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CHITOSAN–SODIUM ALGINATE NANOCOMPOSITES BLENDED WITH CLOISITE 30B AS A NOVEL DRUG DELIVERY SYSTEM FOR ANTICANCER DRUG CURCUMIN

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ABSTRACT: In the present research program, polymer nanocomposites have been used as the drug carrier for delivery systems of anticancer drug. Chitosan (CS) and sodium alginate (ALG) with different ratios were blended with different wt% of Cloisite 30B solution by solvent evaporation method. Cloisite 30B was incorporated in the formulation as a matrix material component which also plays the role of a co-emulsifier in the nanocomposite preparation. Curcumin with different concentrations were loaded with CS-ALG/ C 30B nanocomposites for studying the in-vitro drug delivery systems. Morphology and structure characterization of nanocomposites were investigated by X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM) and Fourier Transmission Infra Red Spectroscopy (FTIR) respectively. The drug release was studied by changing time, pH and drug concentrations. The kinetics of the drug release was studied in order to ascertain the type of release mechanism. Based on the diffusion as well as the kinetics, the mechanism of the drug release from the composite matrix has been reported.
Key words: Chitosan, Sodium alginate, C 30B, Curcumin, Drug delivery

INTRODUCTION

In recent years, biodegradable polymers have attracted attention to be used as biomaterials particularly, for tissue engineering, gene therapy, wound healing and controlled drug delivery systems [1]. The most important advantage of biodegradable polymers is the disappearance of implanted foreign materials from the body as a result of their biodegradation. Most important biodegradable polymers used in biomedical applications are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly (e-caprolactone) (PCL) , poly (3- hydroxybutyrate) (PHB), copolymers of polyglycolide, chitosan, alginate and soy protein [2-5].

Chitosan is a biodegradable polymer obtained by the deacetylation of chitin, which is present in shells of insects and marine crustacean [6]. This is a marine –based polymer. The unique properties of chitosan, among others, are biodegradability, bioactivity, non-toxicity as well as good adhesion and sorption, which largely contribute to its multiple applications [1].Chitosan is also a valuable component of polymer blends and composites [1]. Using an appropriate technological process one may obtain films, fibers, gels and foams as well as chitosan beads of different sizes and morphology. Numerous in vitro studies have analyzed the response to chitosan by smooth muscle cells, macrophages, osteoblasts, chondrocytes, erythrocytes and whole blood. In addition, many studies have been conducted with mouse, rat, rabbit, and canine animal models in order to describe in vivo biocompatibility, biodegradability, drug delivery, DNA delivery, and wound healing using chitosan as a carrier**.** [7-9].

Alginate (ALG) is a water soluble linear polysaccharide extracted from brown sea weed and is composed of alternating blocks of 1-4 linked α-L-guluronic and β-dmannuronic acid residues. ALG has been reported to be mucoadhesive, biodegradable, and biocompatible and has potential for numerous pharmaceutical and biomedical applications such as drug delivery system and cell encapsulation [10].

Alginate micro and nanoparticles can be obtained easily by inducing gelatin with calcium ions [11-13]. Such easy-gelling property can be used to produce a pre-gel consisting of very small aggregates of gel particles, followed by the addition of an aqueous polycationic solution to make a polyelectrolyte complex coating [14].

Over the last few years, medical and pharmaceutical industries have shown an increased interest in biopolymers in general and in alginates in particular. The reason for this increased interest is their usefulness in specific applications, as it enhances efficient treatment of esophageal reflux, creates multiquality calcium fibers for dermatology and wound healing. They are also used for high- and low gel strength dental impression materials. Besides this, alginate is an effective natural disintegrant, tablet binder and offers an attractive alternative for sustained-release systems [15]. It offers advantages over synthetic polymers as it forms hydrogels under relatively mild pH and temperature and is generally regarded as non-toxic, biocompatible, biodegradable, less expensive and abundantly available in nature; in addition, alginate meets the important requirement of being amenable to sterilization and storage. All these advantages make alginates very useful materials for biomedical applications, especially for controlled delivery of drugs and other biologically active compounds and for the encapsulation of cells. Calcium alginate is a natural haemostat, so alginate based dressings are indicated for bleeding wounds [16]. The gel forming property of alginate helps in removing the dressing without much trauma. The biopolymer alginate exhibits, like pectin and others, the effect of ionotropic gelation if multivalent cations diffuse directed from one side into the solution [16].

Curcumin (diferuloylmethane), a polyphenol, is a low molecular- weight active principle of the perennial herb Curcuma longa (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. Curcumin possesses antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial 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. To increase its solubility and bioavailability, attempts have been made through encapsulation in liposomes, polymeric and lipo-NPs, biodegradable microspheres, cyclodextrin, and hydrogels [17-22].

In recent years, various controlled delivery forms, such as polymeric micro/nanospheres, liposomes, micelles, parenteral emulsion, and prodrugs have been investigated to increase its solubility, to minimize the side effects as well as to avoid the use of toxic adjuvant. [23-25].

In the present research program, we wish to report the preparation of a novel nanocomposites formulation, i.e. biodegradable chitosan-alginate (CS-ALG) nanocomposites incorporated with medical clay, Cloisite 30B called CS-ALG/ C 30 B nanocomposites, for oral chemotherapy by using curcumin as a prototype drug due to its excellent therapeutic effects against a wide spectrum of cancers and its great commercial success as the best seller among various anticancer agents. The composites have been characterized using XRD, FTIR and SEM techniques. The kinetics of the drug delivery system have been reported.

EXPERIMENTAL

Materials

Chitosan (CS) (Degree of Deacetylation = 95%) was purchased from India Sea Foods, Kerela, India. Sodium alginate of low viscosity (0.02Pa·s) for a 1% solution at 20°C was purchased from Shanghai Chemical Co. Ltd (China). Curcumin was a generous gift from VINS Bioproducts, Medak, Andhra Pradesh.

Preparation of Chitosan-Alginate Nanocomposites

Both the sodium alginate and chitosan solutions were prepared by dissolving the chemicals in distilled water and 1% acetic acid respectively.

Blend solution of different compositions (i.e. the weight ratios between chitosan and alginate of 100/0, 80/20, 70/30, 60/40, 0/100 (w/w) respectively) were then prepared by casting a mixture of the solutions in a respective weight ratio on a Teflon dish. To this blend solution of CS-ALG (80:20) C 30B of different compositions (1wt%, 3wt% and 5wt %) were added with constant stirring for 4 hours at room temperature to get a homogenous solution. It should be noted that stirring was used to homogenize the mixture prior to pouring onto the dish. The casting was let dry at room temperature for 3 days and was collected for characterization.

Drug Loading

Curcumin-loaded CS-ALG/ C 30B nanocomposites were prepared by emulsion/solvent evaporation method. In short, curcumin of different loadings, i.e., 1 wt%, 3wt%, 5 wt%, 7 wt% and 10wt% were dissolved in ethanol with (80:20) CS-ALG/ C 30B. The formed solution was poured into a labeled Petri dish and allowed to evaporate the solvent overnight at room temperature. This compound was used for drug delivery purposes.

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 1.2 and 7.4) was used as the dissolution media to stimulate gastrointestinal tract (GIT) conditions. A 5 ml aliquot 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 λ_{max} value of 490 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 hydro fusion), 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 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.

(i) Zero - Order Kinetics [29].

$$W = k_1 t \quad \text{..... (1)}$$

(ii) First - Order Kinetics [29,32].

$$\ln (100- W) = \ln 100 - k_2 t \quad \text{..... (2)}$$

(iii) Hixon-Crowel's Cube- Root Equation (Erosin Model) [32].

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

(iv) Higuchi's Square Root of Time Equation (Diffusion Model) [30].

$$W = k_4 t \quad \text{..... (4)}$$

(v) Power Law Equation (Diffusion/ Relaxation model) [31].

$$M_t / M_\infty = k_5 t^n \text{ ..... (5)}$$

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

Fourier Transmission Infra Red Spectroscopy (FTIR)

The FTIR spectrum of the chitosan, alginate, and chitosan-alginate blend was obtained using a BIORAD-FTS-7PC type FTIR spectrophotometer.

X-Ray Diffraction (XRD)

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

Scanning Electron Microscopy (SEM)

The blending of the Chitosan-Alginate composites containing different concentrations were characterized 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° to observe the microstructure of the chitosan-alginate composite blends.

Swelling Studies

Water absorption of the polymer-drug conjugates was measured following ASTM D 570-81. The samples were preconditioned at 50° C for 24h and then cooled in a desiccator before being weighed. The preconditioned samples were submerged in distilled water at 25° C for 24h.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 24h. The total water absorption for 24h 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 hr., W_2= Weight of Dry Composite.

RESULTS AND DISCUSSION

Fourier Transmission Infra Red Spectroscopy (FTIR)

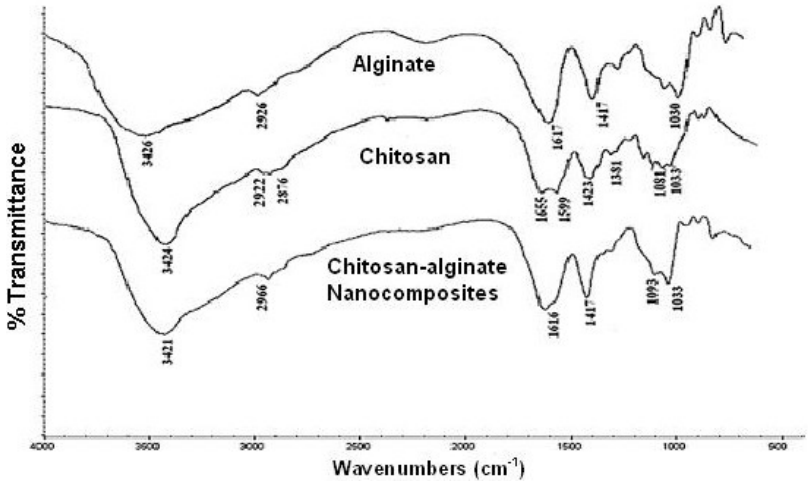


Figure 1. FTIR-Spectra of pure alginate (i.e. the topmost curve), pure chitosan (i.e. the middle curve) and (i.e. the bottommost curve) chitosan / Alginate blends

FT-IR spectra of chitosan (CS), alginate and CS/alginate nanocomposites are shown in Figure 1. In the spectra of CS, the broad band at 3424 cm^{-1} corresponded to the amine and hydroxyl groups; the peak at 2876 cm^{-1} is caused by -OH stretching; the absorption band of the carbonyl (C=O) stretching of the secondary amide (amide I band) at 1655 cm^{-1}, and the bending vibrations of the N–H (N-acetylated residues, amide II band) at 1599 cm^{-1}. The peaks at 1423 and 1381 cm^{-1} belong to the N–H stretching of the amide and ether bonds and N–H stretching (amide III band), respectively. The peaks observed at 1081 and 1033 cm^{-1} are the secondary hydroxyl group (characteristic peak of -CH-OH in cyclic alcohols, C-O stretch) and the primary hydroxyl group (characteristic peak of $-CH_2-$ OH in primary alcohols, C-O stretch). The bands around 1030 cm^{-1} (C-O-C stretching) presenting in the IR spectrum of sodium alginate are attributed to its saccharide structure. In addition, the bands at 1617 and 1417 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups. So in the IR spectrum of CS/ALG nanocomposites, we can observe the asymmetrical stretching of -COO- groups shifted to 1637 cm^{-1} and the symmetrical stretching of - COO- groups shifted to 1415 cm^{-1}. In addition, the absorption band at 1599 cm^{-1} of chitosan shifts to 1559 cm^{-1} after the reaction with alginate, the stretching vibration of -OH and $-NH_2$ at 3424 cm^{-1} shifts to 3448 cm^{-1} and becomes broad.

X-Ray Diffraction (XRD)

Wide-angle X-ray diffraction (WAXD) is a classical method for determining the gallery height (*d*-spacing distance) in clay particles [15]. The *d*-spacing can be determined by the diffraction peak in the XRD patterns, and can be expressed by Bragg's equation ($\lambda = 2d_{001}\sin\theta$), where d_{001} is the interplanar distance of (001) diffraction face, θ is the diffraction position, and λ is the wave length [26]. During intercalation, the insertion of polymer into the organoclay galleries forces the platelets apart and increases the *d*-spacing, resulting in a shift of the diffraction peak to lower angles. In case of Cloisite 30 B, the peak occurs at $2\theta = 4.8^{o}$.

Figure 2 shows XRD pattern of chitosan, Alginate, chitosan-Alginate/MMT (80:20) nanocomposites and MMT.

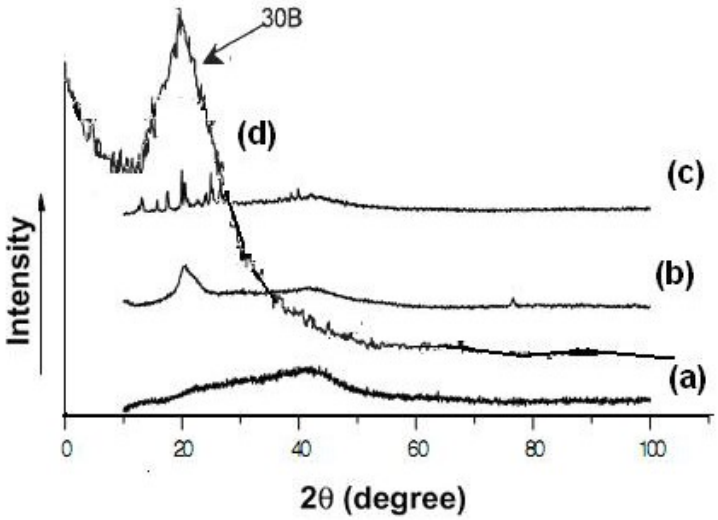


Figure 2. X-ray diffraction of (a) chitosan (b) alginate (c) chitosan-alginate (d) Cloisite 30B

Scanning Electron Microscopy (SEM)

The scanning electron micrograph of a typical drug-loaded alginate bead treated with chitosan is shown in Figure 3. The composite showed surface cracks probably caused by partial collapsing of the polymer network during drying.

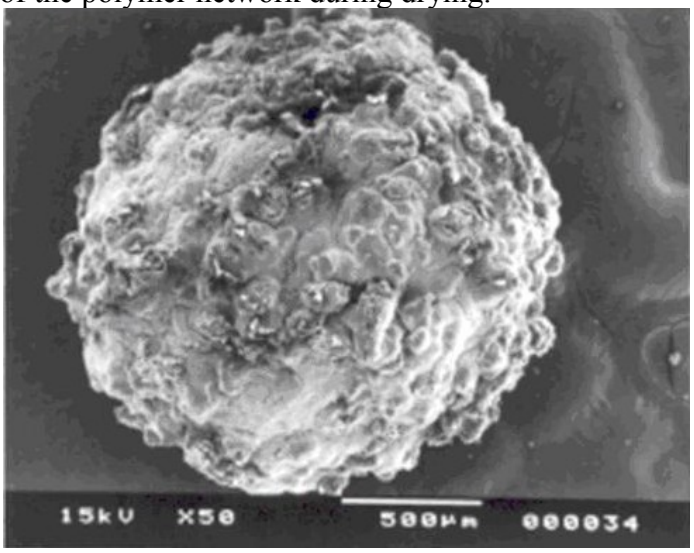


Figure 3. Scanning Electron Micrograph of Curcumin-loaded chitosan-alginate Nanocomposites

Equilibrium Swelling Studies

The swelling behavior of the composites has been investigated. It is generally known that the swelling behavior of the polymer network depends upon the nature of the polymer, polymer solvent compatibility and degree of cross-linking. However, in the case of ionic networks, swelling behavior depends upon mass transfer limitations, ion exchange and ionic interaction [27]. The swelling behavior of the composites is depicted in Figure 4.

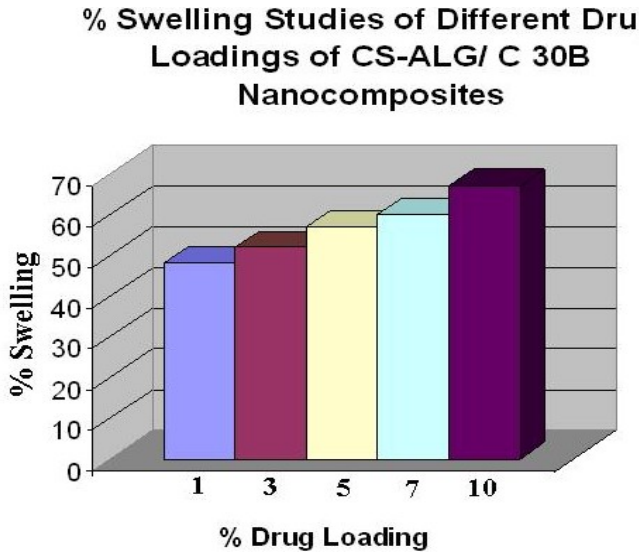


Figure 4. Water Absorption of the chitosan-sodium alginate nanocomposites with different % of drug loadings

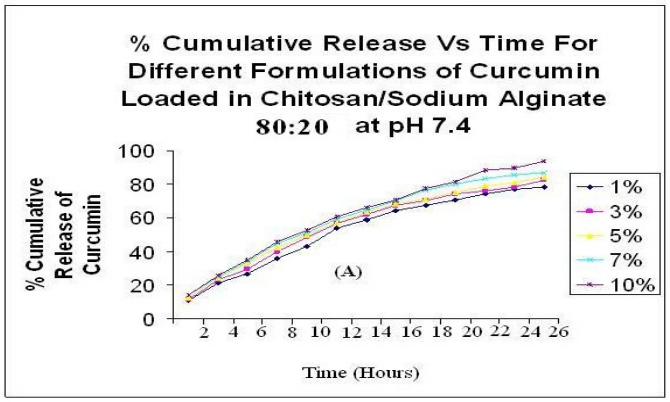
It shows that the swelling increases with time up to a certain level, and then levels off. The swelling parameters are furnished in Figure 4. Due to the hydrophobicity of drug the swelling % increases with increase in the drug loadings.

In-vitro Drug Release

Effect of pH, Time and Drug loading

In order to investigate the effect of pH on the swelling of CS-ALG/C 30B composite (2.5%), we have measured the % cumulative release in both pH 1.2 and 7.4 media. Cumulative release data presented in Figure.5 indicate that by increasing the pH from 1.2 to 7.4, a considerable increase in the cumulative release is observed for all composites. From Figure.5.(A) and (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.

Interestingly, more than 90 wt% curcumin is released from composites at pH 7.4 within 26 hours, whereas less than 80 wt% of the drug is released at pH 1.2 within 26 hours. 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 1.2, leading to curcumin being released more rapidly at pH 7.4 than pH 1.2.



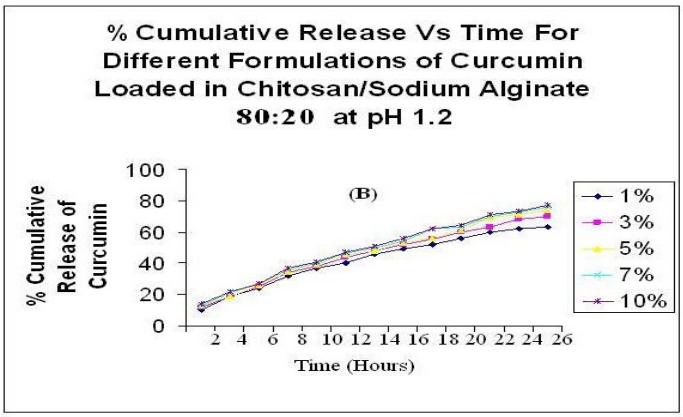


Figure.5. % Cumulative release Vs. Time for different formulation of curcumin loaded in CS-ALG/C 30B composite film in (A) pH 7.4 and (B) pH 1.2 media

Release data (Figure 5) 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, due to 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 vs. time by fitting to an exponential equation of the type as represented below [29].

$$M_t / M_\infty = kt^n$$

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 1. 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 CS-ALG/C 30B (2.5 wt %) constant, ranged from 0.03 to 0.24 in pH 7.4 and 0.01 to 0.18 in pH 1.2 respectively. However, the drug-loaded composites exhibited 'n' values ranging from 0.61 to 1.67 in pH 7.4 and 0.57 to 1.62 in pH 1.2 (Table 1), 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 [27-28]. 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 [29-30].

Table. 1: Release kinetics Parameters of different Formulations at pH 7.4 and pH 1.2

Curcumin	Values of "k"		Values of "n"		Coodination Coefficient, R^2	
(%)	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2
1 wt%	0.03	0.01	0.61	0.57	0.9351	0.9256
3 wt%	0.05	0.04	0.72	0.64	0.9521	0.9337
5 wt%	0.07	0.06	1.50	1.38	0.9676	0.9485
7 wt%	0.16	0.11	1.58	1.43	0.9751	0.9711
10 wt%	0.24	0.18	1.67	1.62	0.9756	0.9726

CONCLUSION

Controlled delivery devices that utilize biodegradable polymers have a significant advantage over competing delivery systems in that there is no need for surgical removal of the device. Further, if the polymer degrades only at the surface, the drug release process is simplified in water diffusion into the bulk is minimized and drug release rate is governed by polymer degradation rate. Novel nanocomposites of chitosan and alginate blended with Cloisite 30B were prepared and characterized by FTIR spectroscopy, X-ray diffractometry and scanning electron microscopy. This blend was loaded with different amounts of anticancer drug curcumin to study the drug release behavior. The swelling studies of the nanocomposites have been reported. The drug was released in a controlled manner. The drug release was monitored by changing time, % drug loading and pH of the medium. It was observed that the release was much more pronounced in the basic medium than the acidic medium. The kinetics of the drug release was investigated and based on the kinetic parameters such as 'k' and 'n' values the probable drug release mechanism has been reported.

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