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Synthesis and Structural characterization of hydroxyapatite nanorods: Antibacterial activity and dermal irritation

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Abstract

Hydroxyapatite (HA) nanoparticles with homogeneous rod morphology were successfully synthesized by the hydrothermal method. The use of cationic and anionic surfactants (cetyltrimethylammonium bromide, CTAB, and sodium dodecyl sulfate, SDS) does not change the morphology, but the length/diameter ratio of HA nanorods did. The structural characterizations revealed well-crystallized samples in the hexagonal phase. The HA samples with a smaller Ca/P ratio presented larger crystalline size. The CTAB- sample present antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus anginosus*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Klebsiella pneumoniae* suggesting that the bacteria nature and surface charge of nanoparticles affect the susceptibility.

Keywords: hydroxyapatite, Nano-rods, antibacterial activity.

1. Introduction

Biomaterials are of great relevance because they are used in different applications such as; soft tissue repairs [1], bioactive coatings [2], column chromatography for rapid fractionation of biomolecules [3], and delivery systems [4,5]. In particular, delivery systems include the delivery of growth antibiotics [6], anticancer drugs [7], enzymes [8] and antigens for slow release vaccination. One of the most studied biomaterials is the hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$), a calcium phosphate [9], due to its chemical composition similarity to the inorganic part of the human bone. As a result, this material presents very high biocompatibility. The biocompatibility refers to the harmony with surrounding tissues of the implanted prosthesis, to fulfill this requirement, toxicity, allergy, inflammation, corrosion, and mechanical failure must be avoided. In this vein, the design of materials for controlling these properties has been a relevant

issue in the last years [10]. Beside hydroxyapatite, there are other elements that have been studied for biomedical purposes. For instance, Magnesium and Titanium have shown good mechanical properties but have failed in compatibility and toxicity [7,11]. Hydroxyapatite (HA) is the most stable ceramic under physiological conditions [12]. However, increasing evidence has suggested some biological effects that require further studies to avoid potential risks to human health. Minimal toxicity is essential for the successful development of pharmaceutical, cosmetic and tissue engineering. Therefore, considerable efforts have been placed on studying the inflammatory response and potential toxicity to cells, tissues, and organisms [11].

On the other hand, the microbial pollution produced by microorganisms has contributed to the problems in industry and in other vital fields. In order to solve these problems, new

antibacterial materials have been demanded and studied. Ceramic powders as zinc oxide [13], magnesium oxide [14], copper oxide [15] and calcium phosphates [16] have presented antibacterial activity. Nowadays, the use of ceramic materials in this area has presented a lot of advantages because of their low toxicity. In fact, Ag-doped ceramics have demonstrated being a long-lasting biocide with high thermal stability and low volatility [17]. Ceramics as hydroxyapatite also present the absorption capacity of proteins, amino acids, and other organic substances which favors the adsorption and antibacterial activity against *E. coli* [18].

According to the literature, *Staphylococcus aureus* (*S. aureus*) is the most common organism which causes nosocomial infections and frequently postsurgical wound infections [19], it is a successful pathogen because of a combination of bacterial immuno-evasive strategies. On the other hand, *Pseudomonas aeruginosa* (*P. aeruginosa*) has also become an important pathogen in nosocomial infection [20,21].

According to ISO 10993-10, irritation tests are used to evaluate the irritation potential of medical devices, biomaterials or their extracts exposed to the eyes, skin or mucous membranes. The tests are performed using an appropriate model and duration of exposure. Byeong k. *et al.* [22] evaluated in rabbits the dermal irritation caused by hydroxyapatite scaffolds, using a subcutaneous injection method and an exposure of 24, 48 and 72 hours showed that there was no significant biological reaction, which concludes his research in promoting the viability of a bone substitute HA.

The design of HA particles with different purposes has been possible by using different synthesis methods. In fact, for obtaining specific morphologies and particle sizes wet chemical methods have been used [23]. Among them, the hydrothermal method, it is considered as a promising approach for large-scale and low-cost production of HA particles, it presents many advantages such as; chemical homogeneity, production of large

crystals with high quality, catalyst-free, inexpensive surfactants [24]. Then, the hydrothermal method is an effective way to obtain single-crystal HA nanorods with narrow-size distribution in diameter and length. The addition of surfactants enhances the nucleation of HA. Second, surfactants could also inhibit the nanorods agglomeration because of the modification of particle surface charge. Surface active agents (surfactants) play an important role in aqueous solution because of their lyophobic and lyophilic group, they are widely used in different industries and laboratories for getting nanoparticles with tunable morphology and different particle size [25]. In this context, different experiments have carried out to elucidate the pathways of morphological transitions. Although surfactants are mainly used in the control of crystallization and morphology, its mechanism is not well-known.

By reducing the HA particles sizes to nano-scale size, HA can be used as a potential material in nano-medicine [26]. HA nanoparticles with nanorods, nanowires, nanotubes morphology have shown excellent biocompatibility [27]. HA nanoparticles can retard the multiplication of cancer cells and be used as an efficient drug delivery agent [28].

In addition to the morphology, the crystalline structure of hydroxyapatite should be considered, since the bioactivity of diverse HA crystals has led to different applications [29]. Yingjun W. *et al.* [30] synthesized HA nanoparticles by low-temperature hydrothermal with uniform morphologies and controllable size. Cationic surfactant, cetyl trimethyl ammonium bromide (CTAB), has been used to regulate the nucleation and crystal growth. Yan L. *et al.* [31] obtained at room temperature fibrous polycrystals of HA and after hydrothermal treatment, nanorods 150 nm in length and 10 nm in diameter using an anionic surfactant, sodium dodecyl sulfate (SDS).

In the present study, HA powders were obtained using CTAB and SDS as cationic and anionic surfactants, respectively. The

powders were studied using structural and morphological characterizations (XRD, FT-IR, Raman spectroscopy, and scanning electron microscopy). The antibacterial properties and dermal irritation of HA were investigated.

Experimental

2.1 Hydroxyapatite synthesis

In the HA syntheses, commercial grade of calcium nitrate hexahydrate $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$ (Mallinckrodt 99%), Di-ammonium and hydrogen phosphate $(\text{NH}_4)_2\text{HPO}_4$, (Baker Analyzed 98.7%) were used in the hydrothermal method as starting materials. Cetyltrimethylammonium bromide (CTAB, ACS reagent, $\geq 99.0\%$) and sodium dodecyl sulfate (SDS, ReagentPlus®, $\geq 98.5\%$) were used as surfactants. $(\text{NH}_4)_2\text{HPO}_4$ solution at 0.5 M was added dropwise to $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$ solution at 0.5 M, during the addition the Ca solution was kept under stirring at 50 °C. Then, the final solution was adjusted at pH=11, using NaOH solution (1.5 M). Subsequently, the final solution was poured into Teflon® lined autoclave at 200 °C for 15 h. The powders obtained were washed with distilled water using a centrifuge (10000 rpm, 15 min. each wash). Finally, these powders were dried in an oven at 120 °C for 8 hrs.

2.2 Characterization techniques

The X-ray diffraction patterns were recorded at room temperature on a Bruker d8 advance eco diffractometer using Cu K α radiation ($\lambda=1.54184$ Å). To determine the crystalline phase of the samples, a 2θ scan ranging from 10 to 60° was used. Infrared spectra were obtained at room temperature with a spectrum two spectrophotometer. The spectrum was recorded at 32 scans in the 4000–450 cm^{-1} range. The SEM micrographs were recorded using a JOEL scanning electron microscope model JSM-78000F operating under high vacuum and low accelerating voltage 5 kV. The tests were performed on a TA

instruments model SDT Q600 from 25 to 1400 °C with a heating ramp of 25 °C/min.

2.3 antibacterial activity

The antibacterial activity of the HA was tested on six bacteria pathogens, three of them Gram-negative; *Pseudomonas aeruginosa* CDBB-B-1011, *Escherichia coli* CDBB-B-1000 and *Streptococcus anginosus* CDBB-B-1311 and three Gram-positive; *Staphylococcus aureus* CDBB-B-1304, *Micrococcus luteus* ATCC 10240, and *Klebsiella pneumoniae* ATCC10031, which were obtained from the National Collection of Microbial Strains and Cell Cultures at the Research Center for Advanced Studies (CINVESTAV-IPN), Mexico.

Before the antibacterial activity test, the strains were cultivated on plates with nutritive agar and incubated 35 ± 1 °C for 24 hours. The colonies were re-suspended in sterile distilled water until obtaining 0.125 of turbidity (McFarland turbidity standard) corresponding to 1×10^8 CFU/mL. The antibacterial activity was determined by the Kirby-Bauer Test [32]. Three sensitive discs of 6 mm in diameter were placed equidistant to each other in the three plates. A control (plate with culture medium only) and two controls; positive and negative. The positive one consisted of a plate with a culture medium, bacterial inoculum and antibiotic in commercial sensitive disc (tetracycline) and the negative control only containing bacterial inoculum and the culture medium.

Three samples of Hap (without surfactant), SDS- (synthesized using 2.5 mol% of SDS) and CTAB- (synthesized using 2.5 mol% of CTAB) were evaluated. For this, sensitive discs were introduced one minute into 0.5 mg/mL solution which contains hydroxyapatite (one solution per sample).

2.3 Dermal irritation procedure.

Young male New Zealand rabbits in the 1.8 – 2.2 kg range were employed. These rabbits were monitored for 14 days at ambient

temperature and humidity. After this period, rabbits with good health were selected for the dermal irritation assay. Commercial feed and water were supplied *ad libitum* during the study. The animals were kept alone in a well-identified stainless steel cage. Rabbits were shaved in the dorsal region (6 cm x 12 cm) using an electric machine.

An amount of 0.5 mg of hydroxyapatite powders was dispersed in one mL of sterile physiological saline solution. Before inoculation, the shaved area was disinfected using alcohol. In the upper region, on the right and left side, an adjuvant (Casein 3.5 g/100 mL and Lactose 5 g/100 mL) was administered (positive control). In the shaved central region, the hydroxyapatite solution was applied and the physiological saline solution (negative control). One mL of HA solution, negative or positive control was used for the intradermal inoculation. The presence of erythema, edema, induration or necrosis was evaluated at 1, 24, 48 and 72 hours after inoculation.

Laws and institutional guidelines were considered according to the Mexican official norms to carry out the experimentation, transportation, and care of the animals in this study. NOM-051-Z00-1995 (policy for humanity in animal treatment and movement), NOM-087-ECOL-1995 (Requisites for the separation, packaging, storage, collection, transportation, treatment, and final disposal of biological infectious hazardous waste generated in establishments providing medical care) and NOM-062-Z00-1999 (Technical specifications for the production, care and use of laboratory animals).

2. Results and discussion

3.1 Structural and morphological characterization

Figure 1 presents the ATR-FTIR spectra of the HA samples. All of the infrared spectra among the HA obtained with and without surfactants presented high similarity. Absorption bands

corresponding to the surfactants were not found, thus they were removed effectively in the washing stage. The broad FT-IR band centered approximately at 1091 cm^{-1} corresponds to the asymmetric stretch vibration mode of the PO_4 group. The band at 550 cm^{-1} corresponds to the symmetric P-O stretching vibration of the PO_4 group. The band corresponding to the OH-group located at 630 cm^{-1} is related to the absorbed water [33,34].

The absorption bands situated around 1406 and 1460 cm^{-1} corresponding to CO_3 , these bands are normally present for non stoichiometric natural and synthetic HA. Mengmeng *et al.* [35] suggested that the hydroxyl ions quantity in the HA network reduces as the carbonate content increases. This phenomenon changes the Ca/P ratio affecting the final properties of the ceramic.

Figure 1 ATR-FTIR spectra of HA, CTAB-, CTAB+, SDS- and SDS+ samples.

Figure 2 displays the X-Ray Diffraction (XRD) patterns of the HA samples. All of the samples crystallized with hexagonal structure (ICDD 74-0565). Thus, surfactants used during the synthesis did not change the crystal structure, however, there is a clear difference in the main diffraction peaks intensities. As matter of fact, the ratio of the peaks corresponding to (002) and (112) planes located at $2\theta = 25.89$ and 32.197° changed as a

function of surfactant concentration and type due to the sample crystal orientation.

Figure 2 XRD patterns HA powders synthesized using surfactant.

The crystallite sizes of the HA powders showing in Figure 2 were determined by using the Scherer equation [36].

$$L = \frac{K \lambda}{\beta \cos \theta}$$

where L is the average crystallite size, λ is the X-ray wavelength in nanometer (nm), β is the peak width of the diffraction peak profile at half maximum height resulting in radians and K is a constant related to particle shape, normally taken as 0.9 [37].

Figure 3 shows that crystallite sizes increase as a function of surfactant concentration. It suggests that small quantities of

CTAB or SDS can affect the crystallite size of HA. Similar finding was reported by Yang *et al.* [38]. They found that average crystallite sizes of luminescent phosphates synthesized by precipitation method increased when the PEG20000 concentration increased. Crystallite size normally reveals the crystallization degree, since larger crystallite sizes are normally associated with better-crystallized powders.

Figure 3 Apparent crystallite sizes of HA samples.

3.2 Morphological studies

The scanning electron microscope is an important structural characterization method for studying powders morphology evolution [25]. Figure 4 shows the SEM images of HA powders. Powders morphology mainly consists of rods morphology with lengths ranging from 100–300 nm in length and 10–20 nm in diameter. Figure 4 depicts that the morphology of HA powders did not change when surfactants used. Although surfactants did not modify the HA morphology, the length/diameter ratio of HA nanorods did.

It is worth noting that crystallite size increases when surfactants were used in the HA synthesis (Figure 3). On the other hand, SDS and CTAB modified the solubility and viscosity during reaction, as a consequence the length/diameter ratio of HA powders were changed slightly. Zhao *et al.* [39] found that the crystalline calcium carbonate formation was due to a preferential aggregation mechanism because of surfactant interaction with starting nanoparticles. On the other hand, Sanosh *et al.* [40] proposed that the OH concentration determines the growth rate and morphology of particles. In this study, the Ca^{2+} and PO_4^{3-} movement during nucleation was restricted due to the pH (11). As a result, the use of the surfactants did not affect the morphology. Nevertheless, using different surfactants at different concentrations becomes very important to understand the HA nucleation.

To strengthen the structural information of HA samples, an Electron Dispersive Spectroscopy (EDS) analysis was carried out. The average quantification of the Ca/P ratio in different zones of the HA samples revealed a small difference among the samples. Thus, the solubility of Ca and P was slightly affected. The CTAB+ and SDS- samples with a smaller Ca/P ratio presented larger crystalline size than their counterparts. Since the Ca/P ratio of these samples is within the 1.67–1.33 range, the samples crystallized in the hexagonal structure of HA.

Figure 4 SEM micrographs of HA, CTAB-, SDS-, CTAB+, SDS+ powders.

SDS- and CTAB+ have a very close composition to tricalcium phosphate (TCP), which has a Ca/P ratio of 1.5. Therefore, these samples are calcium-deficient hydroxyapatite. On the other hand, the larger rods were formed when 2.5 mol% of CTAB was used (CTAB- sample). The shorter rods were obtained using 2.5 mol% of SDS in the HA synthesis (SDS- sample). The L/D ratios of the samples were 3.45, 4.49, 3.1, 2.85, 2.81, for HA, CTAB-, CTAB+, SDS- and SDS+, respectively. These results demonstrate that the cationic surfactant increases the L/D ratio while anionic surfactant reduces it. Han S. *et al.* [41] reported that at above the critical micelle concentration (CMC), there is a micelles transition from spherical to rod-like. The micelles size increases with the increase of CTAB concentration resulting in longer rods of silica.

Figure 5 Size distribution diameter and length of HA samples

The HA samples were evaluated by thermogravimetric and differential thermal analyses (TGA/DTA, see Figure 6). The weight loss of the HA powders is listed in table 1. In the TGA curve, there is weight loss of HA around 25–200 °C (region I) corresponding to adsorbed water on the surface and pores. This process is associated with an exothermic peak in the DTA curve around 200 °C and is present in almost all HA samples. For SDS- an evident shift is shown (around 400 °C), this behavior can be associated with a crystallization degree since this sampled have a lager crystallite size.

The weight loss in the region II (200–700 °C) is ascribed to water molecules chemically adsorbed, this process is also exothermic. In this temperature range, the impurities associated with SDS and CTAB, if existed, could be removed. The weight loss in the region III (700–1200 °C) can be associated with the HA transformation into tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ (β -TCP).

Figure 6 Thermogravimetric (TGA) of HA powders

Zou *et al.* [42] proposed that the synthetic HA with a 1.67 of Ca/P ratio loses its OH- groups gradually around 1200 °C, leading HA into oxyapatite $\text{Ca}_{10}(\text{PO}_4)_6\text{O}$, this change is evidenced by a weight loss in Figure 6.

Figure 7 DTA curve of HA powders

The endothermic peak around 800 °C is associated with HA transformation into tricalcium phosphate. The exothermic peak at 1000 °C is caused by CaO formation. Finally, an exothermic peak around 1100 °C corresponds to hydroxyoxyapatite transformation into tricalcium phosphate. At the end of the DSC

curve, the slope varies among the synthesis conditions. Samples synthesized with CTAB the reaction is slower while samples synthesized with SDS faster. The difference between the samples can be associated with the surface charge of powders promoting by the surfactant type. Cihlar *et al.* [43] pointed that HA decomposition promoted by heat treatment, includes two steps: dehydroxylation producing oxyapatite $\text{Ca}_{10}(\text{PO}_4)_6\text{O}$ (OA) after the appearance of hydroxyoxyapatite $\text{Ca}_{10}(\text{PO}_4)_6\text{O}(\text{OH})_2$ (OHA) as a transition product, resulting calcium phosphates.

Table 1 Weight loss in thermogravimetric analysis

Zone	HA	CTAB-	CTAB+	SDS-	SDS+
A	3.80	4.00	3.57	5.17	3.55
B	1.99	2.28	0.79	2.83	2.72
C	1.13	0.42	0.39	0.38	0.13
Total	6.92	6.70	4.75	8.38	6.40

The HA, SDS- and CTAB- samples presented the better thermal stability. Thus, these samples are promising samples to perform the bacterial sensitivity analyzes and dermal irritation assays.

Raman spectroscopy provides complementary information to the FT-IR and XRD analyses since it describes variations in the mineral crystallinity. Figure 7 shows the Raman spectra of the HA samples. Raman dispersion bands, associated with PO_4^{3-} frequencies, can be observed. The reported values of the normal mode frequencies of the PO_4^{3-} tetrahedron, obtained from the Raman dispersion measurements were identified; $\nu_1 = 961 \text{ cm}^{-1}$, $\nu_2 = 430 \text{ cm}^{-1}$, $\nu_3 = 1039 \text{ cm}^{-1}$ and $\nu_4 = 570 \text{ cm}^{-1}$.

Figure 8 Raman spectra of HA powders.

In crystals, such as HA, the crystalline field induces distortions in the PO_4^{3-} , which change the lengths and angles of intra-tetrahedral bond. Consequently, the intensity and length of the bands depend on the structure and crystallinity of each sample. Aza *et al.* [44] conclude that the different order in the crystalline structure, is differences in crystallinity reflected in different intensities of the bands in the spectrum.

3.2 Antibacterial activity

The antibacterial activity of HA SDS- and CTB- against positive and negative gram bacteria was evaluated using the diameter of the inhibition zone (DIZ), in the disk diffusion assay. DIZ reflects the magnitude of the microorganism susceptibility [45]. Susceptible strains exhibit a larger diameter of inhibition zone, resistant strains smaller diameter of inhibition zone [46]. Only CTAB- did present antibacterial activity against all the tested bacteria. These results suggest that the bacteria nature and surface charge of nanoparticles affect the susceptibility since it is well known that surfactant not only affects morphology but also the particle's charge. In fact, due to the surface charge promoting by SDS-, it presented antibacterial activity against *P. aeruginosa*. These results elucidate the

possibility of changing the antibacterial activity by using surfactants even if the powders morphology does not change. On the other hand, many studies have explained antibacterial tests, some of them reported that the ions present in the nanoparticles react with the HS groups of the proteins and play a vital role in the inactivation of bacteria [47]. Other studies state that peptidoglycan and lipopolysaccharides, in the cell wall of Gram-negative bacteria, are affected by synthetic chemical compounds of nanoparticles. Thus, the cell wall permeability changes, which promotes a respiratory malfunction, and culminates in cell death [48]. Therefore, by changing the surface charge of particles, it is possible modifying the nanoparticles' properties. Moreover, in a nano-sized scale, the study of bacteria-nanoparticles interaction is very important because of the surface-volume ratio.

Figure 9. Inhibition zone diameter of HA powders a) Gram-positive and b) Gram-negative.

On the other hand, some studies have reported that nanoparticles decouple the respiratory electron transport chain (ETC) of oxidative phosphorylation. The latter inhibits enzymes in the respiratory chain and interferes with the membrane permeability to phosphate and protons [49]. Since antibacterial activity mechanisms have not fully elucidated, more studies concerning ceramic nanoparticles should be performed. In this vein, few antibacterial activity mechanisms have been described, among them, the antibacterial activity of silver nanoparticles which states free silver ion interrupts DNA replication, ATP production, reactive oxygen species formation, and damage to cell membranes [50].

3.3 Dermal irritation

No clinical signs of disease were observed in rabbits during the study, neither erythema nor necrosis in the inoculation sites.

The papules formed, in the inoculated dermal regions with the HA solutions and the negative control, were reabsorbed in 10 minutes after inoculation. All the rabbits presented papules in the inoculation sites after one hour of the positive control administration (Table 1). At 24 hours, the size of the papule decreased in the inoculation sites of the positive control, edemas

were observed in all rabbits inoculated with Hap, SDS-, and CTAB- solutions. At 48 hours, only one rabbit treated with (HA) did not present edema. At 72 hours, four rabbits; one inoculated with HA, one with CTAB- and the others with SDS- presented small palpable nodule in the inoculation site, similar findings were reported by Carlo *et al* [51].

Table 2 Inoculation parameters after positive control administration

Sample	Rabbit	1 hour			24 hours			48 hours			72 hours		
		C+	T	C-	C+	T	C-	C+	T	C-	C+	T	C-
CTAB-	24	++++p	0	0	++p	+e	0	+p	+e	0	0	+n	0
	25	++++p	0	0	++p	++e	0	0	+e	0	0	0	0
HA	23	++++p	0	0	++p	++e	0	+p	0	0	0	0	0
	26	++++p	0	0	++p	++e	0	++p	+e	0	0	+n	0
SDS-	27	++++p	0	0	++p	+++	0	0	+e	0	0	++n	0
	28	++++p	0	0	++p	+e	0	0	+e	0	0	+n	0

p = papule, e = edema, n = small palpable nodule

The degree of edema papule and small palpable nodule of skin reaction was graded as 0, +, ++, +++, or ++++

Sepulveda *et al.* [52] evaluated synthetic hydroxyapatite HAP-91® as a dermal filler, they showed that this intradermal implant tends to encapsulate itself and do not cause signs of toxicity but initial inflammatory response only. To determine the characteristics of the formed tissue and infiltration type at the inoculation sites, further studies dealing with histology and immunology are necessary.

Conclusion

Hydroxyapatite nanopowders were successfully obtained by a surfactant-assisted hydrothermal method. By using CTAB and SDS in the HA synthesis larger crystallite size was obtained due to the solubility of precursors modifying the crystal growth. The ratio of the peaks corresponding to (002) and (112) planes located at $2\theta = 25.89$ and 32.197° changed due to the sample crystal orientation. The L/D ratios; 3.45, 4.49, 3.1, 2.85, 2.81, for HA, CTAB-, CTAB+, SDS- and SDS+, respectively demonstrate that CTAB increases the L/D ratio while SDS reduces it. Bacteria nature and surface charge of nanoparticles affect the susceptibility. Since surfactant affects the particle's charge, therefore it is possible modifying the antibacterial activity by using surfactants even if the morphology of the powders does not change. CTAB, HA, SDS caused reversible minimal edema response

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Figures

Figure 1

ATR-FTIR spectra of HA, CTAB-, CTAB+, SDS- and SDS+ samples.

Figure 2

XRD patterns HA powders synthesized using surfactant.

Figure 3

Apparent crystallite sizes of HA samples.

Figure 4

SEM micrographs of HA, CTAB-, SDS-, CTAB+, SDS+ powders.

Figure 5

Size distribution diameter and length of HA samples

Figure 6

Thermogravimetric (TGA) of HA powders

Figure 7

DTA curve of HA powders

Figure 8

Raman spectra of HA powders.

Figure 9

Inhibition zone diameter of HA powders a) Gram-positive and b) Gram-negative.