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A preliminary study on the reactive suspension approach to create bioactive polymer nanocomposites for dental applications

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The modified chemical particle with appropriate combination agents possesses a lesser surface and the organic matrix gives more compatibility with the unprocessed matrix, is a typical strategy for limiting agglomeration problems. The limited time processing was the main sign of the chemical modification and particle purification. The production of nanoparticles directly inside the organic matrix is an alternate technique in situ approach. The present studies focused to make a new nanocomposites matrix with powder and liquid phase PMMA and hydroxyethyl methacrylate and it is processed by in-situ method to compose the hydroxyapatite nanoparticles of $(Ca_{10}(PO_4)_6(OH)_2$. Therefore reactive suspension approach was utilized in this study. During the in-situ process, the precursors like ammonium dihydrogen and calcium nitrate tetrahydrate are used. When the combinations of HA with the influence of the HEMA agent. These precursors and the agents composed the hybrid material with even dispersion. Due to maximum phase interrelations were achieved between the dead filler and polymer matrix base. HA is an inorganic phase that is osteogenic and osteoconductive, comparable to bone-based minerals. Similarly, it promotes polymer-based composites from bioactivity to enhance bone regeneration. The structure and morphology characteristics of PMMA, hydroxyethyl methacrylate and HA processed composites are examined in this work. The mechanical characteristics of the composites are measured in the combinational mode of compression and flexural. Then the bioactivity is investigated by submerging into the simulated bodily fluid (SBF). The mixing among the HA and polymer reduces the water sorption values significantly. After soaking in simulated bodily fluid, the surface of the HA generation was verified into the bioactivity of the processes composites.

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1. Introduction

In recent decades, oral health has grown increasingly essential. Numerous researchers have focused their attention on dental restorative materials in this regard, particularly in the field of acrylic-based resins [1]. There are various research has been accomplished for the resin based composites in the mechanical characteristics, dental based usages and anti-bacterial attributes to enhance the performances of composites [3]

There are solid and liquid components are majorly utilized in the dental applications along with significant properties, less production cost, better stability and simple to use. These components are polymethyl methacrylate and polymethyl methacrylate monomer and it is one of the healing materials [3]. The most ubiquitous usages of these materials led to fabricate the temporal closures and denture bases [2].

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Adding preformed nanoparticles to the blended with liquefied PMMA particles are fine way to improve the mechanical qualities of an acrylic-based denture foundation. Silica, hydroxyapatite, aluminum, titanium, and zirconia are the most popular nanoparticle fillers [5]. Even at extremely low mass fraction levels, nanoparticles can increase resin qualities like as flexural, resist to wear and modulus properties. However, the superiority of the nanofiller is evenly dispersed in the polymeric matrix has a significant impact on these qualities. Agglomerates are generated with their presence of even dispersion in the surface layers of the inorganic nano particles at maximum proportions of filler material. Hence, the lesser size particles with 100 nano meter were combined together in the large cluster to diminish the surface potential. The existence of voids in this agglomeration influences material performance by acting as a favoured location for fracture initiation and failure [4].

To restrict the agglomerated issues is major aspects for improving the compatibility in the unprocessed matrix and also reduces the surface potential when the coupling agents are selected with appropriateness for chemical alteration. The minimum consumption of time for the chemical process and particle distillation are the major disadvantage of this strategy. The production of nanoparticles directly inside the organic matrix is an alternate technique (in situ approach). The aim of this current study is to made new nanocomposite material with powder and liquid phase are PMMA and hydroxyethyl methacrylate and it is processed by in-situ method to compose the hydroxyapatite nanoparticles of $(Ca_{10}(PO_4)_6(OH)_2$. HA is an inorganic phase that is osteogenic and osteoconductive, comparable to bone-based minerals. Similarly, it promotes the polymer-based composites from the bioactivity to enhance bone regeneration [5-8].

Therefore reactive suspension approach was utilized in this study. During the in-situ process, the precursors like ammonium dihydrogen and calcium nitrate tetrahydrate are used. When the combinations of HA with influence of HEMA agent. These precursors and the agents were composes the hybrid material with even dispersion. Due to maximum phase interrelations were achieved between the dead filler and polymer matrix base [11]. These procedures were utilized to avoid the agglomerated particles. This was happened in the general polymers like HA mixed polymer composites by the mechanical process. Then HA powders were blend into the polymer medium led to compose with uneven homogeneous materials. Therefore this method was to create the effective technique to compose the uniform dispersion between the PCL and silicate based glass composites [9-12]. And other combinations are Poly(propylene-fumarate) and HA composites, polyacrylic acid ,and PCL/HA composites [14].

The structure and morphology characteristics of PMMA, hydroxyethyl methacrylate and HA processed composites are examined in this work. The mechanical characteristics of the composites are measured in the combinational mode of compression and flexural with utilizing stimulating body fluid.

2. Materials and Methods

The materials like dibenzoyl peroxide, 3-(trimethoxysilyl) propyl methacrylate, calcium nitrate tetrahydrate and HEMA are purchased with Sigma–Aldrich. Also, this consultant afforded the Tris buffer $(CH_2OH)_3CNH_2$, KCl, NaCl, HCl, $MgCl_2{\cdot}6H_2O$, $K_2HPO_4{\cdot}3H_2O$, Na_2SO_4 and $NaHCO_3$, respectively. The supplier of Jet Ki which is based on dental fabrication also offers the PMMA-based powder. The ethanol, ammonium hydroxide (NH_4OH) and ammonium phosphate ($(NH_4)_2HPO_4$) are purchased from the suppliers of Carlo Erba.

2.1. Production of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$

Table 1 displays the formation of HA with the influence of reagents $(NH_4)2HPO_4$ and $Ca(NO_3)_2.H_2O$, respectively. These reagents were liquefied in the HEMA solution and purified water to formulate a 0.259 M and 0.156 M, solutions. Then $(NH_4)2HPO_4$ at 50 mL concentrations were approached to the 50 mL of $Ca(NO_3)_2$ with maintaining of a 1 hr time period. Similarly, the HEMA medium was stirred at a temperature of 65 degree Celsius. For 1 hour, vigorous stirring was continued, and the pH was checked and balanced at 9 with the addition of NH_4OH [6].

The stability was enhanced in the suspension process, therefore 0.025 mmol of silane was coupled with MSDS to form the HA surface. The processed particles with the evaluation of surface layer were presented in Table 1. These processed particle were composed the enhanced compatibility among the inorganic and organic particles and also protect the precipitation of HA [8]. The following processes like filtration, centrifuge and cleaning are utilized to formulate the HA powder then the dried powders were measured to confirm the 100 percent for yielding [9].

Table 1. Synthesized powders with specific properties of HA.

Code	**$(NH_4)_2HPO_4$ (g)**	**$Ca(NO_3)_2.H_2O$ (g)**	**Theoretical HA (g)**	**Obtained HA (g)**	**HEMA (g)**	**HA content in HEMA (phr)**	**SSA (m_2/g)**
HA	4.12	12.22	5.20	5.47	50	10.94	117.02

To describe the HA powder, the solution was filtered, and the powder was centrifuged and washed with ethanol to remove the organic solvent. After drying, the powder was precisely weighed to ensure that the reaction yield was 100 percent [9].

2.2. Nanocomposites preparation

There are two various weight fractions like 1:2 and 1:3 for blend the PMMA to the suspensions of HEMA and HA with maintaining of 1 phr (BPO) and stirring condition by the influence of radical designer. The unreinforced PMMA and HEMA specimens were composed without HA for the reference matrix. Table 2 showed the processed formulate composites. The thermal and post curing process was accumulated in the matrixes at 60 and 100 degree Celsius for maintain the 1 hr.

Table 2. Composition of the proposed samples.

Code	**PMMA/HEMA weight ratio**	**HA in PMMA-HEMA matrix (phr)**
1:2 HA	0.5	7
1:3 HA	0.33	8

2.3. Powder and composites characterization

PRO diffractometer from the X pert was utilized to carry out the structural behavior for the dried particles. It is employed with 10–90 2theta range along with the action of 1 sec and 0.01 degree for step time and dimensions [10]. A transmission electron microscope (TEM) was utilized to examine the characterization of particles with the dispersion of n-butanol along with dried powders. Then the outcomes were approached with a coating of 200 mesh grid of copper from the support of 30-60 nm thickness of the film and then dried. From the manufacturer of Gemini 2360 of teller, Emmett and brunauer was used to measure the specific powder with the conditioning of 150 degree Celsius vacuum. And also confirm the quality of the silane agent for the formation of HA suspension [11-15].

The processed powders were employed to FT-IR experiments to measure the existence of particles with organic groups. To investigate the overall reflectance mode was influenced with avatar 330 spectrometer. Finally, the overall 64 scans were conducted with their resolutions of 1 cm.

The processed HA particles were further analyzed with thermogravimetry and differential thermal analysis for maintaining the specific heat rate 20 degree Celsius under the one minute for free air with 1000 degree Celsius.

From the influence of HA based nano filler with processed glass transition temperature by the calculating devices of differential scanning calorimetry (DSC). Then the scanning rate was employed with 25 degree Celsius for one minute by the step increasing 0 to 200 degree Celsius. The glass transition temperature ranges were observed by the thermograms' energy jump [16]. The maximum level of HA with 1:3 ratio samples were performed to conduct the compression and 3 point bending experiments by the universal testing machine and flexural for dynamometer of Instron 5500 series [17-19]. The dynamic mechanical thermal analysis test was conducted on the processed samples to calculate the modulus and Tan with maintain 30 to 150 degree Celsius by utilizing of DMA Q800 TA instrument.

The ISO 4049 standard protocol for the flexural strength test was used to conduct the three-point bending test. Five prismatic samples with the dimensions of 25 X 2 X 2 mm were prepared. Just before the measurement, the prepared samples were kept in under water at 37 degree Celsius and 24 hours sustain period. Until fractured samples employed with a 0.75 mm/min rotation is utilized.

As per the ISO 604 standards compression experiments samples were prepared and the dimensions are 10X10X4 mm3. Then the prepared specimens are kept back under the water for maintaining the 37 degree Celsius. The 2mm/min cross head speed was utilized for the testing the samples [20].

The tiny prismatic samples were employed for water sorption experiments and then dipped into desiccators for 37 degree Celsius with maintain of mass (m_1). The wet mass (m_2) was determined by soaked dried samples with sustain of 7 days at 37 degree Celsius. Similarly, those samples were achieved at dry constant mass (m_3).

The water sorption value was calculated by the formula:

$$W_{sp} = \frac{m_2 - m_3}{V} \qquad (1)$$

Where W_{sp} is the water sorption value in μg·mm−3 and V the sample volume.

The bioactivity of the nanocomposites was assessed using in vitro assays. The SBF is a protein-free, pH 7.4 aqueous medium along with inorganic ion elements extremely close to a human being blood plasma, generated in the laboratory using a process devised by Kokubo and Takadama and used by many other writers. Proper amounts of the reagents NaCl, $MgCl_2•6H_2O$, $NaHCO_3$, tris(hydroxymethyl) aminomethane, $CaCl_2$, KCl, Na_2SO_4, $K_2HPO_4•3H_2O$ were dissolved in deionized water. The support of a magnetic stirrer, the buffered solution was sustained at pH 7.4 with 36.5 degrees Celsius and the addition of 1 M HCl and tris [24].

The samples were machined into required dimensions of rectangular shapes and submerged in SBF medium with an SBF/material proportion of 255 ml/g in a revolving incubator at body temperature (37 °C). The samples were examined for 1 hour, 24 hours, 7 and 28 days, with the SBF solution. Then keep changing for 3–4 days in the situation of 7 and 28 days to better simulate the material's in vivo behavior in vitro. Finally each process, the samples were withdrawn from the SBF solution, rinsed with deionized water, and dried at room temperature. The EDS and SEM were utilized to examine the apatite layer on the process samples with the immersion of SBF.

An FT-IR machine from German made of Nicolet 6700 was used to do infrared spectroscopy analysis in ATR. The investigations were carried out in the following sequences among the 4000 and 530 cm1 of spectral arrange. And the resolution has maintained 32 scans with 4 cm. Finally, the crystallized phase on the processed surface was determined [25].

3. Results and Discussion

3.1. Nanocomposites characterization

The inclusion of HA had a substantial impact on the glass transition temperatures with a considerable rise in the nanocomposites compared to the particular non-filled matrix for HA deliberations. The stiffening consequences were caused by relations among the filler and polymer matrix at the interfacial surfaces is responsible for this result (Fig1).

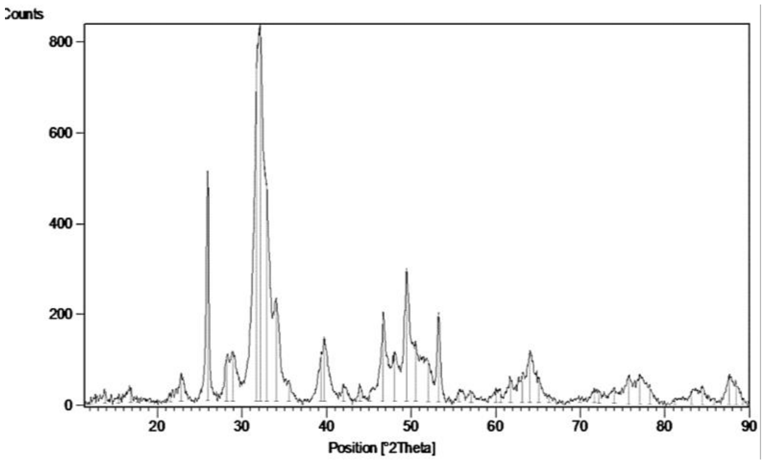

Fig. 1 HA with XRD

From figure 2 of DMTA data, the following mechanical attributes like tan, loss and strength modulus were derived for analyzing the influence of HA with nanocomposites. The increasing the content of stiff fillers on the polymer matrix reduces the movements of the polymer chain led to diminished damping and then Tg values are migrated to elevated temperatures. The tan, loss and strength modulus were determined with DMTA in the zones of elastic and rubbery [21].

The modulus was not matched with a general pattern with lesser temperature which is under the estimation of Tg. The respective sample is a 1:2 ratio of HA. The 1:3 ratio sample with unfilled was greater than the 1:3 filled sample. Then the composites samples procure the maximum modulus with the elastic region and it’s improved by the 10 times of non-processed samples.

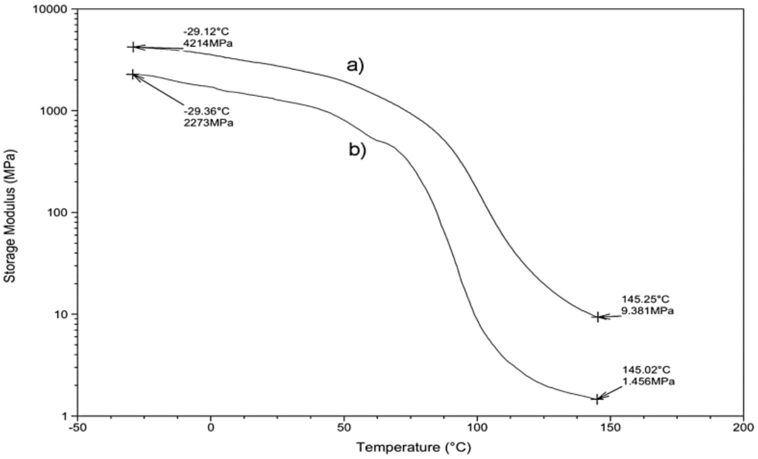

Fig. 2. Processed and Unprocessed HA with modulus

Even at high temperatures, lesser values of Tan in the processed composites were detected, showing that the nanocomposites had less damping with the interface between the packing and the polymer matrix. Considering that the modulus value in the zone of rubbery is mostly controlled with the support of cross-linking. Then improved later with the occurrence of in situ process which is formed by the HA. These processed HA also improves the cross-linking mechanisms.

The flexural modulus with their nanocomposites was increased by 52 percent in the flexural test, which is to be expected given that deformations in the composites are substantially hindered by the nanofiller. However, before reaching the yield point, the nanocomposite fractured and had a decreased flexural strength, which was most likely owing to the existence of mass agglomeration that acted as stress behavior. The solidity modulus of the nanocomposite was over 12 percent higher than that of the unfilled in the compression testing [24, 30].

The glass transition temperature exhibits strength modulus, tan and loss factor for the glass transition temperature. The modulus was not matched with a general pattern with lesser temperature which is under the estimation of Tg. The respective sample is 1:2 ratio of HA. The 1:3 ratio sample unfilled was greater than the 1:3 filled sample. Then the composites samples procure the maximum modulus with the elastic region and it's improved by the 10 times of non-processed samples. Even at high temperatures, lesser values of Tan in the processed composites were detected, showing that the nanocomposites had less damping owing to the crossing point among the stuffing and the polymer matrix [27, 31].

Mooney's empirical equation was utilized to investigate the relationship between expandable strength of modulus in processed polymers and nanoparticle addition predicted the composite material models.

$$\frac{Ec}{Em} = \exp(2.5vp+0.407[p-1]^{1.508} Vp) \qquad (2)$$

The Young's modulus of composite and matrix are Ec and EM. The Vp, p and s are particle weight percentage, ratio of the filer and grouping factor. The 1.35 and 1.91 was represents the lesser which is derived from the minimum dense factor and the sphere factors are ($s = 3\sqrt{2}/\pi \cong 1.35$).

In terms of bioactivity, SEM micrographs demonstrate that immersion in SBF processing after one hr. The processed and non-processed composites samples had without HA indication which is deposited on the surface. The samples were processed at two ratios like 1:2 and 1:3 form of HA to form the required apatite dimensions after the one-day process as shown in Fig.3.

The high degree of bioactivity is linked to a very less period of time required for dispersed apatite surface layer. It is formed from the high specific regions which are severely fine adsorption in the human teeth and bone for the hexagonal nano polycrystal nanorods. Also, it's presented in the OH groups of HEMA and it presents higher absorbance of materials [28-29].

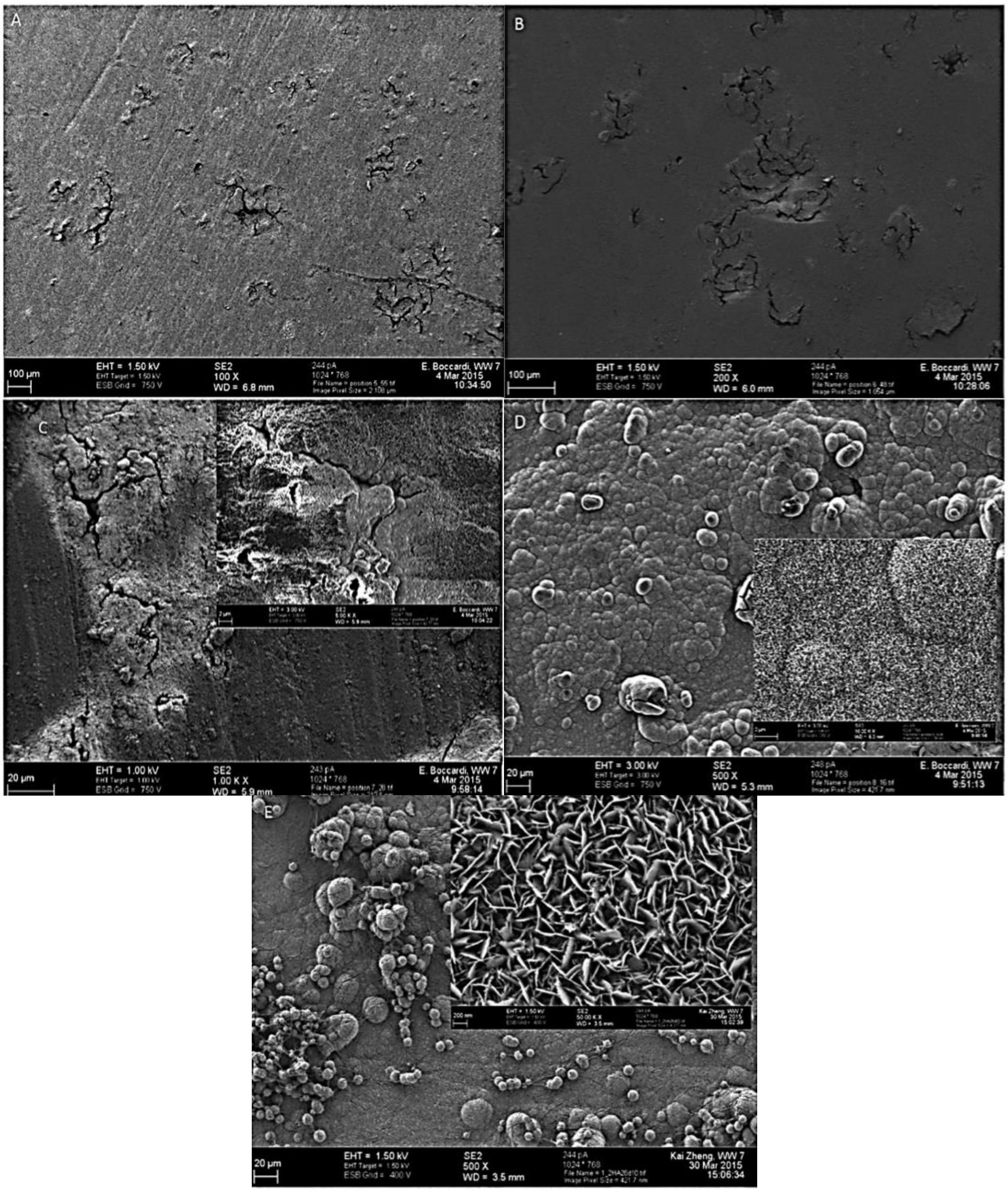

Fig. 3. SEM images of treated HA samples with various working hours (1hr, 24hr, 7 days and 28 days)

Both the processed composite specimens were nearly fully protected by the layer of apatite after immersion for 7 days. But no initiated deposit was not determined in the unprocessed sample. Whereas, total protected processed nano composites surfaces were measured under the 28 days of treatment. The generated layer was identified as hydroxyapatite by EDS analysis, which revealed that it is composed of Ca and P by the presence of an atomic ratio 1.43 to 1.5. And it is similar to the ratio of Ca/P in the natural hydroxyapatite.

The XRD patterns revealed the HA presence with crystallization on the processed sample surface. The lighter peak deflections were observed in the HA-processed nanoparticles in the polymer matrix. From the untreated samples, maximum proportions of amorphous phases are accumulated in improving soaking process in SBF and it is independent for the HEMA and PMMA ratio. At the same time the crystalline hydroxyapatite phases are presented in the increased amount and it was performed with after 28 days treatment as shown in Fig.4.

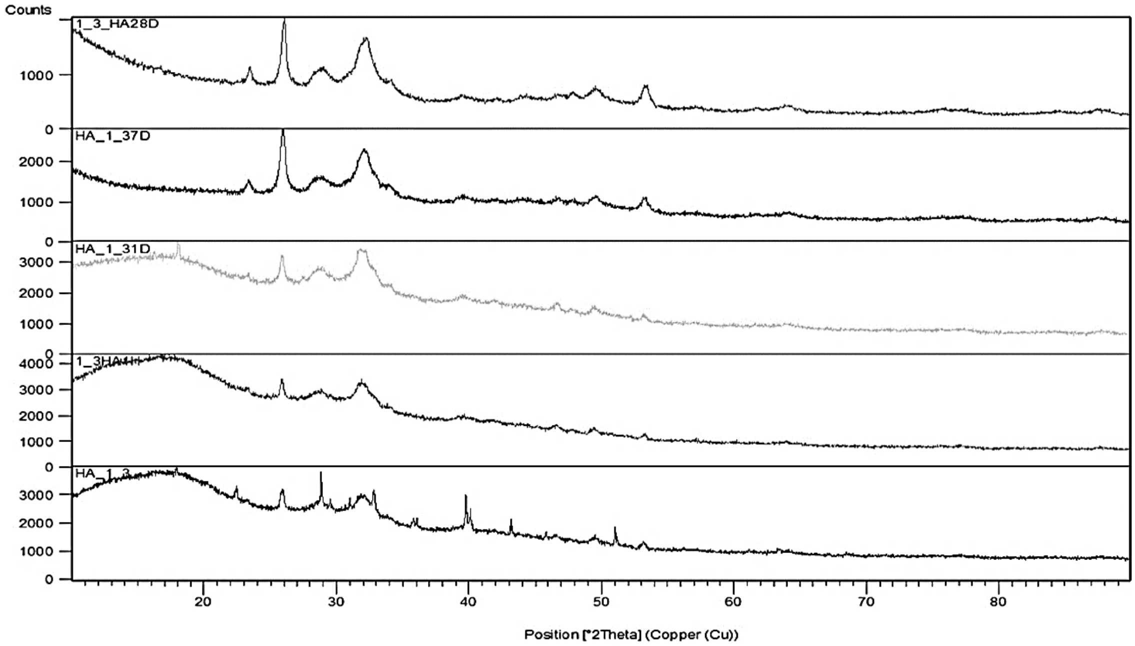

Fig. 4. XRD patterns of 1:3 HA after SBF treatment from the bottom: untreated and after 1 h, 24 h, 7 d, 28 d.

4. Conclusion

The reactive suspension approach was used to effectively create, PHEMA and PMMA polymer combinations were mixed properly by the influence of hydroxyapatite filler. Co-precipitation of nanosized hydroxyapatite was generated by the occurrence of hydroxyethyl methacrylate and which act as the primary solution. The mechanical compounds for the succeeding polymerization process were utilized to produce hydroxyapatite in situ. The synthesis of hydroxyapatite was verified by FT-IR and XRD analyses, and TEM micrographs demonstrated that the attained ratios from 5 to 7 in HA nanorods were generated.

In comparison to the unfilled polymer blends, DSC research revealed a substantial rise in glass transition temperature in the processed samples. The existence of cross-linking spots owing to in situ produced particles was seen in the nanocomposite. At room temperature, an enhanced elastic modulus was attained in mechanical experiments. The addition of hydroxyapatite reduces the water sorption values significantly. Finally, in the composites composed by in situ process of hydroxyapatite, excellent bioactivity findings were also observed.

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