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Convenient approach of nanohydroxyapatite polymeric matrix composites

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ABSTRACT

Polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) were used to prepare polymeric matrix composites of nanohydroxyapatite (HAV and HAP), respectively, by using a biomimetic approach under 100 °C. A comparison with synthetic nanohydroxyapatite prepared by precipitation (SHA) was evaluated. Characterization of the prepared powders by differential thermal analysis (DTA) and thermogravimetric analysis (TGA) was achieved, X-ray diffraction (XRD), scanning electron microscope (SEM) with elemental analysis by (EDS) and inductively coupled plasma-optical emission spectrometer were used. DTA and TG showed the weight loss in the 400–1200 °C was almost attributed to carbonate decomposition which appeared apparently in the polymer associated samples due to the decomposition of polymer matrix. X-ray diffraction confirmed mainly the formation of hydroxyapatite. Specific surface area (BET) and scanning electron microscope and scanning electron microscopy (SEM) confirmed the range of nanosized of the prepared HA samples. The polymer matrix prefers orientation of the particles to rod-like shape. Elemental analysis showed mainly, Ca, P, C and O ions besides Na and Cl. Inductively coupled plasma showed the composition and the Ca/P atomic ratio of all samples ranging between 1.72 and 1.85.

1. Introduction

Bioceramic is a branch of ceramic that supplies various kinds of biomaterials to fit in the field of dental and orthopaedic biomedical applications. The use of bone-substituted materials is imperative to repair and reconstruct diseased or damaged parts in hard tissue such as bone, hips, knees and teeth. The most common materials used in this field are hydroxyapatite. It is considered as ideal biomaterials due to its biocompatibility, bioactivity and chemical reactivity particularly for applications in the bony sites without high mechanical solicitations [1].

Hydroxyapatite (HA) has similar composition and crystalline structure to apatite in human skeletal system, and therefore, it is suitable for bone substitution and reconstruction [1]. Literature survey discusses the use of conventional HA in the form of powder, dense or porous blocks at the macroscale level [2,3]. Recently, nanohydroxyapatite attracts researchers as it plays a significant role in various biomedical applications due to its unique functional properties of high surface area to volume ratio and its ultra fine structure similar to that of the biological bone [4,5]. Extensive efforts have been made to produce synthetic nano-HA materials. Methods that have been used for preparing nano-HA materials included chemical precipitation [6], in some cases followed by spray drying [7], sol–gel approach [8], microemulsion techniques [9], precipitation from complex solutions followed by microwave heating [10], wet chemical methods incorporating a freeze-drying step [11] and mechanochemical synthesis [12]. In our previous work, nano-oxides and hydroxyapatite prepared through the urea-formaldehyde polymeric route [13–15]. This method revealed the possible substitution of the cations from aqueous solution in the structure of the resin formed to produce nanosized and homogenized product after heat treatment.

In the natural bone and teeth, the nanometer-sized HA crystal is embedded in the collagen matrix with an average length of 50 nm, width of 25 nm, and a thickness of only 2–5 nm [16]. In fact, collagen acts as a template in the controlled biomineralization process [17]. However, the poor mechanical properties of the synthesized HA, such as high elastic modulus and low fracture toughness restrict its applications. Thus, HA is used in composite form (HA–polymer). It has found application as sorbent for numerous heavy metals and radionuclides [18–20]. In addition, it can be used to retain useful bioactive properties as well as enhancement in mechanical properties. Meanwhile, the polymer matrix acts as a template that directs crystallization of calcium phosphates. This effect has a great relevance to the understanding of the biomineralization process [21,22]. Where, in the biomineralization process of bone and teeth, the nucleation and growth of inorganic crystals occur in the presence of biological macromolecules. It can interact

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with inorganic crystals by electrostatic and hydrogen bond effects. These effects play an important role in the morphology and grain size of the inorganic crystal. Besides, HA is a more stable phase, i.e. incredibly less resorbable as compared to other bioceramics such as tri-calcium phosphate [23]. The resorbability of HA can slightly be improved with some ceramic oxides and ionic doping agents or by adding polymer to form composite [24,25]. Therefore, synthetic composites have been used in the replacement of the natural bone through the essence of biomimicking approach. Thus, the molecular control over the morphology, size, and anisotropy of the inorganic crystals and their uniform distribution over the organic phase are some of the main goals aimed at in the biomimicking process. Polymers containing polar functional groups such as COOH, CH, CH_2, PO_4H_2, and OH have been found to be useful in this regard. These ionizable side groups provide a greater affinity to positive calcium ions and the nucleation of hydroxyapatite crystals in the solution [2,5].

Keeping the above points in view, we have chosen two types of polymer viz., polyvinyl alcohols (PVA) and polyvinylpyrrolidone (PVP) in this study. Polyvinyl alcohol is a low cost water-soluble synthetic polymer with CH, CH_2 and OH as side group. It is non-toxic, has high tensile strength and flexibility. Polyvinylpyrrolidone is soluble in water and other polar solvents. It is initially used as a blood plasma substitute and later in a wide variety of applications in medicine, pharmacy, cosmetics and industrial production. The imide group (N–C=O) in PVP exists in the collagen molecular structure too. But, the influence of the functional group in collagen on inorganic crystal is difficult to study due to the complicated structure of protein. This leads to the use of PVP as a replacer [26]. They were employed to prepare a composite using a biomimetic approach with mild solution conditions (less than 100 °C) for the synthesis of HA nanopowder. Obtained results were then compared to pure nano-HA elaborated by wet chemical precipitation. We reported here their effect on the thermal behavior, phase formed, size, morphology and the obtained phosphocalcic ratio. The surface-regulating polymers PVA and PVP are used as capping agent to regulate the nucleation and crystal growth of HA crystal.

2. Experimental

2.1. Materials

In situ preparation of hydroxyapatite nanoparticles was carried out in the presence of two different types of polymers (PVA and PVP) named HAV and HAP, respectively, as the flow chart described in Fig. 1. For comparison HA nanoparticles were prepared by wet chemical precipitation method in the absence of polymer named SHA [27].

In order to study the effect of types of polymer on the preparation of nanohydroxyapatite, polyvinyl alcohol (PVA) (Loba Chemicals, India) and polyvinylpyrrolidone (Winlab, UK) were chosen. The first step in the preparation of HA nanoparticles was the preparation of a 1.388 M calcium chloride dihydrate (AR) (Sisco Research Laboratories, India) solution and a 0.833 M solution of di-ammonium hydrogen phosphate (LR) (Arabian Medical & Scientific Lab. Sup. Co., UAE). The two solutions prepared separately.

The calcium chloride dihydrate stock solution was slowly added to the polymer solution with a continuous stirring and heating for approximately 10 min. The pH of the solution was adjusted to 11 by sodium hydroxide. After stirring, di-ammonium hydrogen phosphate solution was added gradually to the above mixture. A milky white coloration was observed almost instantaneously after the addition of phosphate solution. The pH of the solution was adjusted to 11 again. The temperature was risen to 90 °C with constant stirring for 2 h. High values of pH in the solution have been reported to promote HA nucleation through the following reaction [14]:

$$5Ca^{2+} + 3PO_4{}^{3-} + OH^- = Ca_5(PO_4)_3OH$$

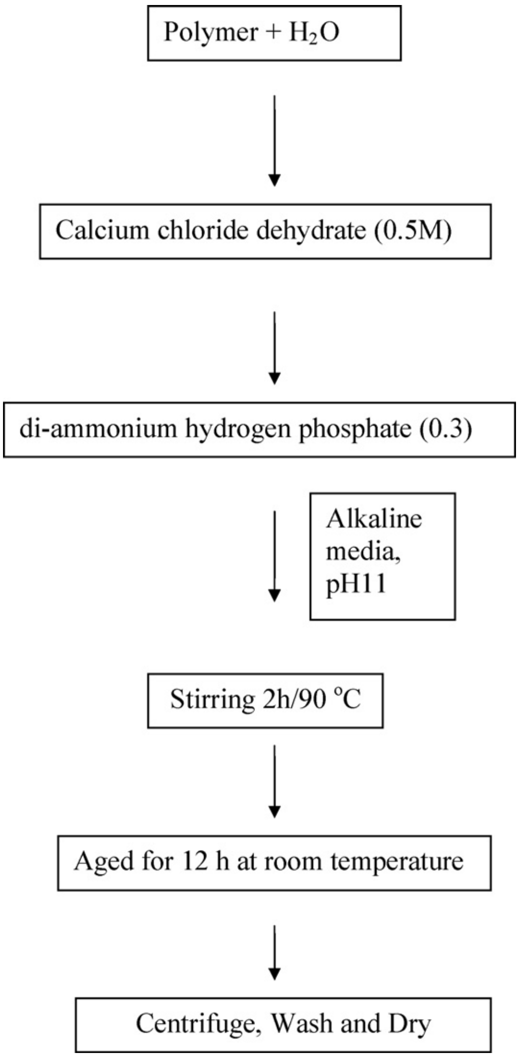

Fig. 1. In situ synthesis of nanohydroxyapatite in polymeric matrix.

The solutions obtained in this manner (with polymer) (HAV and HAP) and that without polymer (SHA) were centrifuged at 4500 rpm for 30 min and washed with distilled water then dried in an oven at 60 °C over night.

2.2. Characterization

The thermal behavior of the as-dried prepared powder samples (SHA, HAV and HAP) was characterized by using differential thermal analyzer Labsys 1600. Phase composition of the powders was determined by the X-ray diffraction method in the range of 2θ from 20 to 90 using (Inel Diffractometer) with Cu Kα radiation. Then the morphology of the powders was studied using scanning electron microscope (SEM) (JEOL JSM 6301f). Surface area measurements (BET) were determined by using NOVA 2000 USA and the crystallite size was calculated. Inductively coupled plasma-optical emission spectrometer (ICP-OES), type CIROS Vision was used to determine the ionic concentration of the prepared powders and the calcium to phosphorous molar ratio. Elemental analysis (EDS) (Oxford Link INCA) was also used in the goal to characterize the ions present too.

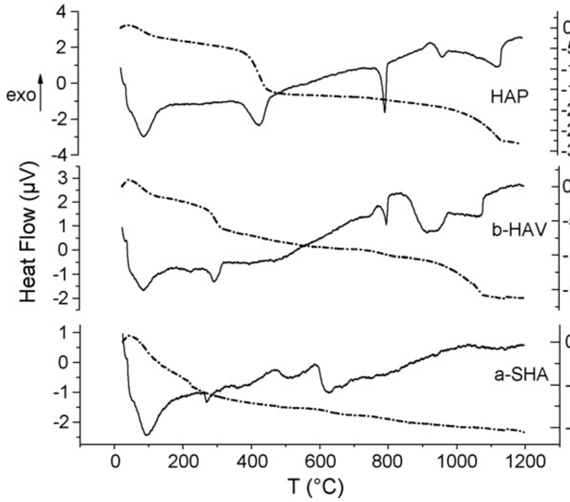

Fig. 2. Thermal behavior of DTA and TG of prepared nanohydrxyapatite samples (a) without polymer (SHA), (b) with PVA (HAV), (c) with PVP (HAP).

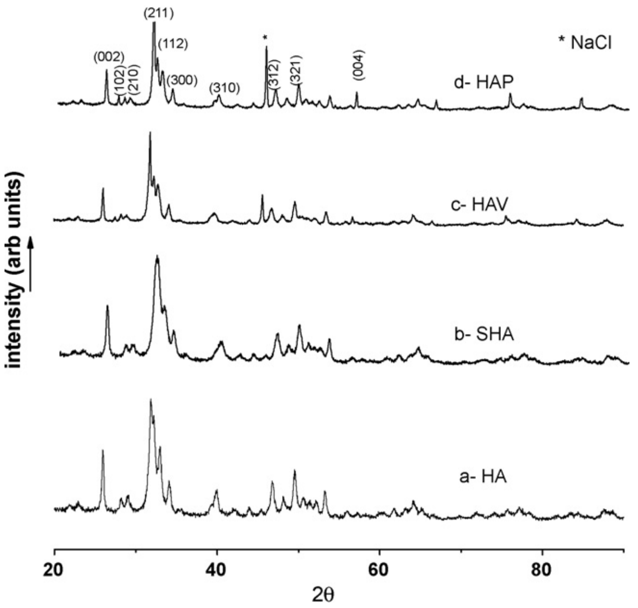

Fig. 3. XRD patterns of nanohydroxyapatite powder samples (a) without polymer (SHA), (b) with PVA (HAV) and (c) with PVP (HAP).

3. Results and discussion

In situ biomimetic process in the presence of polymer that acts as template for inorganic crystals is a promising technique to enhance nucleation and growth [28]. The present investigation describes a simple process for the production of bioresorbable composite bone biomaterial based on nano-HA in conjugation with PVA (HAV) or PVP (HAP) at low temperature. The obtained results compared with nano-HA without polymer prepared by wet chemical precipitation (SHA).

Fig. 2 corresponds to the thermogravimetric analysis (TGA) and differential thermal analysis (DTA) of the prepared samples SHA, HAV and HAP. The total weight loss was apparently increased in the order of SHA, HAV and HAP from 10%, 18% and 28%, respectively after heating up to 1200 °C. This can be attributed to the effect of polymer. The first stage of the weight loss in all the tested samples up to 100 °C with significant broad peak associated in DTA appeared corresponding to the removal of adsorbed water humidity. A second weight loss accompanied by an endothermic peak of DTA appeared from 200 to 500 °C corresponding to the loss of lattice water partly and/or hydroxyl ions (loss until 400 °C), according to other authors [5,29–31] and eventually to carbonate decomposition. Also, the accompanied weight loss of the second stage for samples HAV and HAP should be ascribed to the polymer decomposition matrix. This peak appeared as a small peak at 270 °C for SHA where it is shifted with increasing intensity to 300 °C then to 450 °C for HAV and HAP, respectively. That means the presence of polymer preserves the structure of apatite and prevents the removal of lattice OH to a higher temperature. Actually, for some carbonated apatites, evolution of lattice water has been reported to occur in a wider temperature interval, up to 500 °C [32]. This assignment appears more consistent than that to water evolving as a result of interactions of the apatite constituents [33]. This confirmed that HAV and HAP have a carbonated apatite structure where the polymer structure dissociated and introduced CO_2 in the synthesized samples. Also, we can attribute the presence of CO_2 due to the air-dried adsorption surface layer as suggested by Rey et al. [34]. There was a significant DTA sharp peak appeared at 800 °C for the samples containing polymer HAV and HAP with increasing intensity too, respectively. Ivanova et al. [30] have reported that the peak appearing at 800 °C is due to increasing of HPO_4^{2-} group that was condensate to pyrophosphate (P_2O_7). This group reacted with carbonated apatite to forming β-TCP $Ca_3(PO_4)_2$. This may also be attributed to carbon monoxide CO of the polymer where this peak is completely absent in SHA. The thermal behavior of HA and its carbonate can be supposed to depend on the synthesis technique and route [33].

The XRD pattern of standard HA and synthesized powders SHA, HAV and HAP was shown in Fig. 3. The structure of hydroxyapatite seems without deformation in the three samples but with different degree of crystallinity. The results revealed the presence of all the major HA peaks, (0 0 2), (2 1 1), (3 0 0), (2 0 2), (3 1 0) and (2 2 2), based on the standard XRD pattern card of HA (JCPDS card no. 09-432). No other calcium phosphate phases, such as TCP, or calcium oxide were detected. It can also be evidenced that in samples HAV and HAP where using polymers leads to increased the intensity of the main peaks of HA. However, the crystallographic structure of the HAP is more identical with carbonated apatite, natural bone mineral (biological apatite) [5]. Hence, the prepared HA nanocrystallites in this investigation have more similarity to natural bone mineral in terms of degree of crystallinity and structural morphology. The possible reason for the poor crystalline nature of precipitated HA (SHA) must be emerged from the preparation methodology owing to low temperature procedure [35]. Besides, the sodium chloride peaks can clearly be observed at 2θ 45.5°, 28.4° and coincide with the main peak at 31.9° of apatite that leads to the increase of the intensity of this peak of hydroxyapatite composite. These peaks present only in case of the use of polymer. They can be explained as the presence of any foreign impurities in the chemical precursors may lead to their incorporation into the formed apatitic structures. Whereas, the two peaks at 84° and 88° of apatite inversed their intensity only in case of HAP which attributed to the use of PVP that preferred orientation of the phase formed.

Results of SEM were presented in Fig. 4 where, Fig. 4(a, b) for precipitated HA (SHA) exhibited nanosized crystals with almost uniform particles size. The HA particles prepared were not only stoichiometric but also monodispersive and rough particles were not fused together with other crystals. It can be inferred that majority of the particles were of regular shape and cleaner contours with no agglomeration. On the other hand the presence of polymer in samples HAV or HAP (Fig. 4(c–f)) respectively, prefers the formation of the needle-like HA nanocrystallites that facilitates aggregation along the *c* axis and forms rod-like structure via oriented attachment. While in the absence of polymer, it is difficult to form the rod-like structure. According to Zhang and Lu [26] this is attributed

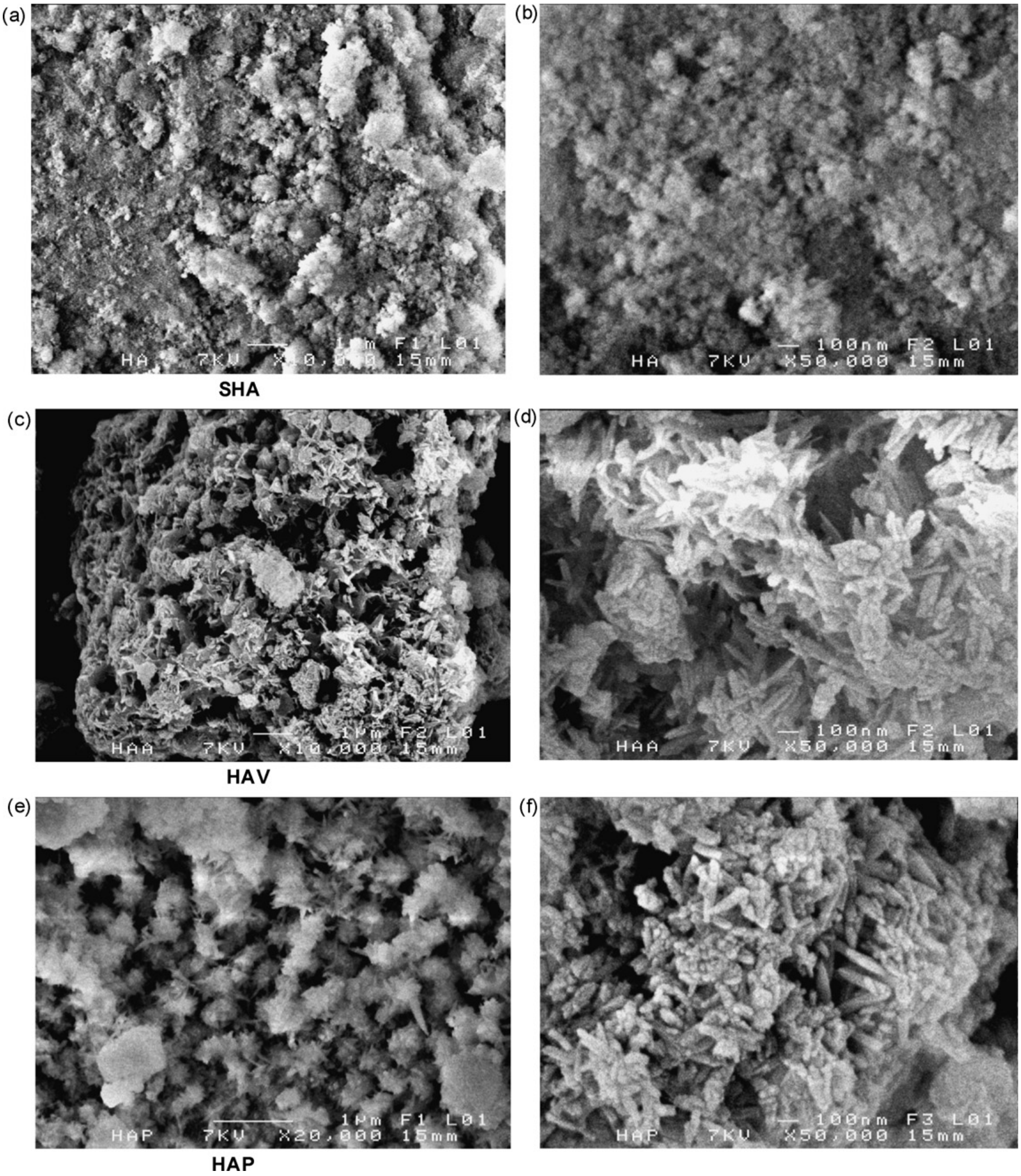

Fig. 4. SEM of prepared nanohydroxyapatite powder samples (a, b) without polymer (SHA), (c, d) with PVA (HAV) and (e, f) with PVP (HAP).

to Ostwald ripening process that occurs in the solution which leads to a typical oriented attachment process (Fig. 4(e, f)) especially on using PVP. The morphology transformation of synthetic HA and oriented attachment process would be accelerated under the reaction condition. Because of the oriented attachment and Ostwald ripening process, some of the small size HA crystals grow to the large rod like HA. Because of the effect of the strong van der Waals attraction, the HA rod-like structure tends to aggregate together and form side-by-side rod like structure along the *c* axis as it is apparent Fig. 4(e) [36]. As a result of the crystallization process of HA controlled by the surface-regulating (PVP) and the oriented attachment process, the synthesized HA crystals are uniform nanorods with a nanometer size.

Results of specific surface area measurements and the equivalent particle size were presented in Table 1. In order to eliminate the effect of type of polymer HA samples prepared both in presence and in absence of polymer were calcined at 500 °C for 2 h and analysed using BET method. From Table 1, we found that the HA crystals prepared were less than 50 nm. HAP sample crystals showed higher surface area than the other two investigated samples. Based on the theory of ionization PVP provides higher number sites for selective adhesion of Ca ions at a later stage will combine with $(PO_4)^{3+}$ initiating the nucleation of nanometer-sized HA particles on organic polymer [36]. Also, we can observe the SHA has nanoparticles too that can be supposed to depend on the synthesis technique and route [33].

Table 1
Specific surface area measurements (BET) and corresponding particle size.

Sample symbol	Surface area (m^2/g)	Equivalent particle size (nm)
SHA	85.25	13.6
HAV	184.12	23.5
HAP	369.62	50.8

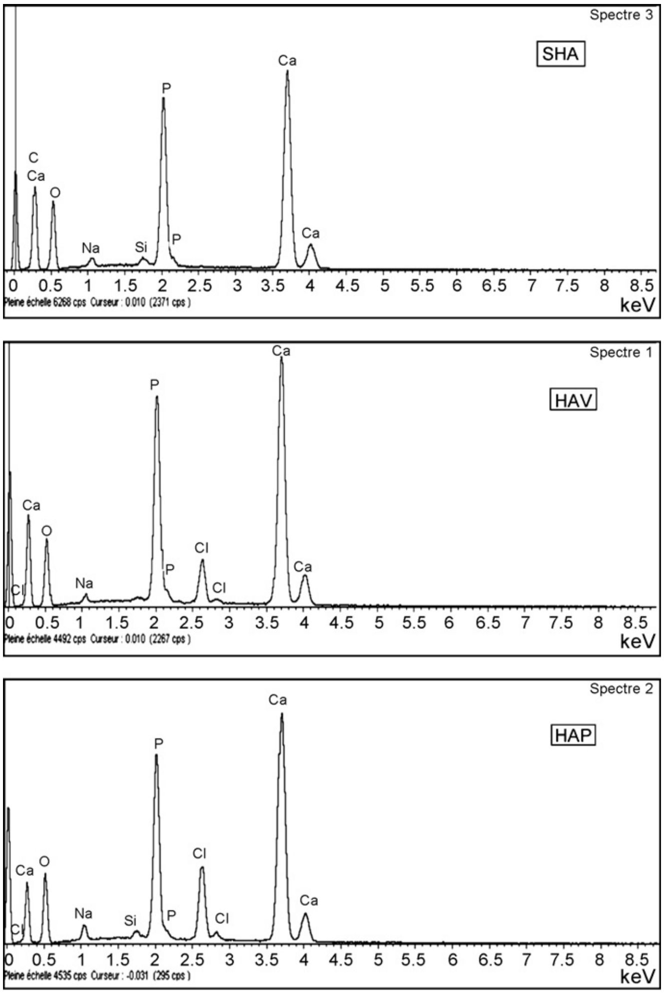

Fig. 5. Elemental analysis (EDS) of prepared nanohydroxyapatite samples; without polymer (SHA), with PVA (HAA) and with PVP (HAP).

The elemental composition was analyzed using elemental analysis (EDS). Fig. 5 showed the spectrum of the prepared samples, which contained Ca, P, C, and O. The overlapping of the secondary peak of Ca with the peak of carbon C can be seen. The concentration of Ca and P elements and the corresponding Ca/P molar ratio of the samples was presented in Table 2 and confirmed by the ICP-OES measurements. These results clearly showed that the Ca/P ratio was strongly affected by the type of polymer used, ranging between 1.72 and 1.85. Particularly, these results showed that the Ca/P ratio ranged from stoichiometric apatite in samples without polymer and close to that of biological apatite with polymer (PVP). By using PVP the Ca/P ratio was high depending on both the species and the functions [5,37]. We can also notice a peak of Na and Cl in Fig. 5 in both samples HAV and HAP containing polymers. This was attributed to the starting precursors used $CaCl_2$ and the alkaline medium where sodium hydroxide was used to adjust the pH. This was coinciding with the results of XRD. Also, we can notice that at low phosphate concentrations the Ca/P ratio was high due to the formation of calcium carbonate [38,39]. As regards data were complementary; from TG analysis, it can be noted that there is a sufficiently good correlation with the data obtained by elemental analysis if the weight loss in 400–1200 °C range is entirely attributed to carbonate decomposition (i.e. CO_2 loss). This assignment appears in agreement with the literature [5,31,32]. For this purpose, various authors have reported that on heating B-type carbonated hydroxyapatites at temperatures from 480 to 800 °C, the changes in the location of carbonate ions occur: some part of them move from B- to A-sites [30,31]. Carbonate loss has been reported to start at about 400–500 °C for samples HAV and HAP containing polymer and to be usually complete from 800 °C up to about 1000 °C, depending on the composition [5,31]. “In vitro” tests will be under investigation.

Table 2
Concentrations (ppm) of the Ca and P elements in the prepared powders and the corresponding Ca/P molar ratios.

Sample symbol	[Ca] (ppm)	[P] (ppm)	Molar (Ca/P)
SHA	38.45	17.441	1.70
HAV	29.51	12.80	1.78
HAP	30.68	12.82	1.85

4. Conclusion

Composites of nanohydroxyapatite with two types of polymer viz, PVA and PVP materials were synthesized by a biomimetic method. The reaction temperature and duration of reaction are also critical toward obtaining controlled morphology and grain size of HA crystals. These results may provide a basis for the preparation of more complicated composite materials. Carbonated apatite phase, which is favorable for the biological response of the bone-substitute material, was obtained even if carbonate source is not introduced in the starting materials. This is attributed to the dissociation of polymer that is evidenced from the results of DTA and TG. Also, it can be attributed due to the adsorption of CO_2 from air. Rod-like nano-HA powder has been prepared using a mild and efficient biomimetic synthesis method. In the crystallization process, the oriented attachment and the effect of polymer were vital for the regulation of the nucleation and crystal growth of rod like HA crystal. On the basis of hydrogen bond effects, the polymer used as a capping agent prompts the fast formation of rod-like HA particles. The reaction temperature and duration of reaction are also critical toward obtaining controlled morphology and grain size of HA crystals. Therefore, it can be concluded from these results that the effect of polymer on HA crystal (forming hydrogen bond at earlier stage of reaction) worked on the crystal morphology but not on the phase composition of product. “In vitro” bioactivity measurements are under investigation.

Acknowledgments

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