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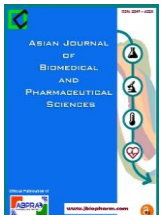

RESEARCH ARTICLE

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Conflict of Interest: None Declared !

Preparation, characterization and drug delivery behavior of novel biopolymer/hydroxyapatite nanocomposite beads

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Abstract
Nano-sized materials possess uniqueness in their properties and designs as compared to their bulk counterparts. For this reason, they have attracted a great deal of attention from the scientific community. Nanocomposite is a composition having a dispersed material that has one or more dimensions, such as length, width and thickness, in the nanometer size range. The nano-sized materials have emerged as suitable alternatives to overcome drawbacks of composite and microcomposite materials. Biodegradable polymer /hydroxyapatite nanocomposites are a novel class of materials which have recently attracted interest as biomaterials and as drug delivery vehicles. The objectives of the present study were the preparation, evaluation and drug delivery behaviour of nanocomposite beads based on biodegradable polymer sodium alginate and inorganic material hydroxyapatite. In the present study, biopolymer / hydroxyapatite nanocomposite beads have been prepared, optimized and studied in parallel. The prepared nanocomposite beads were characterized by means of XRD, zeta sizer, and SEM, for better understanding regarding their composition and surface morphology. Polydispersity index of particles and mean particle sizes of the nanocomposite beads were measured by zeta sizer. XRD reports confirm the nanocrystalline composition and crystallite size. SEM provided the nanocomposites shapes and their surface topography. The average diameters of particles in the nanocomposites were found to be around 200 nm. We used this biopolymer/hydroxyapatite nanocomposite beads to evaluate its drug delivery behaviour using ofloxacin as a model drug. The in vitro drug-release study confirmed that prepared biopolymer/hydroxyapatite nanocomposite beads exhibited extended release period of drug as compared to the pristine biopolymer sodium alginate.

Keywords: Biopolymer, Sodium alginate, Hydroxyapatite, Nanocomposite, Drug release.

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1. INTRODUCTION

Nanocomposites are composite materials in which at least one phase shows dimensions in the nanometer range. These high performance nano-materials exhibit unusual and unique properties for which they are considered as the materials of the 21st century. These materials are of great importance for many industries because major improvements in functional and structural properties in material application are achieved by using composite materials made with nanometer-scale components. The growing demand for nanocomposite material shows a promising future. The application of such material is strongly influencing the industry of medicine and pharmacy [1].

The site specific drug delivery provides an opportunity for optimum pharmacological activity of drug. Recently special attentions have been paid to find novel approaches to control the rate of drug release by means of carrier systems, where the drug should be dispersed in an inert matrix. Polymer based nanocomposites are of great importance as controlled drug delivery vehicles due to their unique structures and properties. These nanocomposites are prepared using inorganic materials including hydroxyapatite, calcium deficient hydroxyapatite [2, 3], mineral clays [4-8], and silica [9]. They possess improved mechanical properties, swelling properties, drug loading efficiency and controlled release behaviour as compared to bulk counterparts.

Hydroxyapatite has been widely used as a biocompatible ceramic in many areas of medicine due to its resemblance to mineral bone. It possesses exceptional biocompatibility and bioactivity properties with respect to bone cells and tissues, probably due to its similarity with the hard tissues of the body. These materials are suitable for bone replacement and for drug release such as antibiotics, growth factors or other substances. Nanosized hydroxyapatite can be used in drug delivery systems like intestinal delivery of insulin or other drugs such as antibiotics [10].

Some of the impressive and recent applications of sodium alginate as polymer have been in the area of biomedical sciences. It is a natural polymer that possesses certain properties such as biocompatibility, biodegradability. Biocompatibility means it will not show any adverse effect inside the body where as biodegradability means its degradation products or materials are metabolizable in the body. Alginate, a linear, naturally occurring polysaccharide extracted from brown sea algae contains D-mannuronic (M) and L-gulcuronic (G) acids which are arranged in homo polymeric MM or GG blocks separated by blocks with an alternating sequence. Sodium alginate can act as drug carriers by controlling the release rate of drug initially loaded in the application of drug delivery systems. The ability of alginate to form two types of gel dependent on pH, i.e., an acid gel and an ionotropic gel, gives the polymer unique properties compared to neutral macromolecules. Alginate shrinks at low pH (gastric environment) and the encapsulated drug. Thus much interest has been shown in its practical prospective as a potential matrix of biodegradable composite which could be synthesized from renewable sources at a very low cost and should be eco friendly[11-20].

Ofloxacin is a new fluoroquinolone with a spectrum of activity similar to other fluoroquinolones with activity which includes *Chlamydia trachomatis*, and *Legionella pneumophila*. Ofloxacin may be less susceptible to the development of resistance from *Staphylococcus aureus* commonly seen with currently available fluoroquinolones. It is also used in chlamydial infections including nongonococcal urethritis in treating mycobacterial infections such as leprosy [21,22].

The present paper reports regarding preparation, evaluation and drug delivery behaviour of sodium alginate-hydroxyapatite nanocomposite beads using ofloxacin as a model drug.

2. EXPERIMENTAL

Sodium alginate (low viscosity) was obtained from Research-Lab Fine Chem. Industries, Maharashtra State, India. Ofloxacin was purchased from Sigma-Aldrich, USA. Other reagents and chemicals were obtained from Deepa industries ltd, Aurangabad, India. All chemicals were used as received, without further purification.

Preparation of hydroxyapatite

Hydroxyapatite was prepared by the wet precipitation method. In one beaker, 7.2 mM solution of Na2HPO_4 was made in double distilled water. In another beaker, 12 mM solution of $CaCl_2$ was made using double distilled water. 200 ml of $CaCl_2$ solution was added slowly through a pipette to 1 liter solution of Na_2HPO_4 with continuous stirring. The solution was adjusted to 7.4 pH using 1N NaOH. The prepared precipitate was allowed to settle down for 24 h. The precipitate recovered was dried and used for nanocomposite preparation. This precipitate was named as hydroxyapatite.

Preparation method of hydroxyapatite-sodium alginate-ofloxacin nanocomposite beads

Firstly sodium alginate solution was prepared at 50^0 C temperature and 300 rpm by using double distilled water (1.5 gm SA + 36ml DDW). After complete dissolution of sodium alginate, $(NH_4)_2HPO_4$ solution (0.6 gm $(NH_4)_2HPO_4$ + 12.5 ml DDW) was added slowly

into the sodium alginate aqueous solution and to this 0.20 gm of ofloxacin was added. The mixture was further stirred for 4 hours to ensure the homogeneity of the system. The prepared solution was added drop wise into 500 ml of 6% Ca $(NO_3)_2.4H_2O$ aqueous solution at 30^0 C with mild stirring (200 rpm) through a 1.2-mm inner diameter needle, using a hypodermic syringe. The pH of the system was maintained in the range 10.3–10.5 using 28% ammonium hydroxide. The milky white nanocomposite beads were formed immediately and further kept for 16 hour. The nanocomposite beads were dried at room temperature for 2 days.

For comparison study, pristine sodium alginate beads, sodium alginate-ofloxacin beads and hydroxyapatite-sodium alginate beads without ofloxacin were also prepared.

Drug incorporation efficiency

The drug incorporation efficiency was determined according to the following equation

IE= W1/W2 × 100

Where, W1 is the actual drug content of the beads and

W2 is the theoretical drug content of the beads.

In vitro drug release

The in vitro drug release tests were carried out using the USP 30 NO.2 dissolution test apparatus fixed with six rotating paddles. The release medium is a simulated intestinal fluid (pH 7.4) or simulated gastric fluid (pH 1.2) (500 ml, 37.5 ± 0.5 °C), and the speed of rotation was 50 ± 1 rpm. 50 mg samples were placed into each of the six cups, and 5 ml solution was collected from the release medium at regular intervals. After each sample collection, the equal amount of fresh release medium at the same temperature was added. The amount of drug released was monitored by a UV Visible spectrophotometer (Shimadzu UV 1800 spectrophotometer) at 294 nm.

Characterization of prepared nanocomposite beads

The intensity of distributions, average diameter, and polydispersity index of particles in the nanocomposite beads were determined by Particle size analyzer (LS230, Beckman Coulter, USA). The surface morphologies and surface topography study of the beads were obtained using a scanning electron microscope (SEM).The powder X-ray diffraction (XRD) analysis was performed using a powder diffractometer with Cu target and Kα (λ=0.154056nm) at 40 kV with a slow scan of 0.3 degree/s in 2θ range 10-50 degree at room temperature.

The crystallite size of the nanocomposite was determined from the XRD study by the Scherrer Equation.

$t= 0.9 \lambda / B.\cos\theta$

Where, t = thickness of crystallite, λ = x-ray wavelength, B = (2θ High) – (2θ Low)

3. RESULTS

Morphology of the nanocomposite beads

Generally, the wet beads were spherical with a diameter of about 5 mm and possessed a smooth surface. After air drying, the diameter of test beads decreased to about 2 mm but still kept the spherical shape. For all batches of nanocomposite beads, there was no variation of the bead size. The large size of wet beads suggested high swelling and water retention capability. The average particle diameter of the nanocomposite beads (hydroxyapatite-sodium alginate-ofloxacin) was found to be 257.6 nm, where as polydispersity index of particles was found to be 0.135. From the intensity of distribution table, it is found that the diameters of 10% particles are below 58.40 nm, 50% particles are below 143.60 nm, 90% particles are below 1292.40 nm.

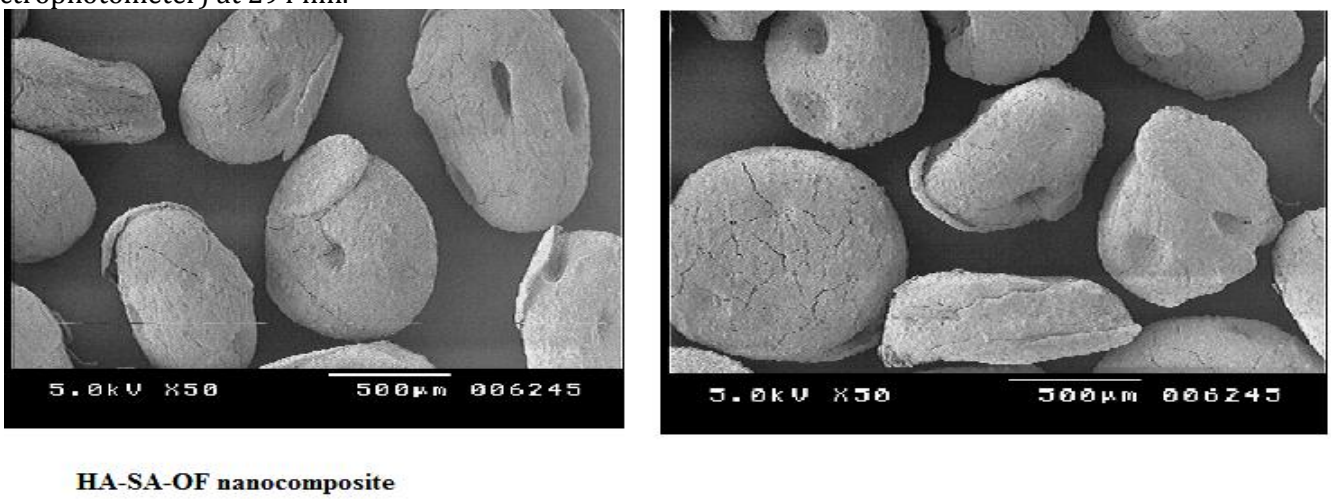

Figure 1: SEM of hydroxyapatite-sodium alginate-ofloxacin nanocomposite

Formulation type	polydispersity Index	Diffusion Const. (cm^2/sec)	Average Particle size(D) in nm	D (10%) in nm	D (50%) in nm	D (90%) in nm
HA-SA-OF	0.135	1.909e-008	257.6	58.40	143.60	1,292.40
HA-SA	0.177	1.639e-008	300.2	36.50	111.90	349.80
SA-OF	0.191	1.399e-008	351.6	35.60	129.80	2,564.40
Pristine SA	0.349	6.044e-009	813.9	237.40	294.10	15,237.60

Table 1: Average particle size (D), Diffusion Constant, polydispersity Index of prepared formulations

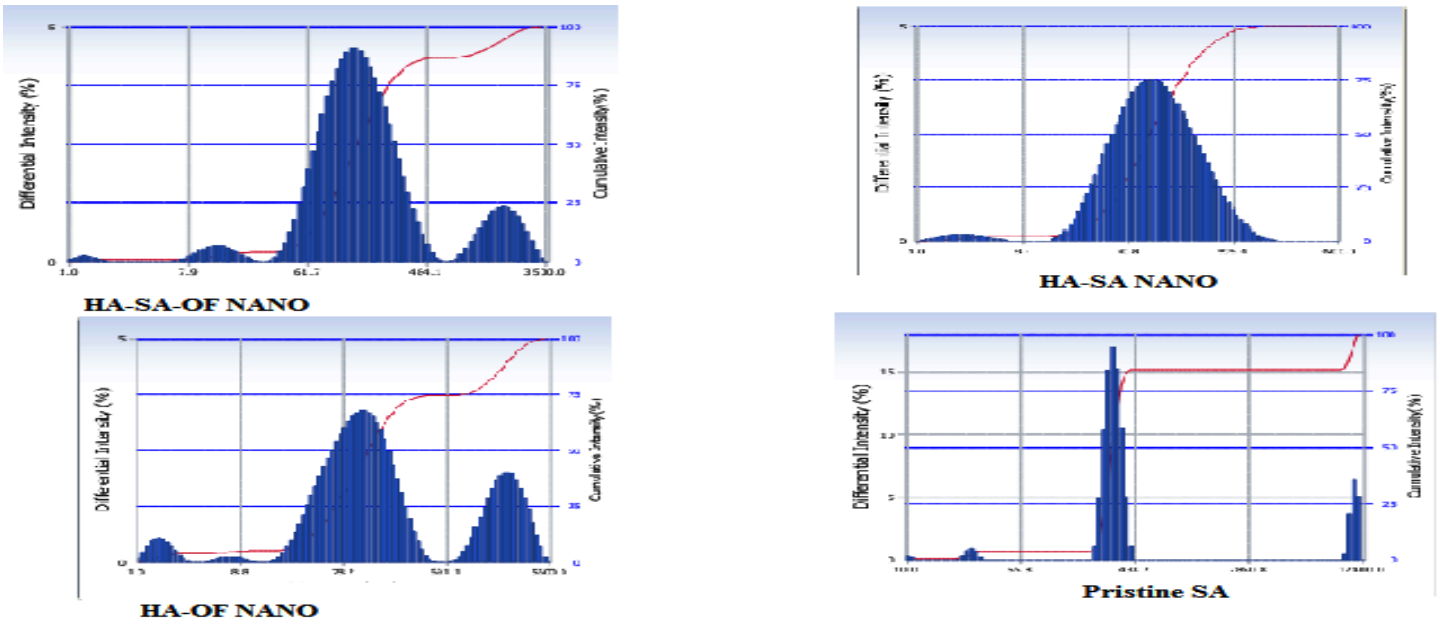

Figure 2: Intensity of particle distributions in nanocomposite beads

Powder X-ray diffraction Analysis:

XRD patterns of polymer-hydroxyapatite nanocomposite beads are shown in figure 3. The XRD result confirms the formation of nanocomposite beads. The crystallite size of the nanocomposite beads was found to be around 200nm from the calculations done by applying Scherrer equation which is somewhat similar with the particle size determined by particle size analyzer (zeta-sizer).

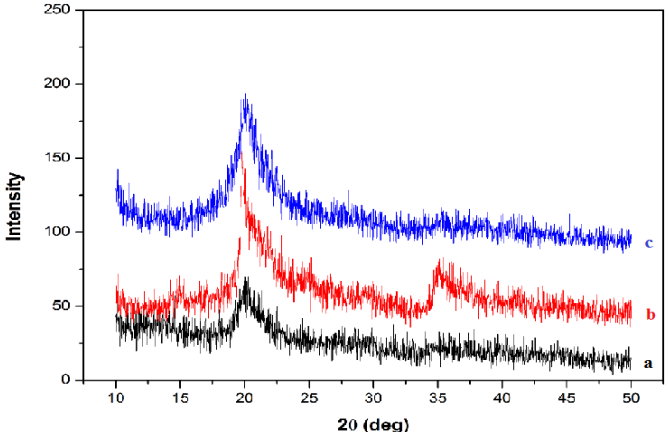

Figure 3: XRD pattern of (a) HA-SA, (b) SA-OF, (c) HA-OF-SA nanocomposite beads

Drug incorporation efficiency

The drug incorporation efficiency was determined according to the following equation

IE= W1/W2 × 100

The incorporation efficiency (IE) is given in Table 2. The IE of the beads increased with the amount of hydroxyapatite and sodium alginate added. The incorporation of drug in beads without hydroxyapatite was 43.23, whereas the corresponding entrapment in the hydroxyapatite and sodium alginate containing beads varied from 55.9 to 93.52.

Nanocomposite code	Incorporation Efficiency (%)
F1	43.23 ± 3.23*
F2	55.9 ± 4.25*
F3	65.43 ± 3.21*
F4	93.52 ± 2.21**

Significantly different at ** $p < 0.01$ and * $p < 0.05$

Table 2: Drug Incorporation Efficiency of Formulations

In vitro drug release study

In vitro release profile of the hydroxyapatite-sodium alginate-ofloxacin, sodium alginate-ofloxacin beads are shown in figure 4. Addition of hydroxyapatite decreases the drug release.

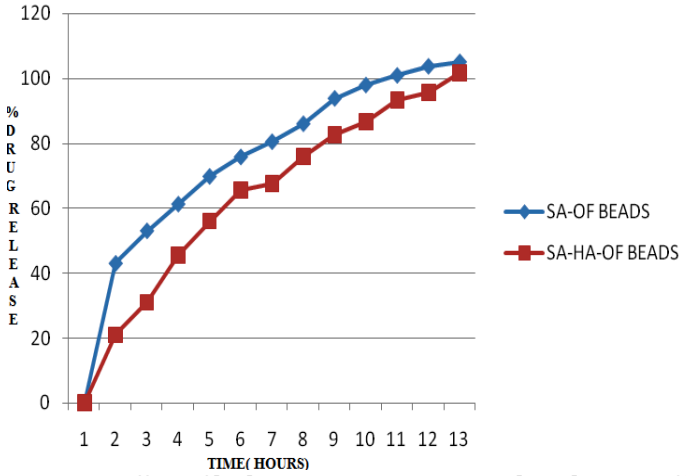

Figure 4: Effect of hydroxyapatite content on the release profile at pH 7.4.

4. DISCUSSION:

The morphology study and surface topography study (SEM, zeta sizer, XRD) revealed the nanocomposite formulation. It is clearly observed that the particle size of the nanocomposite beads (hydroxyapatite-sodium alginate-ofloxacin) is around 200 nm. As the hydroxyapatite content of the nanocomposite beads increased, there was corresponding increase in entrapment efficiency. This may be due to the fact that the higher the hydroxyapatite content the thicker is the coat around the drug, which prevents the loss of drug during the processing of nanocomposite beads. From the in vitro release study, it is confirmed that addition of hydroxyapatite decreases the release of ofloxacin at pH 7.4.

CONCLUSION:

Biodegradable polymer – hydroxyapatite nanocomposites were successfully prepared by incorporating ofloxacin as a model drug. The mechanisms of nanocomposite bead formation, crystallite size, crystallinity, morphology were studied. XRD investigation showed an intermolecular interaction between hydroxyapatite and polymer .The average diameters of particles in the nanocomposites were found to be around 200nm from both XRD and particle size analyzer. The in vitro drug-release study confirmed that prepared biopolymer/hydroxyapatite nanocomposite beads exhibited extended release period of drug as compared to the pristine biopolymer sodium alginate. This study provides a platform for further research on the polymer-hydroxyapatite nanocomposites for drug delivery and biomedical applications.

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