Colloidal Hydroxyapatite/Poly(Acrylic Acid) Hybrids Using Calcium Sucrate and Ammoniumdihydrogen Orthophosphate

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Abstract: This manuscript is concerned with a simple and novel method to synthesize hydroxyapatite-poly(acylic acid) hybrid materials for broad range of applications. In this method, hydroxyapatite nanoparticles are synthesized using calcium sucrate and ammoniumdihydrogen orthophosphate in the presence of poly(acrylic acid). Increase in poly(acrylic acid) concentration in the synthesis medium results in the increase in the hydrodynamic radius of particle size allowing increased hydration. Poly(acylic acid) tends to control both crystallite size and colloidal stability. Increase in poly(acrylic acid) concentration decreases the crystallite size of the products but considerably increases their shelf life as stable colloidal solutions. Thermo gravimetric analysis shows that there are no combustible or volatile impurities present in these samples. This is further supported by FT-IR studies, which show three types of interactions between hydroxyapatite nanoparticles and poly(acrylic acid).

Keywords: Hydroxyapatite, Poly(acrylic acid), Stable colloids, Hybrid materials, Calcium sucrate.

1. INTRODUCTION

Stable dispersions of colloidal hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (HA) nanoparticles are very useful in biomedical fields. Therefore, it is essential to preserve HA nanoparticles as dispersions for a prolonged period in order to use them in wide-range of biomedical applications [1]. Well-stabilized HA colloids are mainly used in the preparation of HA-coated, biocompatible metal prostheses which are prepared by electro-deposition and dip-coating techniques [2]. Furthermore, colloidal HA nanoparticles are used as delivery systems of peptides, proteins, and DNA therapeutics and as a dental adhesive [3, 4]. Water-soluble, biocompatible polymers are used in the stabilization of HA nanoparticles in their colloidal forms. The resulting material is a polymer/HA hybrid. Furthermore, the phase, morphology and particle size of HA in the composite are controlled by the polymer [5]. Therefore, preparation of such hybrid materials is very important to prepare HA for specific applications.

Bone is an excellent example for an organic/HA nanohybrid which is made up of HA nanoparticles and collagen protein fibers [6, 7]. Collagen fibers provide a structural framework for the bone and HA nanoparticles are dispersed in collagen matrix [8, 9]. Therefore, HA nanoparticles are synthesized to use in biomedical applications with structurally and compositionally similar characteristics to natural HA in bones [10]. However, it is not possible to produce artificial bones with synthetic HA alone to mimic natural bones, for them to be used in biomedical applications such as bone remodeling, mechanical stability and carrier for drugs/growth factors [11]. Therefore, synthetic HA nanoparticles are combined with biocompatible and biodegradable organic polymers such as poly(acrylic acid) (PAA) [12, 13], poly(lactic acid) [14], poly(caprolactone) [15], and gelatin [16-18] in order to prepare hybrid materials to suit different biomedical applications [19]. These hybrid materials containing biodegradable polymers are degraded after introducing to the body as bone fillers [11]. Mechanical properties of HA can also be enhanced by combining with the polymers [18, 20]. Therefore, HA-polymer hybrid materials play a vital role in biomedical applications [18, 21].

Hybrid materials should be homogenously dispersed when they are used as bone cements and drug delivery agents. Most of the researchers have re-dispersed HA hybrid materials in different solvents [22, 23]. Generally, PAA is used to deliver drugs individually and also by combining with the other polymers. As such, PAA-HA hybrids also can deliver drugs or growth factors when they are applied in biomedical devices

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[24, 25]. PAA has an ability to bind strongly with hard tissues when it is used as bone cement in various biomedical applications [26, 27]. Chen *et al*. (2008), investigated the mechanical strength of the calcium phosphate cement-PAA hybrid and shown that mechanical properties have been increased when compared to those of the bare calcium phosphate bone cement [26]. PAA has an ability to absorb enormous amount of water and this water absorption by PAA is an essential feature to maintain colloidal stability of HA-PAA hybrid materials [28, 29].

In this study, we have synthesized HA-PAA hybrids, which have very long shelf-life as stable colloids, using a simple and novel method. The colloidal stability of synthesized hybrids is studied by varying the PAA concentration. The preparation method described here is a very simple and an economical method to synthesize HA from readily available minerals such as dolomite and calcite which can be used to prepare calcium sucrate [30-33]. Biological HA nanoparticles have both crystalline and amorphous phases [8, 9, 34], and hence, it is essential to synthesize nanosized HA in mixture of crystalline and amorphous phases for them to be utilized in biomedical applications. PAA can control the formation of crystalline phase and stabilize the amorphous phase of materials [35]. Mantilaka *et al*. (2014) have used PAA as a stabilizer to synthesize amorphous calcium carbonate nanoparticles using calcium sucrate as a raw-material. We have used this concept in synthesizing highly dispersible amorphous HA containing HA-PAA Hybrids [35]. In this manuscript, we describe further insight into the preparation and characterization of highly dispersible hybrids of HA-PAA which also have long shelf-life as stable colloidal dispersions. This long-term stability is mandatory for these hybrid materials to be used in many technological applications as briefed above.

2. EXPERIMENTAL

2.1. Materials Used

Calcium oxide (99%), ammoniumdihydrogen orthophosphate (99%), sucrose (99%), acrylic acid (99%), sodium persulfate (99%) and sodium hydroxide (99%) were purchased from Sigma–Aldrich.

2.2. Preparation of PAA

A stock solution PAA (0.5 M with respect to the repeat unit) was prepared by polymerizing acrylic acid, at 60 ^{o}C, using 1.00 g of $Na_2S_2O_8$ and dissolving the solid-formed in 1 M NaOH solution [35].

2.3. Preparation of Calcium Sucrate Solution

We have already documented this procedure elsewhere [36]. Briefly, 2.80 g of CaO was dissolved in 100 mL of 0.5 M sucrose solution to prepare 0.5 M calcium sucrate solution and it was then stirred for 3 h. Next, the solution was allowed to settle-down and thereafter filtered under suction. The resulted solution is calcium sucrate.

2.4. Preparation of HA-PAA Hybrids

In the preparation of HA-PAA hybrids, 100.0 mL of previously prepared PAA solution was added dropwise to 100.0 mL of the calcium sucrate solution while stirring. Then, 100 mL of 0.3 M ammoniumdihydrogen orthophosphate (ADO) solution was added (until Ca/P ratio of 1.67) to the above reaction mixture using a dropping funnel while stirring. The mixture was further stirred for 12 h and filtered under suction to obtain a precipitate. The precipitate was washed with distilled water for 3 times and was allowed to dry under ambient laboratory conditions. Four samples were prepared by varying the concentrations of 100 mL of PAA in samples S1 to S4 to have concentrations, respectively, of 0.5 M, 0.1 M, 0.05 M and 0.025 M. The key steps of formation of HA-PAA hybrids are shown in Figure **1**.

2.5. Characterization of HA-PAA Hybrids

Dried samples were carefully milled to a fine powder and thoroughly mixed to homogenize and their powder XRD spectra were recorded with a Siemens D5000 X-ray Powder Diffractometer with Cu Kα radiation of wavelength k = 0.1540562 nm. The X-ray diffraction (XRD) studies were performed to estimate the crystallite size and to identify the crystal forms of the synthesized HA-PAA hybrids. The resulted XRD patterns were analyzed using the ICDD PDF 2 database. HA-PAA hybrids were further analyzed from the Fourier Transform Infrared (FT-IR) spectroscopy. The FTIR spectra of the products were recorded on a Shimadzu IR Prestige 21 instrument with the KBr pellet method at dry mass ratio of 1:40 product:KBr. Morphologies of the products were observed with the help of Hitachi SU6600 Scanning Electron Microscope (SEM). Thermo Gravimetric Analysis (TGA) was performed to investigate the weight percentages of PAA and water in HA-PAA products using a Scinco STA N-650 instrument, which was performed at a heating rate of 10 °C/ min in air flow. Particle size distribution of the HA-PAA hybrids were analyzed using Cilas Nano DS Sn 110 Particle Size Analyzer.

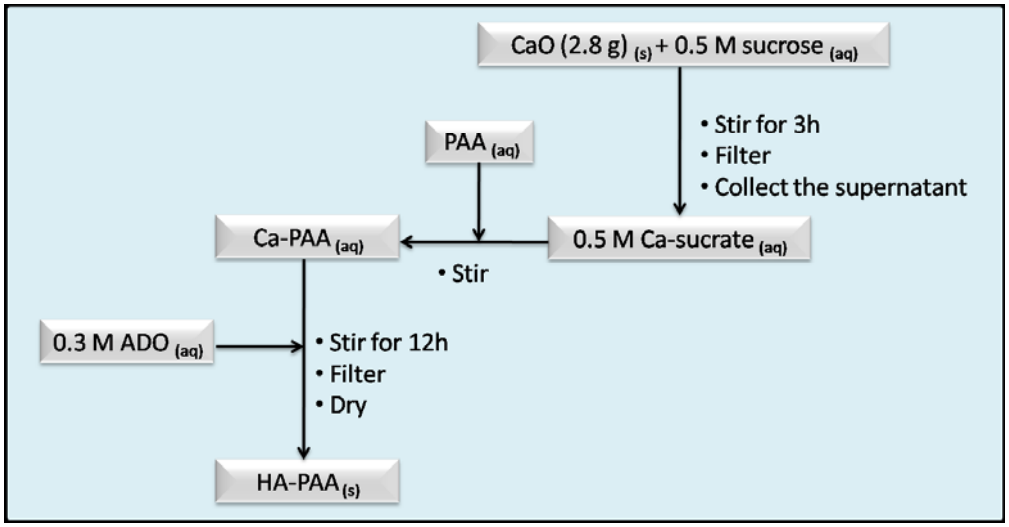

Figure 1: A Process flow diagram for the synthesis of HA-PAA hybrid, where PAA is polyacrylate and ADO is ammoniumdihydrogen orthophosphate.

Prepared hybrids were dispersed in water and were allowed to settle down at the required lengths of times, under ambient laboratory conditions. The settling times were used to investigate the colloidal stability of the HA-PAA hybrids.

3. RESULT AND DISCUSSION

3.1. Synthesis of HA-PAA Hybrids

We have documented the importance of calcium sucrate as a precursor for the synthesis of $CaCO_3$ and HA nanoparticles, elsewhere [30-33, 36]. When the sodium salt of PAA solution is mixed with the calcium sucrate solution, Ca^{2+} ions and carboxylate groups of PAA form chelating complexes [35]. These coordinately bonded Ca^{2+} ions provide essential sites for the formation of HA, thus, colloidal particles of PAA-Ca^{2+} are produced which is observed as turbidity. Once the ADO solution is added dropwise to the mixture of PAA and calcium sucrate, the turbidity of the solution is increased, and later, a white colored precipitate is obtained. The resulted product is HA-PAA hybrid as confirmed by several characterization techniques *(vide-infra)*.

3.2. XRD Studies of HA-PAA Hybrids

The XRD results of the prepared HA-PAA samples are given in Figure **1**. The XRD patterns of all HA-PAA samples, prepared with different amounts of PAA, can be assigned to those of hydroxyapatite (JCPDS card no. 72-1243). The XRD peaks of HA-PAA samples are found at 2θ values of 25.8°, 31.7°, 39.7°, 46.6°, 49.4° and 53.2° which are due to reflections from the basal planes of (002), (211), (130), (222), (213), and (004), respectively. The broad diffraction peaks of XRD patterns are observed for samples synthesized with the highest amount of PAA, while, the narrow diffraction peaks are obtained for those synthesized with the lowest amounts of PAA. Increase in broadness of the XRD peaks represents the decrease in the crystallite size of HA crystals. That is because; PAA has an ability to control the crystal growth and the crystallite size of HA. The mean crystallite sizes of the HA-PAA hybrid materials are calculated according to the Debye–Sherrer formula using (211) plane [37]. The calculated crystallite sizes of HA synthesized in the presence of 0.5 M, 0.1 M, 0.05 M, and 0.025 M, PAA solutions are 5 nm, 8 nm, 11 nm, and 15 nm, respectively.

These results clearly indicate that PAA has the ability to control the growth of nuclei to form HA products with less crystallite size of HA. Therefore, prepared HA-PAA hybrid materials can be easily used during bioengineering and biomedical applications of bone tissues.

3.3. FTIR Studies of HA-PAA Hybrids

Figure **3** shows the FT-IR spectra of all HA-PAA hybrid samples, which are prepared with different PAA amounts. The spectra of all prepared HA-PAA hybrid samples are very similar with respect to their band positions. The bands centered at 468 cm^{-1}, 564 cm^{-1} and 600 cm^{-1} are related to the bending vibrations of the phosphate groups of HA [38]. The band centered at 1035 cm^{-1} and 1101 cm^{-1} are related to the stretching vibration of the P-O bonds which are present in the phosphate groups [38]. The bands at 874 cm^{-1} and

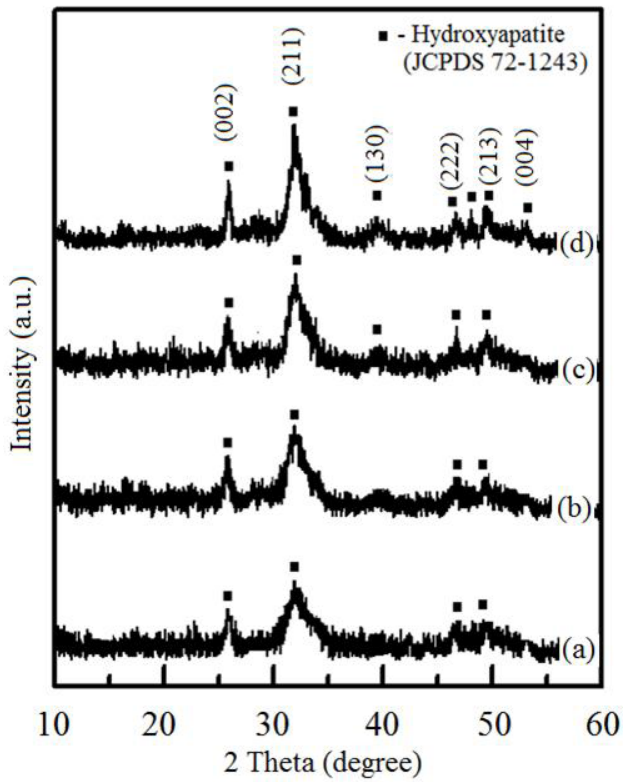

Figure 2: XRD pattern of HA-PAA hybrid synthesized with 100 mL of (a) 0.5 M (b) 0.1 M (c) 0.05 M (d) 0.025 M, PAA solution.

1458 cm^{-1} are attributed to the C-O bonds of PAA which are hydrogen bonded with HA [39-41]. The band at 1410 cm^{-1} is related to the scissoring and bending modes of C-H bonds which are in -CH_2-, and -CH-CO- groups, respectively. The new band appeared at 1560 cm^{-1} can be assigned to be due to the dissociated carbonyl group [39], which is due to the formation of chelate bonds between carboxylate group and Ca^{2+} in HA. The broad band around 3400 cm^{-1} and the sharp band at 1655 cm^{-1} can be assigned to the stretching vibrations of combined water in the HA-PAA hybrids [36]. The band at 3550 cm^{-1} is attributed to the bending mode of the hydroxyl groups of HA-PAA hybrid materials [42].

There are three types of interactions that are present in HA-PAA hybrids. They are, (1) H-bonds between surface OH groups of HA with carboxylate groups of PAA, and others (2) H-bonds between surface phosphate groups of HA with carboxylate groups of PAA and (3) chelation between surface calcium atoms of HA with carbonyl oxygen atoms of carboxylate group of PAA [40]. Bhowmik *et al*. (2007) reported that all of those interactions can be investigated by molecular dynamics simulations [43]. According to the Bhowmik *et al.*, strength of those bonds is higher when the samples prepared under *in-situ* methods when compared to those prepared by *ex-situ* methods.

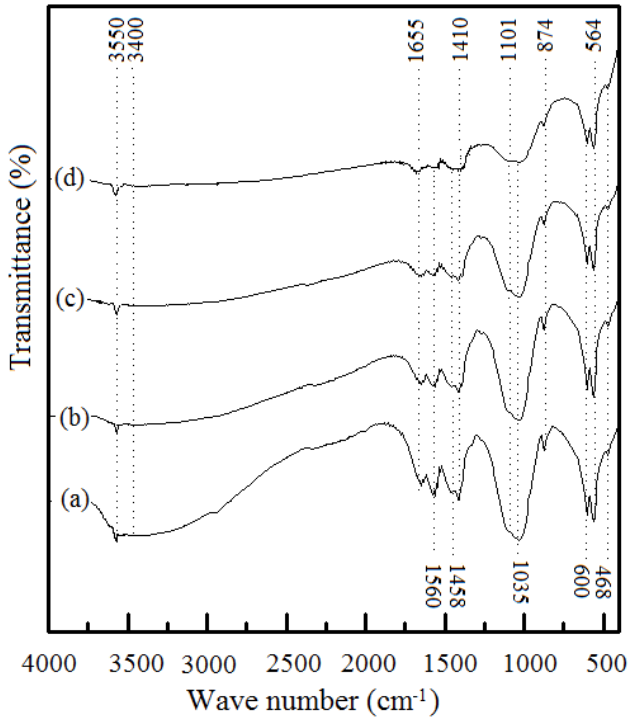

Figure 3: FTIR Spectra of HA- PAA hybrids synthesized with 100 mL of (a) 0.5 M (b) 0.1 M (c) 0.05 M and (d) 0.025 M, PAA solution.

3.4. Thermal Analytical Studies of HA-PAA Hybrids

Figure **4** shows the TGA curves of prepared powders of HA-PAA hybrids which have been prepared with different PAA amounts. The mass-loss of HA-PAA hybrid materials at around 100 °C is attributed to the removal of adsorbed water from HA-PAA hybrids. The mass-loss from 200 °C to 350 °C has been observed in all TGA curves, which can be assigned to the combustion of PAA polymer in the hybrid materials. The resulted percentages were 29%, 18%, 10% and 6% which are related to the HA samples prepared with 0.5 M, 0.1 M, 0.05 M, and 0.025 M of PAA solutions, respectively. There are no any other considerable mass-losses in the TGA curves indicating high purity of these hybrid materials in terms of burnable or volatile materials.

3.5. Particle Size Distribution Studies of HA-PAA Hybrids

The particle size distribution of hybrids is an essential character for the determination of colloidal stability of the hybrid. The particle size distribution

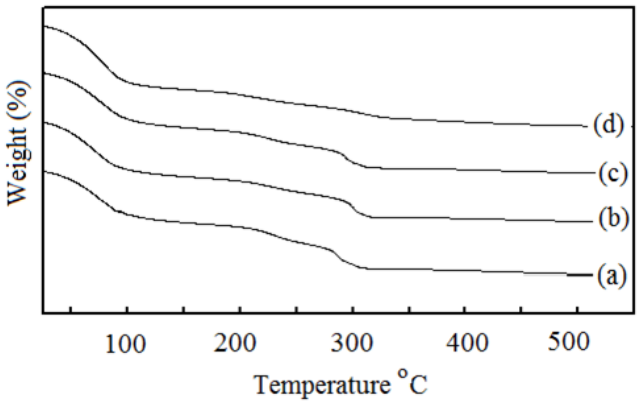

Figure 4: TGA curve of HA- PAA hybrid synthesized with 100 mL of (a) 0.5 M (b) 0.1 M (c) 0.05 M (d) 0.025 M, PAA solution.

curves of the hybrid using DLS technique are given in Figure **5**. The average particle sizes of HA colloids prepared using 0.5 M, 0.1 M, 0.05 M and 0.25 M PAA solutions are 377.5 nm, 277.5 nm, 83 nm and 29.5 nm respectively. The particle sizes of hybrid materials have been increased with the PAA concentration. According to the particle size distribution studies, the highest average particle size with the highest distribution is obtained with the HA-PAA hybrid which has been prepared with the highest PAA concentration. Lowest particle size and its distribution obtained with the sample which has been prepared with the lowest PAA concentration. That is because, the radius of the hybrid increases with the PAA concentration and ability of absorption of water by the hybrid increases with PAA concentration. Hence, if the hybrid contains highest amount of PAA, that then has a greater ability to absorb higher quantity of water from aqueous medium and to swell and thereby to increase their radius.

3.6. Colloidal Stability of HA-PAA Hybrid

Study of colloidal stability of hybrid materials is an essential component when it used as a drug delivery agent or a growth factor during biomedical applications. Therefore, in this study, distilled water was used as a dispersing medium for HA-PAA hybrid materials for the study of colloidal stability of the hybrid materials (Figure

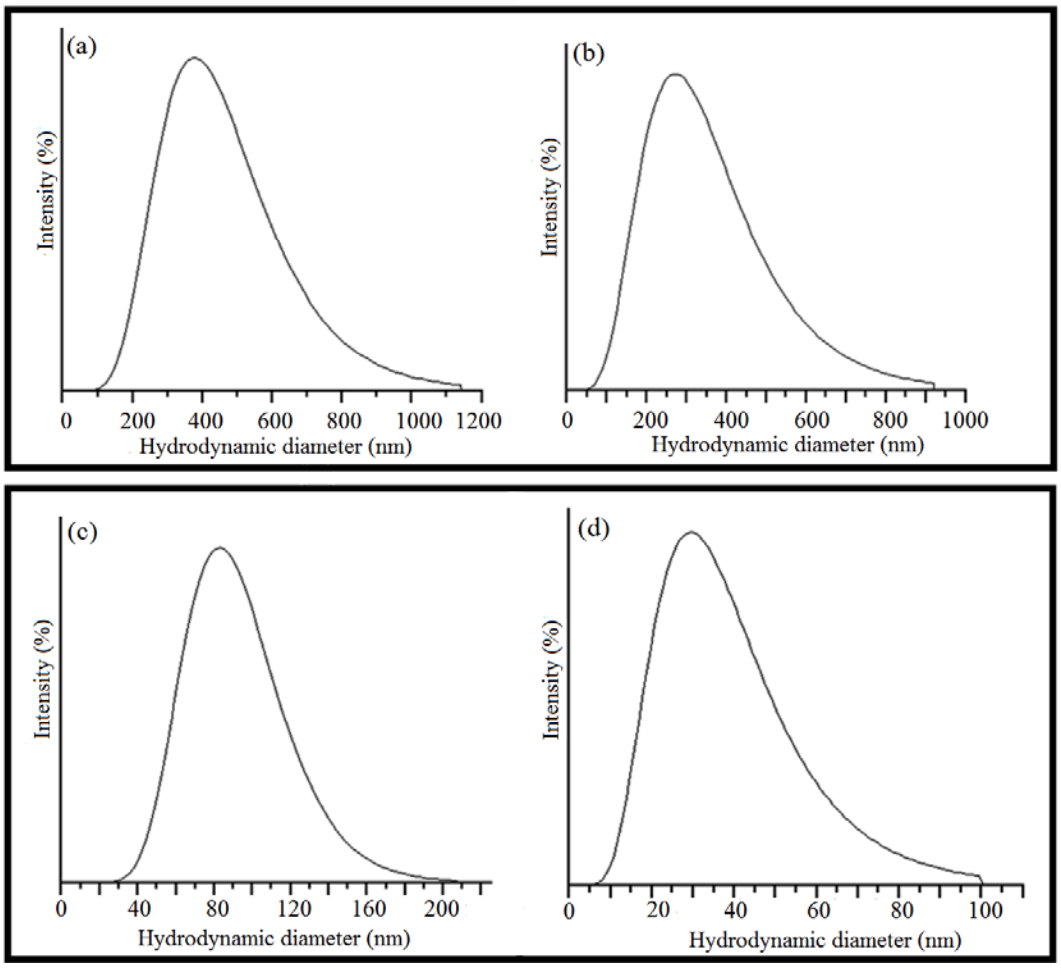

Figure 5: Particle distribution curve of HA- PAA hybrid synthesized with 100 mL of (**a**) 0.5 M (**b**) 0.1 M (**c**) 0.05 M (**d**) 0.025 M, PAA solution.

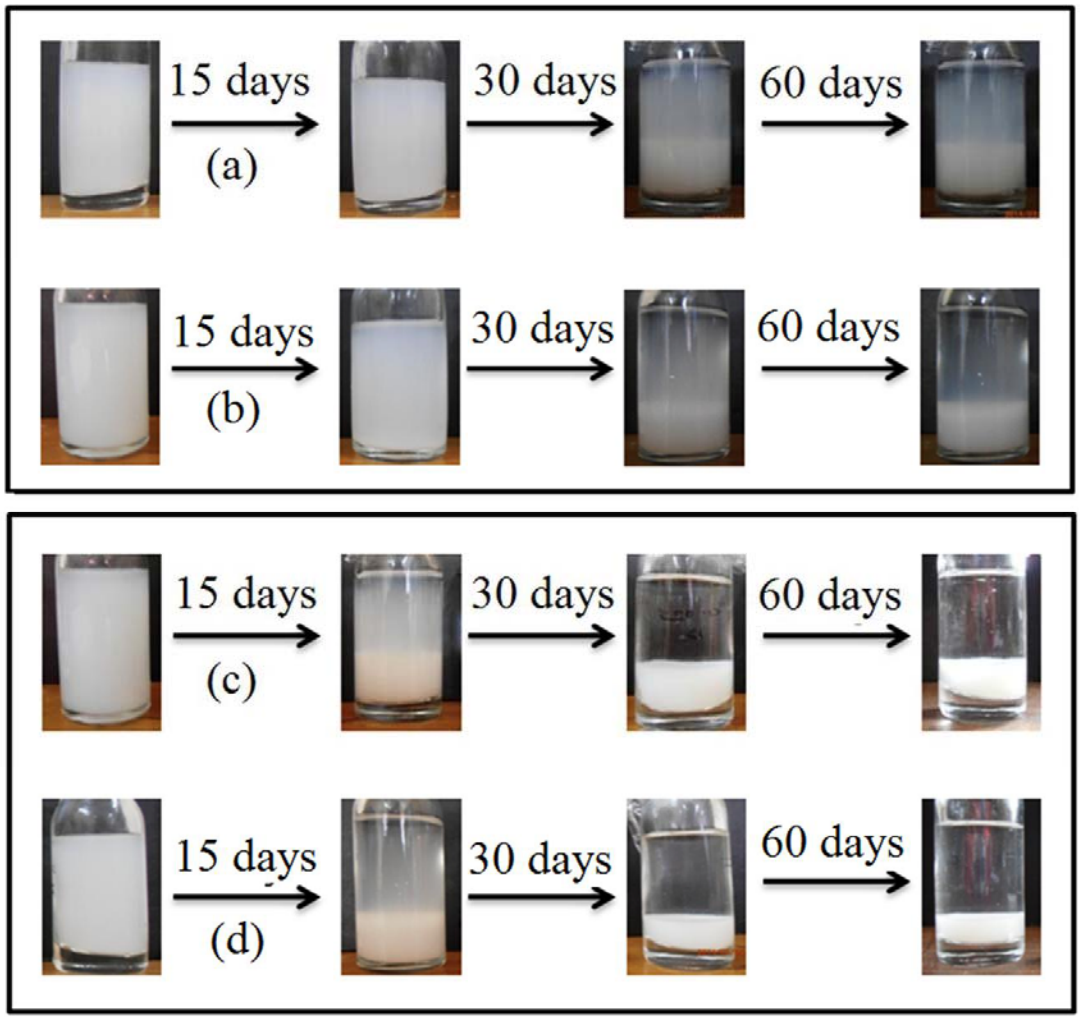

Figure 6: Photographs of colloidal stability study of HA-PAA hybrid synthesized with 100 mL of (**a**) 0.5 M (**b**) 0.1 M (**c**) 0.05 M (**d**) 0.025 M, PAA solution.

6). The prepared colloids were allowed to sediment and the sedimentation time was measured. According to Figure **6**, Samples with highest colloidal stability are “a” and “b” which are prepared by using PAA solutions with 0.50 M and 0.10 M concentrations whereas the samples with lowest colloidal stability are “c” and “d”. Sample “a” is more stable than sample “b”. In similar manner, sample “c” is stable than the sample “d”. Therefore, it can be conclude that the stability of colloidal nanoparticles increases with increasing PAA concentrations. That is because PAA has an ability to absorb water and disperse by increasing their volume and settling time. Thus, the water absorption ability and increment of size of PAA is essential for the colloidal stability of HA-PAA hybrid materials. This is because when PAA concentration is high, that supports to form greater number of hydrogen bonds along with water which is responsible for the colloidal stability and also for the control of the aggregation of HA by the PAA polymer.

3.7. Morphology of HA-PAA Hybrid

Figure **7** shows SEM images of colloidal HA-PAA nanocomposite prepared using 0.5 M PAA solution which shows the highest colloidal stability as discussed in previous section. According to our previous study [36], synthesized HA nanoparticles are of needle shape when prepared under ambient conditions (25 °C). However, particles in the HA-PAA nanocomposite have become spherical in shape. This is because, the synthesized HA covered by PAA and the PAA polymer is imposing on the nucleation and growth of HA particles. Also, their interactions in all directions have made the particles more spherical than elongating in one direction. Figure **8** shows SEM images of powdered HA-PAA nanocomposite prepared using 0.5 M PAA solution. Herein, particles have become irregular in shape due to aggregation while preparation of HA-PAA powder from the colloid.

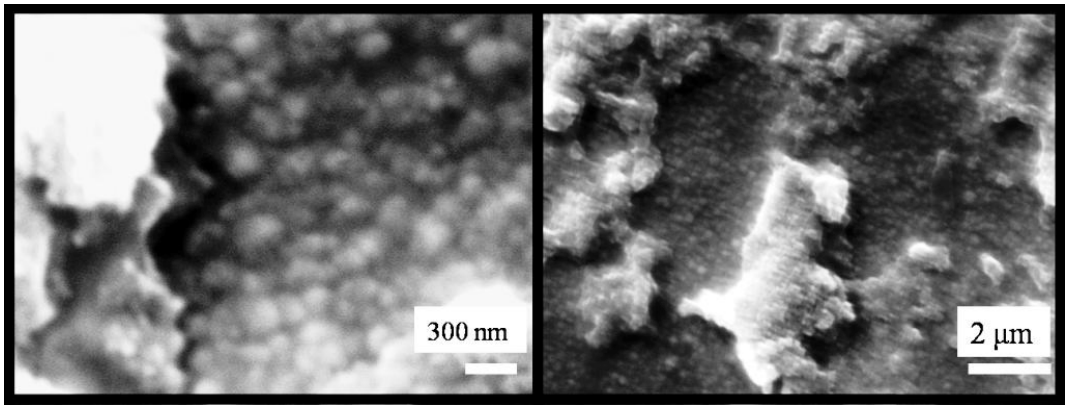

Figure 7: The SEM images of colloidal HA- PAA hybrid material synthesized with 100 mL of 0.5 M PAA solution at two different magnifications.

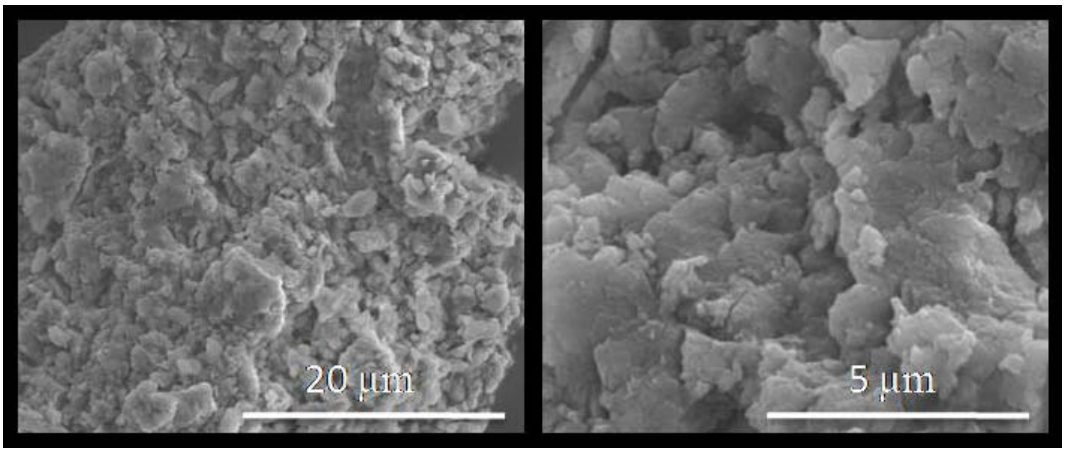

Figure 8: The SEM images of powdered aggregates of HA-PAA hybrid material synthesized with 0.5 M PAA solution at two different magnifications.

4. CONCLUSIONS

Stable colloids of HA-PAA hybrid materials are synthesized starting from calcium sucrate and ammonium dihydrogen orthophosphate, for first time. Increase in poly(acrylic acid) concentration decreases the crystallite size of the products but considerably increases their shelf life as stable colloidal solutions. Hydroxyapatite nanoparticles in the hybrid have been stabilized by poly(acrylic acid) from three interactions as, (1) H-bonds between surface OH groups of HA with carboxylate groups of PAA, and others (2) H-bonds between surface phosphate groups of HA with carboxylate groups of PAA and (3) chelation between surface calcium atoms of HA with carbonyl oxygen atoms of carboxylate group of PAA. The water absorption ability and increment of size of PAA is essential for the colloidal stability of HA-PAA hybrid since higher PAA concentrations support to form greater number of hydrogen bonds along with water which is responsible for the colloidal stability and also for the control of the aggregation of HA by the PAA polymer. The devised simple method for the synthesis of stable colloidal hydroxyapatite nanoparticles is easily up-scalable.

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