

Full Length Research Paper

Gelatin blended with nanoparticle cloisite30B (MMT) for control drug delivery of anticancer drug paclitaxel

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Gelatin/montmorillonite (MMT) hybrid nanocomposite was directly prepared by blending gelatin with cloisite 30B in aqueous solution by solvent evaporation method. MMT was incorporated in the formulation as a matrix material component which also plays the role of a co-emulsifier in the nanocomposite preparation. Paclitaxel (PTX) with different concentrations were loaded with Gelatin/MMT nanocomposites for studying the in-vitro drug delivery systems. The composites were characterized by using FTIR, SEM, and XRD methods. 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 was investigated.

Key words: Gelatin, Cloisite 30B, Controlled drug delivery, Paclitaxel

INTRODUCTION

Drug delivery research is clearly moving from the micro- to the nanosize scale. Nanotechnology is therefore emerging as a field in medicine that is expected to elicit significant therapeutic benefits. The development of effective nanodelivery systems capable of carrying a drug specifically and safely to a desired site of action is one of the most challenging tasks of pharmaceutical formulation investigators. They are attempting to reformulate and add new indications to the existing blockbuster drugs to maintain positive scientific outcomes and therapeutic breakthroughs. The nanodelivery systems mainly include nanoemulsions, lipid or polymeric nanoparticles, and liposomes. Nanoemulsions are primarily used as vehicles of lipophilic drugs following intravenous administration. On the other hand, the ultimate objective of the other nanodelivery systems is to alter the normal biofate of potent drug molecules in the body following their intravenous administration to markedly improve their efficacy and reduce their potential intrinsic severe adverse effects

Today, nanoparticle-mediated drug delivery and drug targeting are intensively researched. Within the growing field of nanomedicine, drug delivery accounts for more 50 % of all publications and patent filings worldwide

(Wagner et al., 2006), whereas nano particulate delivery is still mostly part of basic research. The need for nanoparticles as biodegradable and non toxic drug delivery system was formulated first time in 1978 by Marty (Marty et al., 1978). Since then, numerous synthetic and natural polymers were adopted for the production of biodegradable nanoparticles. Poly-ε-caprolactone (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their co-polymers poly(lactide-co-glycolide) (PLGA) are the most widely used starting materials (Hans et al., 2006). Natural polymers used are proteins (albumin and gelatin) (Kaul et al., 2005) and polysaccharides (dextran, alginate, and chitosan) (Chorny et al., 2004). Scholes and co-workers in an excellent article summarized the requirements for an ideal targeting system as follows: i) biocompatibility, biodegradability, and low antigenicity, ii) protection of the drug, iii) maintenance of the integrity till the target is reached, iv) avoidance of side effects, v) membrane passage, vi) target recognition and association, vii) controlled drug release, and viii) elimination upon drug release (Scholes et al., 1997).

Among several natural polymers gelatin offers some advantageous material properties. Due to its proteinaceous nature it is readily accessible for chemical modifications either of the bulk material or the finished nanoparticles via the functionalities of the amino acid

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residues (Djagny et al., 2001). Beside these technological aspects, gelatin is known for its good biodegradability and biocompatibility (Yamamoto et al., 2001; Stevens et al., 2002) accompanied with low immunogenicity (10-11) (Schwick et al., 1969; Kuijpers et al., 2000). These beneficial characteristics are not only expressed in long clinical usage of gelatin as plasma expander (Tabata et al., 1998) and as sealant for vascular prosthesis (Kuijpers et al., 2000) but also led to the acceptance of gelatin as "Generally Recognized as Safe" (GRAS) substance in the area of food additives by the U.S. Food and Drug Administration (FDA). The application of gelatin in controlled release devices for bioactive molecules like protein or plasmid DNA was recently reviewed (Young et al., 2005).

In the present research program, we wish to report the preparation of a novel nanocomposite formulation, i.e. biodegradable gelatin-MMT nanocomposites, for oral chemotherapy by using paclitaxel 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 were characterized using FTIR, XRD and SEM techniques. The kinetics of the drug delivery system have been reported and based on the kinetic parameters the plausible mechanism has been suggested.

MATERIALS AND METHODS

Materials

Paclitaxel was obtained from the National Cancer Institute (Bethesda, MD). Gelatin (Type B, extracted from bovine skin) was purchased from Sigma Chemical (St. Louis, MO). The Cloisite 30B was procured from Southern Clay Products, USA, and glutaraldehyde (25% in water) was purchased from Sigma Chemical Co. (St. Louis, MO); All other samples were of Analytical Grade.

Preparation of Nanocomposites

Ten grams of gelatin powder was soaked in 50 ml deionized water and heated at 70 °C to obtain a homogeneous solution by adding 5gm of glycerin as the plasticizer. Calculated amount of Cloisite 30B (MMT) (1%, 2.5%, and 5%) was added to this slurry. The mixture was stirred for 8 hours at room temperature till a homogenous solution was formed. Then from 0.1 to 0.5gm of glutaraldehyde was added to the solution. The product was poured into the specially self-made mold and dried at ambient temperature for several days.

Drug Loading

Paclitaxel-loaded gelatin/MMT nanocomposites were prepared by adding glycerin and glutaraldehyde by emulsion/solvent evaporation method. In short, paclitaxel of different loadings, i.e. 5wt%, 10wt%, 15wt%, 20wt% with gelatin/MMT were mixed with glycerin and glutaraldehyde. The formed nanocomposites was

allowed to evaporate overnight at room temperature to harden the nanocomposites. This nanocomposite thus prepared was used to 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 3.4 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 Paclitaxel content at a fixed time interval. The dissolution media was replenished with a fresh stock solution. The amount of Paclitaxel released was analyzed using a UV spectrophotometer (Systronics, India) at the λ_{\max} value of 232 nm.

Characterization

Fourier Transmission Infra Red Spectroscopy (FTIR)

The FTIR spectrum of the gelatin blends were monitored using a BIORAD-FTS-7PC type FTIR(UK) spectrophotometer

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 K α radiation (USA) at a generator voltage of 40 kV and a generator current of 100 mA. Samples were scanned from $2\theta = 1-10^\circ$ at a scanning rate of $2^\circ/\text{min}$.

Scanning Electron Microscopy (SEM)

The blending of the gelatin nanocomposites containing different concentrations was characterized using SEM (440, Leica Cambridge Ltd., Cambridge, UK). The 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 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 gelatin composite blends

Swelling Studies

Water absorption of the polymer-drug conjugates was measured following standard 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.

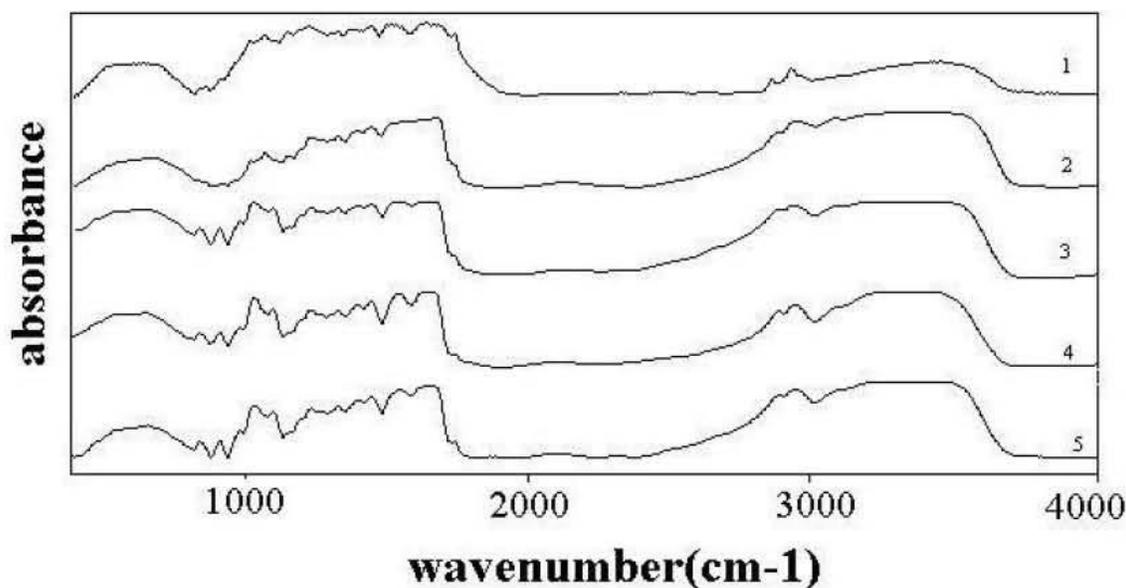


Figure 1. FTIR spectra for (1) gelatin powder (2) gelatin film (3)gelatin film containing glycerin (4) gelatin film containing glycerin and 0.01%GTA (5)gelatin film containing glycerin and 0.12%GTA

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

Where, W1=Weight of Swollen composite after 24 hr. W2= Weight of Dry Composite

RESULTS AND DISCUSSION

Fourier transmission infra red spectroscopy (FTIR)

The FTIR spectra exhibited by gelatin powder differ from those exhibited by gelatin film (Figure 1) specially in the amide I (about 1650cm^{-1}) amide II (about 1550cm^{-1}) and amide III (about 1240cm^{-1}) regions. Compared to the spectra for gelatin powder, the gelatin films contain GTA shows higher intensity amide I and amide II bonds it means the extent of order in GTA gelatin films may be higher than that in gelatin powder. The intensity of the amide III bond has been associated with triple helical structure. The intensity of amide III bond for the GTA gelatin films is higher than that for the gelatin powder. It seems that the GTA gelatin films have more intermolecular associates as a result of GTA cross linking.

With increasing in the GTA concentrations the intensity of peaks increase at about 1110cm^{-1} , which related to C-O vibration, showing that cross linking has occurred. Absorption in the region of at $1000\text{-}1100\text{cm}^{-1}$ is attributed to C-O vibration due to carbohydrates in collagen and carbohydrates are required in the formation of pentosidine crosslink.

X-ray diffraction analysis

The XRD patterns of pristine MMT and composites are shown in Figure 2. Original MMT exhibits a sharp peak at $2\theta = 6^\circ$, and through the Bragg's equation: $\lambda = 2d \sin\theta$; 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 disappears, 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 SEM photographs of gelatin and blended with 5% MMT are shown in Figure 3.

The fracture surface of gelatin exhibits a smooth laminated structure made up of a thin parallel layer, reflecting its brittleness. Comparatively, the fracture surface of composite seems coarse, indicating an improved toughness.

Swelling studies

Table 1 shows the swelling variation of the films as a function of GTA concentration following different time of storage in distilled water. The swelling percentage of

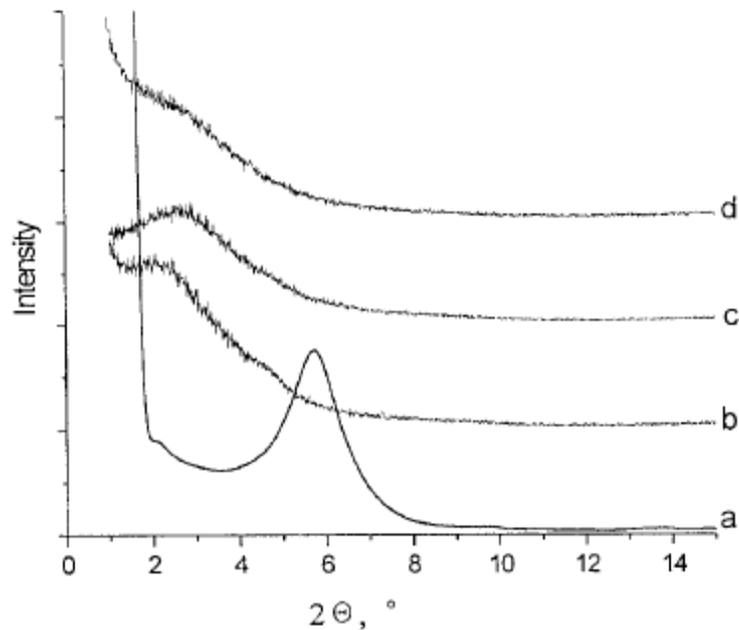


Figure 2. Wide angle X-ray diffraction (a) MMT, (b) 1% MMT, (c) 2.5% MMT, (d) 5% MMT, (e) 7.5% MMT

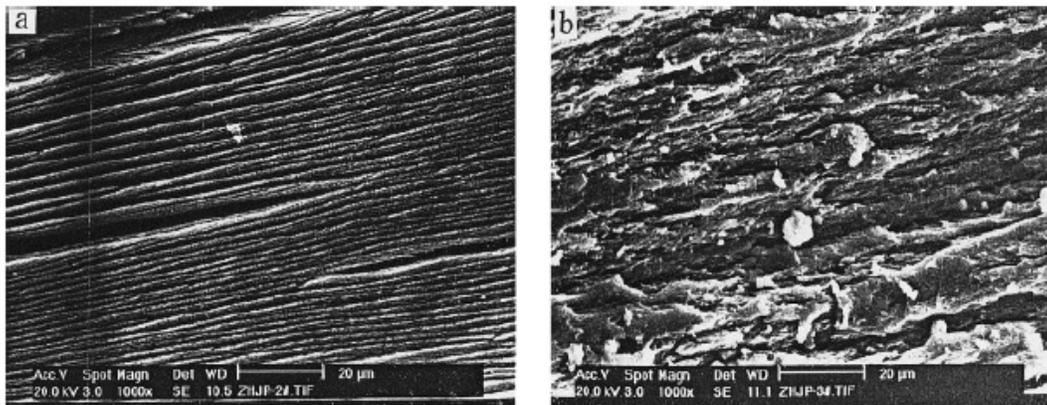


Figure 3. SEM photograph of (a) Gelatin and (b) blended with 5% MMT

uncross-linked gelatin film is about 390% after 5 min. Swelling measurement of longer times are hindered by the solubility of the film which begins to dissolve in the water. In general, increasing GTA concentration proves to decrease the swelling percentage and increase the time of films solubility. There is not a significant variation when GTA concentration has increased more than 0-16(%w) and the swelling percentage reaches to a maximum value.

In-vitro drug release

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 (Langer, 1990, Rathbone et al., 1999). The biodegradable polymer can contribute largely to this technology by adding its own characters to the drugs. In this connection, some biodegradable polymer like gelatin

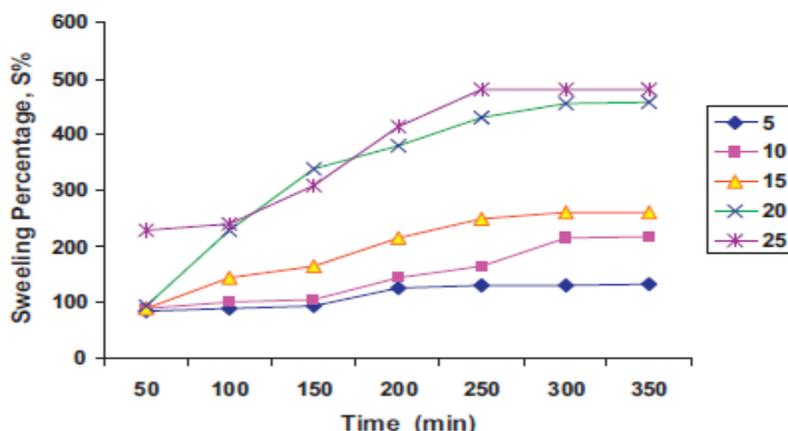
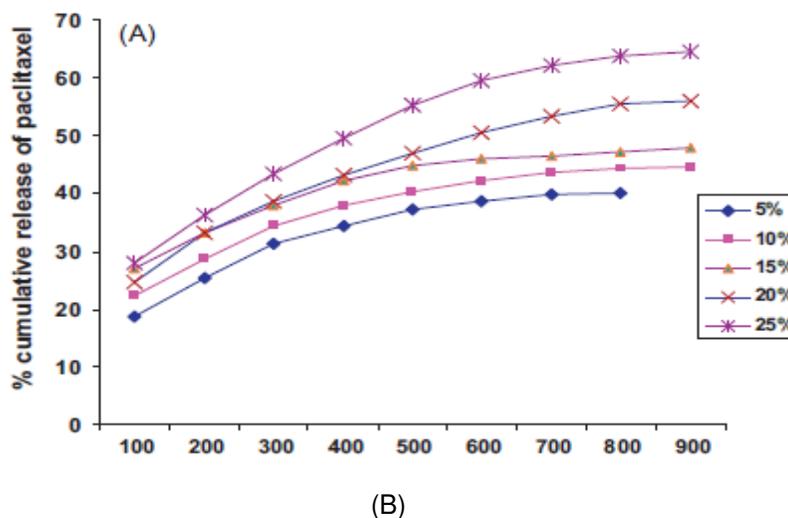


Figure 4. Swelling release of gelatin with different drug loadings in buffer solution pH 7.40



(B)

is commonly used as this polymer can be prepared in the 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(17) (Lewis, Chasin, and Langer, 1990). There are some formulations for the drug delivery systems, for example, films, gels, porous matrices, microcapsules, micro spheres, nanoparticles, polymeric micelles and polymer linked drugs(18-19) (Heller, 1987; Li and Vert, 1999; Li, Vert, Scott, and Gilead, 1995).

Effect of pH

In order to investigate the effect of pH on the swelling of composite gelatin we measured the % cumulative release in both pH 3.4 and 7.4 media. Cumulative release data presented in Figure 5 indicate that by increasing the pH from 3.4 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, paclitaxel is being released more rapidly at pH 7.4 than at pH 3.4, the release half times t_{50} (time required for releasing 50 wt% of drug) for 10%, 20%, 30%, 40%, 50% drug loading are 2.8, 1.8 and 1.7 h at pH 7.4, and 6.0, 5.0 and 4.4 h at pH 3.4, respectively are shown in Figure 7(A) and (B). More than 80 wt% Paclitaxel is released from composites at pH 7.4 within 8 h, whereas less than 44 wt% of the drug is released at pH 1.2 within 4 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 3.4 leading to ofloxacin being released more

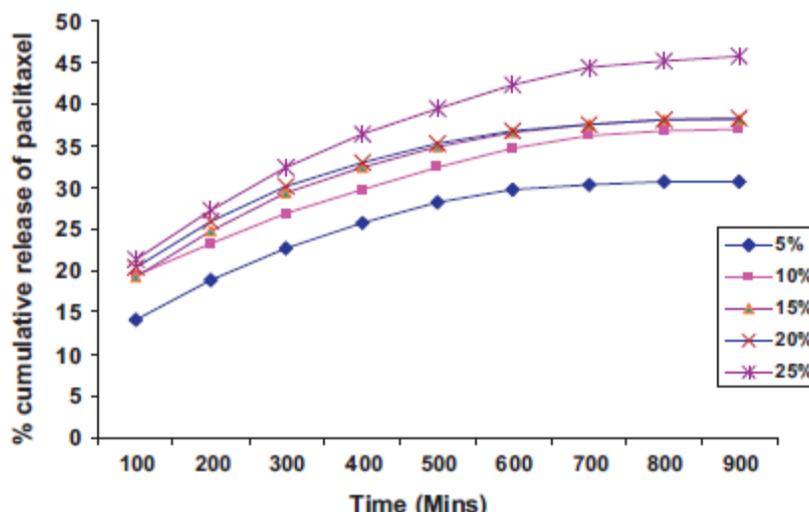


Figure 5. % Cumulative release of paclitaxel for different formulations loaded with gelatin (A) pH 7.4 and (B) pH 1.2

rapidly at pH 7.4 than 3.4.

Effect of drug loading

Figure 5 displays the release profiles of drug from composites at different amounts of drug loadings. Release data show 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 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 kinetics 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(20) (Xu & Sunada, 1995).

$$W = k_1 t$$

(ii) First-order kinetics (20-21) (Singla & Medirata, 1988; Xu & Sunada, 1995).

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

(iii) Hixon–Crowel's cube-root equation(22) (Erosin model) (Singla & Medirata, 1988).

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

(iv) Higuchi's square root of time equation (diffusion model)(23) (Higuchi, 1963).

$$W = k_4 t$$

(v) Power law equation (diffusion/relaxation model)(24) (Kulkarni, Soppimath, and Aminabhavi, 1999).

$$M_t/M_\infty = k_5 t^n$$

$M_t/M_\infty = k_5 t^n$ 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 diffuses through and is release 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.

Drug release kinetics was analyzed by plotting the cumulative release data vs. time by fitting to an exponential equation of the type(24) (Kulkarni et al., 1999) as represented below. $M_t/M_\infty = Kt^n$ 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 nine formulations and these data are given in Table 2.

Table 1 The effect of GTA and Time on swelling(%w)of gelatin films

GTA (wt%)	5 min	30 min	60 min	24 h
0	388.98	nd	nd	cs
0.02	250.76	800.65	nd	cs
0.04	276.60	809.33	866.403	nd
0.06	357.82	1266.32	1485.38	768.5731
0.08	476.06	1081.12	1051.13	559.5377
0.1	263.01	368.99	377.7	247.6847
0.12	169.62	261.72	285.18	357.93
0.14	158.09	237.57	237.03	315.55
0.16	169.17	214.91	217.37	220.88
0.18	155.95	196.44	215.89	223.74
0.2	159.28	183.32	185.68	202.20

Table 2. Release kinetics parameters of different formulations at PH 1.2

Sample code	k	n	Co-ordination-coefficient, r
7.4 pH			
5%	0.08	.50	0.964
10%	0.04	.55	0.968
15%	0.07	1.68	0.961
20%	0.25	1.55	0.955
25%	0.29	1.66	0.963
1.2 pH			
5%	0.24	.48	0.979
10%	0.02	0.54	0.944
15%	0.19	1.55	0.978
20%	0.04	1.67	0.969
25%	0.20	1.88	0.991

The values of k and n have shown a dependence on the, % drug loading and polymer content of the matrix. Values of n for composites prepared by varying the amounts of drug containing 10, 20 and 30 wt% and keeping gelatin constant, ranged from 0.57 to 0.88 suggesting shift of drug transport from Fickian to anomalous type. However, the drug-loaded composites exhibited n values ranging from 0.96 to 1.57 (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 (Kulkarni et al., 1999). 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 (Aminabhavi and Naik 1998; Lyu et al., 2005; Ritger and Peppas 1987, Sahoo, 2010, Sahoo 2010 and Sasmal 2010).

CONCLUSION

Novel nanocomposites of gelatin with MMT (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 paclitaxel 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 suggested.

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