CHAPTER -VI

SUMMARY AND CONCLUSIONS

Hydroxyapatite is an imperative biomaterial which finds its applications as bone substitute and implant coating due to its high similarity to the inorganic materials like bone and tooth. The clinical success of this hydroxyapatite is strongly dependent on its various properties such as biocompatibility, solubility, fracture toughness and so on. These properties are mainly controlled by tuning the morphology and size of the as-synthesized powders. Due to these properties, there is a great interest and demands to synthesize HAP with a special focus on the size and morphology. Thus, the present work is based on the synthesis of hydroxyapatite by using various green chelating agents as templates for various biomedical applications.

Firstly, the synthesis of hydroxyapatite was achieved by using the malic acid as template. The malic acid used here is obtained both from the commercially available one and as well as from the natural source. The malic acid extracted from the natural source of apple was further characterized by Fourier transform infrared spectroscopy, Proton nuclear magnetic resonance spectroscopy and Carbon-13 nuclear magnetic resonance spectroscopy. The effect of chelating agent on the phase purity, crystallinity and morphology of the as-synthesized HAP powders were systematically investigated. The phase purity of the as-synthesized HAP powders was characterized by the various analytical techniques such as FT-IR, ^{13}C-NMR and X-ray diffraction analysis . The morphology of the as-synthesized HAP powders was investigated with the aid of the Scanning electron microscopy.

The elements present in the HAP samples was analysed using Energy dispersive x-ray analysis. Thermo gravimetric analysis, Differential thermal analysis and Differential scanning calorimetric techniques were employed to evaluate the thermal stability of the as-synthesized HAP powders. The antibacterial activity of the as-synthesized HAP powders against the two gram negative bacteria E. coli and Klebsiella has been performed. From the above experimental results it was found that the HAP powders obtained using the natural source of malic acid as a chelating agent is of phase pure, with a well-defined morphology having discrete particles with less agglomeration than the HAP powders obtained from the commercially available malic acid. Further, the reduced particle size and with good antibacterial activity can be achieved only from the apple extract as the source of the chelating agent.

Followed by this, secondly the synthesis of hydroxyapatite powders was attained by using the oxalic acid as the chelating agent. Here the oxalic acid obtained from the commercial and as well as from the natural source acts as a template. For the natural source of oxalic acid, the vegetable tomato was used. The oxalic acid present in the tomato extract was also characterized by the FT-IR, ^{1}H-NMR and ^{13}C-NMR. Further, the consequence of the chelating agent on the phase purity, crystallinity and morphology of the as-synthesized HAP powders were systematically examined. The phase purity of the as-synthesized HAP powders was characterized by the numerous analytical techniques such as FT-IR, ^{13}C-NMR and XRD. The morphology and the elemental studies were done using SEM/EDAX analysis. The thermal stability of the as-synthesized HAP samples was studied using TGA, DTA and DSC techniques. The antibacterial activity of the HAP samples

against the two gram negative bacteria E. coli and Klebsiella were also explored. From the above experimental results it was found that the uniform morphology, reduced size and less agglomerated HAP particles were gained only from the natural source of tomato extract than the one achieved from the commercially available oxalic acid. The crystallinity, particle size, morphology and the excellent antibacterial activity were strongly dependent on the natural source of the chelating agent used.

Similarly, the synthesis of hydroxyapatite using sucrose as the template green chelating agent was also prepared. The sucrose used in this method has been acquired from various sources, three from the natural (pineapple, carrot and sugarcane) and one from the commercially available source were exploited in this work to attain a controlled crystallinity, particle size as well as uniform morphology. The spectral characterizations involving the FT-IR for the functional group analysis of sucrose both from the commercial, natural source and the as-synthesized HAP powders, ^{1}H-NMR for the identification of the protons in the commercial and the natural source for the sucrose, ^{13}C-NMR for the identification of carbon atoms in the sucrose and in the as-synthesized HAP powders, liquid chromatography/mass spectrometry for the determination of the hydrolysed products of the sucrose and XRD techniques for the phase identification of the HAP powders were performed. The morphology of the HAP particles were assessed thoroughly using a scanning electron microscope equipped with EDAX. Additionally the thermal stability of the as-synthesized HAP powders using the green chelating agent sucrose was completed by adapting the TGA, DTA and DSC techniques. Further, the antibacterial activity of the as-synthesized HAP powders against the two gram negative bacteria E. coli

and Klebsiella was also carried out. The tentative results indicate that the obtained HAP using the natural source as a chelating agent is of phase pure, with a well-defined morphology having discrete particles without any agglomeration and also bearing good antibacterial activity than the HAP obtained from the commercially available source. Further, the reduced particle size can be achieved from the sugarcane stem as the source of the chelating agent.

Finally, the HAP nanorods with excellent antibacterial properties have been successfully synthesized by using the green template method. Here the tartaric acid is used as the green template for the overall synthesis of HAP which has been achieved from the extracts of natural fruits such as banana, grapes and tamarind and also from the commercially available one. The extracted tartaric acid from the natural sources and the commercially available one has been characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR studies. The effect of the chelating agent on the phase purity, crystallinity and morphology of the as-synthesized HAP powders were characterized by various analytical techniques such as FT-IR, ^{13}C-NMR, XRD and SEM analysis. The elements present in the HAP samples were recognised by studying the EDAX analysis. The thermal stability of the as-synthesized HAP powders by using the natural extract was investigated by TGA, DTA and DSC techniques. The antibacterial activity of the as-synthesized HAP powders against the two gram negative bacteria E. coli and Klebsiella was also studied. From the above said results it was concluded that the phase pure HAP nanorods with uniform size distribution, reduced crystallinity, crystallite size and very good antibacterial activity has been achieved only by using the natural source of tartaric acid than the commercially available one.

Among the three natural sources the results obtained for the tamarind extract was found to be the best when compared to the other two natural sources.

The present work on the synthesis of HAP powders using various chelating agents as template has led to the following conclusions;

The role of the addition of a chelating agent from various sources on the purity, crystallinity and morphology of the HAP particles obtained by the green template synthesis method was investigated.

The green templates used were malic acid, oxalic acid, sucrose and tartaric acid.

The extracted malic acid, oxalic acid, sucrose and tartaric acid from the various natural sources were found to be pure and coincide with the commercially available one as evident from the results of FT-IR, ^{1}H-NMR and ^{13}C-NMR.

The FT-IR and ^{13}C-NMR results emphasize that HAP particles synthesized by using the green template method were found to be pure and free from any organic moieties.

The LC-MS results reveal the variation in the concentration of the hydrolysed products of sucrose.

The uniform morphology and less agglomerated HAP particles were obtained from the natural source of malic acid, oxalic acid, sucrose and tartaric acid than the commercially available one.

The crystallinity, crystallite size and the morphology of HAP are strongly dependent on the natural sources of chelating agents and were evident from the XRD and SEM results.

The fairly well-defined HAP with reduced size can be achieved using the malic acid, oxalic acid, sucrose and tartaric acid as green chelating agents derived from the extract of apple, tomato, sugarcane stem and tamarind, respectively.

SEM images showed that among the HAP particles prepared by various natural sources, an uniform sized and well-defined HAP nanorods were obtained only by using the tamarind extract as the green template.

The EDAX pattern for the HAP synthesized from the commercial malic acid. oxalic acid, sucrose and tartaric acid clearly shows the existence of principal constituents such as Ca, P and O. Whereas in the case of HAP obtained from the natural source demonstrates the entrapment of minerals such as Mg, Zn and Na along with the main constituents of HAP like Ca, P and O, respectively.

The thermal studies such as TGA, DTA and DSC also confirm the stability of the as-synthesized HAP powders.

The antibacterial results reveal that all the as-synthesized HAP powders exhibited a strong antibacterial activity against the two gram negative bacteria such as E. coli and Klebsiella. Among which the best antibacterial activity was obtained with the HAP nanoparticles prepared by using the tamarind extract as the green template.

This green template approach towards the synthesis of HAP particles has many advantages such as ease with which the process can be easily scaled up, biodegradability, biocompatibility and economic viability and can be used as an impending material for various biomedical applications with improved antibacterial and bioactivities.