s). The morphology and size of NPs were examined using transmission electron microscopy (TEM: EM 109, ZEISS, Germany, with the accelerating voltage of 100 kV) and field

Fig. 1. The schematic of the modification process by using amino acids.

emission scanning electron microscopy (FE-SEM: Hitachi, S-4160). The FE-SEM and TEM images were analyzed using image analysis software (NIH Image J) in order to determine the size of prepared NPs. The thermal gravimetric analysis (TGA: Rheometric scientific 1998, USA) was used to determine the weight loss of the samples from room temperature to 800 °C by the heating rate of 10 °C min⁻¹.

3. Results and discussion

In order to improve the dispersion of Mg-FHA NPs and enhance their compatibility with the polymer matrix, the NPs were surface modified using natural amino acids. To confirm that a chemical reaction has occurred between the surface of the Mg-FHA NPs and the amino acids and also, to identify the resultant functional groups, FT-IR analysis was applied before and after surface modification. The FT-IR spectra corresponding to the initial and the surface modified Mg-FHA NPs are represented in Fig. 2. A major band related to the phosphate group was noticed over the range of 1000–1100 cm⁻¹. In fact, it has been observed that the PO₄⁻³ absorbance appears within the range of 950–1100 cm⁻¹ (symmetric P–O stretching vibration) and 550–620 cm⁻¹ (the vibrational mode of phosphate group) [1,17]. The bands observed at 850 cm⁻¹ and 1550–1450 cm⁻¹ indicated the existence of CO₃⁻² groups. The mentioned bands have been reported to be associated with biological HA and the broader bands at 3500 cm⁻¹ can be attributed to the adsorbed water [18]. Moreover, several weak bands related to –OH and sp³ CH stretching were detected around 3400 cm⁻¹ and 2853–2983 cm⁻¹, respectively [14]. Two strong bands around 1400 and 1570 cm⁻¹ revealed the

Fig. 2. The FT-IR spectra of (a) pure Mg-FA NPs, Mg-FA NPs modified by (b) isoleucine, (c) valine, (d) tyrosine, (e) phenylalanine, (f) methionine and (g) leucine.

symmetric and asymmetric stretching vibration for deprotonated carboxylate group of amino acids. Due to the change in the bond lengths and angles, a minor shift appeared in the carboxylate group; this is because the cation chelation is dependent on its type [19]. Therefore, the symmetric and asymmetric stretching bands of the carboxylate group were observed at 1406 cm⁻¹ and 1449 cm⁻¹, respectively.

Compared to the unmodified Mg-FHA NPs, two new broad absorption bands around 1520 and 1601 cm^{-1} could be found in the FT-IR spectra of amino acids-modified Mg-FHA NPs that could be attributed to the primary amines. Absorption at 3400–3500 cm^{-1} proved the presence of N–H groups on amino acids modified Mg-FHA NPs. Regarding the chelation of amino acids, the FT-IR analysis exhibited carboxylate (COO^-) and amine (NH) groups in the amino acids-modified Mg-FHA NPs spectra. It is worth noting that free amino acids existing as zwitterions (NH_3^+ and COO^-) showed different FT-IR spectra compared to those of modified Mg-FHA NPs; this stems from the fact that the amino acids present in modified Mg-FHA NPs are not in the form of zwitterions [20].

The carboxylate group of the amino acids-modified Mg-FHA NPs was deprotonated and bound to NPs through the neutral COO^- group. The occurrence of a chemical reaction between the COO^- groups of amino acids and the surface of Mg-FHA NPs led to an upward shift in $\nu_{\text{C=O}}$ compared to the same group in the free amino acids. As for the modified Mg-FHA NPs of the FT-IR spectra, the characteristic bands were observed in the region between 1406 cm^{-1} and 1449 cm^{-1} , which were less than those for the carboxylate group (1550–1610 cm^{-1}). Hence, it can be concluded that the oxygen of the carboxylate group was involved in cation coordination. Meanwhile, no shift was observed in the vibrational mode of amino groups, thereby signifying that the amino groups were not involved in cation coordination. A low intensity band observed in the FT-IR spectra at $\sim 436 \text{ cm}^{-1}$ was assigned to $\nu_{\text{M-O}}$ stretching vibration [16,21]. The FT-IR spectra bands revealed that a chemical reaction had occurred in the acidic position of the amino acid and Mg-FHA NPs, thereby causing the chemical bonding of amino acid molecules to the surface of NPs. Consequently, the surface of Mg-FHA NPs was efficiently modified by amino acids. According to FT-IR observations, the proposed interactions between amino acids-modified Mg-FHA NPs are illustrated in Fig. 3.

To evaluate the effect of surface modification, the dispersibility of modified Mg-FHA NPs in chloroform was compared to that of unmodified Mg-FHA NPs, as shown in Fig. 4.

Table 1 demonstrates the behavior of the unmodified and modified Mg-FHA NPs in polar (water) and weakly polar (chloroform) solvents. The results indicated that the unmodified Mg-FHA NPs entirely precipitated after 0.5 and 1 h in chloroform and water, respectively. It is known that the FHA NPs tend to be agglomerated in organic solutions due to the inter-particle hydrogen bonding as well as van der Waals interaction [22]. By surface modification, the sedimentation time for NPs was dramatically changed with respect to the unmodified NPs. The sedimentation time of all samples in water was longer than that of modified NPs in chloroform solvent. It can be assumed that the modified NPs represent different behaviors in different solvents according to the principle of similar compatibility [6]. Based on these results, it can be proposed that the modified NPs are appropriate for the physiological environments as they can be well-dispersed in both polar and weakly polar solvents [6,22].

The surface modification process can increase the compatibility between the unmodified Mg-FHA NPs and the organic solvents. After the modification of the NPs surface, the sedimentation time was dramatically increased in comparison with unmodified NPs. According to Fig. 4, the sedimentation time for the modified Mg-FHA NPs was increased by increasing the molecule length of amino acids or chemical reactivity between the amino acids and NPs surface. In the case of phenylalanine and tyrosine-modified Mg-FHA NPs, higher stability was achieved as compared to other modifiers. This arose from the enhanced stability of phenylalanine and tyrosine-modified Mg-FHA NPs in the chloroform solution due to the higher grafting of NPs. Phenylalanine and tyrosine possess a large group preventing NPs agglomeration. Furthermore, the phenyl group in phenylalanine and tyrosine can behave in an improved manner with the organic solvent. Consequently, the phenylalanine-modified Mg-FHA NPs can be efficiently dispersed in the chloroform solvent. This also shows that the grafted amino acids on the surface of Mg-FHA NPs would yield to the steric repulsive forces among particles preventing their aggregation in the organic solvents. In addition, the hydrophilicity of the NPs surface could be changed to extremely high

Fig. 3. The suggested interactions of Mg-FA NPs surface modified with amino acids.

hydrophobicity. Thus, it is expected that utilizing a polymeric solution containing the modified Mg-FHA NPs would produce a nanocomposite with homogeneous distribution; this results in the larger specific surface area of bioceramic NPs reinforcements exposed to the biofluids and facilitates the protein adsorption and cell attachment [5].

The average crystallite size of the Mg-FHANPs, as calculated by the Scherrer formula (Eq. 1), was found to be ~ 32 nm, which was also confirmed by TEM observations. The characteristic bands of Mg-FHA NPs, as shown in Fig. 5, were related to the standard card of fluorapatite (JCPDS, No. 15-0876). There were no band corresponding to the impurities and the modification process did not affect the crystalline phase of Mg-FHANPs. The reason is that the characteristic bands remained practically identical for all the samples. On the other hand, the characteristic bands of modified Mg-FHA NPs

were gradually broadened compared to that of unmodified ones owing to the appearance of the amino acid as an organic molecule [14].

The morphology of the modified Mg-FHA NPs was studied by FE-SEM and TEM observations. Fig. 6 shows the FE-SEM images of unmodified and modified Mg-FHA NPs with different amino acids. The unmodified Mg-FHA NPs had an agglomerated morphology with inhomogeneous shapes while the amino acids-modified Mg-FHA NPs were properly dispersed in the solution. As illustrated in Fig. 6, the modified Mg-FHA NPs displayed a spherical morphology configuration with a uniform distribution [5,14,23]. The amino acids-modified Mg-FHA NPs exhibited a smaller and uniform particle as compared to the unmodified Mg-FHA NPs. In fact, as a result of surface modification, the surface energy of NPs was decreased, leading to the lower extent of agglomeration. The FE-SEM images of the phenylalanine and the isoleucine-modified Mg-FHA NPs demonstrated that there was less agglomeration in NPs and they presented more uniform particles in comparison with other modifiers, probably due to the large size of these organic molecules. Therefore, they made a long steric hindrance around the Mg-FHA NPs, preventing their agglomeration to a large extent.

However, due to the presence of similar functional groups on the surface of modified NPs, they tend to be superficially agglomerated. The modified NPs are connected to each other by hydrogen or van der Waals bonds. Therefore, the surface modification of NPs by amino acids can increase agglomeration of nanoparticles in solid state. But, they can easily

Fig. 4. The dispersibility of modified Mg-FA NPs in chloroform was compared to that of pure Mg-FA NPs.

Fig. 5. The XRD patterns of (a) pure Mg-FA NPs, Mg-FA NPs modified by (b) isoleucine, (c) valine, (d) tyrosine, (e) phenylalanine, (f) methionine and (g) leucine.

Table 1
Effect of hydrolytic conditions on dispersion of nanoparticles.

Solvents	Sedimentation time						
	Pure NPs	Ile- NPs	Val-NPs	Tyr-NPs	Phe-NPs	Met-NPs	Leu-NPs
Water	2 h	18 h	16 h	1 h	24 h	1 h	24 h
Chloroform	30 min	3 h	2 h	3 h	3 h	1 h	2.5 h

Fig. 6. The FE-SEM images of (a) pure Mg-FA NPs, Mg-FA NPs modified by (b) isoleucine, (c) valine, (d) tyrosine, (e) phenylalanine, (f) methionine and (g) leucine.

disperse in an organic solvent or a polymeric matrix. It can be overcome by dispersion into organic solvents such as alcohol and chloroform (as can be seen in Fig. 4) [12–15].

As illustrated in Fig. 4, they can be dispersed into organic solvents such as alcohol and chloroform. To prepare samples for TEM observations, the amino acids- modified Mg-FHA NPs were dispersed in alcohol. Fig. 7 shows the TEM images of the unmodified (a) and the isoleucine-modified Mg-FHA NPs (b). According to Fig. 7a, the particle size of Mg-FHA NPs was in the range of 25–35 nm, in agreement with the calculated crystallite size by XRD analysis using the Debby–Scherrer equation (Eq. 1). Due to the large specific surface area and high surface energy, Mg-FHA NPs tended to be extremely agglomerated as shown in Fig. 7a [14,15,24]. Fig. 7b shows the TEM image of the surface modified-Mg-FHA NPs. It illustrates that dispersion was improved as pointed by the presence of individual particles on the TEM image. The

particles showed a spherical-shape structure without signs of aggregation. The particle size of modified Mg-FHA NPs was 15 ± 2 nm. Moreover, the existence of enclosed shadows around the modified NPs suggested the presence of the grafted organic layer.

Fig. 8 represents the TGA curves of the unmodified and modified Mg-FHA NPs, where the temperature range was adjusted between 25 °C and 800 °C. As can be seen in Fig. 8a, it was found that about 3.38% of the total mass of the unmodified Mg-FHA NPs was lost mainly from the evaporation of the adsorbed water on the surface of NPs. However, the modified Mg-FHA NPs revealed an increase in the mass loss. The mass loss for the samples modified by isoleucine, tyrosine, phenylalanine, leucine, methionine and valine were 6.07, 4.42, 3.56, 3.44, 1.1 and 1.06 mass%, respectively. The TGA mass loss of modified Mg-FHA NPs was increased because of the presence of the absorbed amino acid molecules on the surface

Fig. 7. The TEM images of (a) pure and (b) isoleucine-modified Mg-FA NPs.

Fig. 8. TGA thermograms of (a) pure Mg-FA NPs, Mg-FA NPs modified by (b) isoleucine, (c) tyrosine, (d) phenylalanine, (e) leucine, (f) methionine and (g) valine.

of NPs. The results showed that isoleucine molecules could be more effectively adsorbed on the surface of NPs, after which tyrosine had also an appropriate chemical reaction with the surface of NPs. Accordingly, the trend of the molar mass loss may be ordered as isoleucine, leucine, tyrosine, phenylalanine, valine and methionine. Based on these results, the findings of sedimentation test can be understandable. It should also be noted that the tyrosine and phenylalanine molecules possessed an appropriate steric hindrance as well as a suitable link with the hydroxyl group on the surface of NPs.

After modification by different amino acids, the Mg-FHANPs were changed from a hydrophilic state to a hydrophobic one [12–15]. The Mg-FHANPs covered by the simple amino acids could be well-dispersed in the non-polar and weak polar organic solvents such as chloroform and acetone as well

as polymer matrices. During the preparation process, condensation took place to form C–O bonds between the coupling agent amino acid and the surface of the Mg-FHA NPs. To obtain a homogeneous distribution of the Mg-FHA NPs in polymer matrix, the surface of the Mg-FHA NPs had to be characteristically hydrophobic to achieve close affinity to the organic matrix. Consequently, the Mg-FHA NPs were treated by simple amino acids as the coupling agent to functionalize their surface [14,15].

During the process, the amino acid reacted with the surface of Mg-FHA NPs. Each OH group of amino acid molecule, as a coupling agent, could react with hydroxyl groups on the surface of the Mg-FHA NPs. Therefore, the coupling agent molecules were grafted on the Mg-FHA NPs surface. Furthermore, the organic chains of amino acid could reach steric hindrance between inorganic NPs to reduce the agglomeration of Mg-FHA NPs.

4. Conclusions

The surface modification of Mg-FHA NPs was successfully carried out using an easy, rapid and environmentally friendly technique. To this end, different types of known natural amino acids were employed through the sonication method in the aqueous media. Based on the results, a chemical reaction took place between superficial hydroxyl groups of the Mg-FHA NPs and the amino acids molecules, causing the formation of amino acids as coupling agents on the surface of NPs. The amino acids were linked with the hydroxyl groups of the Mg-FHA NPs surface through the hydrogen bonding. Therefore, the dispersion of modified hydrophobic Mg-FHA NPs was significantly improved as compared to the unmodified ones in the organic solvents. The results proved that the surface modification with amino acids could be an appropriate

technique to obtain the homogeneous dispersion of NPs. It should be noted that the length of amino acids or their chemical reactivity with the surface is a key factor affecting the dispersion of NPs in the organic environments. According to the results, improvement of dispersion and a higher stability in the weakly polar solvent were achieved for the Mg-FHA NPs modified by phenylalanine and tyrosine with respect to other modifiers. Isoleucine molecules could also be adsorbed more effectively on the surface of Mg-FHA NPs. Because of the appropriate compatibility of the modified Mg-FHA NPs with the polymeric solution, it was expected that the polymeric solution containing the modified Mg-FHA NPs would yield to the formation of a uniform nanocomposite in which the bioceramic NPs with homogeneous distribution and larger specific surface area could be exposed to the biofluids to promote the protein adsorption, cell attachment and other bio-applications for tissue engineering.

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References

- [1] M. Kheradmandfard, M.H. Fathi, Preparation and characterization of Mg-doped fluorapatite nanopowders by sol–gel method, *J. Alloy. Compd.* 504 (2010) 141–145.
- [2] F. Mohandes, M. Salavati-Niasari, Z. Fereshteh, M.H. Fathi, Novel preparation of hydroxyapatite nanoparticles and nanorods with the aid of complexing agents, *Ceram. Int.* 40 (2014) 12227–12233.
- [3] I. Cacciotti, A. Bianco, M. Lombardi, L. Montanaro, Mg-substituted hydroxyapatite nanopowders: synthesis, thermal stability and sintering behaviour, *J. Eur. Ceram. Soc.* 29 (2009) 2969–2978.
- [4] M. Razavi, M.H. Fathi, O. Savabi, S.M. Razavi, F. Heidari, M. Manshaei, D. Vashaei, L. Tayebi, In vivo study of nanostructured diopside ($\text{CaMgSi}_2\text{O}_6$) coating on magnesium alloy as biodegradable orthopedic implants, *Appl. Surf. Sci.* 313 (2014) 60–66.
- [5] M. Kharaziha, M.H. Fathi, H. Edris, Effects of surface modification on the mechanical and structural properties of nanofibrous poly(ϵ -caprolactone)/forsterite scaffold for tissue engineering applications, *Mater. Sci. Eng. C Mater. Biol. Appl.* 33 (2013) 4512–4519.
- [6] M. Zhang, L. Ye, Y. Gao, X. Lv, J. Chang, Effects of hydrolysis on dodecyl alcohol modified β - CaSiO_3 particles and PDLLA/modified β - CaSiO_3 composite films, *Compos. Sci. Technol.* 69 (2009) 2547–2553.
- [7] Z. Hong, P. Zhang, C. He, X. Qiu, A. Liu, L. Chen, X. Chen, X. Jing, Nano-composite of poly(L-lactide) and surface grafted hydroxyapatite: mechanical properties and biocompatibility, *Biomaterials* 26 (2005) 6296–6304.
- [8] O. Wilsonjr, J. Hull, Surface modification of nanophase hydroxyapatite with chitosan, *Mater. Sci. Eng. C* 28 (2008) 434–437.
- [9] L. Borum-Nicholas, O.C. Wilson, Surface modification of hydroxyapatite. Part I. Dodecyl alcohol, *Biomaterials* 24 (2003) 3671–3679.
- [10] X.J. Wang, Y. Li, J. Wei, K. de Groot, Development of biomimetic nano-hydroxyapatite/poly (hexamethyleneadipamide) composites, *Biomaterials* 23 (2002) 4787–4791.
- [11] L. Borum-Nicholas, O.C. Wilson, Surface modification of hydroxyapatite. Part II. Silica, *Biomaterials* 24 (2003) 3681–3688.
- [12] S. Mallakpour, M. Dinari, V. Behranvand, Ultrasonic-assisted synthesis and characterization of layered double hydroxides intercalated with bioactive N,N'-(pyromellitoyl)-bis-l- α -amino acids, *RSC Adv.* 3 (2013) 23303–23308.
- [13] S. Mallakpour, M. Dinari, Insertion of novel optically active poly(amide-imide) chains containing pyromellitoyl-bis-l-phenylalanine linkages into the nanolayered silicates modified withl-tyrosine through solution intercalation, *Polymer* 52 (2011) 2514–2523.
- [14] S. Mallakpour, R. Aalizadeh, A simple and convenient method for the surface coating of TiO_2 nanoparticles with bioactive chiral diacids containing different amino acids as the coupling agent, *Prog. Org. Coat.* 76 (2013) 648–653.
- [15] S. Mallakpour, E. Nikkhoo, Production and characterization of nanocomposites based on poly(amide–imide) containing 4,4'-methylenebis(3-chloro-2,6-diethylaniline) using nano- TiO_2 surface-coupled by 3-aminopropyltriethoxysilane, *Prog. Org. Coat.* 76 (2013) 231–237.
- [16] M. Salavati-niasari, F. Davar, Z. Fereshteh, Synthesis of nickel and nickel oxide nanoparticles via heat-treatment of simple octanoate precursor, *J. Alloy. Compd.* 494 (2010) 410–414.
- [17] M.H. Fathi, A. Hanifi, V. Mortazavi, Preparation and bioactivity evaluation of bone-like hydroxyapatite nanopowder, *J. Mater. Process. Technol.* 202 (2008) 536–542.
- [18] M.H. Fathi, E.M. Zahrani, Fabrication and characterization of fluoridated hydroxyapatite nanopowders via mechanical alloying, *J. Alloy. Compd.* 475 (2009) 408–414.
- [19] A. Barth, The infrared absorption of amino acid side chains, *Prog. Biophys. Mol. Biol.* 74 (2000) 141–173.
- [20] P.R. Reddy, M. Radhika, P. Manjula, Synthesis and characterization of mixed ligand complexes of Zn (II) and Co (II) with amino acids : relevance to zinc binding sites in zinc fingers, *J. Chem. Sci.* 117 (2005) 239–246.
- [21] Z. Fereshteh, M. Salavati-niasari, K. Saberyan, S.M. Hosseinpour-mashkani, F. Tavakoli, Synthesis of nickel oxide nanoparticles from thermal decomposition of a new precursor, *J. Clust. Sci.* 23 (2012) 577–583.
- [22] H.J. Lee, S.E. Kim, H.W. Choi, C.W. Kim, K.J. Kim, S.C. Lee, The effect of surface-modified nano-hydroxyapatite on biocompatibility of poly(ϵ -caprolactone)/hydroxyapatite nanocomposites, *Eur. Polym. J.* 43 (2007) 1602–1608.
- [23] H. Tanaka, M. Futaoka, R. Hino, K. Kandori, T. Ishikawa, Structure of synthetic calcium hydroxyapatite particles modified with pyrophosphoric acid, *J. Colloid Interface Sci.* 283 (2005) 609–612.
- [24] R.Y. Hong, J.H. Li, L.L. Chen, D.Q. Liu, H.Z. Li, Y. Zheng, J. Ding, Synthesis, surface modification and photocatalytic property of ZnO nanoparticles, *Powder Technol.* 189 (2009) 426–432.