Indian Journal of Science and Technology

Supplementary Article

Nanocomposites Based on Polymer and Hydroxyapatite for Drug Delivery Application

M. Sundar Raj[1*], V. H. Arkin[2], Adalarasu[3] and M. Jagannath[4]

[1]Asst. Professor, Biomedical Engineering, Bharath University, Chennai - 600 073, India; sundarmbe@gmail.com

[2]R & D Engineer, URILAB Systems- Diagnostics, New Delhi- 110 081; arkinvh@gmal.com

[3]Professor, Department of ECE, PSNACET, Dindigul – 624 622; adalbiotech@gmail.com

[4]Prof & Head - BME, SMK Fomra Institute of Technology, Chennai – 600 103; jagan.faith@gmail.com

Abstract

This research paper tells about sustained delivery of small molecular drug ciprofloxacin using hydroxyapatite hybrid poly (vinyl alcohol) nanocomposites. Hydroxyapatite - poly (vinyl alcohol) nanocomposites are used as a vehicle to carry drug, protein, implantable materials etc. In situ synthesized hydroxyapatite hybrid poly (vinyl alcohol) matrix was characterized using different characterization techniques like XRD, SEM and FTIR. Small molecular drug ciprofloxacin was loaded into hydroxyapatite hybrid poly (vinyl alcohol) nanocomposites. The morphology of drug free and drug loaded hydroxyapatite concoction poly (vinyl alcohol) nanocomposites is observed using SEM. *In-vitro* drug release is quantified using UV/VIS spectrophotometer.

Keywords: Hydroxyapatite (HAP), Poly (Vinyl Alcohol) (PVA), Ciprofloxacin, Nanocomposites, Sustained Release, Drug Delivery, X-ray Diffraction (XRD), Scanning Electron Microscope (SEM).

1. Introduction

Conventional form of drug delivery is not target specific and release of drug to the target site is not sustained. Also conventional drugs cause side effect and alternation in circulating the drug level. Novel drug delivery will give steady and complete drug release by polymer hybrid hydroxyapatite (HAP) that will follow a membrane diffusion controlled release mechanism [1]. In the past decade nanocomposites designing and fabrication form different biodegradable polymer and bioactive materials is an essential step to engineer bone tissue [2]. Mineral phase of bone and teeth has the chemical and structural similarity of bioactive ceramic hydroxyapatite, chemical formula $Ca_{10}(PO_4)_6(OH)_2$ also gained much attention because of its biocompatibility, bioactivity, osteoconductivity and osteoproductivity [3, A. 4]. The Ca^{++} ions in hydroxyapatite (inorganic) hybrid with –OH group of PVA (organic) will give more mechanical strength and osteoconductivity [5, 6, 7]. Because of large surface to volume ratio, will give the properties of porous structure, bioresorbability and ability to take in drug molecule in the surface [8, 9, 10]. Ciprofloxacin $C_{17}H_{18}FN_3O_3$, a small molecular drug (MW 331.3415) is one of the broad spectrum antimicrobial carboxyfluoroquinoline agents which have been demonstrate excellent in vitro activity against both gram-positive and gram-negative bacteria [11, 12, 13, 14, 15]. *Staphylococcus aureus, staphylococcus epidermidis, pseudomonas aerrginosa and proteus mirabilis* are the few pathogens that cause osteomyelitis which can be treated by ciprofloxacin, since the minimal inhibitory concentration of ciprofloxacin is low as 0.25-2 µg/ml [16, 17]. Hydroxyapatite hybrid PVA nanocomposites are synthesized using biomimetic approach gives a good microporous size to carry drug with sustained and pulsatile release [18].

In this paper, the adsorption and release behavior of ciprofloxacin (Figure 1) which could interact with

*Corresponding author:
M. Sundar Raj (sundarmbe@gmail.com)

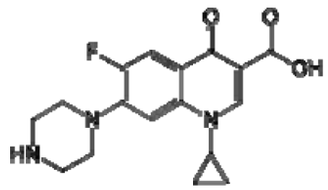

Figure 1.1 Chemical structure of ciprofloxacin.

hydroxyapatite hybrid poly (vinyl alcohol) nanocomposites was studied. Adsorption and release behavior of the drug is performed using *in vitro* study. Characterization of HAP hybrid PVA nanocomposites is validated using XRD, SEM, and bonding of chemical compounds are analyzed using FTIR. *In vitro* adsorption and release of the drug is validated using UV- VISIBLE spectrophotometer.

2. Materials

$Ca(NO_3)_2 . 4H_2O$ (calcium nitrate tertrahydrate) and $(NH_4)_2HPO_4$ (di-ammonium hydrogen phosphate) were purchased from Merck, India. Polyvinyl alcohol was procured from Sigma Aldrich, India. Ciprofloxacin obtained from a pharmacy, deionized water is obtained from Bharath University, Chennai, India.

3. Synthesis of Polyvinyl Alcohol – Hydroxyapatite Nanocomposites

Solution of 0.4M calcium nitrate tetrahydrate ($Ca(NO_3)_2 . 4H_2O$) was added in deionized water (200 ml). The pH of the solution (200 ml 0.4M calcium nitrate tetrahydrate) should be maintained above 10. To maintain above ≥10 pH add ammonia and water in the ratio 1:2. Prepare 200 ml of 0.5% poly (vinyl alcohol) (MW 31,000-50,000 Da) solution at temperature 40 - 50^{0}C. The prepared 200 ml 0.4M calcium nitrate tetrahydrate ($Ca(NO_3)_2 . 4H_2O$) is added to 0.5% 200 ml PVA solution drop wise with stirring. After complete addition, stir both the solution calcium nitrate tetrahydrate ($Ca(NO_3)_2 . 4H_2O$) and poly (vinyl alcohol) for about 24hr at 30±2^{0}C. 24hr stirring will allow the binding of –OH groups of PVA with the Ca^{++} ions. Solution of 0.156M di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$ was added in 200 ml deionized water. The pH of the solution (200 ml 0.156M di-ammonium hydrogen phosphate) should be maintained above ≥10.5. To maintain above ≥10.5 add ammonia and water in the ratio 1:1. Completely dissolved 200 ml 0.156M $(NH_4)_2HPO_4$ was added to ($Ca(NO_3)_2 . 4H_2O$) hybrid PVA solution at about 30±2^{0}C with constant stirring. Complete addition of $(NH_4)_2HPO_4$ to ($Ca(NO_3)_2 . 4H_2O$) hybrid PVA solution the mixture was washed with deionized water to remove most of the impurities, water soluble salts and to neutralize the pH. After three days of washing the final product is kept at hot air oven for a overnight at about 75-80^{0}C. The oven dried mixture ($Ca(NO_3)_2.4H_2O$) – PVA - $(NH_4)_2HPO_4$) are characterized by using XRD, SEM and FTIR characterization techniques.

4. Preparation Methodology for 0.15M NaCl

To prepare 0.15M NaCl solution,

Mass = required morality x required volume x molecular weight of the compound / 1000

M = 0.15 x 1000 x 58.44 / 1000

M = 8.766 g in 1000 ml.

Take a 1000 ml beaker and mix 8.766 g of NaCl into 1000 ml double distilled water. This will give 0.15M NaCl solution

5. Preparation of SBF (Synthetic Body Fluid)

5.1 Materials Required

Sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl), disodium hydrogen phosphate dehydrate $(Na_2HPO_4)2H_2O$, magnesium chloride ($MgCl_2$) $6H_2O$, calcium chloride dehydrate ($CaCl_2$) $2H_2O$, sodium sulfate (Na_2SO_4), were from Merck, India and tris (hydroxymethyl) aminomethane (TRIS) $((CH_2OH)_3CNH_2$ were purchased from HiMedia Laboratories Pvt. Ltd.

6. Preparation Methodology for SBF (Synthetic Body Fluid)

For preparing 1 litter of SBF (Synthetic Body Fluid), first add 700 ml deionized water in 1 liter beaker. 40 ml of 1M HCl is required to prepare 1 liter of SBF. Add first five reagents according to the order given in the tabular column Table 1 to 700 ml deionized water. Before the addition of sixth reagent add 15 ml of 1M HCl to 700 ml deionized water. Sixth, seventh and eight reagent are added subsequently and raise the temperature to 37 ^{0}C. The remaining 25 ml 1M HCl solution is added to subsequent titration to

maintain the pH of about 7.4 at 37 ^{0}C. Add another 300 ml of deionized water to make it to 1000 ml during the titration process. This 1 liter of prepared SBF is stored in 5 ^{0}C for about a month without degradation.

7. Adsorption of Ciprofloxacin onto Hydroxyapatite – Poly (Vinyl Alcohol) Nanocomposites

0.5 g of hydroxyapatite PVA nanocomposites powder is mixed with 0.10% ciprofloxacin solution to investigate the adsorption kinetics. The mixed combination of 0.10% drug (ciprofloxacin) and 0.5 g of HAP-PVA is agitated at 120 rpm/min in a shaker and incubated at 25^{0}C for 24 hours. This is done to achieve the adsorption equilibrium. Time intervals, samples were withdrawn from the suspension and centrifuged at 2000 rpm for 10 min and the supernatant is measured at 277 nm to find the amount of drug adsorbed by the nanocomposites.

8. In vitro Drug Release

To achieve in vitro sustained delivery of drug (ciprofloxacin) is performed using 0.15M NaCl as the release media or SBF (Synthetic Body Fluid) as the release media. Here we use 0.15M NaCl as the release media, because long period in vitro drug release study is performed in 0.15M NaCl medium. 100 mg of CFX-HAP-PVA is loaded into test tube and then 4 ml of 0.15M NaCl is added. Then use the shaker to shake the test tube at 25^{0}C. After certain time intervals, the test tube is centrifuged at 2000rpm/min for about 10 min. After centrifugation the 3 ml of supernatant was withdrawn for the validation by U-V VISIBLE spectrophotometer, wavelength of 277 nm to find the amount of ciprofloxacin (CFX) released. Every 3 ml of withdrawal of supernatant, add new 3 ml of 0.15M NaCl fresh release media.

9. Results and Discussion

9.1 XRD Studies

In Figure 1.2, a broad diffraction peak at 2 theta value shows the conformation of HAP-PVA composites. The diffraction peak analysis was carried out using Debye Sehere's equation: Average crystallite size (Xs) = 0.9λ/FWHMcosθ, where 0.9 is the constant related with crystalline shape, λ is the wave length of the radiation, FWHM (Full width half maximum) peak width in radians at half of the intensity maximum. By calculating different peaks of (002), (211), and (310), the average crystalline size is about 30-34 nm.

9.2 SEM and EDX Studies

Figure 1.3 shows the SEM (Scanning electron microscope) image of oven dried hydroxyapatite hybrid poly (vinyl alcohol). The image shows a thick film like structure,

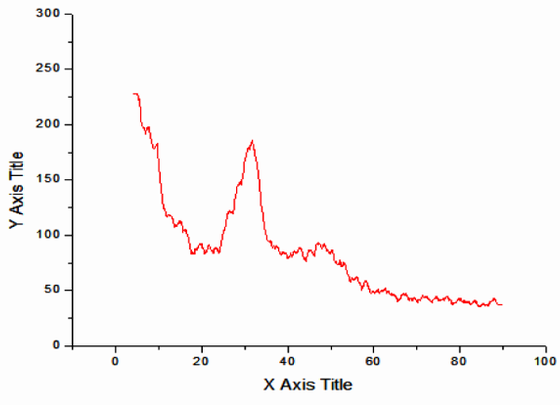

Figure 1.2 XRD of oven dried PVA-HAP composite.

Table 1. The composition of the reagent required to prepare SBF

Order	Reagent	Amount (gpl)
1.	Sodium chloride (NaCl),	6.546 g
2.	Sodium bicarbonate ($NaHCO_3$),	2.268 g
3.	Potassium chloride (KCl)	0.373 g
4.	Disodium hydrogen phosphate dehydrate (Na_2HPO_4)$2H_2O$	0.178 g
5.	magnesium chloride ($MgCl_2$) $6H_2O$	0.305 g
6.	Calcium chloride dehydrate ($CaCl_2$) $2H_2O$	0.368 g
7.	Sodium sulfate (Na_2SO_4),	0.071 g
8.	Tris(hydroxymethyl)aminomethane $(CH_2OH)_3CNH_2$	6.057 g

exhibiting particle size of about (100-200 nm) in polymeric matrix.

9.3 EDX Analysis

In the given EDX (Energy dispersive X-ray spectroscopy) Figure 1.4 shows the presence of Ca and P in the precipitated PVA-HAP composite. The given HAP-PVA composite gives the stochiometric ratio of about Ca: P ± 1.62 shows in the elemental analysis.

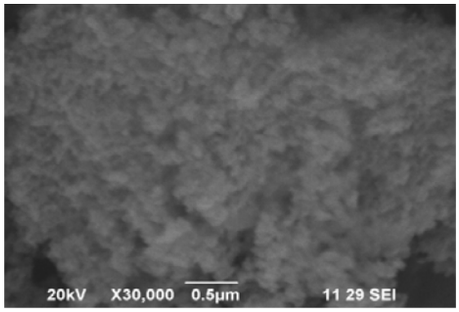

Figure 1.3 SEM image of oven dried PVA-HAP composite.

9.4 SEM Image with Microsphere

Figure 1.5 (a), (b), shows the SEM studies of the oven-dried HAP-PVA composite powder that confirmed with microspheres having diameter of about the range of 1-2 µm.

9.5 FTIR Studies

Figure 1.6 reveals the FTIR (Fourier transform infrared spectroscopy) spectra of oven dried hydroxyapatite hybrid poly (vinyl alcohol). The spectra shows the absorbance bands at 3415.93 cm^{-1} correspond to the presence of hydroxyl groups in the system. Absorbance bands at 1647.21 and 1382.96 cm^{-1} reveals the presence of C=O and CH_2 asymmetric bending and bands at 1099.43 and 565.14 cm^{-1} shows the presence of phosphate group in the hydroxyapatite hybrid PVA nanocomposites system.

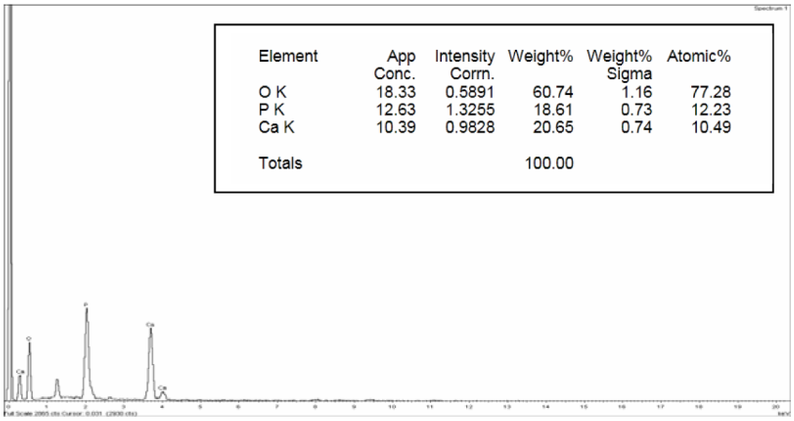

Figure 1.4 Shows the EDX of as precipitated PVA-HAP composite.

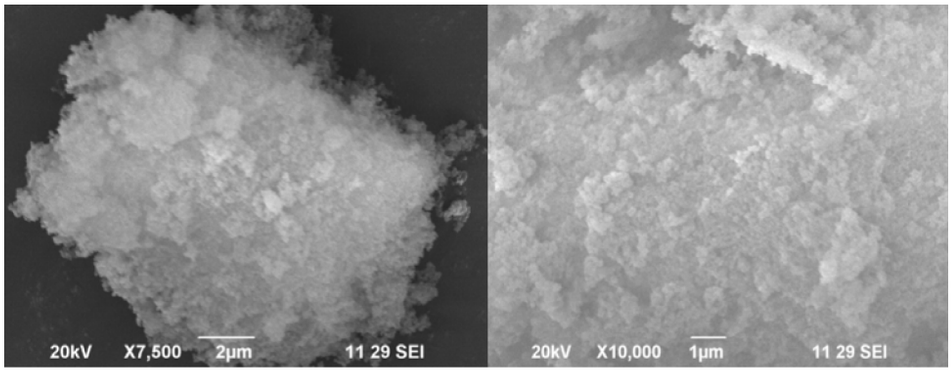

Figure 1.5 (a), (b), SEM images shows the HAP-PVA microsphere.

9.6 In vitro Ciprofloxacin Release

Carrying and delivery of active drugs to the infection site was frequently extended to resorbable and even to soluble biomedical polymer. HAP and PVA has the ability to absorb and release the drug ciprofloxacin also has the biocompatible and nanocomposites which gives more advantage [19]. The given Figure 1.7 shows the release kinetics of ciprofloxacin from HAP-PVA oven dried nanocomposites using 0.15M NaCl. Normal hydroxyapatite will show a burst release in the initial stage. Coating of HAP by PVA shows the sustained release of about 70% drug in 7days and 12 hours. This shows that bioceramic material hydroxyapatite hybrid with biopolymer PVA nanocomposites help to adsorb small molecular drug and gives shows a sustained release in in-vitro analysis.

10. Conclusion

Novel drug loaded hydroxyapatite (HAP) coated poly (vinyl alcohol) (PVA) microsphere have been synthesized by biomimetic method and adsorption – release properties of drug ciprofloxacin was investigated. Interaction of drug between ciprofloxacin and hydroxyapatite hybrid poly (vinyl alcohol) surface and the release profile obtained from UV analyses. Further this work can be carried out by in vitro anti bacterial testing and coating of CFX-HAP-PVA in to materials like titanium alloy for controlled and targeted drug release by in vivo study can be done.

11. Acknowledgement

This research is supported by Crystal Research Center and Pharmaceutical Chemistry, Bharath University, Chennai, India.

12. References

1. Wang X, Hui A et al. (2010). Towards sustained delivery of small molecular drugs using hydroxyapatite microsphere as the vehicle, Advance Power Technology, vol 21(3), 268–272.
2. Wahl D A, and Czernuszka J T (2006). Collagen hydroxyapatite composites for hard tissue repair, European Cells and Materials, vol 11, 43–56.
3. Degirmembasi N, Kalyon D M et al. (2006). Biocomposites of nanohdyroxyaaptite will collegen, and poly (vinyl alcohol), Colloids and Surfaces B: Biointerfaces, vol 48(1), 42–49.
4. Asron A S, Henning S et al. (2010). Poly vinyl alcohol collegen hydroxyapatite biocomposites, nano fibrous scaffold: mimicking the key feature of natural bone at the nanoscale level, Polymer, vol 51(4), 868–876.
5. Taguchi T, Kishida A et al. (1998). Hydroxyapatite formation on/in poly(vinyl alcohol) hydrogel matrices using a novel alternate soaking process, Chemistry Letters, vol 27, No. 8, 711–712.
6. Pan Y S, Xiong D S et al. (2007). Friction characteristics of poly (vinyl alcohol) hydrogel as an articular cartilage biomaterial, Key Engineering Materials, vol 330–332, 1297–1300.
7. Noguchi T, Yamamuro T et al. (1991). Poly (vinyl alcohol) hydrogel as an artificial articular cartilage: evaluation of

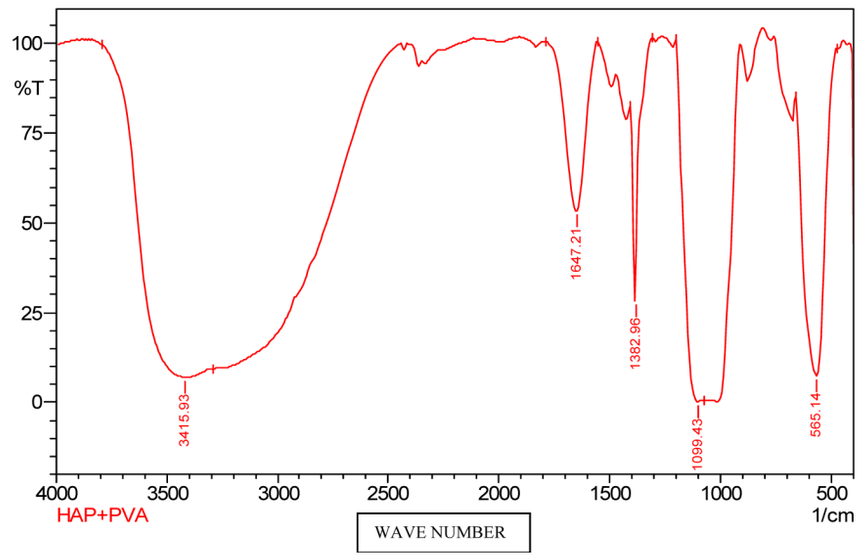

Figure 1.6 FTIR spectra of oven-dried HAP-PVA particle.

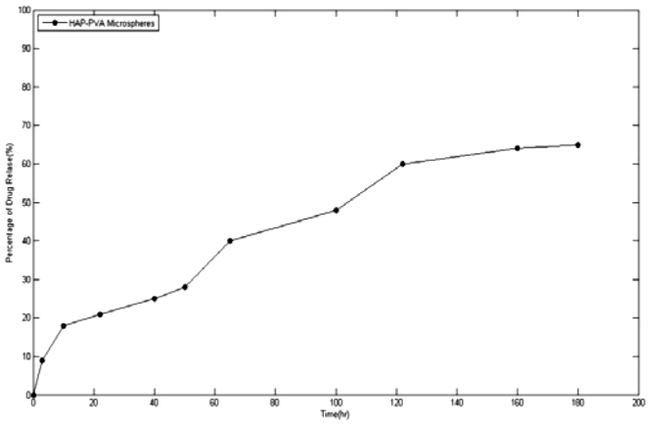

Figure 1.7 In vitro drug release of CFX adsorbed HAP-PVA

biocompatibility, Journal of Applied Biomaterials, vol 2(2), 101–107.

8. Paul W, and Sharma C P (2003). Ceramic drug delivery: a perspective, Journal of Biomaterials Application, vol 17(4), 253.
9. Paul W, and Sharma C P (2006). Nanoceramic materials: Biomedical applications, American Journal of Biochemistry & Biotechnology, vol 2, 41–48.
10. Barroug A, and Glimche M (2002). Hydroxyapatite crystals as a local delivery system for cisplatin: adsorption and release of cisplatin in vitro. Journal of orthopaedics, vol 20(2), 274–280.
11. Chow A W, Hall C B et al. (1992). General guidelines for the evaluation of new anti-infective drugs for the treatment of respiratory tract infections, Clinical Infectious Diseases, vol 15(Supplement 1), S62–S88.
12. Mylotte J M, Ksiazek S et al. (1994). Bentley Rational approach to the antibiotic treatment of pneumonia in elderly, Drug & Aging, vol 4(1), 21–33.
13. Cunha B A (1995). The antibiotic treatment of community-acquired, athipical, and nosocomial pneumonias, The Medical clinics of North America, vol 79(3), 581–597.
14. Vogel F (1995). A guide to the treatment of lower respiratory tract infections, Drugs, vol 50(1), 62–72.
15. Ortqvist A (1995). Antibiotic treatment of community-acquired pneumonia in clinical practice: a European perspective, Journal of Antimicrobial Chemotherapy, vol 35(1), 205–212.
16. Lambert H P, and O'Grady F W (1992). Antibiotic and Chemotherapy, 6th Edn., Churchill Livingstone, Edinburgh, 245–262.
17. Armstrong E P, Elsberry V A (1997). Bone and joint infections, Dipiro J T, Talbert R L, Yee G C, Matzke G R, Wells B G, Posey L M (Eds.), Pharmacotherapy. A Pathophysiologic Approach, 3rd Edn, Appleton & Lange, Stamford, 2221–2235.
18. Gupta S, Nayar S et al. (2008). Effect of particle morphology on drug release from poly (vinyl alcohol)- hydroxyapatite nanocomposites, Kapecele J. Soluble biomedical polymers, polim med vol 7(3), 191–221.
19. Shiffman M L, and Keith F B (1990). Pathogenesis of ceftriaxone – associated biliary sludge, In vitro studies of calcium – ceftriaxone binding and solubility, Gastroenterology, vol 99(6), 1772–1778.