SYNTHESIS AND CHARACTERIZATION OF GELATIN-CHITOSAN NANOCOMPOSITE TO EXPLORE THE POSSIBLE USE AS DRUG DELIVERY VEHICLE

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Abstract

We prepared hybrid nano-composites by blending chitosan-Gelatin with Cloisite 30B in aqueous solution. These composites were characterized by using FTIR, SEM and XRD analysis. Various groups present in the chitosan-Gelatin blend were monitored from FTIR spectra. The morphology, homogeneity and crystallinity of the blends were analyzed from SEM and XRD data. The results indicated that an intercalated or partially exfoliated nano-composite could be achieved, and the properties of the composite were significantly improved.

Keywords: Chitosan, Gelatin, Cloisite 30 B, Drug delivery

Introduction

Gelatin has lots of biodegradability, biocompatibility, non-toxicity and of low cost. Gelatin can be used as a valuable biopolymer in tissue engineering (Nanda P K 2007) (e.g., wound-dressing and bone scaffolding), but its poor mechanical properties (especially in the wet state) limits its application as a structural biomaterial. Thus the reinforcement of gelatin materials becomes a challenge for the researchers. There are attempts like vapor crosslink (Draye J P 1998) , orientation technique(Maeda T 1996, Fakirov S 1996, Fakirov S 1997) and gelatin-based composites filled with hydroxyapatite(Zhao W Y 1996) tricalcium phosphate (Bigi A 1998) and carbonfiber(Lin F H 1998) which have shown good outcomes. However it has been noted that the strength was still not high enough, especially in the

wet state. Thermal properties of gelatin have been improved mainly through an orientation technique (Wan Y 2000). Although there is still a long way to go before the application of high-performance gelatin or gelatin-based composites, but the development of polymer-layered silicate nanocomposites provided us with some new opportunities.

Coming to a natural polymer chitosan can be derived from the shells of shrimp and other sea crustaceans, including *Pandalus borealis*, Chitosan is produced commercially by deacetylation of chitin. The chitin is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. The molecular weight of commercially produced chitosan is between 3800 and 20,000 Daltons. A common method for the synthesis of chitosan is the deacetylation of chitin using sodium hydroxide in excess as a reagent and water as a solvent.

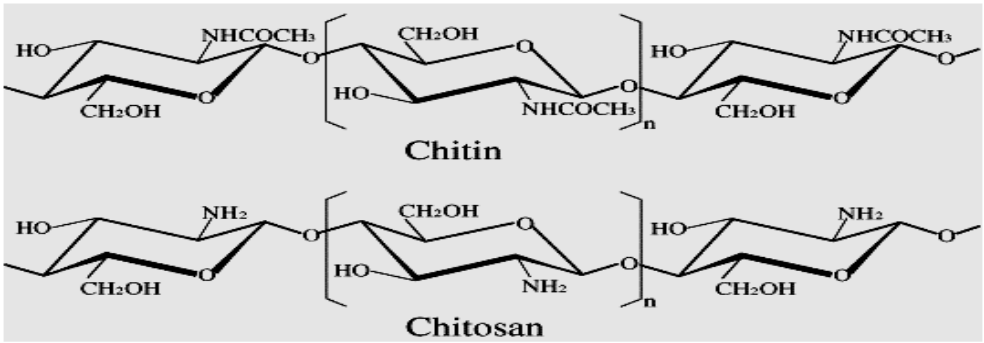


Fig 1-Chitin and chitosan

Cloisite 30B is methyl, tallow, bis-2 hydroxyethyl, quaternary ammonium, where tallow is 65% C18, 30% C16, and 5% C14. These Clay minerals are widely used materials in drug products as delivery agents. Montmorillonite (MMT) can provide mucoadhesive capability for the nanoparticle to cross the gastrointestinal (GI) barrier. MMT is also a potent detoxifier, which belongs to the structural family of 2:1 phyllosilicate. MMT could absorb dietary toxins, bacterial toxins associated gastrointestinal disturbance, hydrogen ions in acidosis and metabolic toxins such as steroidal metabolites associated with pregnancy. Hence blending chitosan –Gelatin with Cloisite 30 B can enhance the drug releasing property of the composite.

This research deals with blending of chitosan-Gelatin with Cloisite 30B which is organically modified sodium in MMT with quaternary ammonium salt (the organic modifier in Cloisite 30B The blends were characterized using FTIR, SEM, and XRD methods.

Experimental Study

Materials

Gelatinwas purchased from Sigma Chemical. (St.Louis, MO). Chitosan (CS) (Degree of Deacetylation 98% determined by 1H-NMR and

Molecular Weight 13.45 _104 Da) was purchased from India Sea Foods, Kerela, India. The procurement of Cloisite 30B was from Southern Clay Products.

Preparation of Nanocomposites

The aqueous solution of Chitosan which was 2 wt % prepared by dissolving 20 g of chitosan powder in 1000 mL of acetic acid solution (1%, v/v). After chitosan was dissolved, the solutions were filtered with cheese cloth by vacuum aspiration to remove foam and any undissolved impurities. One gram of gelatin powder was soaked in 50 ml deionized water and heated at 70^0 C to obtain a homogeneous solution. Both polymer solutions were mixed .and then stirred it for 3-4 hrs. Cloisite 30 B with different clay compositions (1 wt %, 2.5 wt % based on chitosan-Gelatin) were prepared by dispersing appropriate amounts of clays into 10 mL of 1% acetic acid solution and vigorously stirring for 24 hours. The mixture was stirred continuously for 4 h and then cast onto level Teflon coated glass plates. After drying at room temperature for at least 72 hours, the films were peeled from the plates.

Characterisation

Fourier Transmission Infra-Red Spectroscopy (FTIR)

The FTIR spectrum of the chitosan-gelatin film was obtained using a BIORAD-FTS-7PC type FTIR spectrophotometer.

X-Ray Diffraction (XRD)

In order to know the change in gallery height of the blend the blend was investigated by WAXD experiments, which were carried out using an X-ray diffractometer (BEDE D-3 system) with Cu K_ radiation at a generator voltage of 40 kV and a generator current of 100 mA. Samples were scanned from 2_ = 1-1000 at a scanning rate of 2 0/min.

Scanning Electron Microscopy (SEM)

The characterization of Chitosan-Gelatin film was done by using SEM (440, Leica Cambridge Ltd., Cambridge, UK). The powdered specimens were placed on the Cambridge standard aluminium specimen mounts (pin type) with double-sided adhesive electrically conductive carbon tape (SPI Supplies, West Chester, PA). The specimen mounts were then coated with 60% Gold and 40% Palladium for 30 seconds with 45 mA current in a sputter coater (Desk II, Denton Vacuum, Moorestown, NJ). The coated specimens were then observed on the SEM using an accelerating voltage of 20 kV at a tilt angle of 30^0 to observe the microstructure of the Chitosan-gelatin-C-30B composite blends.

Swelling Studies

The swelling property study was done by immersing the disk samples (approximately 0.5 g) in three different swelling solutions: water, pH 4.0 buffer solution, and pH 10.0 buffer solution. The samples were placed in the

swelling solution and the weight of the swollen samples was measured against time after the excess surface water was removed by gently tapping the surface with a dry piece of filter paper. The degree of swelling (*H*) for each disk sample at time *t* was calculated using Equation where wt and w0 are the sample's weight at any given time, and in the dry state, respectively.(Fig2)

$$H = \frac{w_t - w_0}{w_0}$$

Fig.2

Results

Fourier transmission infrared spectroscopy

In Figure 3 the characteristic peaks of chitosan were located at 3450 cm^{-1} for the hydroxyl group and 1592 cm^{-1} for the amino group. The peak at 1656 cm^{-1} was due to carbonyl stretching vibration of remaining acetamide group in chitosan. b) AlAO vibrations at 915, 624, 842, and 792 cm^{-1} confirm the presence of C 30B in the dispersion. The SiAO stretching peaks can be seen at 1086 and 1034 cm^{-1} and finally SiAO bending peaks at 520 and 467 cm^{-1}1.27,28 X

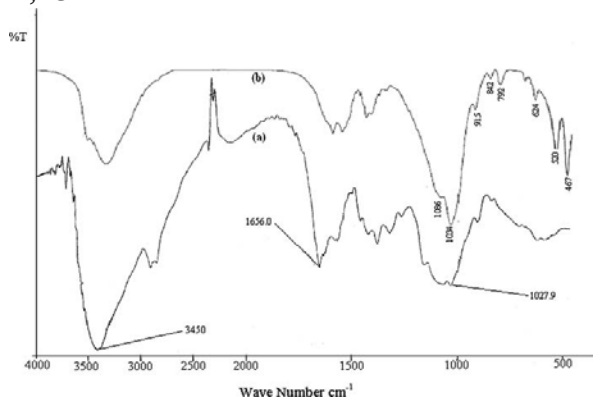
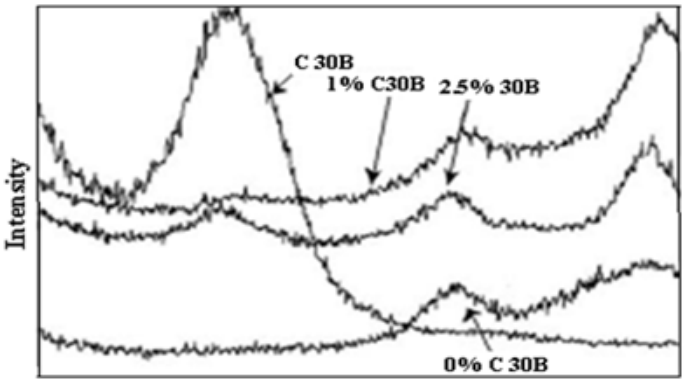


Figure 3. FTIR spectra of (a) Chitosan and (b) Chitosan/C30 B composite film

X-ray diffraction analysis

When Cloisite 30B was added to the chitosan solution, irrespective of amount, the peaks remained atF3 the same position (2y ¼ 4.8_) (Fig. 4), indicating that no intercalation had occurred and that microscale composite-tactoids were formed. AS Cloisite 30B is the organically modified sodium in MMT with a quaternary ammonium salt, so it became organic and its hydrophobicity increased, and hence, it was very difficult to disperse Cloisite 30B in the chitosan aqueous solution and to form an intermolecular reaction between clay and chitosan despite the presence of the hydroxyl group in the gallery of Cloisite 30B. Strong polar interactions, especially hydrogen

bonding, critically affected the formation of intercalation and exfoliated hybrids.



XRD spectra of a) Chitosan and (b) Chitosan/C30 B composite film

Figure 4 XRD of chitosan/C30 B composite film

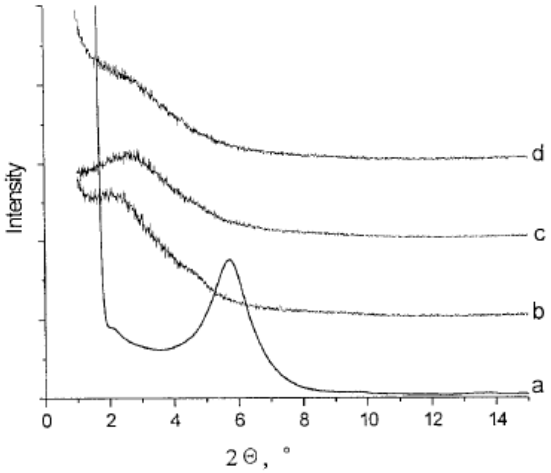


Fig 5.XRD patterns of pristine MMT and composites (a) MMT; (b) 1% MMT; (c) 2 % MMT(d) 5% MMT

XRD patterns of pristine MMT and composites are shown in (Figure 5). Original MMT exhibits a sharp peak at 2θ= 6°, and through the Bragg's equation: λ= 2*d* sin θ; *d*001 is 1.47 nm. XRD patterns of composites change dramatically in comparison with pristine MMT. All diffraction peaks shift toward lower angle values, become broad, and even disappear, indicating that intercalation or exfoliation structures have been formed. For intercalation composites, the interlayer spacing increases from 1.47 to 4.42 nm due to the insertion of gelatin molecules into the sheets of MMT. The absence of the diffraction peak reveals the exfoliation structure.

Scanning electron microscopy (SEM)

The microstructure obtained by SEM for the chitosan and its composites prepared by solvent casting, showed that particles are relatively well dispersed in the chitosan matrix. Figure 6 showed that as the concentration of the nanoclay increases from 0 to 2.5% the homogeneity of the surfaces also increases. In particular, 2.5% C 30B was superior to individual polymers

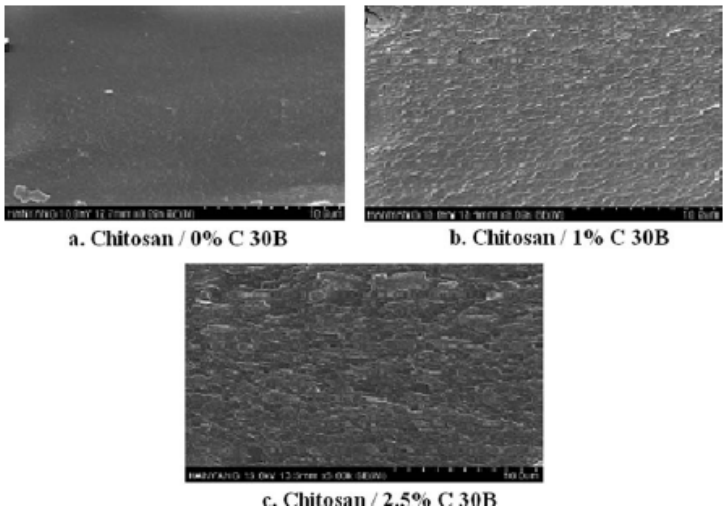


Figure 6. SEM of chitosan/C30 B composite films

SEM of Gelatin

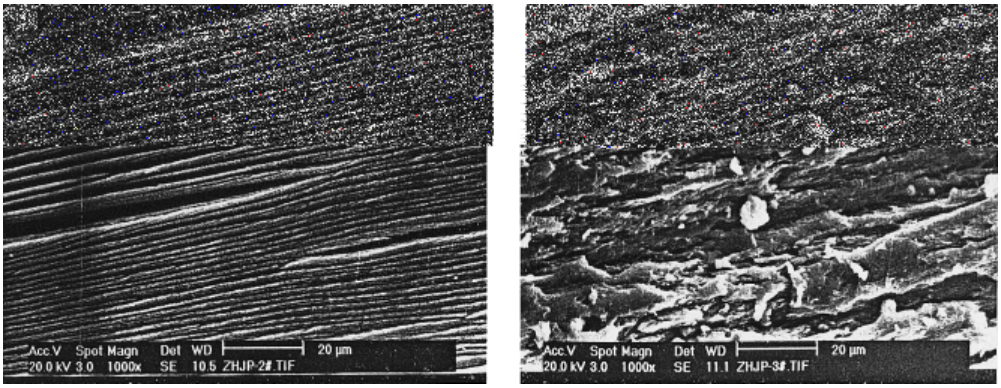


Fig 7. SEM of Gelatin Blended with Cloisite 30 B (a. 1%MMT,b. 2%MMT)

The morphology of fracture surfaces of gelatin and composite are shown in(Figure 7). The fracture surface of gelatin exhibits a smooth laminated structure. Comparatively, the fracture surface of composite seems coarse, indicating an improved

Discussion & Conclusion

The blends have been characterized using various physicochemical methods. From the FTIR spectra the different pendant groups present in the composites have been ascertained. The morphology as well as the compatibility of the blends has been studied using SEM and XRD methods. The compatibility & the mechanical properties were improved by using Cloisite 30B , which was used in our blend to produce nanocomposite.

Chitosan is biodegradable, biocompatible, and nontoxic in nature. Hence, it is being used as a biopolymer of first choice for controlled drug delivery system. The blending of Chitosan-Gelatin with Cloisite 30B was carried out to delay the drug release for a longer duration of time so that the toxicity of the drug will be minimum with increased effectiveness. From the XRD data it is clear that only tactoids are formed.. Swelling studies predicted the diffusion of the drugs from the matrix. The percentage of swelling increases with increase in the percentage of drug loading. The drug release depends upon the nature of the polymer matrix as well as pH of the media. Hence, chitosan-Gelatin blended with cloisite 30B is a better drug carrier than the neat chitosan-Gelatin film.

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