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RESEARCH ARTICLE

Design and Characterization of Novel Biodegradable Polymer-Clay-Hydroxyapatite Nanocomposites for drug delivery applications

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ABSTRACT

In recent years, biodegradable polymer based nanocomposites have been used promisingly for various drug delivery systems and biomedical applications as compared to synthetic polymer composites because of the biocompatible and biodegradable behavior of natural polymers. These biodegradable polymer nanocomposites also show improved mechanical properties, swelling behavior, drug loading efficiency and controlled release behavior as compared to polymer matrices. The objective of the present study was to design and characterize nanocomposites based on hydroxyapatite and biodegradable polymer chitosan and montmorillonite clay. The nanocomposites were successfully prepared and their structures were characterized by powder x-ray diffraction (XRD), particle size analyzer (Beckman coulter), scanning electron microscopy (SEM), and fourier transmission infrared spectroscopy (FT-IR) techniques. FT-IR studies provided the evidence of molecular interaction among the constituents of the nanocomposite. SEM provided the shapes and surface topography of polymer nanocomposites. Particle size analyzer provides the intensity distributions and average size and polydispersity index of particles. XRD provided the information on the degree of hybrid structure generated and crystal size. The average diameters of particles in the nanocomposites were found to be below 500nm. This work represents the design of novel clay–chitosan–hydroxyapatite nanocomposite with improved properties that has potential applications in novel drug delivery.

KEY WORDS: hydroxyapatite, chitosan, cloisite 10A, nanocomposite

1. INTRODUCTION

Polymer nanocomposite materials have emerged as suitable alternatives to overcome limitations of microcomposites for drug delivery [1]. In recent years the advances in synthesis techniques and the ability to characterize materials on atomic scale has lead to a growing interest in nanometer-size materials. Polymer nanocomposites combine these two concepts, i.e., composites and nanosized materials. Polymer nanocomposite materials are materials that consist of particles of one compound with a mean diameter in the nanometer range (1-1000nm) dispersed throughout material commonly modified inorganic clay dispersed within an organic polymer [2].

Chitosan is a naturally occurring polymer which finds a wide variety of pharmaceutical applications due to its biodegradability, biocompatibility, nontoxicity and mucoadhesion. It is also very cheap. It has found pronounced application in multi-particulate drug delivery like microparticles and nanoparticles [3- 7]. Chitosan is a modified natural carbohydrate polymer by the alkaline deacetylation of chitin. The primary amine groups render special properties that make chitosan very useful in pharmaceutical applications [8].

Montmorillonite is complex colloidal magnesium aluminum silicate clay. It is available in several grades for different applications and for different formulations. It was discovered in Montmorillon of France, named by Mauduyt

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in 1847. Montmorillonite, a member of the Smectite family, is having 2 tetrahedral sheets sandwiching a central octahedral sheet. The particles are plate-shaped with an average diameter of approximately 1 micrometer. It is the main constituent of the volcanic ash weathering product, bentonite [9-11].

Montmorillonite clays and their modified forms find wide range of applications, in various areas of science, like drug delivery systems due to their natural abundance and the propensity with which they can be chemically and physically modified for drug delivery applications [12-13]. Montmorillonite has been extensively applied for prolonged release of drugs as it can retain large amounts of drug due to its high cation exchange capacity. The surface adsorption of various drugs like griseofulvin, indomethacin and prednisone to montmorillonite clay improves the dissolution rate. The hydrophilic and swelling properties of montmorillonite in aqueous media help to facilitate the wetting of hydrophobic drug substances. This clay ultimately improves the bioavailability of drugs.

Cloisite® 10A is a natural montmorillonite modified with a quaternary ammonium salt. The organic modifier is 2MBHT: dimethyl, benzyl, hydrogenatedtallow, quaternary ammonium. The modifier concentration is 125 meq/100g clay.

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ is chemically similar to the mineral component of teeth and bones. It normally supports bone in growth and osteointegration when used in orthopedic, dental and maxillofacial applications. It can be produced from biogenic materials like coral, seashell, eggshell, body fluids and synthetic methods. It is suitable for delivery system as it is biocompatible. It also can adsorb a variety of chemical species. As there is weak interaction between the drugs and the hydroxyapatite particles, the drug release from hybrid is very fast [14, 15, 16]

2. MATERIALS AND METHODS

Materials:

Chitosan (86% deacetylation,) was commercially obtained from Research-Lab Fine Chem. Industries, Maharashtra State, India. Cloisite 10A was purchased from Southern Clay Products, USA. Other reagents and chemicals were obtained from Deepa industries ltd, Aurangabad, India. All chemicals were used as received, without further purification.

Methods:

Preparation method of cloisite 10A-chitosan composite

Cloisite 10A-chitosan composite is prepared in 4 steps.

Firstly, Cloisite 10A(10 wt %) suspension was prepared by adding double distilled water under vigorous stirring for 24 hours and kept for another 24 hours for swelling. Then chitosan (90 wt %) solution is prepared by dissolving it in 1 %(w/v) acetic acid to prepare 0.5 %(w/v) solution.

The prepared chitosan solution was added slowly into the cloisite 10A suspensions under stirring for 12 hour at 60 ^{0}C. The solution was then poured into a petri dish and dried in an oven at 80 ^{0}C for 48 hour to form thin sheets. This film was named as chitosan-cloisite 10A [17].

Preparation of hydroxyapatite

Hydroxyapatite was prepared by the wet precipitation method in six steps.

In one beaker, 7.2 mM solution of Na_2HPO_{4n} was made in DI water. In another beaker, 12 mM solution of CaCl2 was made using DI 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 [18].

Preparation method of hydroxyapatite-chitosan composite

A hydroxyapatite-chitosan composite was prepared in 3 steps.

Firstly, 40 wt% of freshly prepared hydroxyapatite precipitate was added to 60 wt% of chitosan solution under stirring for 12 hour at 60 ^{0}C. This solution was then poured into a petri dish and dried in an oven at 80 ^{0}C for 48 hour to form thin sheets. This film was named as chitosan-hydroxyapatite.

Preparation method of cloisite 10A-chitosan-hydroxyapatite composite

Cloisite 10A-chitosan- hydroxyapatite composite is prepared in 4 steps. 50 wt % of chitosan solution was made dissolving it in 1 %(w/v) acetic acid. Then, 10 wt % of previously prepared cloisite 10A suspension was added to it followed by 40 wt % hydroxyapatite. The prepared mixture was stirred properly for 12 hour under vigorous stirring.This solution was then poured into a petri dish and dried in an oven at 80 ^{0}C for 24 hour to form thin sheets. This film was named as cloisite 10A-chitosan-hydroxyapatite.

Preparation of chitosan film

Pure chitosan film is also made in the same process in order to compare with these composites.

This film was named as chitosan film. All the prepared films were characterized using different characterization tools like XRD, FT-IR, SEM, and Particle size analyzer.

3. CHARACTERIZATION

The nanocomposite films were evaluated by powder XRD, particle size analyzer (Beckman coulter), SEM, and FT-IR techniques.

FT-IR spectroscopy

Infrared spectroscopy is one of the most powerful analytical tools, which provides the possibility of chemical identification. The important advantages of infrared spectroscopy over the other usual methods of structural analysis (X-ray diffraction, electron spin resonance, etc.) are that it provides information regarding the structure of a molecule, without tiresome evaluation methods. FT-IR is based upon the simple principle that a chemical substance shows selective absorption in the infrared region giving rise to absorption bands called an IR absorption spectrum, over a wide wavelength range. Different bands will be present in the IR spectrum, which will correspond to the characteristic functional groups and bonds present in a chemical substance. Thus an IR spectrum of a chemical substance is a fingerprint for its identification. IR spectrum of polymer nanocomposite shows the presence of both nanomaterials and polymers (depending upon the polymer chain) at various frequencies.

The infrared absorption spectra of the formulated films were obtained using a FT-IR spectrophotometer (PerkinElmer Spectrum Version 10.03.02).

Particle size analysis

Particle size analyzer (LS230, Beckman Coulter, USA) provides the information regarding the intensity of distributions, average diameter, and polydispersity index of particles in the composite films.

Scanning electron microscope (SEM)

The surface morphologies of the films were obtained using a scanning electron microscope (SEM). The scanning electron microscope (SEM) is one of the most versatile instruments widely applied to surface microstructure imaging. SEM is a type of electron microscopy that images the sample surface of a solid specimen by using a focused beam of high-energy electrons. The signal contains information about surface topography, external morphology, chemical composition, crystallographic information, and electrical conductivity.

X-Ray Diffraction study (XRD)

X-ray diffraction is one of the most important characterization tools used in solid state chemistry and materials science. X-rays are electromagnetic radiation having a wavelength of about 1 Å (10-10 m), which is about the same size as an atom.

The 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 10^0-50^0 at room temperature.

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

$t = K * \lambda / B*\cos \Theta$

Where

K = constant dependent on crystallite shape (0.89)

B = FWHM (full width at half max) or integral breadth
= (2θ High) – (2θ Low)

t = thickness of crystallite

λ = x-ray wavelength (usually 1.54056 Å)

B is the difference in angles at half max, and Θ = Bragg angle

4. RESULTS AND DISCUSSION

FT-IR spectroscopy

The objective of FT-IR analysis was to investigate the molecular interaction between chitosan sheets, and prepared nanocomposite sheets.

The FT-IR spectra of chitosan sheet, hydroxyapatite, cloisite 10A, cloisite 10A-chitosan, chitosan-hydroxyapatite, cloisite 10A-chitosan-hydroxyapatite are shown in figure 1-6.

In the FT-IR spectra of cloisite 10A, sharp band at 3550 cm^{-1} corresponds to N–H stretching where as peaks at 2900 cm^{-1} and 2950 cm^{-1} corresponds to aliphatic C–H stretching. The peaks observed at 1590 cm^{-1} corresponds to N-H bending vibration and peak between 1000 cm^{-1} to 1100 cm^{-1} correspond to C-H stretching vibration. In the spectra of chitosan, the broad band near 3500 cm^{-1} corresponded to the amine and hydroxyl groups; the peak at 2950 cm^{-1} is caused by -OH stretching. The peaks observed at 1080 cm^{-1} and 1042 cm^{-1} are the hydroxyl groups (characteristic peak of -CH-OH in cyclic alcohols, C-O stretch) and the primary hydroxyl group (characteristic peak of -CH2- OH in primary alcohols, C-O stretch). In the FT-IR spectra of nanocomposite, the broad band at 3510 cm^{-1} corresponds to the -OH and NH_2 of chitosan ,which has been shifted to 3440 cm^{-1} in the nanocomposite , moreover the broadening of peak has also been reduced suggesting good interaction between hydroxyapatite, cloisite 10A and chitosan.

Particle size analysis

Particle size analyzer provides the intensity distributions and average size and polydispersity index of particles. The average particle diameter of the nanocomposite was found

to be 394.6 nm, where as polydispersity index of particles was found to be 0.275. From the intensity of distribution table, it is found that the diameters of 10% particles are below 79.70 nm, 50% particles are below 196.80 nm, 90% particles are below 4134.20 nm.

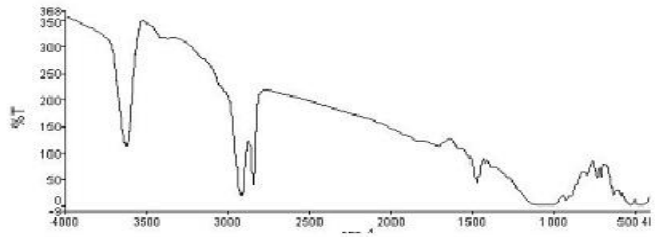

Figure 1: FT-IR spectra of cloisite 10A

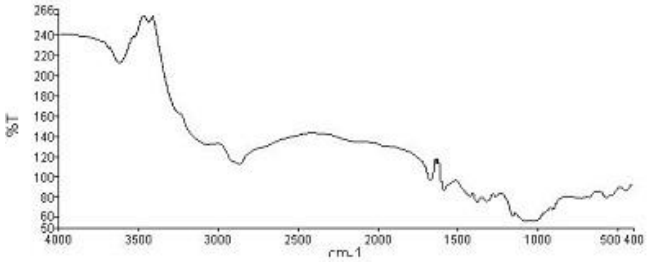

Figure 2: FT-IR spectra of chitosan sheet

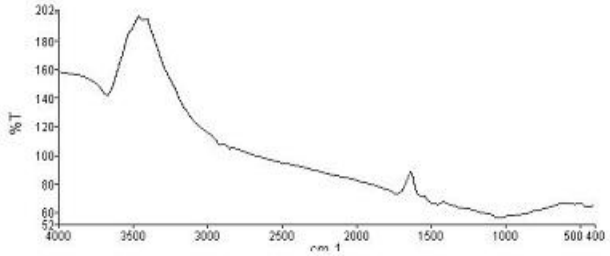

Figure 3: FT-IR spectra of cloisite 10A-chitosan

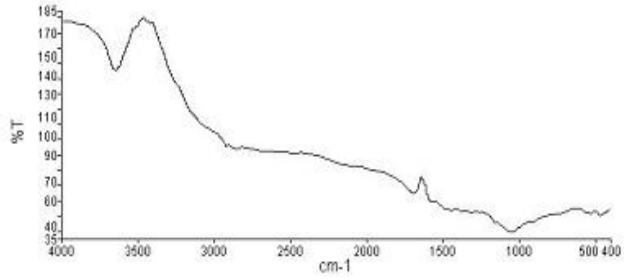

Figure 4: FT-IR spectra of cloisite 10A-chitosan-hydroxyapatite

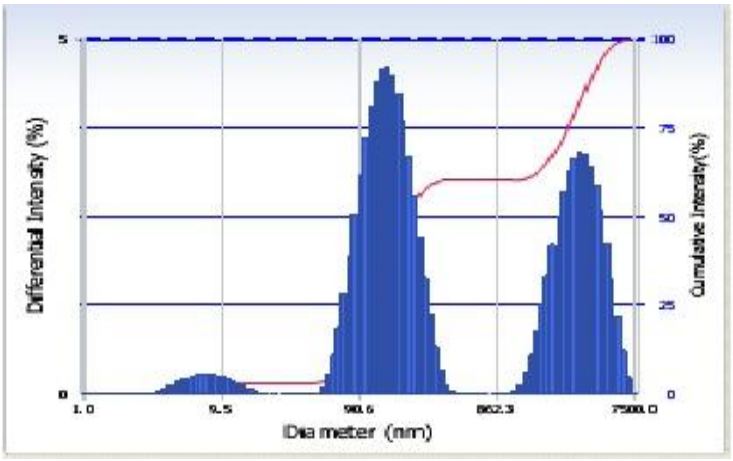

Figure 5: Intensity of particle distributions in nanocomposite formulation

X-ray Diffraction study (XRD)

XRD patterns of cloisite 10A- chitosan-hydroxyapatite nanocomposite are shown in Figure 6. The XRD result confirms the formation of nanocomposites. The crystallite size of the nanocomposite was found to be around 400nm by the Scherrer Equation.

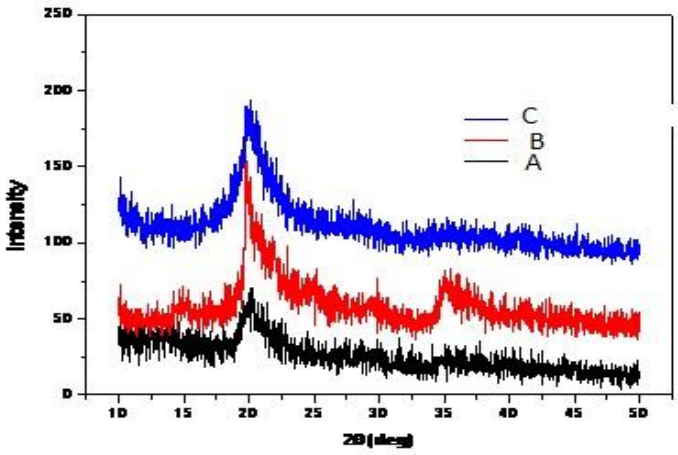

Figure 6: XRD graph of chitosan (A), chitosan-cloisite 10A (B) , and cloisite 10A- chitosan-hydroxyapatite (C) nanocomposite

Morphology

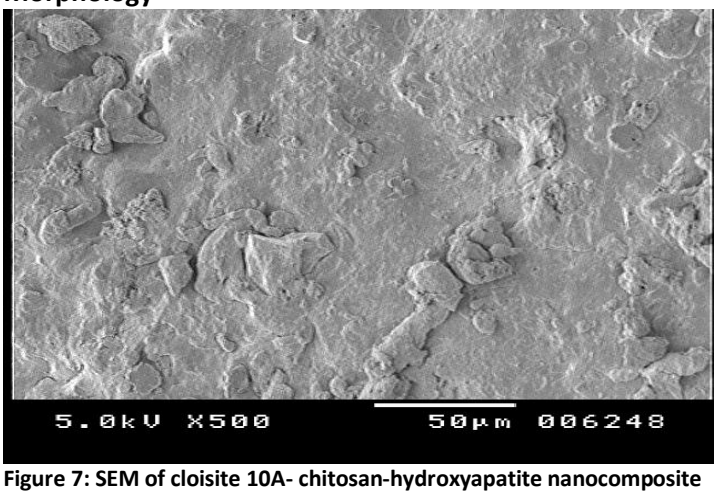

Figure 7: SEM of cloisite 10A- chitosan-hydroxyapatite nanocomposite

The surface morphologies of the prepared composites were examined using a scanning electron microscope (SEM). The nanostructured composite formation between hydroxyapatite, clay and polymer was identified by SEM analysis.

CONCLUSION

Chitosan-cloisite10A-hydrodyapatite nanocomposites were successfully prepared by solution mixing technique. The mechanisms of nanocomposite formation, crystallite size, crystallinity, morphology were studied. XRD and FTIR investigations showed an intermolecular interaction between clay and polymer .The average diameters of particles in the nanocomposites were found to be around 500nm from both XRD and particle size analyzer. This study provides a platform for further research on the polymer-clay nanocomposites for drug delivery and biomedical applications.

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Conflict of Interest: None Declared

