Synthesis and Characterization of Chitosan/Cloisite 30B Film for Controlled Release of Ofloxacin

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ABSTRACT: In this research program, chitosan film was prepared by blending chitosan with Cloisite 30 B at different concentrations 0 wt %, 1 wt %, and 2.5 wt %. The blends were characterized by Fourier transmission infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis. From the FTIR spectra the various groups present in chitosan/C 30 B blend were monitored. The homogeneity, morphology, and crystallinity of the blends were ascertained from SEM and XRD data, respectively. The most suitable form of blend was taken and used as a carrier for the controlled release of ofloxacin. The swelling studies have been carried out at different drug loading. Drug release kinetics was analyzed by plotting the cumulative release data versus time by fitting to an exponential equation which indicated the occurrence of non-Fickian type of kinetics. The drug release was investigated at different pH medium and it was found that the drug release depends upon the pH medium as well as the nature of matrix. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 2588–2594, 2012

Key words: chitosan; ofloxacin; controlled drug delivery; kinetics

INTRODUCTION

Chitosan (CS) is a biopolymer that has received great attention in a variety of applications because of their biodegradability and biocompatibility.[1] It is derived from chitin, which is the second most abundant biomass on earth next to cellulose. Because of its excellent film-forming property, chitosan can be used effectively as a film-forming material to carry active ingredients, such as mineral or vitamin for food packaging applications,[2] and hydrophilic or hydrophobic drugs for drug delivery applications.[3] These unique properties make it an attractive carrier for biomedical applications. Of late, chitosan has been widely applied in biomedical fields as a carrier for drug delivery, wound dressing, etc.[4,5]

The drug delivery system was developed for the purpose of bringing, up taking, retaining, releasing, activating, localizing, and targeting the drugs at the right time period, dose, and place.[6] The biodegradable polymer can contribute largely to this technology by adding its own characters to the drugs. In this connection, some biodegradable polymers, such as Chitosan, PLA, PCL, are commonly used as these polymers can be prepared in moderate conditions, has a similar stiffness of the body and has an appropriate biodegradability and low crystallinity enough to be mixed well with many kinds of drug.[7] There are some formulations for the drug delivery systems, e.g., films, gels, porous matrices, microcapsules, microspheres, nanoparticles, polymeric micelles, and polymer-linked drugs. The physical interactions are usually preferred for binding of the drug to the polymer avoiding damage to the molecular structure of the drug unless it will lead to the loss of bioactivity.[8]

Although the drug delivery system (DDS) concept is not new, great progress has recently been made in the treatment of a variety of diseases. Targeting delivery of drugs to the diseased lesions is one of the most important aspects of DDS. To convey a sufficient dose of drug to the lesion suitable carriers of drugs are needed. Nano and microparticle carriers have important potential applications for the administration of therapeutic molecules, controlled drug delivery technology represents one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care. These delivery systems offer numerous advantages compared with conventional dosage forms, which include improved efficacy, reduced toxicity, and improved patient compliance and convenience. Such systems often use macromolecules as carriers for the drugs. By doing so, treatments that would not otherwise be possible are now in conventional use. This field of pharmaceutical technology has grown and diversified rapidly in recent years.

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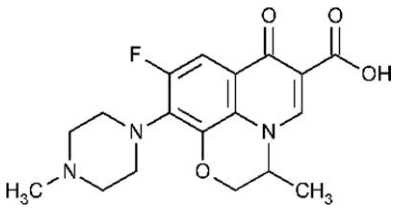

Figure 1 Structure of Ofloxacin.

Orally administered as well as implantable delivery systems containing chitosan as a drug carrier have been prepared to effect sustained release of the drug.[9,10] Modulation of drug release has been achieved by drug-chitosan complexation involving ionic[11–13] or covalent interactions.[14,15] While the focus for ionic interactions of chitosan involves the amino groups of its glucosamine residues, covalent interactions often involve other sites as well (e.g., the CH_2OH moieties), also positively charged chitosan is easy to interact with negatively charged glycosaminoglycans in the extracellular matrix.

On the other hand, montmorillonite (MMT) can provide mucoadhesive capability for the nanoparticle to cross the gastrointestinal (GI) barrier.[16] MMT is also a potent detoxifier, which belongs to the structural family of 2 : 1 phyllosilicate. MMT could absorb dietary toxins, bacterial toxins associated with gastrointestinal disturbance, hydrogen ions in acidosis, and metabolic toxins, such as steroidal metabolites associated with pregnancy.[17]

Ofloxacin, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl- 1-piperazinyl)-7-oxo-7H-pyrido {1,2,3-de]-1,4-benzoxazine- 6-carboxalic acid[18] is a second generation fluorinated quinolone, a pyridone carboxylic acid derivative, which exert a broad-spectrum having antimicrobial effect in a variety of systemic infections.[19–21] It blocks bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase IV. Inhibition of DNA gyrase prevents the relaxation of positively super coiled DNA that is required for normal transcription and replication.[22] Ofloxacin is commonly used in clinics but its bioavailability and pharmacokinetic profile needs to be described in local population and environments. Figure 1 shows the structure of ofloxacin.

In this research program, chitosan has been blended with Cloisite 30B which is organically modified sodium in MMT with quaternary ammonium salt (the organic modifier in Cloisite 30B is methyl, tallow, bis-2 hydroxyethyl, quaternary ammonium, where tallow is 65% C18, 30% C16, and 5% C14) for the controlled release of ofloxacin. The blends have been characterized using FTIR, SEM, and XRD. This composite (2.5% Cloisite 30B) has been compounded with ofloxacin and the control release of the drug has been evaluated. The swelling kinetics as well as the drug delivery systems using ofloxacin has also been studied at different pH and drug loading.

EXPERIMENTAL

Materials

Chitosan (CS) (Degree of Deacetylation = 95% determined by ^{1}H-NMR and Molecular Weight 13.45 $\times$ 10^4 Da) was purchased from India Sea Foods, Kerela, India. Cloisite 30B was procured from Southern Clay Products. Ofloxacin was received as gift sample from Ranbaxy, India. Acetic acid, NaH_2 PO_4, NaOH, and other chemicals were used as analytical grade and purchased from Sigma Aldrich Company.

Preparation of chitosan films

A chitosan aqueous solution of 2 wt % was 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 cheesecloth by vacuum aspiration to remove foam and any undissolved impurities. Nanoclay solutions with different clay compositions (1 wt %, 2.5 wt % based on chitosan) were prepared by dispersing appropriate amounts of clays into 10 mL of 1% acetic acid solution and vigorously stirring for 24 h. Afterward, 200 mL of chitosan solution was added slowly into pretreated clay solutions. The mixtures were stirred continuously for 4 h and then cast onto level Teflon-coated glass plates. After drying at room temperature for at least 72 h, the films were peeled from the plates.

Drug loading

Ofloxacin of different loadings, i.e., 10 wt %, 20 wt %, 30 wt %, 40 wt % and 50 wt % were added to the Chitosan/Cloisite 30 B (2.5 wt %) clay solution and stirred for 1 h and then the polymer-drug conjugates were kept at room temperature for drying.

Dissolution experiments

Dissolution experiments were performed at 37°C using the dissolution tester (Disso test, Lab India, Mumbai, India) equipped with six paddles at a paddle speed of 100 rpm. About 900 mL of phosphate buffer solution (pH 3.4 and 7.4) was used as the dissolution media to stimulate gastrointestinal tract (GIT) conditions. A 5 mL aliquot (polymer–drug conjugate) was used each time for analyzing the ofloxacin content at a fixed time interval. The dissolution media was replenished with a fresh stock solution. The amount of ofloxacin released was

analyzed using a UV spectrophotometer (Systronics, India) at the λ_{max} value of 287 nm.

Drug release mechanism from matrices

From time to time, various authors have proposed several types of drug release mechanisms from matrices. It has been proposed that drug release from matrices usually implies water penetration in the matrix, hydration, swelling, diffusion of the dissolved drug (polymer hydrofusion), and/or the erosion of the gelatinous layer. Several kinetic models relating to the drug release from matrices, selected from the most important mathematical models, are described over here. However, it is worth mentioning that the release mechanism of a drug would depend on the dosage from selected, pH, nature of the drug and, of course, the polymer used.

i. Zero-Order Kinetics.[23]

$$W = k_1 t \quad (1)$$

ii. First-Order Kinetics.[23,24]

$$\ln (100 - W) = \ln 100 - k_2 t \quad (2)$$

iii. Hixon-Crowel's Cube-Root Equation (Erosin Model).[24]

$$(100 - W)^{1/3} = 100^{1/3} = k_3 t \quad (3)$$

iv. Higuchi's Square Root of Time Equation (Diffusion Model).[25]

$$W = k_4 t \quad (4)$$

v. Power Law Equation (Diffusion/Relaxation model).[26]

$$M_t/M_\infty = k_5 t^n \quad (5)$$

M_t/M_∞ is the fractional drug release into dissolution medium and k_5 is a constant incorporating the structural and geometric characteristics of the tablet. The term 'n' is the diffusional constant that characterizes the drug release transport mechanism. When $n = 0.5$, the drug diffused and released from the polymeric matrix with a quasi-Fickian diffusion mechanism. For $n > 0.5$, an anomalous, non-Fickian drug diffusion occurs. When $n = 1$, a non-Fickian, case II or Zero-order release kinetics could be observed.

CHARACTERIZATION

FTIR spectral analysis

The Fourier transmission infrared spectra (FTIR) was obtained through a Perkin–Elmer Spectrum RX1 FTIR spectrometer at Hanyang University, South Korea.

X-ray diffraction (XRD)

The change in gallery height of the blend was investigated by WAXD experiments, which were carried out using a X-ray diffractometer (BEDE D-3 system) with Cu Ka radiation at a generator voltage of 40 kV and a generator current of 100 mA. Samples were scanned from $2\theta = 1$–10° at a scanning rate of 2°/min.

Scanning electron microscopy

The chitosan films (taking acetic acid as a solvent) were characterized using SEM (440, Leica Cambridge, Cambridge, UK). The chitosan/C 30B composite film specimens were placed on the Cambridge standard aluminum 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 s 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° to observe the microstructure of the chitosan/C 30B blends.

Swelling studies

Water absorption of the polymer-drug conjugates (Chitosan/2.5 wt % of C 30B with required amount of drug, i.e., 10, 20, 30, 40 and 50%) were measured following ASTM D 570-81. The samples were preconditioned at 50°C for 24 h and then cooled in a desiccator before being weighed. The preconditioned samples were submerged in distilled water at 25°C for 24 h. The samples were removed and dried with a paper towel before weighing. Water absorption was calculated as a percentage of initial weight. The soluble material loss was checked by weighting the specimens after drying them in an oven at 50°C for another 24 h. The total water absorption for 24 h was calculated including the soluble material loss.

$$\% \text{ Swelling} = \frac{W_1 - W_2}{W_2} \times 100$$

where, W_1 = Weight of Swollen composite after 24 h, W_2 = Weight of Dry Composite.

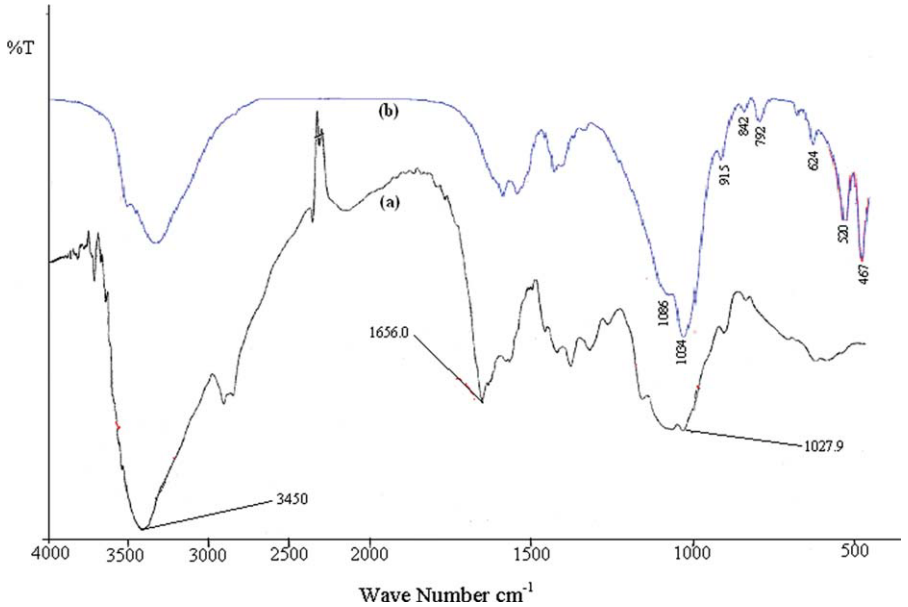

Figure 2 FTIR spectra of (a) Chitosan and (b) Chitosan/C30 B composite film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Fourier transmission infra red spectroscopy

In Figure 2 the characteristic peaks of chitosan (graph a and graph b) 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.

In graph (b) Al—O vibrations at 915, 624, 842, and 792 cm^{-1} confirm the presence of C 30B in the dispersion. The Si—O stretching peaks can be seen at 1086 and 1034 cm^{-1} and finally Si—O bending peaks at 520 and 467 cm^{-1}.[27,28]

X-ray diffraction analysis

When Cloisite 30B was added to the chitosan solution, irrespective of amount, the peaks remained at the same position ($2\theta = 4.8°$) (Fig. 3), 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.[29]

Scanning electron microscopy (SEM)

SEM has been employed for the observation of the surface morphology of the chitosan blended with different concentrations like 0, 1, and 2.5% of C 30B. 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 4 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.

Swelling studies

Here, the percentage of swelling increases with increase in the percentage of drug loading in chitosan composites (Fig. 5). This is clearly because of the hydrophilic nature of the drug.

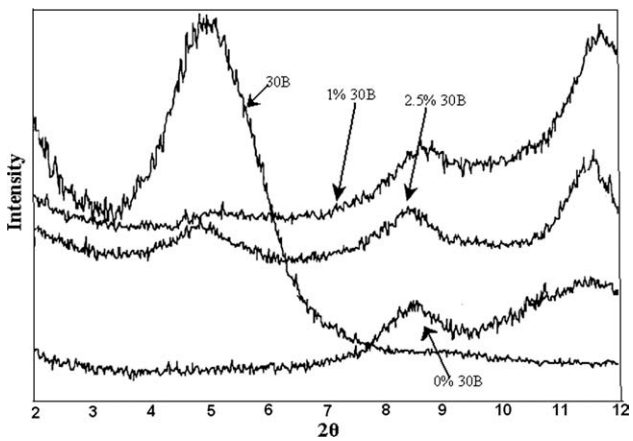

Figure 3 XRD of chitosan/C30 B composite films.

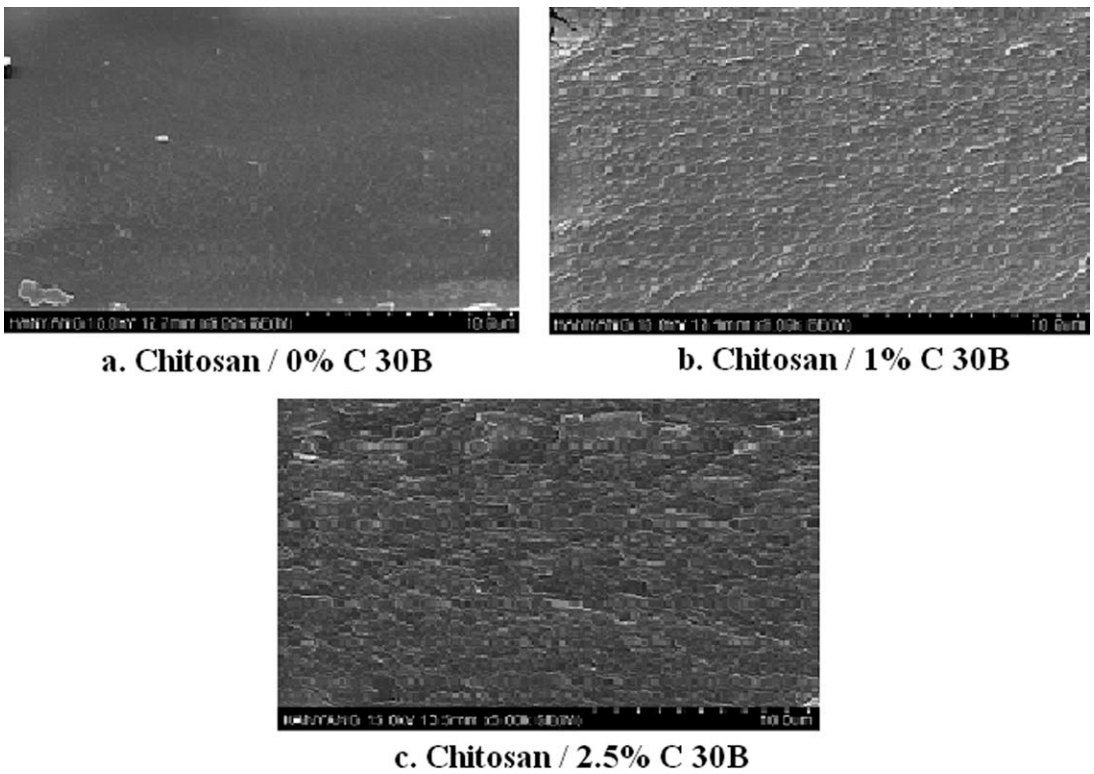

Figure 4 SEM of chitosan/C30 B composite films.

In vitro drug release

Effect of pH, time, and drug loading

To investigate the effect of pH on the swelling of chitosan/C 30B composite (2.5%), we have measured the % cumulative release in both pH 2.3 and 7.4 media. Cumulative release data presented in Figure 6 indicate that by increasing the pH from 2.3 to 7.4, a considerable increase in the cumulative release is observed for all composites. From Figure 6 (a,b), it is seen that the 50% drug-polymer composites have shown longer drug release rates than the other composites. Thus, drug release depends upon the nature of the polymer matrix as well as pH of the media. This suggests that the drugs in the blend can be used to be suitable for the basic environment of the large intestine, colon, and rectal mucosa for which there are different emptying times.

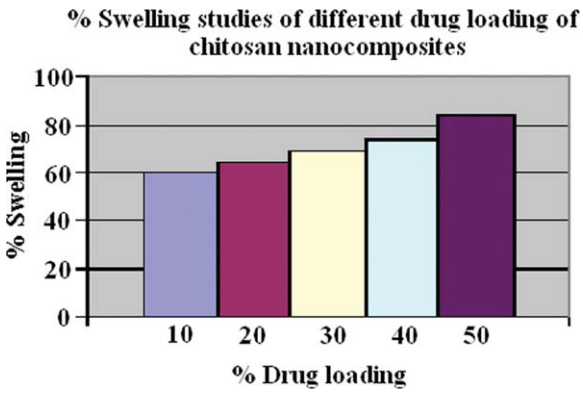

Figure 5 Water Absorption of the chitosan/C30 B composite films with different % of drug loadings. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Interestingly, more than 90 wt % ofloxacin is released from composites at pH 7.4 within 10 h, whereas less than 75 wt % of the drug is released at pH 2.3 within 10 h. This suggests that the drugs in the composites can be used to be suitable for the basic environment. Further the electrostatic interaction of composites is more easily broken at pH 7.4 than at pH 2.3, leading to ofloxacin being released more rapidly at pH 7.4 than pH 2.3.

Release data (Fig. 6) showed that formulations containing highest amount of drug (50%) displayed fast and higher release rates than those formulations containing a small amount of drug loading. The release rate becomes quite slower at the lower amount of drug in the matrix, because of the availability of more free void spaces through which a lesser number of drug molecules could transport.

Drug release kinetics

Drug release kinetics was analyzed by plotting the cumulative release data versus time by fitting to an exponential equation of the type as represented below.[23]

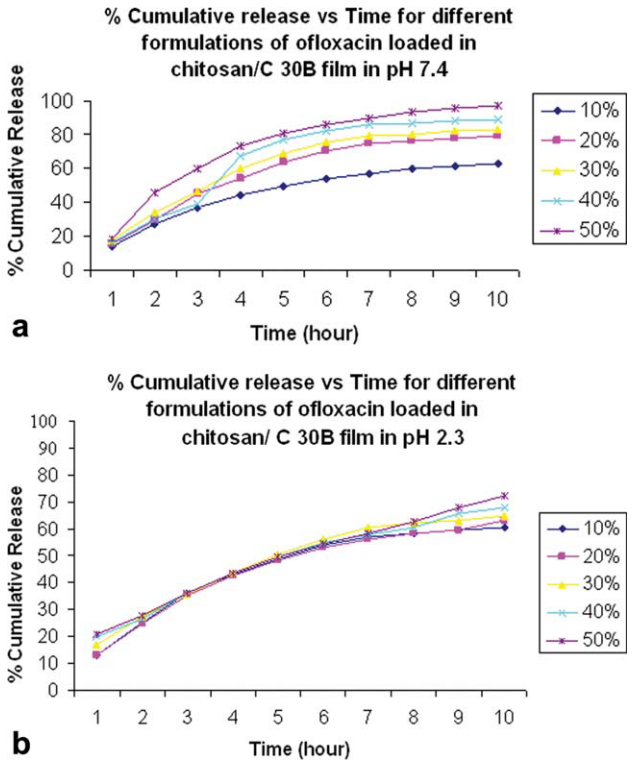

Figure 6 (a) % Cumulative release vs. time for different formulation of ofloxacin loaded in chitosan/C30 B composite film in pH 7.4; (b) % Cumulative release vs. time for different formulation of ofloxacin loaded in chitosan/ C30 B composite film in pH 2.3 media. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$$M_t/M = \mathrm{kt}^{\mathrm{n}}$$

Here, M_t/M_∞ represents the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and n is an empirical parameter characterizing the release mechanism. Using the least squares procedure, we have estimated the values of n and k for all the five formulations and these data are given in Table I. The values of k and n have shown a dependence on the, % drug loading and polymer content of the matrix. Values of k for composites prepared by varying the amounts of drug containing and keeping Chitosan/C 30 B (2.5 wt %) constant, ranged from 0.20 to 0.36 in pH 7.4 and 0.20 to 0.32 in pH 2.3, respectively. However, the drug-loaded composites exhibited n values ranging from 0.85 to 1.71 in pH 7.4 and 0.93 to 1.50 in pH 2.3 (Table I), indicating a shift from erosion type release to a swelling controlled, non-Fickian type mechanism. The values of n more than 1 has also been recently reported.[25,26] This may be due to a reduction in the regions of low micro viscosity inside the matrix and closure of microcavities during the swollen state of the polymer. Similar findings have been found elsewhere, wherein the effect of different polymer ratios on dissolution kinetics was investigated.[24,30]

CONCLUSIONS

Chitosan is biodegradable, biocompatible, and non-toxic in nature. Hence, it is being used as a biopolymer of first choice for controlled drug delivery system. The blending of Chitosan 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. The blends have been characterized using various physico-chemical 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. From the XRD data it is clear that only tactoids are formed. Chitosan blended with 2.5% of Cloisite 30 B was found to be the better carrier for controlled release of ofloxacin. 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. The kinetics of the drug release has been investigated. The values of "k" and "n" have been computed. On the basis of the values of "n" a non-Fickian kinetics has been predicted. Hence, chitosan blended with cloisite 30 be is a better drug carrier than the neat chitosan film.

TABLE I
Release Kinetics Parameters of Different Formulations at pH 7.4 and pH 2.3

	K		n		Co-ordination-coefficient, R^2	
Sample code	pH 7.4	pH 2.3	pH 7.4	pH 2.3	pH 7.4	pH 2.3
10 wt %	0.20	0.20	0.90	0.93	0.9425	0.9308
20 wt %	0.20	0.20	0.89	0.95	0.9677	0.9316
30 wt %	0.23	0.21	0.85	0.99	0.9788	0.9558
40 wt %	0.25	0.24	0.98	1.50	0.9856	0.9736
50 wt %	0.36	0.32	1.71	1.44	0.9888	0.9910

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