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Synthesis, characterization and *in vitro* drug delivery of nanostructured Fe-doped hydroxyapatite bioceramics

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ABSTRACT

Bioactive nanocrystalline Fe doped hydroxyapatite (Fe: HAP) were successfully prepared through auto-combustion route. The synthesized samples were characterized by various physico-chemical methods. Thermal stability investigated using thermo-gravimetric and differential thermal analysis (TG-DTA). Structural characterizations of the samples were carried out by the X-ray diffraction technique (XRD). Phase formation of Fe-HAP was determined by fourier transform infrared spectroscopy (FTIR). The obtained multifunctional Fe doped HAP samples were performed as a drug delivery carrier to investigate the drug storage/release properties using norfloxacin as a model drug. The results reveal that the multifunctional Fe-HAP exhibits the typical hexagonal structure, and have crystallite size of 30–25 nm. The drug storage/release test indicates that the Fe-HAP shows higher drug loading amount and cumulative release rate to those of pure HAP. In summary, this work has demonstrated the potential to produce Fe-HAP as a carrier for drug delivery for bone regeneration and other biomedical applications.

Keywords: Hydroxyapatite, Biomaterials, Porous material, Nanocrystallites

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INTRODUCTION

Bone tissue engineering does study on bioceramics as one among many basics used medically. HAP a nano-composite had been identified as the substitute for grafting of the bones. In addition it also has the versatility of applications in engineering too. [1]. A recent researches, the researchers, had given an attention to magnetic nanoparticles, confining about the usage, on applicative and commercial basis, into the biomedicine sector [2-5]. Drug delivery agent is one applications, [6] and then if enlisted is hyperthermia treatment of cancer [7]. Nanoparticles, as found, are basically semi-crystalline structures, if considered with the dimension, ranges between 10 and 100 nm. Properties of it when characterized are size uniformity, surface area and adsorption kinetics. These can finally be refrained while in the preparation process with its purpose that too specific. Most of the magnetic nanoparticles are found to be used in medicine and biotechnology fields both [8, 9]. When mentioning about the applicative part of the nanoparticles it must get introduced to the blood stream. This does exposes nanoparticles to the bio-safety even. Passivation – the method or the process, especially for the surface of the magnetic nanoparticles can be utilized to augment the biocompatibility of the same. If the HAP with its properties and applications needs to get developed then magnetic ions must be incorporated into it.

Iron (Fe), though being the metal, it is traced that it is an essential element having its presence in bone and teeth [10]. Considering different biological process, it is needy micronutrient and also an important component of metalloproteins. Almost 60-70% of Iron is present in hemoglobin processing as circulating erythrocytes. The Fe in the intestinal lumen survives as ferrous and ferric salts [11]. Hence when it is bipological functioning of the human body Fe does play an important role. Research paper on the subject reads that the ions present in the iron are toxic to human body functioning through all the sources [12-14], but the reports suggests that the Fe doped HAP are having many separate biomedical applications [15]. The researchers have made available many studies over the synthesis of HAP bioceramics [16-18]. Some researchers even conclude to the insertion of Fe into the HAP [19, 20]. However, from literature review, no report on synthesis of Fe doped HAP by auto-combustion method.

The present work is focused on the synthesis of an innovative biocompatible Fe ion doped HAP by auto-combustion method and thorough examination of the effect of Fe ions on the synthesis, including TG-DTA, XRD and FTIR. In vitro drug storage and release properties also investigated on these systems by using norfloxacin as a model drug.

MATERIALS AND METHODS

Nanostructured Fe doped HAP samples having the general formula, $Ca_{10-2x}\,Fe_{2x}\,(PO_4)_6\,(OH)_2$ was synthesized by auto-combustion method. The synthesis was carried out with the calcium nitrate, iron nitrate and diammonium hydrogen phosphate as the Ca, Fe and P sources, citric acid used as fuel. The raw materials used in the experiment were all of analytical purity. The molar ratio of metal nitrates and phosphate to citric acid was taken as 1:2. Firstly, stoichiometric amount calcium nitrate, iron nitrate and diammonium hydrogen phosphate were dissolved in doubly distilled water with vigorous stirring. Citric acid was weighted stoichiometrically and dissolved in distilled water. The above solution mixed under constant stirring and pH adjusted 9 to 9.5 using ammonia. A homogeneous clear solution was achieved. This clear solution was stirred vigorously and heated up to 80°C for 4 h to obtain gel. Further, dry Fe-HAP powder was obtained by heating of gel at 300-400°C. This dried Fe-HAP was further sintered at 700-1000°C for 4 h and used for characterization (Scheme 1).

The thermal decomposition behaviour and required sintering temperature of citrate precursor to phase formation of the sample was checked by thermogravimetric analysis on the SDT Q-600 instrument by heating the powder after auto-combustion in air atmosphere from 20° to 1000°C at the rate of 10°C per min. The X-ray diffraction analysis was used to know phase purity of synthesized material by using PW3710 (Model- Philips) with CuKα radiation (λ = 1.5406Å). JASCO FT/IR-6100 type 'A' spectrometer was used for FTIR study.

2.1 Drug loading experiment:

If the material is biocompatible and biodegradable and have capacity to uptake and release drug in controlled manner then this material have applications in biomedicines [21]. For that purpose, norfloxacin (widely used antibiotic) was chosen as the model drug. In particular, different concentrations (5, 10, 15%) of norfloxacin powder were prepared by dissolving it into 100 mL of de-ionized water. Afterwards, 1 g of HAP sample was added to above said drug solutions. The resultant suspensions were stirred for different time (20, 40, 80, 120 minutes) and different temperatures (40, 50, 60, 70°C). Then after, the resultant solution was kept intact for 24 h. The solution was centrifuged in order separate the precipitate. The drug loaded on HAP sample was assessed by knowing the difference in concentration of drug before and after loading. Drug loading (%) on HAP was evaluated by following equation:

$$\text{Drug loading (\%)} = [(A-B)/A] \text{ X } 100 \quad (1)$$

Where, A is the initial and B is the final norfloxacin concentration in aqueous solution.

Drug loading on iron doped hydroxyapatite was done in the same way where instead of hydroxyapatite Fe-HAP was used. 1 g of calcined Fe-HAP samples were added to norfloxacin drug solution and stirred at optimized temperature and for optimized time.

2.2 In vitro drug release experiment:

Time period of 200 hours was used to evaluate in vitro drug release. 100 mg of drug-loaded HAP and Fe doped HAP samples added into a capped glass bottle contains phosphate-buffered (50 mL) saline (PBS) having pH =7.4 and incubated at 37°C. The stationary mode was used to study drug release. To calculate the released amount of

drug, 5 mL of sample were centrifuged and replaced with 5 mL of fresh PBS medium. Afterwards, the actual concentration of norfloxacins in the supernatant liquid was measured at 274 nm using spectrophotometer.

RESULTS AND DISCUSSION

3.1 TGA-DTA Analysis:

The $Ca_{10-2x}Fe_{2x}$ $(PO_4)_6(OH)_2$ systems heated at the rate 10°C/minute from 0 to 1000°C, is given in Fig. 1. The TGA curve shows thermal decomposition of Fe-HAP (x= 0.2) samples below 200°C due to removal of absorbed water. Decomposition 250–400°C is due to thermal decomposition of organic residue. The weight loss along with endothermic peak at 700-750°C indicates that decomposition of $CaCO_3$ following the reaction [22].

$$CaCO_3 \rightarrow CaO + CO_2 \uparrow \quad (2)$$

3.2 XRD Analysis:

The X-ray diffraction study and phase purity of synthesized HAP and Fe doped HAP bioceramic samples were examined by XRD analysis. Fig.2 shows X- ray diffraction pattern of pure HAP sample annealed at different temperatures (750-1000°C) for 4 h. These samples exhibit almost similar patterns with each other except changes in peak height and peak width with increases in temperature. Fig. 3 shows X- ray diffraction pattern of Fe-HAP (x=0.1 to 0.4) samples calcined at 1000°C for 4 hours. The peaks in XRD patterns were indexed to (002), (211), (300), (202), (310), (311), (113), (222), (312), (213), (321), (410), (402), (004). These polycrystalline HAP and Fe-HAP nanoparticles exhibit single phase hexagonal structure (JCPDS 09-0432) [23]. Debye Scherrer's formula was used to estimate average crystallite size of synthesized Fe-HAP samples [24-26].

$$\tau = 0.94 * \lambda / \beta * \cos\theta \quad (3)$$

Where, crystallite size (τ) depends upon wavelength λ (1.54 Å for CuKα) of the X- ray and the FWHM (β) of the XRD peak at 2θ. The average crystallite size obtained for Fe-HAP samples [x = 0.0 to 0.4] 30 to 25 nm.

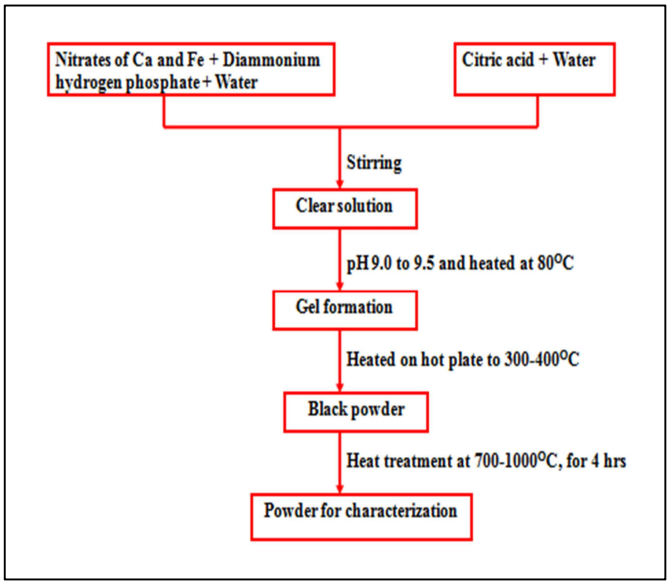
3.3 FTIR Analysis:

FTIR scanning of pure HAP and $Ca_{10-2x}Fe_{2x}(PO_4)_6(OH)_2$ system with (x=0.1, 0.2, 0.3, 0.4) calcined at 1000°C are given in the Fig. 4 and found to be very similar to those observed by D. Gopi et al and C. Yang et al [27, 28]. There are distinct differences between the spectra of the HAP and Fe doped HAP. Therefore, one can say there were some changes were taking place in the chemical structure due to the addition of Fe. All samples shows bands corresponding to HAP, which is confirmed by FTIR spectra. The peak located approximately at 3571 cm^{-1} and 632cm^{-1} corresponds to the stretching vibration and vibrational mode of OH^- respectively. The strong doublet band or shoulder at 960-1100 cm^{-1} was assigned to P-O stretching vibration of the phosphate group ($PO4^{3-}$). The band at about 473 cm^{-1} (weak) and 570-601 cm^{-1}(doublet) corresponds to the phosphate bending vibration and $PO4^{3-}$ bending mode respectively. A small band at around 1650 cm^{-1} was possibly due to absorbed water (bending mode). The FTIR result demonstrates that the synthesized pure HAP and Fe-HAP samples were in high purity state, similar results were reported by C. Yang et al [28].

3.4 Drug loading:

The drug concentration and drug to bioceramics ratio are responsible factor for drug uptake efficiency of bioceramics. The drug loading percentage is directly proportional to these parameters and attains maximum value at a particular level Fig. 5 (A) shows that variation of drug loading on HAP sample with change in drug concentration. 80% of drug is loaded for 5% drug concentration and loading of drug increases up to 87% when concentration of drug increases to 10% then after, it remains constant at 20% drug concentration. Fig. 5 (B) shows effect of temperature on loading of drug. A maximum 80% drug loading is observed in case of 5% drug concentration at 60°C with 40-min stirring time. As temperature increases there is increase in drug loading (%) afterwards, it attains constant value. Fig. 5 (C) shows bar graph of changes in drug loading on HAP sample with different stirring time. Therefore, 40-min stirring time and 60°C are considered as optimized parameters for drug loading.

Four Fe doped HAP samples were used for drug loading each having 5% concentration of norfloxacins. The percentage of drug loading for different Fe-HAP samples (x=0.1 to 0.4) was shown in Fig. 6. From the bar graph it is concluded that there is no significant change occur in the percent drug loading with the incorporation of iron.



Scheme 1: Flowchart of sol-gel auto combustion synthesis of Fe-HAP bioceramics

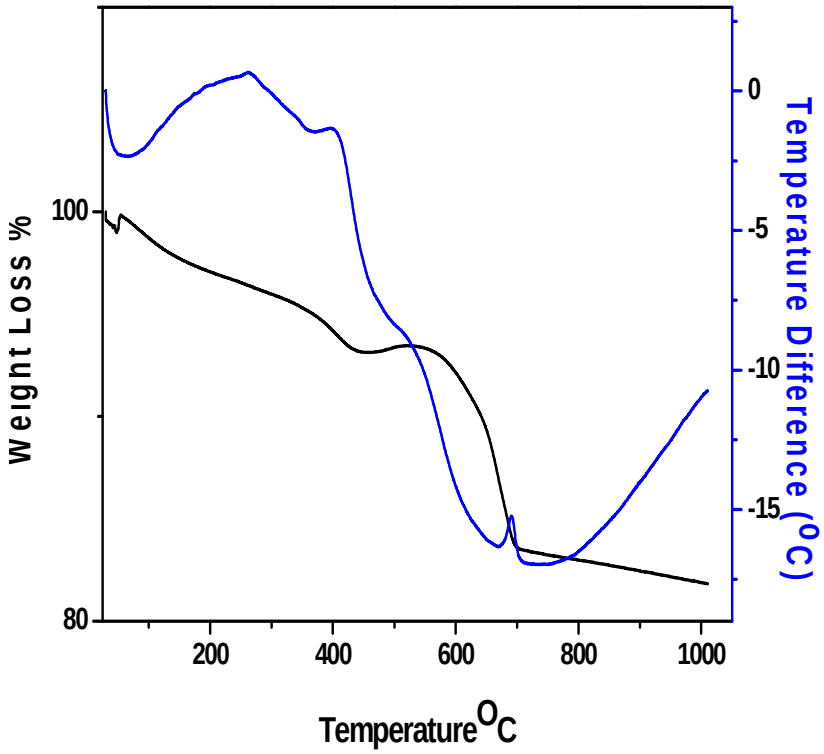


Fig. 1 TGA-DTA curve of $Ca_{10-2x}Fe_x(PO_4)_6(OH)_2$ System (x=0.2)

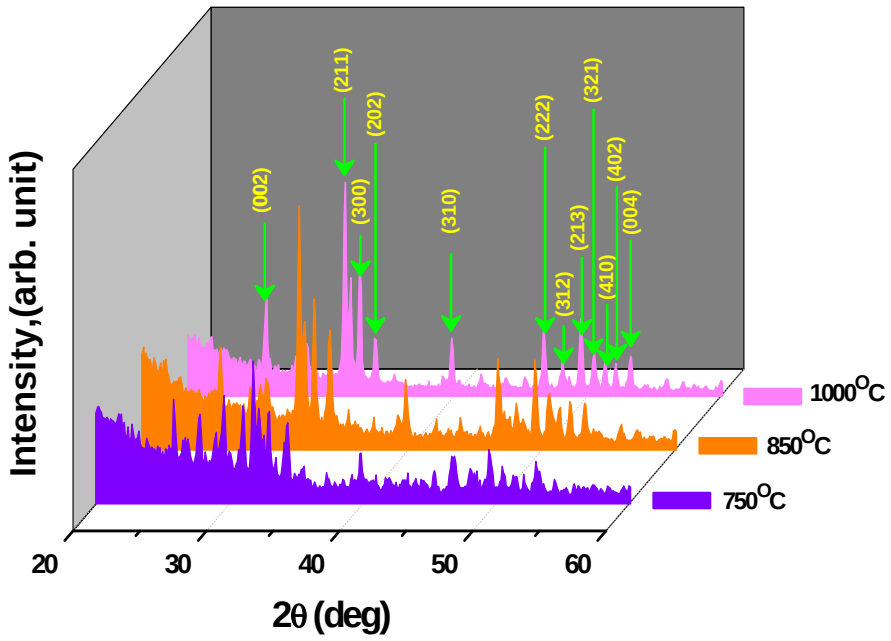


Fig. 2 XRD patterns of the pure HAP powders

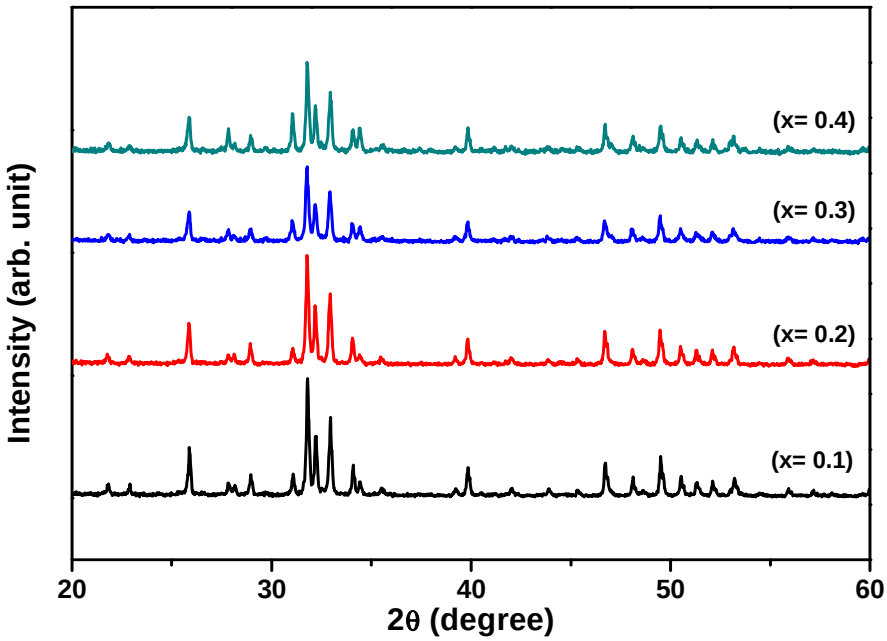


Fig. 3 XRD pattern for $Ca_{10-2x}Fe_x(PO_4)_6(OH)_2$ System ($0.1 \leq x \leq 0.4$)

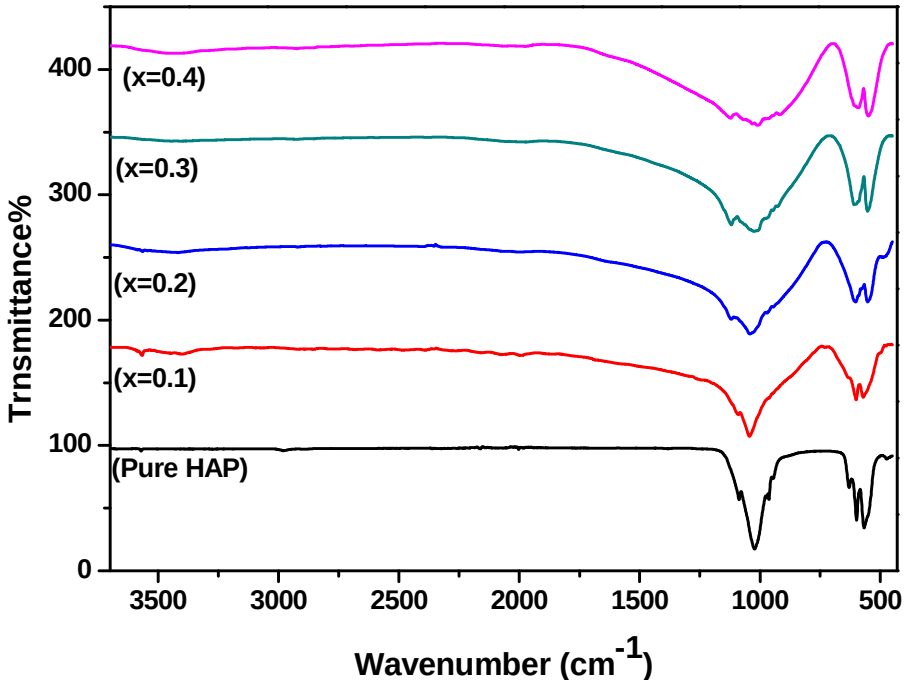


Fig.4 FTIR spectrum of $Ca_{10-2x}Fe_{2x}(PO_4)_6(OH)_2$ system

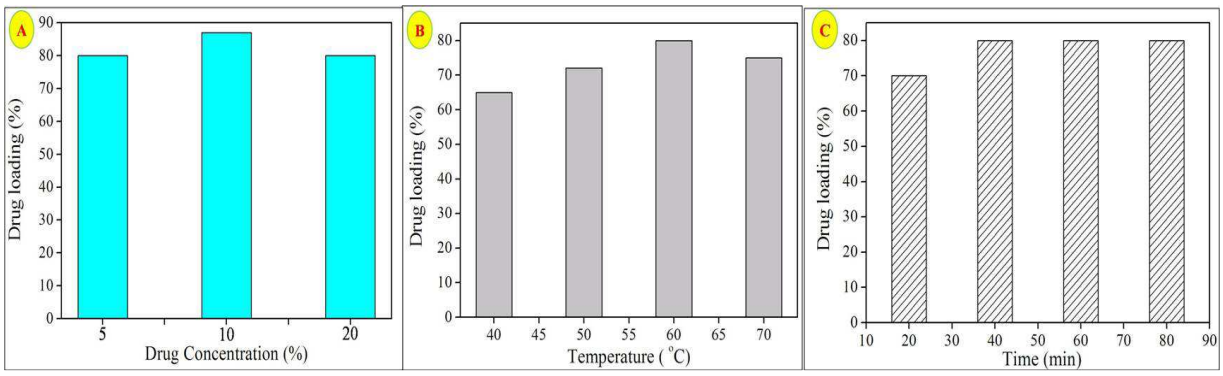


Fig. 5 Drug loading behavior on HAP at different Drug concentration (A) different temperature (B) and at different stirring time (C)

3.5 *In vitro* drug release:

Norfloxacin drug was loaded on HAP sample to examine the efficiency of the drug delivery. Fig. 7 shows different drug release profiles. The approximate drug release (%) in 200 h were found to be 60, 48 and 40% from 15, 10 and 5% of drug-loaded samples, respectively. The steady release of drug is observed for particular time; afterwards it is more or less constant. At initial time drug release behavior is high, then after, it decreases to attain some constant value. The possible reason for initial high release of norfloxacins is desorption of norfloxacins molecules from surface of the sample. The molecules of norfloxacin do not show any powerful interaction with HAP sample. HAP sample absorbs the surrounding fluid in to it during the in vitro drug release analysis, due to this exclusion and dissolution of norfloxacins is observed. More amounts of norfloxacins get exposed to the fluid because of breaking of large particles of drug into smaller particles. The complete desorption of loosely adsorbed norfloxacins and incorporation between norfloxacin drugs with HAP nanoparticles leads to slow drug release.

The in vitro drug release profile from the $Ca_{10-2x}Fe_{2x}(PO_4)_6(OH)_2$ systems is shown in Fig. 8. The percentage of norfloxacins drug released from Fe doped HAP is increases with this composition in pure HAP. Estimated percentages of drug released in 200 h were found to be 40, 44, 50 and 54% from x=0.1 to x=0.4 samples, respectively. The steady release of drug is observed for particular time; which confirm the release of drug from Fe-

HAP in controlled manner. Norfloxacin release behavior from Fe-HAP is increases with Fe composition (x=0.1 to 4.0). The results reveal that the release of drug from Fe doped HAP is superior to that of pure HAP optimum temperature and time. In vitro drug delivery experiment for HAP based bioceramics materials are also reported by other scientist for proteins and different antibiotics at different conditions and time [29, 30].

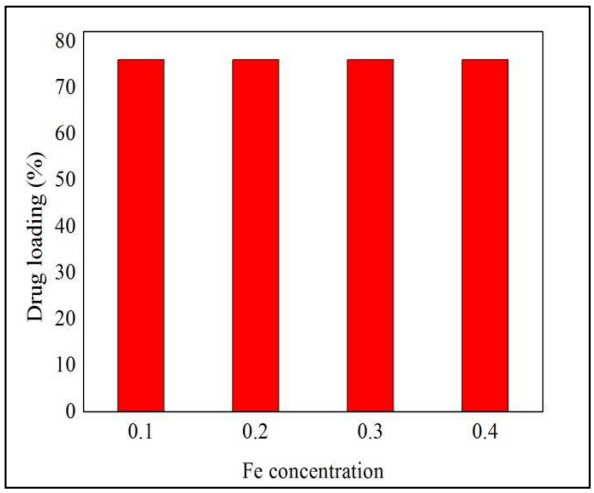


Fig. 6 Drug loading behavior on Fe-HAP system

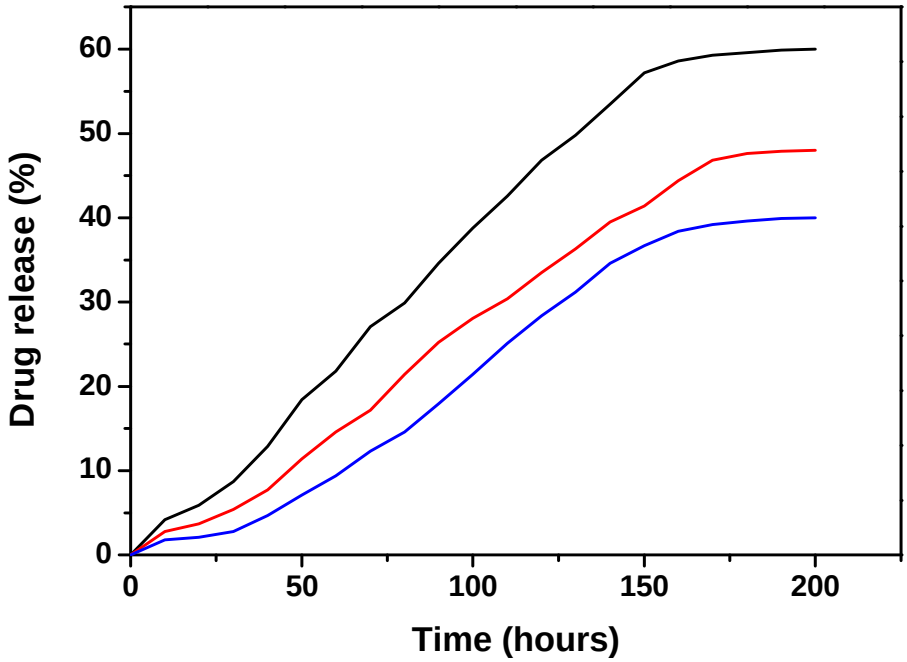


Fig. 7 Drug release percentage from HAP

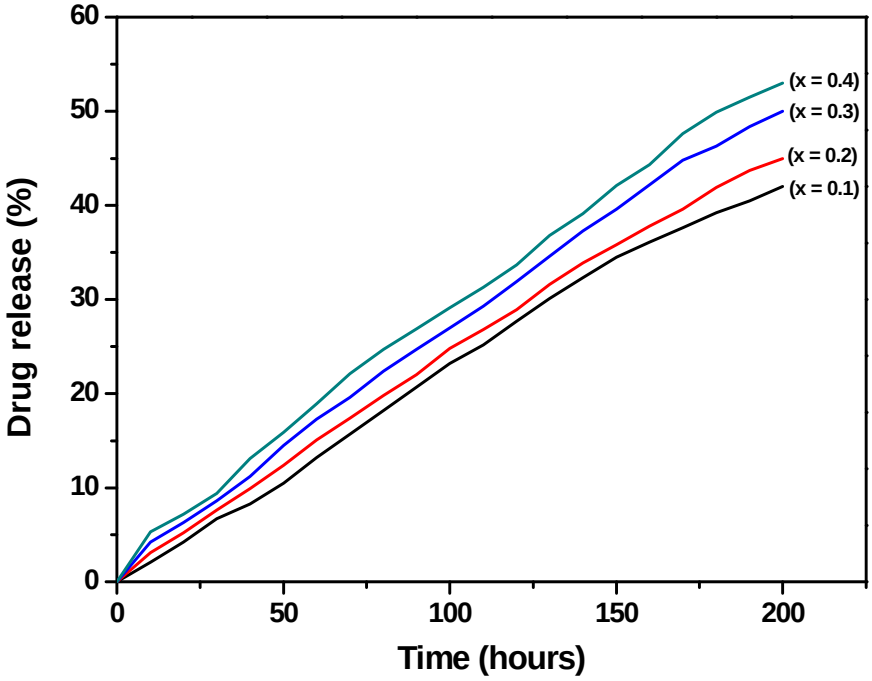


Fig. 8 Drug release percentage from $Ca_{10-2x}Fe_{2x}(PO_4)_6(OH)_2$ (x = 0.1 to 0.4)

CONCLUSION

Hydroxyapatite (HAP) and Fe doped hydroxyapatite nanoparticles with improved and unique applications in tissue engineering were synthesized by innovative auto-combustion synthesis route. Our results show that this method is particularly well suited for the formation of nano HAP and Fe doped HAP without any other phases. Crystallite size obtained from XRD was found to be 30 to 25 nm. The present investigation well demonstrated that HAP and Fe doped HAP could efficiently adsorb and controlled release of norfloxacins, which suggested its extended applications for other antibiotics.

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