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Synthesis and Characterization of Chitosan/Cloisite 30B(MMT) Nanocomposite for Controlled Release of Anticancer Drug Curcumin

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Subject: Polymer Science

Abstract

Chitosan/Cloisite 30 B (MMT) hybrid nanocomposites were prepared by blending chitosan with cloisite 30B in aqueous solution. The nanocomposites were characterized by using FTIR, SEM and XRD analysis. From the FTIR spectra the various groups present in the chitosan blend were monitored. The homogeneity, morphology and crystallinity of the blends were ascertained from the SEM and XRD data, respectively. The results indicated that an intercalated or partially exfoliated nanocomposite has been formed, and the properties of the composite were significantly improved. The drug release kinetics was investigated using the anti cancer drug curcumin . Drug release kinetics was analyzed by plotting the cumulative release data vs. time by fitting to an exponential equation which indicated the 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 polymer matrix.

Key words: *Chitosan, Cloisite 30B, Curcumin, Drug delivery, Kinetics.*

1. Introduction

Natural polymers have potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biodegradability. In recent years, biodegradable polymeric systems have gained importance for design of surgical devices, artificial organs, drug delivery systems with different routes of administration, carriers of immobilized enzymes and cells, biosensors, ocular inserts, and materials for orthopedic applications [1]. These polymers are classified as either synthetic (polyesters, polyamides, polyanhydrides) or natural (polyamino acids, polysaccharides) [2-4]. Polysaccharide-based polymers represent a major class of biomaterials, which includes agarose, alginate, carageenan, dextran, and chitosan.

Chitosan [_(1,4)2- amino-2-d-glucose] (Fig.1) is a cationic biopolymer produced by alkaline N-deacetylation of chitin, which is the main component of the shells of crab, shrimp, and krill [5-8]. Chitosan is derived from the shells of shrimp and other sea crustaceans, including *Pandalus borealis*. Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi [9]. The degree of deacetylation (%DD) can be determined by NMR spectroscopy, and the %DD in commercial chitosans ranges from 60 to 100%. On average, the molecular weight of commercially produced chitosan is between 3800 and 20,000 Daltons [10]. 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. This reaction pathway, when allowed to go to completion (complete deacetylation) yields up to 98% product. The amino group in chitosan has a pKa value of ~6.5, which leads to a protonation in acidic to neutral solution with a charge density dependent on pH and the %DA-value. This makes chitosan water soluble and a bioadhesive which readily binds to negatively charged surfaces such as mucosal membranes. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable [11]. Purified quantities of chitosans are available for biomedical applications. Chitosan and its derivatives, such as trimethylchitosan (where the amino group has been trimethylated), have been used in nonviral gene

delivery. trimethylchitosan, or quaternised chitosan, has been shown to transfectbreast cancer cells, with increased degree of trimethylation increasing the cytotoxicity; at approximately 50% trimethylation, the derivative is the most efficient at gene delivery [12]. Oligomeric derivatives (3-6 kDa) are relatively nontoxic and have good gene delivery properties. Chitosan's properties also allow it to be used in transdermal drug delivery; it is mucoadhesive in nature, reactive (so it can be produced in many different forms), and most importantly, its positive charge under acidic conditions [13-14]. This positive charge comes from protonation of its free amino groups. Lack of a positive charge means chitosan is insoluble in neutral and basic environments. However, in acidic environments, protonation of the amino groups leads to an increase in solubility. The implications of this are very important to biomedical applications. This molecule will maintain its structure in a neutral environment, but will solubilize and degrade in an acidic environment [15]. This means chitosan can be used to transport a drug to an acidic environment, where the chitosan packaging will then degrade, releasing the drug to the desired environment. One example of this drug delivery has been the transport of insulin.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 [16-20].

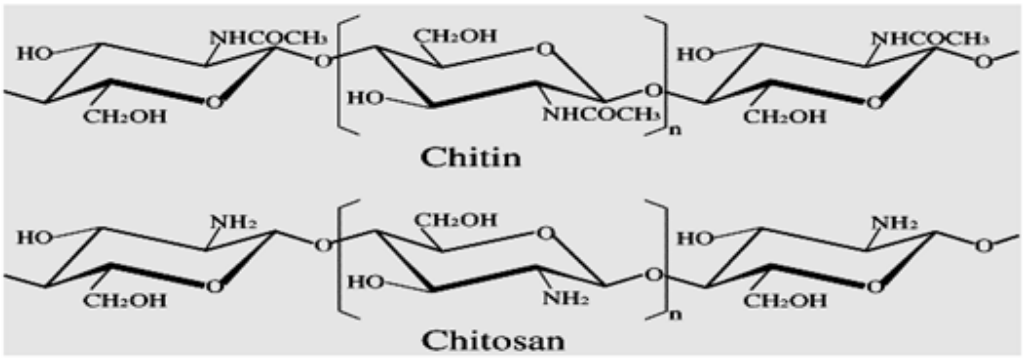

Figure 1. Chitin and chitosan

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 healthcare. 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 This is indeed a challenging field of research with unlimited future prospects [21-23].Developing chitosan /C 30B nanocomposites (CS/C 30B) by inserting chitosan chains into interlayers of silicate can improve its mechanical properties. In recent years, polymer nanocomposites have received considerable interest because of their superior thermal and mechanical properties, as compared with the polymer itself. Polymer-clay nanocomposites are a class of hybrid materials composed of organic polymer matrices and nanoscale organophilic clay fillers Cloisite 30B is methyl, tallow, bis-2 hydroxyethyl, quaternary ammonium, where tallow is 65% C18, 30% C16, and 5% C14. Clay minerals are widely used materi-als in drug products as delivery agents [6-7]**.** 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 associatedgastrointestinal disturbance, hydrogen ions in acidosis and metabolic toxins such as steroidal metabolites associated with pregnancy . Hence blending chitosan with Cloisite 30 B can enhance the drug releasing property of the composite [11-15].

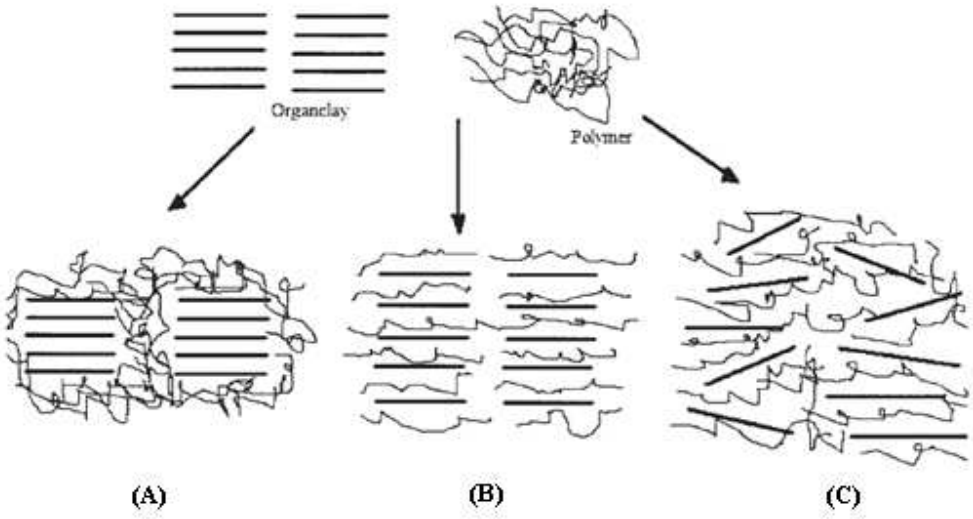

Figure 2. Schematic of possible composite structures obtained when mixing polymer with Organoclays: (A) tactoids, (B) intercalation and (C) exfoliation

When nanoclay is mixed with a polymer, three types of composites (tactoids, intercalation, and exfoliation) can be obtained (Fig. 2). In the case of tactoids, complete clay particles are dispersed within the polymer matrix and the layers do not separate. Mixing a polymer and organoclay forms a micro-scale composite, with the clay serving only as a conventional filler. Intercalation and exfoliation are two ideal nano-scale composites. Intercalation occurs when a small amount of polymer is inserted between the layers of the clay, thus expanding the interlayer spacing and forming a well-ordered multilayer structure. In exfoliation, the layers of the clay are separated completely and the individual layers are distributed throughout the polymer matrix. The formation of intercalation or exfoliation depends on the types and amounts of nanoclay used.

Curcumin (diferuloylmethane) (Fig. 3), a polyphenol, is a low molecular- weight active principle of the perennial herb Curcumalonga (commonly known as turmeric). Recent evidence suggests that curcumin is a highly pleotropic molecule that interacts physically with its diverse range of molecular targets including transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation and apoptosis [18]. Curcumin possesses antioxidant antiinflammatory, [19-21] anticarcinogenic, and antimicrobial [14-15] properties, and suppresses proliferation of a wide variety of tumor cells. Several clinical trials dealing with cancer have addressed the pharmacokinetics, safety, and efficacy of curcumin in humans. Despite extensive research and development, poor solubility of curcumin in aqueous solution remains a major barrier in its bioavailability and clinical efficacy. Being hydrophobic in nature, it is insoluble in water but soluble in ethanol, dimethylsulfoxide, and acetone [24]. To increase its solubility and bioavailability, attempts have been made through encapsulation in liposomes, polymeric and lipo-NPs, biodegradable microspheres, cyclodextrin and hydrogels [25-30].

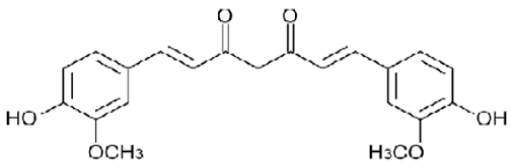

Figure 3. Structure of curcumin (1*E*,6*E*)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione

In the present research program, chitosan has been blended with Cloisite 30B for the controlled release of curcumin. The blends were characterized using FTIR, SEM, and XRD methods. The kinetics of the drug delivery systems using curcumin was studied at different pH conditions of drug loading and plausible mechanism has been suggested for the drug release.

2. Experimental

2.1. Materials

Chitosan (CS) (degree of deacetylation 98% determined by 1H-NMR and Molecular Weight 13.45 x 10^4 Da) was purchased from India Sea Foods, Kerela,India. Cloisite 30B was procured from Southern Clay Products. Curcumin was received as gift sample from VINS Bioproduct medak, Andhrapradesh, India. Acetic acid, NaH_2 PO_4, NaOH, and other chemicals were used as analytical grade and purchased from Sigma Aldrich Company.

2.2. Preparation of chitosan films

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 cheese cloth by vacuum aspiration to remove foam and any undissolved impurities. Cloisite 30 B 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. . 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 h, the films were peeled from the plates.

2.3. Drug loading

Curcumin of different loadings, i.e., 1 wt %, 2.5 wt %, 5 wt %, 7.5 wt % and 10 wt %,15% 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.

2.4. Dissolution experiment

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 curcumin content at a fixed time interval. The dissolution media was replenished with a fresh stock solution. The amount of curcumin released was analyzed using a UV spectrophotometer (Systronics, India) at the *k* max value of 420 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 below

2.5. Drug release kinetics:

To study the release kinetics, data obtained from in vitro drug release studies were plotted in various kinetic models: zero order (Equation 1) as cumulative amount of drug release vs time, first order (Equation 2) as log cumulative percentage of drug remaining vs time, and Higuchi's model (Equation 3) as cumulative percentage of drug released vs square root of time.

$C=K_0 t$… (1)

Where K_0 is the zero order rate constant expressed in units of concentration / time and t is the time in hours. A graph of concentration vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axes [31]

$Log\ C = log\ C_0 - Kt/2.303$… (2)

Where C_0 is the intial concentration of drug, k is the first order constant, and t is the time [32].

$Q = kt½$…(3)

Where K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time [33].

2.6. Mechanism of drug release:

To evaluate the mechanism of drug release from CS/C 30B film data of drug release were plotted in Korsmeyer et al's [34] equation (Equation 4) as log cumulative percentage of drug release vs log time and the exponent n was calculated through the slope of the straight line.

$Mt/ M\infty = kt^n$… (4)

Where M_t/M_∞ is the fractional solute release, t is the release time, k is a kinetic constant characteristics of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers [34]. For cylindrical matrix tablets, if the exponent n = 0.45, then the drug release mechanism is Fickian diffusion and if 0.45 <n <0.89, then it is non-Fickian or anomalous diffusion. An exponents value of 0.89 is indicative of case-II Transport or typical zero-order release [35].

However, it is worth to mention 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.

Figure 4. Photos of chitosan Film (a) chitosan film with 1% curcumin, (b) chitosan film with 2% curcumin, (c) chitosan fim with 5% curcumin, (d) chitosan film with 7% curcumin

3. Characterization

3.1. FT-IR Spectral Analysis

The Fourier Transmission Infrared Spectra (FT–IR) were obtained through a Perkin Elmer Spectrum RX1 FT–IR spectrometer at Hanyang University, South Korea.

3.2. 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 $2\theta = 1–10^{o}$ at a scanning rate of 2^{o}/min.

3.3. Scanning Electron Microscopy (SEM)

The chitosan films (taking acetic acid as a solvent) were characterized using SEM (440, Leica Cambridge Ltd, Cambridge, UK). The CS/C 30B nanocomposite film 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 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 CS/C 30B blends.

3.4. Swelling Studies

In order to study the swelling behaviour, the disk samples (approximately 0.5 g) were immersed 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 (5):

$$H = \frac{w_t - w_0}{w_0} \quad (5)$$

where w_t and w_0 are the sample's weight at any given time, and in the dry state, respectively.

4. Results and Discussion

4.1. Fourier Transmission Infrared Spectroscopy(FTIR)

In Figure 5 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) Al-AO vibrations at 915, 624, 842, and 792 cm^{-1} confirm the presence of C 30B in the dispersion. The Si-AO stretching peaks can be seen at 1086 and 1034 cm^{-1} and finally Si-AO bending peaks at 520 and 467 cm^{-1}.

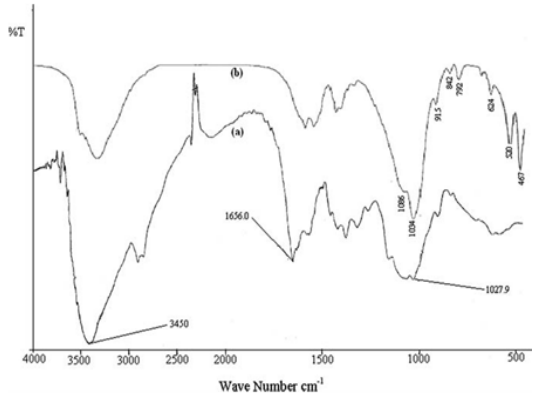

Figure 5. FTIR spectra of (a) Chitosan and (b) Chitosan/C30 B composite film

4.2. X-ray Diffraction Analysis

When Cloisite 30B was added to the chitosan solution, irrespective of amount, the peaks remained at the same position (2θ=4.8°) (Fig. 6), 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.

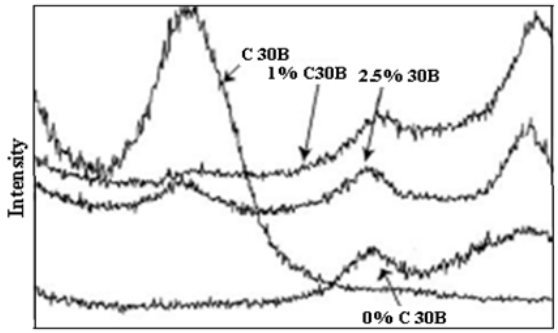

Figure 6. XRD of chitosan/C30 B composite film

4.3. 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 7 shows that as the concentration of the nanoclay increases from 0 to 2.5%, the homogeneity of the surfaces also increases.

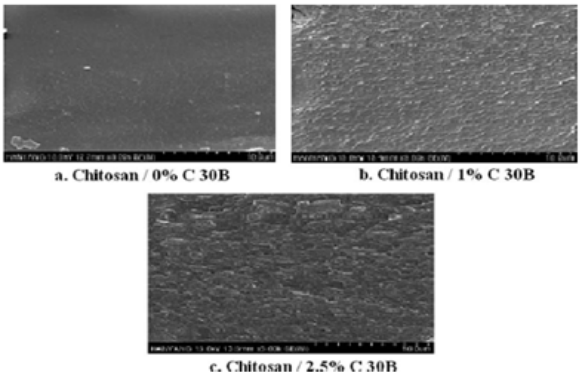

Figure 7. SEM of chitosan/C30 B composite films

4.4. Swelling Kinetics

For most of the drug delivery applications it is important to know the swelling kinetics of the composites, which will be used as drug release agents, because this process has a direct impact on drug release/delivery. There are a few rigorous theories dealing with swelling kinetics; and some authors have proposed that swelling can be described by a second order kinetics [36], as indicated in equation (6):

$$\frac{dH}{dt} = k(H_\infty - H)^2 \quad (6)$$

where k is the swelling rate constant. By integration of Equation (6), and applying the initial conditions, we have Equations (7) and (8):

$$\frac{t}{H} = \frac{1}{k_\infty} + \frac{t}{H_\infty} \quad (7)$$

$$k_\infty = kH_\infty^2 \quad (8)$$

where k_∞ is the equilibrium swelling rate constant. When the swelling kinetics corresponds to a second order kinetics, equation (7) is a linear relationship, and H_∞ and k corresponds to the slope and intercept of the line, respectively [37]. Table 1 summarizes the second order swelling rate constants (k) obtained in this experiment. The results obtained in this study agree with this behaviour.

Considering that the swelling process is affected by specific relations between the molecules of the swelling medium and the polymer pendant groups, one can expect many kinds of polymer-solution interactions, and probably a complex kinetics. The decrease in the swelling rate when the C 30B concentration increases (k increases) suggests that specific interactions between the nanoclay layers loaded with chitosan and the medium are weaker when compared to the polymeric network without C 30B.

Table 1. Values of the swelling rate constant *k* for the CS/C 30B films swelled in different aqueous media

C 30B concentration [wt%)]	k (acid medium)	k (water)	k (basic medium)
0	0.028	0.025	0.020
1	0.038	0.046	0.050
2.5	0.085	0.077	0.088

4.5. In vitro Drug Release: Effect of pH, time, and drug loading

To investigate the effect of pH on the swelling of CS/C 30B composite (2.5%), we have measured the % cumulative release in both pH 5.8, pH 7.0 and 7.4 media. Cumulative release data presented in Figure 8, Figure 9 and Figure 10 indicates that by increasing

the pH from 5.8 to 7.4, a considerable increase in the cumulative release is observed for all composites. From Figure 8, 9 and 10 it is seen that the 10% drug-polymer composites have shown longer drug release rates than the other composites.

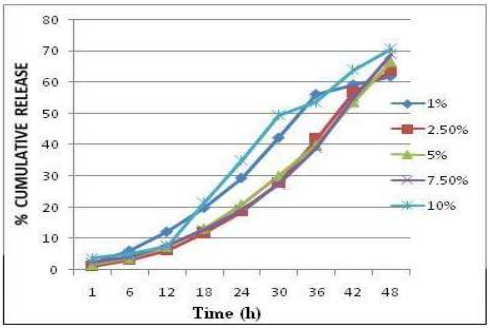

Figure 8. % cumulative release vs time of curcumin loaded in CS/ C-30B composite at pH 5.8 media

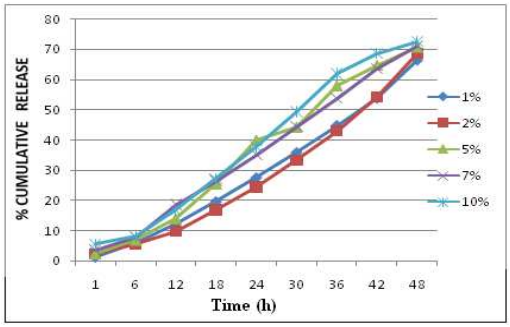

Figure 9. % cumulative release vs time of curcumin loaded in CS/ C-30B composite at pH 7.0 media

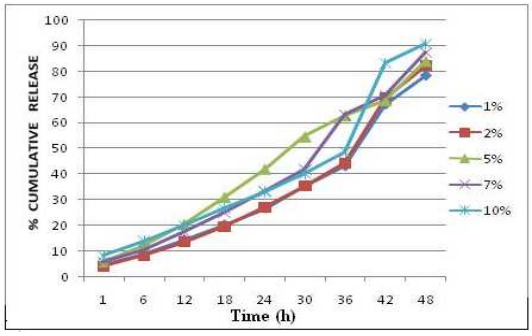

Figure 10. % cumulative release vs time of curcumin loaded with CS/ C-30B composite at pH media 7.4

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

$$Mt = kt^n \quad [35]$$

Here, Mt/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 2. The values of k and n have shown a dependence on the, % drug loading and polymer content of the matrix. Values of k were computed by varying the amount of drug and keeping chitosan/C 30 B at (2.5 wt %).The values of k ranged from 0.05 to 0.12 at pH 7.4, 0.04 to 0.14 in pH 7.0 and 0.04 to 0.14 in pH 5.8 respectively .The values of n were computed to be 0.9 to 1.4 in case of pH 7.4, 0.9 to 1.5 at pH 7.0 and 0.8 to 1.8 at pH 5.8 respectively 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 [3,4]. 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 reported, wherein the effect of different polymer ratios on dissolution kinetics was investigated [11-12].

Interestingly, more than 90 wt % curcumin was released from composites at pH 7.4 within 48h, whereas less than 70 wt % of the drug is released at pH 5.8 within 48 h. This suggests that the drugs with the composite can be used suitable for cancer treatment and also the side effects of anticancer drug curcumin can be minimized.

Table-2. Release kinetic parameters of different formulation at pH 5.8, pH 7.0, and pH 7.4

Curcumin (%)	values of “k”			values of”n		
	pH 5.8	pH 7.0	pH 7.4	pH 5.8	pH 7.0	pH 7.4
1 wt%	0.05	0.04	0.11	1.1	1.1	1
2.5 wt%	0.07	0.05	0.04	1.2	1.5	1.3
5 wt%	0.12	0.08	0.04	1.1	1.1	1.5
7.5 wt%	0.05	0.14	0.04	1.4	0.9	1.5
10 wt%	0.11	0.13	0.13	0.9	1.2	0.8

5. Conclusion

Chitosan is a biodegradable, biocompatible, and nontoxic marine based polysaccharide. Because of its excellent properties it is being used as a biopolymer of first choice for controlled drug delivery system. Curcumin is the ‘active’ ingredient of the Indian/Asian curry spice Turmeric. To put this technically, curcumin is the principal curcuminoid in turmeric. Curcuminoids are polyphenols. Curcumin can suppress tumor initiation, promotion and metastasis. Extensive research over the last 50 years

has indicated it can prevent and treat cancer. Cloisite 30B is a nontoxic mucoadhesive nanomaterial. 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 were characterized using various physicochemical methods such as FTIR, SEM, XRD and swelling studies. 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 curcumin. 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 was investigated by varying the pH of the medium. 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 30B is a better drug carrier than the neat chitosan film.

"Cite this article"

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References

1. D. Sahoo, S. Sahoo, P. Mohanty, S. Sasmal, P.L. Nayak, Chitosan: a new versatile biopolymer for various applications, J.designed monomer and polymer. 12 (2009) 377-404.

2. D. Sahoo, P.L.Nayak, Release Behaviour of Chloramphenicol From N-Acyl Derivatives of Chitosan, NanoTrends: A Journal of Nanotechnology and Its Applications. 12 (2012) 01-09.

3. V.K. Malesu, D. Sahoo, P.L.Nayak, Chitosan–Sodium Alginate Nanocomposites Blended with Cloisite 30B As a Novel Drug Delivery System for Anticancer Drug Curcumin, International Journal of Applied biology and Pharmaceutical Technology. 2 (2011) 402-411.

4. D. Sahoo, P.L.Nayak, Synthesis and Characterization of Chitosan Nanocomposite Film for controlled Release of Ofloxacin, Journal of Applied Polymer Science. 123 (2011) 2588-2594 .

5. P.L.Nayak, D. Sahoo, Chitosan-alginate composites blended with cloisite 30B as a noveldrug delivery system for anticancer drug paclitaxel, International Journal of Plastics Technology. 15(1) (2011) 68-81.

6. A.K. Singla, A. Garg, D. Aggarwal, Paclitaxel and its formulations, International Journal of Pharmaceutics. 235 (2002) 179-192.

7. A.K. Singla, M. Chawla, Chitosan: Some Pharma-ceutical and Biological Aspects-an Update, Journal of Pharmacy and Pharmacology. 53 (2001) 1047- 1067.

8. D. Sahoo, S. Sahoo, J. Das, T.K. Dangar, P.L. Nayak, Antibacterial Activity of Chitosan Cross Linked with Aldehydes and Blended with Cloisite 30 B, NanoTrends: A Journal of Nanotechnology and Its Applications. 10 (2011) 1-9.

9. S. Sahoo, A. Sasmal, D. Sahoo, P.L. Nayak, Synthesis and Characterization of Chitosan-Polycaprolactone Blended with Organoclay for Control Release of Doxycycline, Journal of Applied Polymer Science. 118 (2010) 3167-3175 .

10. D. Sahoo, P.L. Nayak, Chitosan: The Most Valuable Derivative of Chitin, in: Biopolymers: Biomedical and Environmental Application, Scrivener Publishing LLC,USA, 2011, pp.129–166, ISBN: 9780470639238).

11. V.K. Malesu, D. Sahoo, P.L.Nayak, Synthesis and Characterization of Chitosan-Polyvinyl Alcohol Blend for Controlled Release of the Anticancer Drug Curcumin, Journal of Biomaterials and Nanobiotechnolog. 2 (2011) 414-425.

12. K. Vimala, Y.M. Mohan, K. Varaprasad, N.N. Redd, S. Ravindra, N.S. Naidu, K.M. Raju, Fabrication of Curcumin Encapsulated Chitosan-PVA Silver Nanocomposite Films for Improved Antimicrobial Activity, Journal of Biomaterials and Nanobiotechnology. 2 (2011) 55-64.

13. A. Sahu, U. Bora, N. Kasoju, P. Goswami, Synthesis of novelbiodegradable and self-assembling methoxy poly (ethylene glycol)-palmitatenanocarrier for curcumin delivery to cancer cells, ActaBiomater. 4 (2008) 1752-61.

14. Z. Ma, A. Shayeganpour, D.R. Brocks, A. Lavasanifar, Samuel J. High performance liquid chromatography analysis of curcumin in rat plasma:application to pharmacokinetics of polymeric micellar formulation of curcumin, Biomed Chromatogr. 21 (2007) 546-52.

15. I. Brouet, H. Ohshima, Curcumin, an anti-tumour promoter and antiimflammatory agent, inhibits induction of nitric oxide synthase inactivated macrophages, BiochemBiophysRes Commun. 206 (1995) 533-40.

16. A. Goel, B.A. Kunnumakkara, B.B. Aggarwal, Curcumin as "Curcumin": from kitchen to clinic (commentary), Biochem Pharmacol. 75 (2008) 787-809.

17. A.R. Kulkarni, K.S. Soppimath, T.M. Aminabhavi, Controlled Release of Diclofenac Sodium from Sodium Alginate Beads Crosslinked with Glutaraldehyde, PharmaceuticaActaHelvetiae. 74 (1999) 29-36.

18. T. Sekine, K. Yoshida, F. Matsuzaki, T. Yanaki, M. Yamaguchi, A novel method for preparing oil-in water-in-oil type multiple emulsions using organophilic montmorillonite clay mineral, Journal of Surfactants and Detergents. 2 (1999) 309-15.

19. M. Dikshit, L. Rastogi, R. Shukla, R.C. Srimal, Prevention of ischaemia induced biochemical changes by curcumin and quinidine in the cat heart, Ind J Med Res. 101 (1995) 31-50.

20. C.V. Rao, A. Rivenson, B. Simi, B.S. Reddy, Chemoprevention of coloncarcinogenesis by dietary curcumin, a naturally occurring plant phenoliccompound, Cancer Res. 55 (1995) 259-66.

21. Y. Kiso, Y. Suzuki, N. Watanabe, Y. Oshima, H. Hikino, Antihepatotoxic principles of Curcuma longa rhizomes, Planta Med. 49 (1983) 185-7.

22. H.H. Tonnesen, J. Karlsen, Studies on curcumin and curcuminoids. VI.Kinetics of curcumin degradation in aqueous solution, Z LebensmUntersForsch. 180 (1985) 402-4.

23. A. Kunwar, A. Barik, R. Pandey, K.I. Priyadarsini, Transport of liposomaland albumin loaded curcumin to living cells; an absorption andfluorescence spectroscopic study, BiochimBiophysActa. 1760, (2006) 1513-20.

24. S. Bisht, G. Feldmann, S. Soni, R. Ravi, C. Karikar, A. Maitra, Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): anovel strategy for human cancer therapy, J Nanobiotechnol. 5 (2007) 3.

25. K. Sou, S. Inenaga, S. Takeoka, E. Tsuchida, Loading of curcumin intomacrophages using lipid-based nanoparticles. Int J Pharm. 352 (2008) 287-93.

26. V. Kumar, S.A. Lewis, S. Mutalik, D.B. Shenoy, Venkatesh, Udupa N.Biodegradable microspheres of curcumin for treatment of inflammation, Ind J PhysiolPharmacol. 46 (2002) 209-17.

27. S. Salmaso, S. Bersani, A. Semenzato, P. Caliceti, New cyclodextrin bioconjugates for active tumour targeting. J Drug Target. 15 (2007) 379-90.

28. P.K. Vemula, J. Li, G. John, Enzyme catalysis: tool to make and breakamygdalin hydrogelators from renewable resources: a delivery model forhydrophobic drugs, J Am ChemSoc. 128 (2006) 8932-8.

29. T.P. Hadjiioannou, G.D. Christian, M.A. koupparis, Quantitative calculations in Pharmaceutical Practices and Research New Dehli, NY- VCH publishers Inc, 1993, pp. 345- 348.

30. D.W. Bourne, Pharmacokinetics. In: G.S.Banker, C.T.Rhodes, eds. Modern Pharmaceutical, 4th ed. New York, NY, Marcel Dekker Inc, 2002, pp.67-92.

31. G. Xu, H. Sunada, Influence of Formation Changes on Drug Release Kinetics. Chemical & Pharmaceutical Bulleti. 43 (1995) 483-487.

32. L. Ritger, N.A. Peppas, A Simple Equation for Disposition of Solute Release-II, Journal of Controlled Release. 5 (1987) 37-42.

33. T. Higuchi, Mechanism of Sustained Action Medication. Theoretical Analysis of Rate of

Release of Solid Drugs Dispersed in Solid Matrices, Journal of Pharmaceutical Sciences. 52 (1963) 1145-1149.

34. R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, N.A. Peppas, Mechanism of solute release from porous hydrophilic polymers, Int. J. Pharm. 15 (1983) 25-35.
35. J. Siepmann, N.A. Peppas, Modeling of drug release from delivery system based on hydroxypropyl methylcellulose (HPMC), Adv Drug Deli Rev. 48 (2001) 139-157.
36. J.W. Lee, S.Y. Kim, S.S. Kim, Y.M. Lee, K.H. Lee, S.J. Kim, Synthesis and characteristics of interpenetrating polymer network hydrogel composed of chitosan and poly(acrylic acid). Journal of Applied Polymer Science 73 (1999) 113–120.
37. B. Gibbs Jackson, Mechanism-Based Target Identification and Drug Discovery in Cancer Research, Science. 287 (2000) 1969-1973.