

XRD, thermal, haemolysis and dna binding studies of l-arginine functionalized hydroxyapatite nano-particles

Abstract

Hydroxyapatite (HAP), an important bio-mineral, finds numerous applications in the clinical field. As amino acids exhibit synergetic effects on structure, morphology and several biological properties of HAP, an attempt was made by the present authors to synthesize pure and different amount of L-arginine functionalized HAP nano-particles by the surfactant mediated approach. The samples were characterized by using different techniques, viz., powder X-Ray Diffraction (powder XRD), Fourier Transform Infrared (FT-IR) spectroscopy, Transmission Electron Microscopy (TEM) and Thermo-Gravimetry Analysis (TGA). The Williamson and Hall (W-H) analysis was performed on the powder XRD patterns, which indicated the particle size in nano-meter range and the strain associated with nano-particles changed with functionalization of HAP by L-arginine. FTIR study indicated the presence of various functional groups due to the functionalization of L-arginine with HAP. TGA results indicated slightly poor thermal stability of L-arginine functionalized HAP samples with comparison to pure HAP. The DNA binding studies indicated improved DNA binding on L-arginine functionalization of HAP. The haemolysis studies were also carried out.

Keywords: Hydroxyapatite, Functionalization, Powder XRD, FTIR, TGA, DNA binding, Haemolysis

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Abbreviations

HAP, Hydroxyapatite; FT-IR, Fourier Transform Infrared; TEM, Transmission Electron Microscopy; W-H, Williamson and Hall; XRD, X-Ray Diffraction; TGA, Thermo-Gravimetry Analysis; dsDNA, Double-Stranded Deoxyribonucleic Acids; OD, Optical Density; ssDNA, Single Stranded DNA

Introduction

Hydroxyapatite or HAP ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is a well known bio-mineral and finds numerous applications in medicinal field as well as industrial field, for example, coatings in dental implants,¹ bone implants facilitating bone formation,^{2,3} chromatographic separation of protein and DNA,⁴ protein purification,⁵ gene delivery,⁶ catalysis,⁷ gas sensing,⁸ H_2O_2 fuel cell,⁹ etc. In addition, HAP inhibits the proliferation and apoptosis of cancerous cells.¹⁰⁻¹² Several books and review papers are available on HAP and its applications.¹³⁻¹⁷ It is non toxic, anti inflammatory and highly bio-compatible material. Marchi et al.¹⁸ have examined the in vitro interaction of human fibro-blast cells with HAP as well as alumina and observed better bio-compatible nature of HAP compared to alumina.

Presently, the targeted gene and drug delivery is on the prime focus in medicinal research. As early as in 1970s, the defected DNA was replaced by normal DNA in a patient suffering from genetic disorder.¹⁹ In succession to this, in the last 25 years, several successful gene transfer experiments have been reported in the treatment of various diseases and a few noteworthy recent attempts are in the diseases like Parkinson,²⁰ Chronic granulomatous,²¹ and several retinal diseases.²² Recently, promising results are also recorded in gene therapy for treatment of HIV.²³ Technically, the gene therapy is accomplished

by using either viruses or biological nano-particles as gene delivery vectors.⁶ However, the viral/former therapy may sometimes cause immune response that leads to dangerous infections.²⁴ and hence it will be safer to use non-viral and bio-compatible vectors for gene therapy.^{6,25,26} In this regard the HAP has been preferred as a better alternative due to its high bio-compatible nature.²⁷ Therefore, the nano-particles of HAP finds novel applications in medicine, for instance, HAP as non-viral vectors for efficient *In vivo* gene delivery.²⁸ celecoxib-loaded HAP-chitosan nano-composite for colon cancer treatment.²⁸ effect of HAP uptake into hepatoma cells for inhibitory effect on liver cancer metastasis.²⁹ and mineralization of DNA into nano-particles of HAP.³⁰ However, relatively low efficiency of gene delivery is one of the major problems for using the HAP in clinical trials. Recently, some researchers have revealed that hydrophilic L-arginine with guanidyl group $(\text{CH}_2)_3\text{NHC}(\text{NH}_2)^+$ could enhance the gene delivery efficiency of HAP.³¹

The functionalization of the HAP nano-particles by amino acids has synergetic effects on their structural, morphological and surface properties.³² Functionalizing HAP with amino acids has resulted in to high protein adsorptive capacity.³² The effects of various amino acids on the particle size and morphology of HAP nano-particles along with their physical characterizations are reported.³²⁻³⁵ In the earlier studies from our laboratory, Tank et al.³⁶ have carried out antimicrobial and haemolysis study of cobalt doped nano HAP to confirm its safe application in the medical field. After considering the benefits of HAP nano-particles as a gene delivery agent, in this investigation the authors aim to synthesize pure and functionalized HAP nano-particles with different amount of amino acid L-arginine and characterized them by Powder XRD, TEM and TGA, haemolysis and DNA binding to identify their possible biological applications.

Materials and methods

The HAP nano-particles were synthesized by the surfactant mediated approach. Calcium nitrate hexa-hydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), potassium dihydrogen phosphate (KH_2PO_4), Triton X-100, aqueous ammonia (all AR grade) were used as precursors. Initially, 2 ml of Triton X-100 was mixed with 100 ml of 0.3 M calcium nitrate hexa-hydrate aqueous solution and, thereafter, the 100 ml of 0.18 M potassium dihydrogen phosphate aqueous solution was added and the mixture was treated with aqueous ammonia to set the pH 9 with constant stirring and heating at 60°C temperature. This resulted into milky white solution with precipitates. The precipitates were recovered by filtration (Whatman filter paper No. 1) and washed with the mixture of ethanol and de-ionized water and then dried in air at room temperature. The L-arginine functionalized HAP (Ar-HAP) nano-particles were synthesized by adding 100ml different concentrations of L-arginine aqueous solutions, viz., 0.3 M, 0.6 M, 0.9 M and 1.2 M concentration, to the solution of calcium nitrate hexa-hydrate before the addition of surfactant and, thereafter, the same experimental procedure was repeated. Here, calcium to phosphate ratio was maintained 1.67, while calcium to amino acid ratio was selected as 1:1, 1:2, 1:3 and 1:4 and the synthesized samples were labelled as 1-Ar-HAP, 2-Ar-HAP, 3-Ar-HAP and 4-Ar-HAP, respectively, and the pure sample as HAP.

Characterization techniques

The Powder X-ray Diffraction (XRD) study was carried out on Bruker AXS D8 Advance setup using Cu ($K\alpha 1$) radiation and the data were analyzed by software Powder X. The average crystallite size and strain were calculated by applying Williamson and Hall analysis to the powder XRD patterns. The Transmission Electron Microscope (TEM) analysis was conducted to determine the morphology and size of synthesized nano-particles using TECNAIKA20 (Philips) setup operating at 200 kV potential. Fourier Transform Infrared (FTIR) spectra were recorded on Thermo Scientific Nicolet 1510 in KBr media in the range of 400cm^{-1} to 4000cm^{-1} . Thermo-Gravimetry Analysis (TGA) was carried out on LINSEIS 1400 in atmosphere of air at heating rate of 15°C/min.

DNA extraction

The DNA isolation was performed as per the process reported by Chudasama & Thaker.³⁷ The bacterial cells were pelleted by centrifugation and re-suspended in 500µl of 10 mM Tris-EDTA (ethylene-diamine-tetra-acetic acid) buffer and, thereafter, treated with 30µl SDS (sodium dodecyl sulfate 10% W/V) and 2µl proteinase K (10mg/ml). This was mixed well and incubated for 1 h at 37°C. Thereafter, 20µl of cetyltrimethyl ammonium bromide (10%, W/V) and 100 µl of NaCl (5M) were added, and incubated for 10 minutes at 65°C. The DNA was purified by two 1:1 extractions in which, (i) chloroform: Isoamyl Alcohol (24:1) and (ii) Phenol: Chloroform: Isoamyl Alcohol (25:24:1) were used and then it was precipitated with iso-propanol, washed with ethanol (70%), and dissolved in tris-EDTA buffer. The quality and concentration of the DNA was confirmed by measuring optical density at 260/280 nm ratio. The experiment was carried out in the aseptic medium.

DNA binding

For the DNA binding test, 50 mg of pure and L-arginine functionalized HAP (Ar-HAP) samples were taken. The DNA binding generally involves three steps, viz., equilibrium, load, and elution. The columns were attached to a 60°C circulating water bath and rinsed twice with 1 ml of 0.12 M phosphate buffer of pH 6.8 before use. The samples were pre-equilibrated in 2 ml of 0.12 M phosphate buffer of

pH 6.8. Thereafter, 2 ml of well mixed hydrated HAP nano-particles sample was added to the column and allowed to settle for 15 minutes. The equilibration of the column was accomplished by washing it three times with 0.12 M phosphate buffer of pH 6.8. A known concentration of dsDNA (Double-Stranded Deoxyribonucleic Acids) was used to test the binding with pure HAP nano-particles and Ar-HAP nano-particles. The nucleic acid sample was incubated in 200µl of 0.12 M phosphate buffer of pH 6.8 at 60°C for at least 20 minutes before applying it to the HAP/Ar-HAP column. The binding with HAP and/Ar-HAP was allowed for 20 minutes. The DNA was eluted with a 1 ml of 0.40 M phosphate buffer of pH 6.8 and centrifuged at 10000 rpm for 10 minutes. The resulting supernatant was collected and used as DNA source. In the pallet, 1 ml of 1.0 M phosphate buffer of pH 6.8 was added to remove the remaining DNA from the materials. The 0.40 M and 1.0 M fractions containing dsDNA were mixed. The pooled fractions were extracted by equal volumes of phenol-chloroform-isoamyl alcohol (25:24:1) and then precipitated with isopropanol, washed with ethanol (70%), and dissolved in 50µl tris-EDTA buffer. The DNA fragments were separated by electrophoresis through 1.0% agarose gel. The concentration of the DNA was confirmed by measuring optical density at 260 nm by using SPECORD 200 PLUS spectrophotometer.

Haemolytic activity

The haemolytic activity of pure HAP and Ar-HAP nano-particles was investigated with fresh human blood collected in anticoagulant containing tube. 50mg of samples were immersed in 1 ml extraction medium of sterilized physiological saline buffer and incubated at 37°C for 30 minutes under static condition. The human blood was diluted by 6 ml sterilized physiological saline and 200µl of this diluted blood was then added to the tubes containing 50 mg HAP and Ar-HAP samples in sterile saline solution and incubated for 60 min in water bath. Thereafter, each tube was centrifuged and then the absorbance of the supernatant was measured at 545 nm. Here, the negative control was sterilized physiological saline, and the positive control was distilled water. The haemolytic ratio (Z) was calculated using the following formula.

$$z = (D_t - D_{nc}) \times \frac{100}{(D_{pc} - D_{nc})} \quad (1)$$

Where, Z is haemolytic ratio and D_t , D_{nc} and D_{pc} are the average absorbance of samples, negative controls and positive controls, respectively. When the values of Z are >5, 2-5 and 0-2, the corresponding haemolytic grade is haemolytic, slightly haemolytic and non-haemolytic, respectively.

Results and discussion

The effect of amino acid functionalization on HAP was studied in terms of crystallinity by several workers.^{32,33,35} and found that the crystallinity of HAP decreased with functionalization of amino acid. Figure 1 shows the powder XRD patterns of pure HAP and Ar-HAP samples. One can observe that the powder XRD patterns are not altered much for pure and 1-Ar-HAP samples. However, separate peaks at 29°C and 34.3°C start appearing in 3-Ar-HAP sample and become more clear in 4-Ar-HAP sample, which are indicated by asterics in the figure. This indicates that the functionalization by L-arginine does not distort HAP much up to 1-Ar-HAP sample and further functionalization is expected to add a separate phase, which can be assigned to the possible formation of brushite (JCPDS-04-0740). The brushite phase is expected to occur due to positively charged guanidine

group of L-arginine competing with the calcium ion for binding with the anions, which leads to the deficiency of calcium and the presence of ample amount the $(\text{HPO}_4)_2$ ions gives the brushite (CaHPO_4) phase. Due to small particle size and broadening of the XRD patterns it is not possible to resolve other peaks for brushite and L-arginine in the XRD patterns. The brushite phase formation in HAP is well discussed by Tank et al.³⁸ The analysis of the powder XRD patterns indicated monoclinic structure.

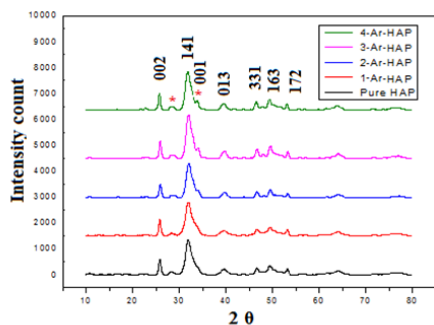


Figure 1 Powder XRD patterns of pure and L-arginine functionalized HAP samples.

Usually, the broadening of peaks in the powder XRD patterns is due to (i) the reduction in the crystallite domain, (ii) the presence of domains with lattice spacing differing from one to another and (iii) the presence of elastic deformations and stacking faults within the domains. To evaluate the contribution of crystallite size and micro-strain to the peak broadening of the powder XRD patterns, the present authors have employed the Williamson and Hall (W-H) analysis to the powder XRD patterns of (Figure 1). The W-H method considers both the limited crystallite size and the presence of crystallographic distortion or strain leading to the Lorentzian intensity distribution and can be written as follows:

$$\beta \cos \theta = k \frac{\lambda}{L} + 4 \eta \sin \theta \quad (2)$$

Where, β = Full width and half maximum (FWHM), L = Crystallite size, η = Strain, $k = 0.9$ and $\lambda = 1.54178 \text{ \AA}$. The average particle size and strain was evaluated from the plots $\beta \cos \theta$ versus $4 \sin \theta$ by finding the intercept on the y-axis and the slope of the fitted linear plot.^{39,40}

Figure 2a & 2b shows the Williamson and Hall plots of the above relation for pure HAP and 4-Ar-HAP nano-particle samples, respectively, where, the slope indicates the value of strain. The average crystallite size and strain has been calculated for different samples and given in Table 1. It is found that L-arginine functionalization reduces the particle size of HAP nano-particles, while the strain is found to be increasing in the 1-Ar-HAP sample. But, for the sample 2-Ar-HAP the particle size and the strain both are increased, which may due to the presence of large number of positively charged guanidine group of L-arginine on the surface of HAP and their mutual interaction give rise of strain on the surface of HAP. On further increasing the proportion of L-arginine the particle size or crystallite size suddenly decreases and the strain increases due to more number of guanidine groups interacting with the surface and reducing the particle size and increasing correspondingly the strain. Finally, for the 4-Ar-HAP sample the particle size increases and the strain decreases; which may be attributed to the presence of a separate phase. The effect of

functionalization of L-arginine on crystallinity is studied by defining the degree of crystallinity as:

$$\text{Crystallinity} = 100 \times \frac{C}{A + C} \quad (3)$$

Where, C is the area of the peaks in the diffraction pattern, i.e., the crystalline area, and A is the area between the peaks and the back ground, i.e., the amorphous area. The values of C and A were calculated by using Powder X software. Table 1 gives the values of crystallinity and it is found that the crystallinity slightly decreases with increasing the L-arginine functionalization in HAP nano-particles. This corresponds to earlier results of functionalization of HAP by amino acids.³⁰ However, for 4-Ar-HAP sample again higher degree of crystallinity is observed due to development of another phase as marked in the powder XRD pattern. The TEM (Figure 3a) images of amino acid functionalized HAP were earlier reported by several authors.^{32,33} Figure 3b exhibits the TEM image of 1-Ar-HAP sample and indicates the needle type morphology with reduced particle size. While, (Figure 3c-3e) show the TEM images of 2-Ar-HAP, 3-Ar-HAP and 4-Ar-HAP samples, respectively, which show the spherical type morphology with particle size in 10 to 20 nm range. One can mark from the TEM images that the decrement in particle size as well as change in the morphology is occurring as the concentration of L-arginine increases. The needle type morphology exhibits higher growth rate in c-direction. The positively charged guanidine group of L-arginine binds with the negative hydroxyl group of HAP exposed on the surface in such a way that it produces some kind of capping and reducing the preferential growth in one direction and resulting in to spherical type morphology.

Figure 4 shows the FTIR spectra of pure and L-arginine functionalized HAP samples. It confirms the presence of all the functional groups related to HAP. The absorption peaks located in the range of $3404\text{--}3440 \text{ cm}^{-1}$ are due to the stretching mode of O-H. The characteristic band of the PO_4 group of HAP was clearly observed in the spectrum. The vibrations of the asymmetric stretching mode of PO_4^{3-} were marked by $1034\text{--}1022 \text{ cm}^{-1}$, while a small hump at 950 cm^{-1} exhibiting the stretching mode of PO_4^{3-} . The medium absorption close to 600 cm^{-1} in the spectra was due to the O-P-O bending mode.³³ Presence of the stretching modes of NH_3 and CO_2 were observed nearby 1455 cm^{-1} and 1460 cm^{-1} . While an absorption observed at 1644 cm^{-1} represents the asymmetric stretching mode of NH_3 ; which was getting overlapped with vibration of H_2O at 1630 cm^{-1} .³⁴ resulted in to the broadening of the peak. A weak hump observed at $2900\text{--}2800 \text{ cm}^{-1}$ revealed the existence stretching mode of C-H bond.³⁴ which was absent in the FTIR spectrum of pure HAP. Hence, the FTIR spectra confirm the successful functionalization of the HAP by the L-arginine.

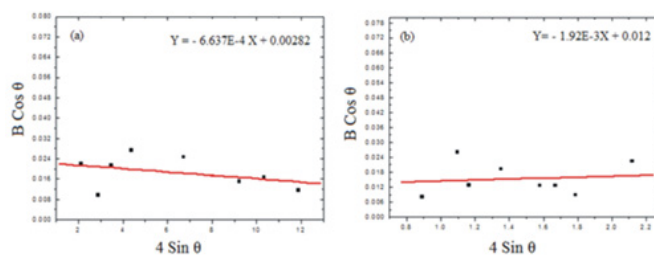


Figure 2 W-H plots of (a) Pure HAP and (b) 4-Ar-HAP.

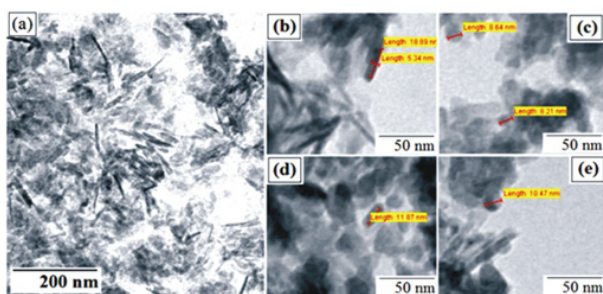


Figure 3 TEM images of (a) Pure HAP (b) 1-Ar-HAP (c) 2-Ar-HAP (d) 3-Ar-HAP (e) 4-Ar-HAP.

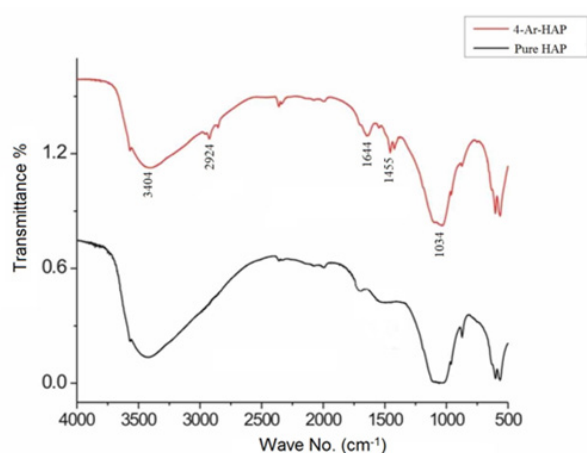


Figure 4 FTIR spectra of (a) Pure HAP (b) 4-Ar-HAP.

The thermal study of pure HAP and Ar-HAP nano-particles was carried out by using thermo-gravimetry to investigate the thermal stability of the samples. The thermal stability is an important parameter for physiological applications. The sample must remain thermally stable at physiological temperatures for safe and long term application. HAP is thermally very stable and reported well earlier.⁴¹ Also, the thermal stability of calcium deficient HAP functionalized with different amino acids was reported.³² Table 2. Figure 5 shows the thermo-grams of pure and Ar-HAP samples. The samples exhibit the weight loss up to 600°C due to liberation of weakly and strongly absorbed water molecules.⁴¹ The further weight loss from 600°C to 1200°C was due the gradual dehydration of HAP which is briefly discussed by Murray et al.⁴² However, the enhanced weight loss in the Ar-HAP samples with comparison to pure HAP sample proves the presence of L-arginine. As L-arginine being organic material getting destabilized faster on heating and giving less thermal stability to the host material. Also, the residual mass of the samples at the end of the TGA at 1200°C is highest in the pure HAP and decreases progressively on increasing the amino acid L-arginine content in the samples, which further confirms the successful functionalization of the amino acid with HAP. This is further suggests the linking of guanidine group of the L-arginine to the crystal structure of HAP.

In vivo the haemolysis, i.e., the destruction of red blood cells, can leads to large number of serious diseases including anaemia and jaundice.⁴³ Therefore, it is essential to check the haemolysis response of a biomaterial for safer medical management. Recently, the haemolysis of pure HAP and cobalt, manganese, silver and

zinc doped HAP has been reported.^{36,38,41,44} and found to be non-haemolytic and safe to use medically. In the present investigation, the haemolysis study was carried out for pure HAP and Ar-HAP nano material samples. Table 3 shows the Optical Density (OD) values of different samples for haemolysis study. The result shows that as the functionalization of L-arginine increases in HAP, the haemolytic ratio (Z) decreases from slightly haemolytic to non-haemolytic grade. The least haemolytic ratio was observed in the sample 2-Ar-HAP.

Particularly, the HAP structure has attracted the attention of researchers for the application of protein purification and DNA separation. There are two different protein binding sites on the surface of HAP, i.e., C and P sites. The C site is rich in Ca^{2+} and exposed on the surface due to the dissolution of OH^- in an aqueous solution and hence it strongly binds to the negatively charged COO^- group of amino acid, whereas, the P site is rich in PO_4^{3-} and interacts with positively charged groups, e.g., NH_3^+ group.^{33,45} The functionalization of HAP with amino acids can alter the surface charges due to the presence of COO^- , NH_3^+ and side groups in amino acids. The aspartic acid incorporation on the HAP surface enhances the adsorption of basic proteins owing to additional COO^- groups on HAP due to aspartic acid.⁴⁶ The HAP is extensively used for the separation of ssDNA (Single Stranded DNA) and dsDNA (Double Stranded DNA). Andrews-Pfannkoch et al.⁴⁷ examined the effects of various binding parameters. The isotherm analysis suggested that the electrostatic forces are the main driving force of dsDNA binding to HAP. Because of the hydrophobic bases and the negative charge associated with the phosphate backbone, the dsDNA molecule binds to HAP with hydrophobic and electrostatic interactions.

In the present investigation, the bacterial double stranded genomic DNA (linear) was selected for binding to the pure HAP and Ar-HAP nano-particles. The agarose gel electrophoresis confirmed that the pure HAP and Ar-HAP samples were successfully bound with DNA and formed L-arginine-HAP-DNA complex. As shown in Figure 6, lane 1 contains the control DNA, while the lanes 2-5 contain the pure HAP, 1-Ar-HAP, 2-Ar-HAP, 3-Ar-HAP and 4-Ar-HAP sample-DNA complexes, respectively; demonstrating that the dsDNA is completely bound with pure HAP and functionalized HAP. The concentration of dsDNA was measured by 260nm wavelength and compiled in Table 4 which shows that the DNA is maximum bound with 1-Ar-HAP nano-particle sample compared to the 4-Ar-HAP, 3-Ar-HAP, 2-Ar-HAP and Pure HAP nano-particles. With comparison to the pure HAP, the DNA binding efficacy is more in functionalized samples, which may due to the enhanced interaction between DNA and hydrophilic L-arginine with a guanidine group on the surface of HAP. The comparative decrease in the DNA binding on increasing L-arginine content may be due to large number of its binding on HAP surfaces and the presence of large number of COO^- and NH_3^+ groups and side chains inviting further competition within themselves to bind with DNA and only some of them are successful in achieving that; while for sample 1-Ar-HAP the situation seems to be quite conducive and optimum nature of DNA binding is obtained. In general, the L-arginine functionalized HAP possesses better DNA binding efficacy than the pure HAP. One can conclude from the study that the 1-Ar-HAP sample is the best one with the maximum DNA efficacy for *in vitro* applications, but it possesses slight haemolytic property. While the samples 2-Ar-HAP, 3-Ar-HAP and 4-Ar-HAP possess slightly less DNA efficacy but exhibit non haemolytic nature with safer medical use.

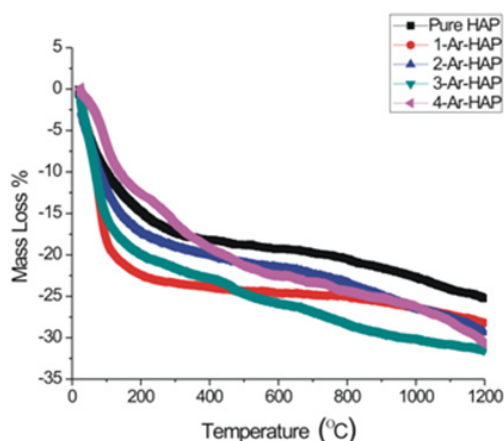


Figure 5 Thermo gram of (a) Pure HAP, (b) 1-Ar-HAP, (c) 2-Ar-HAP, (d) 3-Ar-HAP, (e) 4-Ar-HAP.



Figure 6 Agarose gel electrophoresis of pure and L-arginine functionalized HAP/DNA complexes. Lane: (C) control (1) Pure HAP (2)1-Ar-HAP (3)2-Ar-HAP (4)3-Ar-HAP (5)4-Ar-HAP.

Table 1 Crystallite size, Strain and Crystallinity using W-H method.

Sr. No	Sample Name	Crystallite Size	Strain (10^{-3})	Crystallinity
1	Pure HAP	49 nm	0.16	61%
2	1-Ar-HAP	6 nm	3.1	56%
3	2-Ar-HAP	13nm	4.83	52%
4	3-Ar-HAP	7 nm	6.41	53%
5	4-Ar-HAP	11 nm	1.91	59%

Table 2 Thermal stability of HAP and L-arginine functionalized HAP nano-particles.

Weight Loss (Δm) (%) In Different Temperature Range	Pure HAP	1-Ar-HAP	2-Ar-HAP	3-Ar-HAP	4-Ar-HAP
Δm (%) Room Temperature-600 °C	19	24	21	25	28
Δm (%) 600 °C-1200 °C	6	4	8	6	3
Final Residual Δm (%) at 1200 °C	75	72	71	69	69

Table 3 OD value determined in haemolysis test of pure HAP and L-Arginine functionalized HAP.

Sr. No	Sample Name	D_t	D_{pc}	D_{nc}	Ratio Z
1	Arginine	0.124	2.064	0.06	2.9±0.5
2	Pure HAP	0.122	2.064	0.06	2.8±0.2
3	1-Ar-HAP	0.109	2.064	0.06	2.4±0.2
4	2-Ar-HAP	0.084	2.064	0.06	1.2±0.1
5	3-Ar-HAP	0.098	2.064	0.06	1.9±0.1
6	4-Ar-HAP	0.092	2.064	0.06	1.6±0.1

Table 4 Quantification of control DNA, Pure HAP and arginine functionalized bound DNA.

Sr. No.	Sample Name	DNA concentration $\mu g/ml$
1	Control	280
2	Pure HAP	94
3	1-Ar-HAP	180
4	2-Ar-HAP	133
5	3-Ar-HAP	135
6	4-Ar-HAP	150

Conclusion

Pure and Ar-HAP nano-particles were successfully synthesized by using surfactant mediated approach. All the samples indicated the nano-crystalline nature with monoclinic crystal structure confirmed by the powder XRD analysis. For higher concentration of L-arginine

the presence of brushite phase was noticed. The W-H analysis performed on the powder XRD patterns suggested the presence of strain, which changed with increase in concentration of L-arginine. With comparison to pure HAP the functionalized HAP exhibited less crystallinity and average particle size or crystallite size. The TEM images confirmed the nano size of particles with morphological

change from needle to spherical due to the functionalization of L-arginine on HAP surfaces and modifying the growth. Presence of all the functional group related to HAP were confirmed by the FTIR. Further, the successful functionalization of L-arginine on HAP was marked by the existence of C-H, CO₂ and NH₃ vibrations in the FTIR spectrum. Thermal analysis suggested the decrease in thermal stability of HAP due to the presence of L-arginine and progressively decreased with increase in the content of L-arginine. However, the thermal stability of HAP and L-arginine functionalized HAP were above the physiological temperature suggested the safer use at physiological temperatures. Haemolysis study suggested that the samples turned to non-haemolytic from slight haemolytic on increasing L-arginine content. The DNA binding results showed that all the samples could successfully bind the DNA, but 1-Ar-HAP showed the maximum binding efficacy. This finds further applications in gene therapeutic treatment.

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