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EDTA assisted Synthesis of HA Nanoparticles and Cobalt-HA Nanocomposites

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ABSTRACT

Nano biomaterials such as polymers, ceramics and metals are widely used in bone for regenerative therapies, bone grafts and tissue engineering as well as for temporary or permanent implants to stabilize fractures and replace joints. Bioceramics are specifically developed to replace parts of living system due to its biocompatibility'. Hydroxyapatite (HA) CajQ (PO) (OHH used as a bone substitute material with stoichiometric composition i.e., Ca P(ratio) 1.67 which is similar to the mineral phase of the human bone. In this present work, (EDTA) ethylene diamine tetra acetic acid assisted as a capping agent for the synthesis of nano hydroxyapatite and cobalt substituted hydroxyapatite nanocomposites by sol gel method. The synthesized nano-HA powder and Co-HA nanocomposites are further characterized by using FTIR. XRD. SEM, EDAX and anti-bacterial activity. XRD analysis of HA Nanoparticles and Co-HA Nanocomposites, shows the strongest 20 values at 32.1, 25.91 and at 32.1, 10.1, 16.6 respectively. SEM morphology' predicts an elongated spherical morphology for HA Nanoparticies and Co-HA Nanocomposites. The elemental composition was confirmed with EDAX analysis and the FTIR spectrum indicates the functional groups of the synthesized compounds. The antibacterial activity was analyzed for both the gram-positive and the gram-negative bacteria's.

Keywords: Bioceramics. Biocompatibility, Nanocomposites, HA. EDTA Anti- bacterial.

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1. Introduction

Nanotechnology has allowed the preparation of nano structured biomaterials, giving way to the third generation of bioceramics, focused on the enhanced bioactivity and the initial physiological trace inducing an enhanced cell to respond at the molecular level in order to regenerate tissues due to their similarity to the inorganic component of human bone tissues.[1] A nanobioceramic is defined as ceramic less than 100 nm in at least one direction.[2] Nanobioceramics are highly biocompatible, stable at a physiological environment, and corrosion-resistant and have remarkable higher specific surface area and volume ratio and contain a higher quantity of grain boundaries than the conventional counterparts, offering better surface properties such as topography, energy, roughness, and wettability which potentially favour cell response.[3,4]

Hydroxyapatites are a naturally occurring mineral form of calcium apatite and also it is the hydroxyl end member of the complex apatite group, with the general formula (CasfPCUMOH). It crystallizes in the hexagonal ciystal system. The nano-HA's has outstanding properties like bio-compatibility, bioactivity, oesto conductivity, non-toxicity and non-inflammatory nature.[5] Nano sized HA NPs is more efficient in osteoblast adhesion and proliferation and improved mineralization[3] and are better for cell proliferation and induction of apoptosis in some cancer cells.[6-8] It was found that the spherical and rod-like shapes of HA NPs showed remarkably less cytotoxicity as compared with the needle and plate-like shapes.[9] Nanocomposites are materials that incorporate nano sized particles into a matrix of standard material. The result of the addition of nanocomposites, there's a drastic improvement in properties that can include mechanical strength, toughness and electrical or thermal conductivity. The effectiveness of the nanocomposites is such that the amount of material added is normally between 0.5%, 5% or 50% by weight.[10] Here, Cobalt-substituted HA shows catalytic activity for oxidative dehydrogenation of alcohols.[11] Thus, the Synthetic HA's and metal substituted HA's, frequently are used as reference materials in bio mineralization and biomaterial studies.[12] For control and reference purposes, it is important to have available pure and stoichiometric ratio of calcium and phosphate compounds or nearly stoichiometric. A number of novel processing routes for preparation, such as dry, wet, hydrothermal, sono chemical, and microwave assisted process.[13] Among these, microwave synthesis method offers several advantages including shorter synthesis time, rapid heating, acceleration of chemical reaction, narrow particle distribution, non-polluting and throughout volume heating.[14] Also, organic modifiers are used for the morphology and size controlled synthesis. In this the modifier used is ethylene diammine tetraacetic acid (EDTA - $C_{10}H_{16}N_2O_3$), a member of the polyamino carboxylic acid family of ligands, is widely used as capping agent as well as reducing agent for the synthesis of a variety of nanomaterials. The chelating agent EDTA is a compound of massive use worldwide with household and industrial applications, being one of the anthropogenic compounds.

In this context, a large amount of highly pure, homogeneous and highly crystalline HA's and Co-HA nanocomposites was synthesized by sol-gel method from aqueous solution of calcium hydroxide and ortho-phosphoric acid, along with the usage of the capping agent EDTA and then rigorously characterized by Fourier- transform infrared spectroscopy (FTIR), powder X-ray diffraction (XRD), scanning electron microscopies (SEM), and Energy-dispersive X-ray spectroscopy (EDAX). The anti-bacterial activity was also analyzed. The chemical composition and other properties of the compounds qualify it as a standard reference material.

2. EXPERIMENTAL PROCEDURE

2.1 Materials

All the chemicals; calcium hydroxide, ortho-phosphoric acid was purchased by [Merck], EDTA were purchased from [NICE] and Cobalt Nitrate Tetrahydrate from were used directly without any further purification. Ethylene diamine tetra-acetic acid (EDTA) ($C_{10}H_{16}N_2O_3$) served the purpose of capping agent or as a surfactant The pH value of the precursor powders were maintained at 12 by the ammonium solution from [NICE]. All the solutions were made up with distilled water.

2.2 Synthesis

2.2.1 EDTA Assisted to form HA Nanoparticles

It is a sol-gel method where the combination of Calcium Hydroxide [$Ca(0H)_2$]and Ortho-phosphoric acid [H_3PO_4] are used as the source of calcium and phosphate ions and also the inclusion of the surfactant EDTA (Ethylene diaminetetra-acetic acid) which also acts as a capping agent. 1M of calcium hydroxide [$Ca(OH)_2$] and 0.1M of EDTA [$C_{10}H_{16}N_2O_3$] are dissolved together in distilled water which is continuously stirred by magnetic stirrer. Meanwhile, 0.6M of ortho-phosphoric acid [H_3PO_4] is dissolved and added in drop-wise to the same solution for the formation of gelatinous white precipitate. The ratio of the mixtures Ca:P should be of 1.67. The pH should be maintained at 12 which can be achieved by the addition of ammonia solution. A continuous stirring should be maintained for 30 minutes and solution is kept aging for 24 hrs. After the aging of the precipitated solution, it is centrifuged and it is washed with distilled water to remove the impurities and filtered using whatman filter paper. This gel precipitate is dried at oven for 26 hrs and the dried precipitate is finally grounded by using mortar and pestle. Thus, EDTA assisted HA is obtained.

2.2.2 EDTA assisted to form Co- HA nanocomposites

It also follows the similar way with all the above-mentioned compounds, since it forms cobalt HA nanocomposites. Thus the main inclusion is Cobalt Nitrate Tetrahydrate [$Co(NO_3)_2\ xH_2O$]. Here, 1M is the combination of 3.7 g of cobalt ions and 3.7g of calcium ions along with 0.1M of EDTA to the distilled water. It should be noted that the ratio of (Co+ Ca) : P=1.67. As this also forms a gel precipitate which is in purple color. The change in colour is due to the addition of cobalt compound to the synthesis. This gel precipitate is dried at oven for over-night and the dried precipitate is finally grounded by using mortar and pestle. Thus, EDTA assisted HA nanocomposites are obtained.

3. RESULTS AND DISCUSSION

3.1 Structural Characterization

3.3.1 Ray Diffraction (XRD) analysis

The XRD characterizes and determines the ciystal materials in unit cell dimensions. It also measures the purity of the sample. The analysis of the control group and the synthesized product using X-ray diffraction of HA Nanoparticles shows that the synthesized product had sharper ciystal peak and 20 value showed the strongest peak at 32.1° of planes (112) and at 25.9° of plane (002). From analysis of the synthesized product using JCPDS (powder diffraction standards) CARD, the synthesized product was identified as hydroxyapatite. Similarly, for XRD of Co-HA Nanocomposites shows the strongest peak at 32.1° of planes (112) and 16.6° of planes (101).

Using Scherrer's formula, the crystallite size of the HA powder obtained was calculated by,

$$d = k\lambda / \beta \cos\theta$$

where, d is the average crystallite size of the obtained HAp, A is the wavelength of X-rays (A =0.154056 nm for CuKa radiation), β is the full-width at half-maximum intensity of reflections in radian, and θ is Bragg's diffraction angle in degrees. The average crystalline size of the sample obtained and is shown in Table 1.

3.1.2 Scanning Electron Microscope (SEM) Analysis

The SEM is widely used to identify phases based on qualitative chemical analysis and/or crystalline structure. It is also used for the precise measurement of veiy small features and objects down to 50 nm in size. The samples have elongated spherical morphology with the average particle size of about 34 nm to 55 nm for HA prepared with EDTA respectively as shown in Fig. 2. The driving force for these morphologies may be attributed to the pH and as well as the addition of capping agents. Fig.3. signifies the structure of HA nanocomposites.

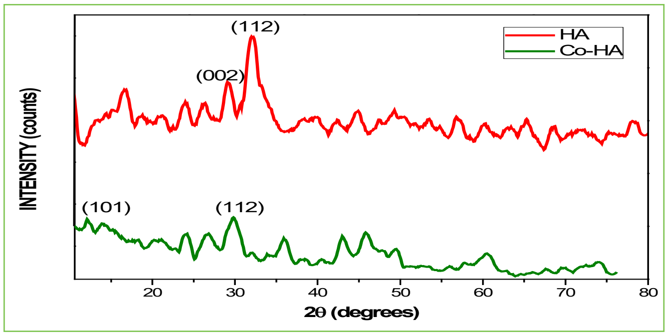

Fig. 1: XRD graph of HA and Co-HAnanocomposites

3.1.3 Energy Dispersive X- Ray Analysis (EDX)

EDX is an analytical technique used for the elemental analysis or chemical characterization of a sample. It enhances catalyst quality, poisoning, quality control raw material and end product and elemental distribution. In this analysis, it is proven that the sample is a calcium hydroxyapatite by the indication of peaks. [Fig. 4.] whereas [Fig. 5.] proves the hydroxyapatite nanocomposites by indicating corresponding hydroxyapatite peaks along with cobalt, as it all together confirms the formation of nano-composites.

3.1.4 FTIR analysis

The FTIR analysis of the sample showed the quality verification of incoming/outgoing materials and also the Microanalysis of small sections of materials to identify contaminants.[15] Here, the analysis showed the peak at 565.14 cm^{-1} referring to strong C-Br stretching of alkyl halide functional group. The broad peak at 1033.85 cm^{-1} refers to strong C-OH stretching of sulfoxide functional group. The peak at 3201.83 cm^{-1} refers to the stretching of O-H functional group and peak at 1597.06 cm^{-1} shows strong C=0 bending of amide functional group. The variable intensity peak at 2819.93 cm^{-1} shows the aldehydic functional group. Peak at 3429.43 cm^{-1} refers to amine N -H stretching functional group.

Table 1: Structural parameters of HA-nanoparticles

2θ (deg)	FWHM (deg)	B (rad)	CRYSTALLINE SIZE, D (nm)	MICRO STRAIN $\varepsilon \times 10^{-3}$m	DISLOCATION DENSITY 6 $\times 10^{-3}$m
32.1791	1.52750	16.08	5.656	6.40	0.3125
25.9139	0.92780	12.95	9.181	3.94	0.1186

Table 2: Structural parameters of Co-HA nanocomposites

2θ (deg)	FWHM (deg)	B (rad)	CRYSTALLINE SIZE, D (nm)	MICRO STRAIN $\varepsilon \times 10^{-3}$m	DISLOCATION DENSITY 6 $\times 10^{-3}$m
32.1333	2.78332	30.23	4.98	7.26	4.02
16.6000	5.33611	37.50	3.90	9.27	6.56

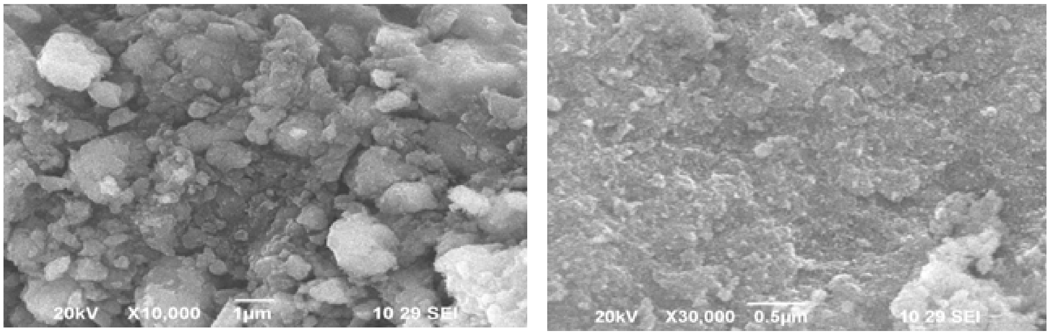

Fig. 2: SEM images of EDTA-HA nanoparticles

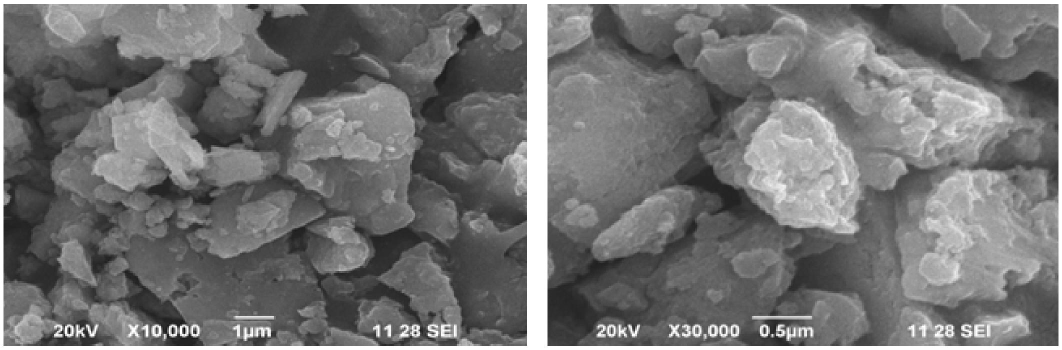

Fig. 3: SEM images of EDTA-(Co-HA) nanocomposites

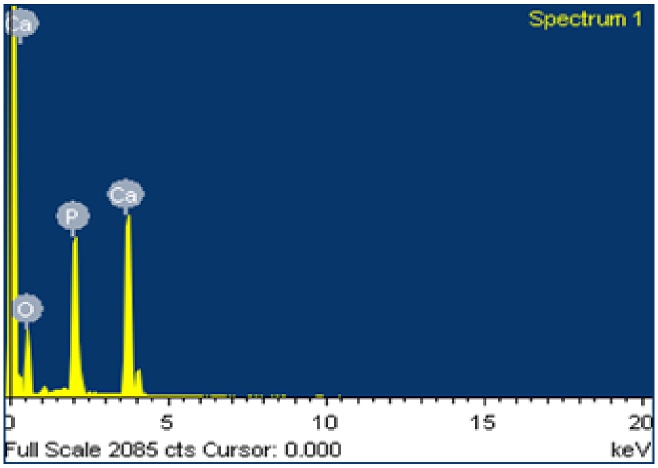

Fig.4. EDX images of HA

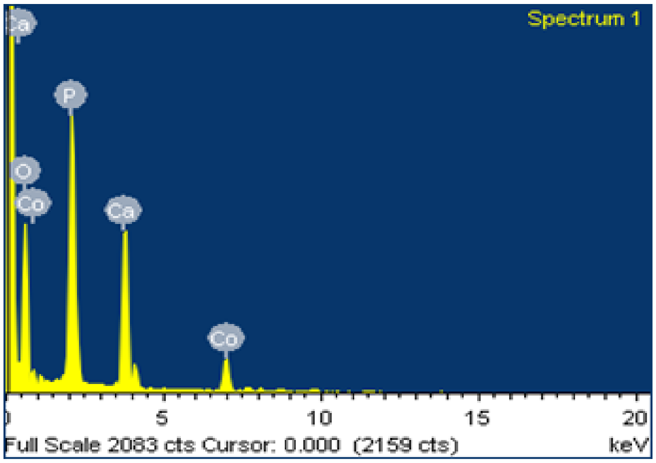

Fig.5. EDX images of Co-HA Nanocomposites

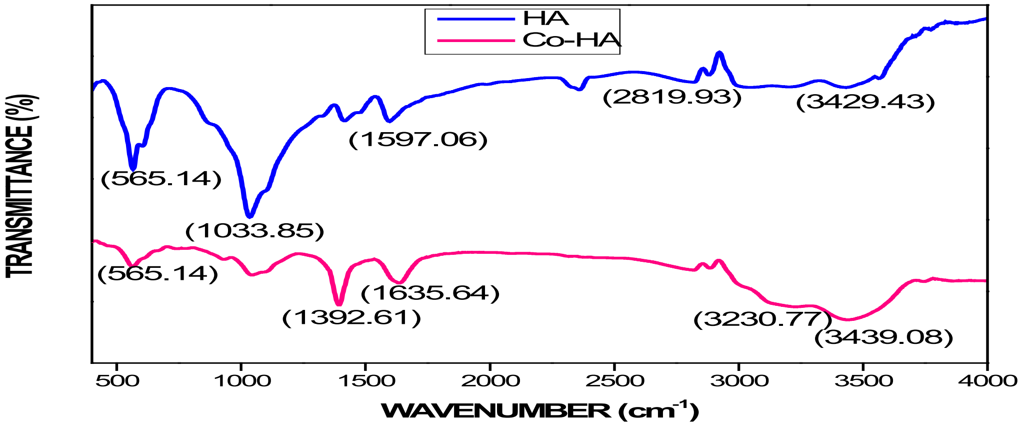

Fig. 6: FTIR graph for HA and Co-HA nanocomposites

3.2 Spectroscopic Characterization

3.2.1 FTIR analysis

The given sample was tested for antimicrobial activity by well diffusion method. Liquid Mueller Hinton agar media and the Petri plates were sterilized by autoclaving at 121°C for about 30 minutes at 15 lbs pressure. Under aseptic conditions in the laminar airflow chamber, about 20ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media, 18 hrs culture of Gram positive microorganisms such as Bacillus cereus (MTCC 430), Staphylococcus aureus (MTCC 3160), Gram negative microorganisms such as E. coli (MTCC 1698) and Klebsiellapneumoniae (MTCC10309) obtained from IMTECH , Chandigarh were swabbed on the surface of the agar plates. Well was prepared by using cork borer followed with loading of 100 pi of each sample to the distinct well with sterile distilled water as negative control and Levofloxacin as positive control. The sample loaded plates were then incubated at 37° C for 24 hours to observe the zone of inhibition and is shown in Fig. 7 & Fig. 8 also tabulated in the Table. 5.

Table 3: FTIR verification of HA nanoparticles

FREQUENCY (cm^{-1})	BENDING AND STRETCHING	FUNCTIONAL GROUP
565.14	C-Br Stretching	Alkyl Halide
1033.85	S=O Stretching	Sulfoxide
1597.06	N-H Bending	Amine
2819.93	C-H Stretching	Aldehyde
3201.83	O-H Stretching	Alcohol
3429.43	N-H Stretching	Primary Amine

Table 4: FTIR verification of Co-HA nanocomposites

Frequency (cm^{-1})	Bending and Stretching	Functional group
565.14	C-Br Stretching	Alkyl Halide
1041.56	C-O Stretching	Vinyl Ether
1392.61	O-H Bending	Alcohol
1635.64	C=C Stretching	Conjugated Alkene
2883.58	C-H Stretching	Alkanes
3230.77	O-H Stretching	Alcohol
3439.08	N-H Stretching	Primary Amines

Table 5: Antibacterial activity of HA and Co-HA

			Zone of Inhibition in Diameter(mm)				
S. No.	Microorganisms	Control	HA		Co-HA		Std. Antibiotic Levofloxacin
		(100 µl)	50 µl	100 µl	50 µl	100 µl	
1	Bacillus cereus	Nil	30	35	10	14	26
2	Staphylococcus aureus	Nil	15	19	11	15	27
3	Escherichia coli	Nil	20	23	11	16	27
4	Klebsiellapneumoniae	Nil	28	30	12	14	25

Fig. 7: Anti-bacterial activity of HA nanoparticles

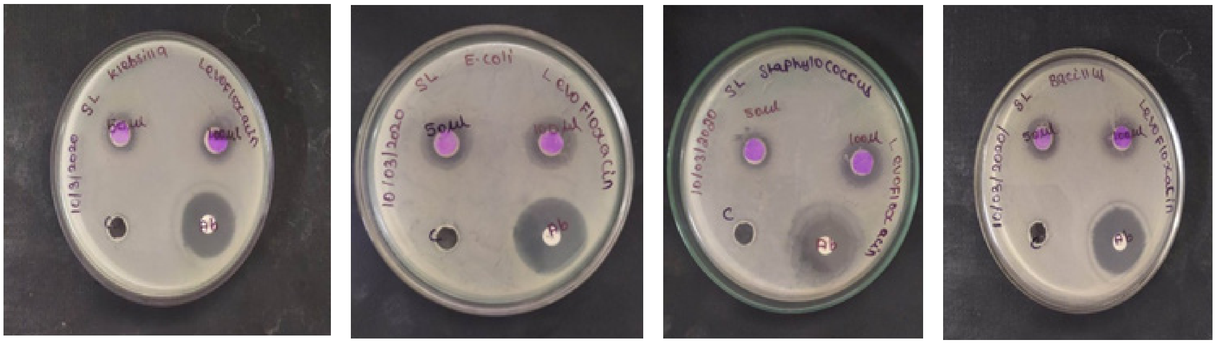

Fig. 8: Antibacterial activity of Co-HA nanocomposites

4. CONCLUSION

We have successfully synthesized Hydroxyapatite nanoparticles and Cobalt substituted Hydroxyapatite by sol- gel method using EDTA as organic modifier. Here the role of EDTA is it acted as a capping agent for the formation of pure hydroxyapatite. As the EDTA and the pH plays a vital role in the morphology and the structure of the HA's. Powder XRD and SEM analyses confirmed the phase purity and nano-metric dimension of the samples. The particle size of HA powder was found to be strongly dependent on the presence of EDTA. The particle size of the EDTA assisted HA sample ranges from 34 to 55 nm. EDX confirmed the chemical composition, which matches the compositions of HA's. Similarly, by taking spectroscopic analysis using FTIR it is proven that the presence of functional group. Antibacterial analysis proves the excellent antibacterial activity against the gram positive microorganisms such as Bacillus cereus and Staphylococcus aureus and Gram negative microorganisms such as E.coli and Klebsiellapneumonia.

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