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Research Article

Formulation and Evaluation of Chitosan-Polyaniline Nanocomposites for Controlled Release of Anticancer Drug Doxorubicin

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ABSTRACT

In this present work, chitosan (CS) crosslink with polyaniline (PANI) with montmorilonite (MMT) called as (CS-PANI/MMT) and CS crosslink with PANI without MMT called as (CS-PANI) were prepared by employing the solution casting method. Further the formation of nanocomposites CS-PANI/MMT and CS-PANI were investigated using XRD, FTIR, SEM and tensile strength. Water uptake and swelling ratio of the CS-PANI and CS-PANI/MMT were found to decrease with increase in concentration of clay. Mechanical properties of the CS-PANI and CS-PANI/MMT were assessed in terms of tensile strength and extensibility using texture analyzer. Increase in tensile strength and reduction in extensibility was reported with increase in the nanoclay content. In vitro drug release study on CS-PANI and CS-PANI/MMT indicated pronounced sustained release of doxorubicin by the incorporation of clay particles in the CS polymer matrix. Overall CS-PANI/MMT nanocomposite films exhibited improved mechanical and sustained drug release properties than CS-PANI.

Keywords: Chitosan, Polyaniline, Montmorillonite nanocomposites, Doxorubicin, Drug delivery.

INTRODUCTION

Health care is a challenging subject for medical science. Today the advanced technology and development science are promoting to modern medical and pharmaceutical science[1]. This is a new opportunity to understand, prevent and control the serious diseases by different type of modern techniques. In 21st century controlled drug delivery system is a great challenge for medical science and pharmaceutical science. Recently the combination of nanotechnology and polymer science has supported to pharmaceutical science for preparation and synthesis of new drug carrier for new drug molecules. Wide spectrums of nontoxic, biocompatible natural and synthetic polymers are available for drug carrier[2-4].

Many researchers have developed chitosan based nanocomposites. It was found enhancing drug carrier ability. It is also low cost and eco-friendly. Commonly biopolymer chitosan and their derived products are proved to be biodegradable with unique properties. Some reporter has described chitosan as abundant natural polysaccharide[5-7]. The chitosan based nanocomposites polymers are exhibited unique properties due to the formation of inter molecular hydrogen bond in between amino groups and hydrogen group. It has also been reported that the mechanical, water barrier, and miscibility of biodegradable properties are affected by the ratio of chitosan and MMT. Recently some researcher reported, that starch based nanocomposites are influenced by mechanical properties and water resistance strength.

Additionally the swelling behaviour of drug carrier biodegradable polymer is depending on environment condition at the time of drug released. However, the change of environment is depended on pH, temperature or ionic strength etc respectively. Drug release is achieved when the polymer matrix swells and because many of the potentially most useful pH-sensitive polymers matrix swell at high pH values and collapse at low pH values[6,7-9]. In this study CS-PANI with MMT and CS-PANI polymer composites were prepared for controlled release of anticancer drug doxorubicin. The CS-PANI and CS-PANI/MMT films were evaluated in terms of SEM, FTIR, XRD, Mechanical, physical, TGA, swelling and invitro release study of an anticancer drug Doxorubcin.

Experimental

Materials

Chitosan and Polyaniline were purchased from Himedia. Doxorubicin was purchased from Hindustan scientific, Cuttack, India. C30B was purchased from Southern Clay, USA. All other reagents and chemicals were used as the analytical grade. Millipore water was used in the entire experimental work.

Preparation of CS-PANI/MMT blends and their nanocomposites

The CS-PANI/MMT films were prepared using CS, MMT and PANI. CS solution was prepared by dissolving CS in a 2% (v/v) aqueous acetic acid solution, followed by centrifuging to remove the insoluble material. MMT was first swelled in 2% (v/v) aqueous acetic acid solution and then added to CS solution with continuous stirring (300 rpm) at 60°C for 4 h. PANI was added as a conducting polymer agent. CS-PANI/MMT films were prepared using different ratios of CS, PANI and MMT as shown in Table

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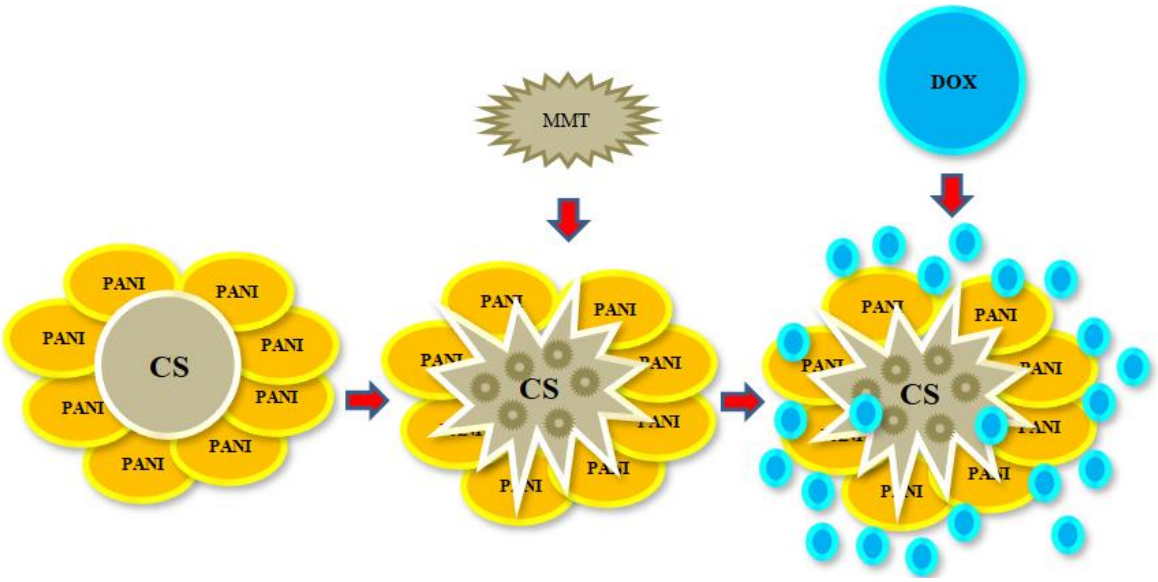

Figure 1: Schematically presented DOX release from chitosan-PANI-MMT.

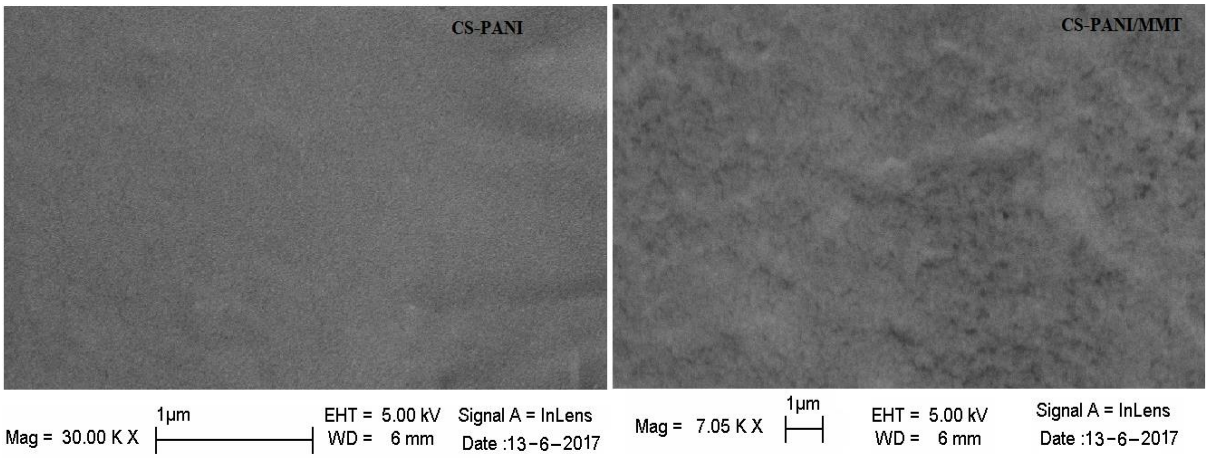

Figure 2: SEM of CS-PANI and CS-PANI/MMT-4.

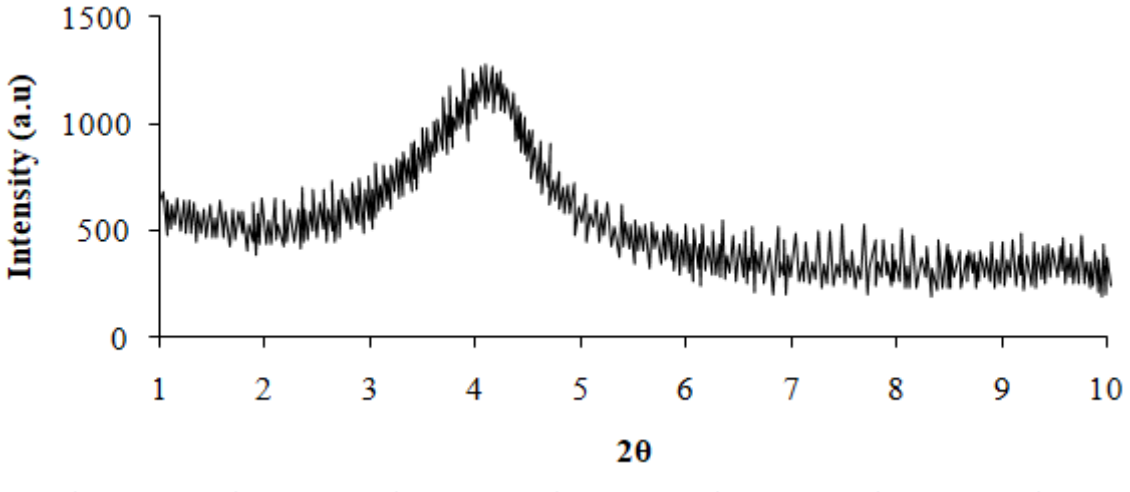

Figure 3:(a) XRD of MMT

These were coded as CS-PANI/MMT-2, CS-PANI/MMT-3, CS-PANI/MMT-4 representing different wt % of MMT content (1%, 5%, 10%) respectively[10,11]. The formulation codes for different films are shown in Table 1and figure-1.

Drug loading

Drug content pre weighed sample of film was placed in 100 ml of phosphate buffer (pH 7.4) and agitated on

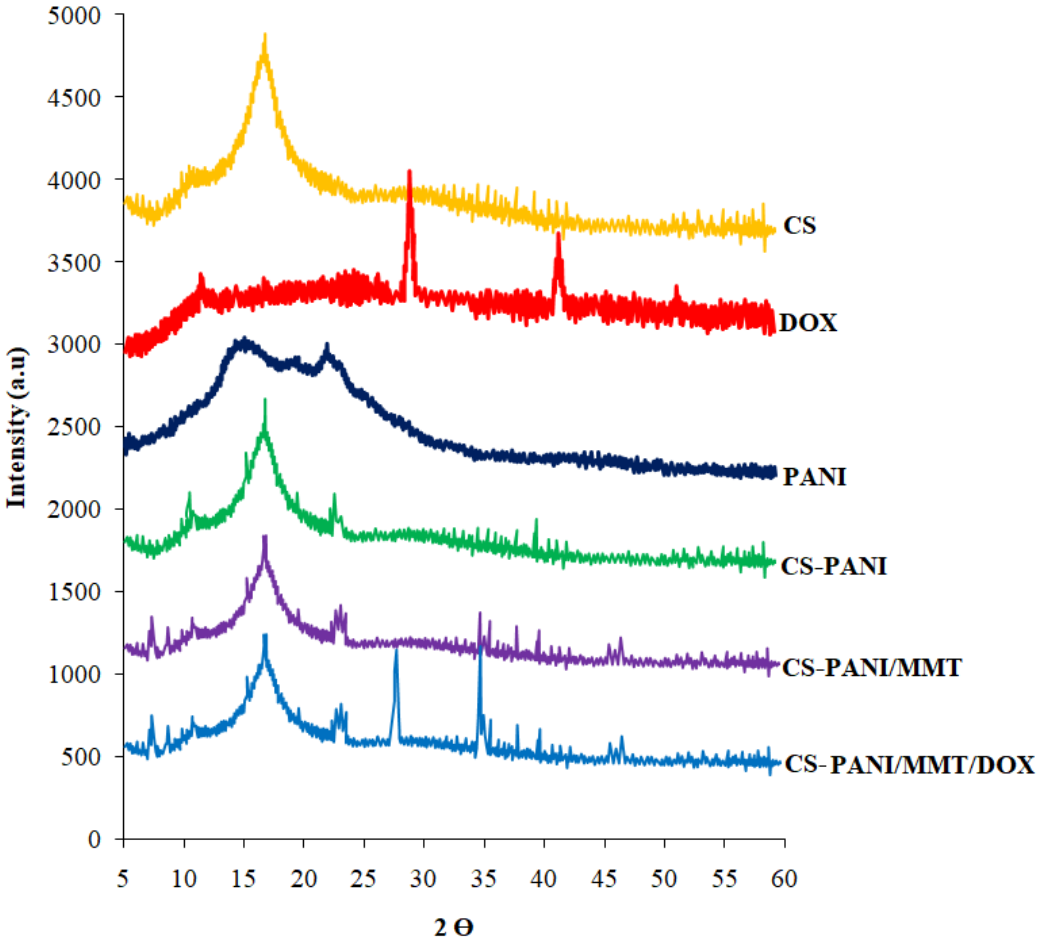

Figure 3: (b) XRD of CS, and DOX, CS-PANI, CS-PANI/MMT-2, CS-PANI/MMT-3, CS-PANI/MMT-4

Table 1: Sample preparation of CS-PANI-MMT nanocomposite.

Sample	CS (in gms)	PANI	MMT (wt%)
CS-PANI-1	2.5	1	-
CS-PANI /MMT-2	2.5	1	1
CS-PANI /MMT-3	2.5	1	5
CS-PANI /MMT-4	2.5	1	10

mechanical shaker for 6 h. Solution was filtered and analyzed spectrophotometrically at 224 nm.

Loading efficiency

$$LE = \frac{W_a}{W_d} X\ 100\%$$

Loading content

$$LC = \frac{W_a}{W_{np}} X\ 100\%$$

Where LC: Loading content;

LE: Loading efficiency,

W_a: quantity of drug found in the drug-loaded nanocomposite,

W_d: quantity of drug beginning added into the system

W_{np}: quantity of drug-loaded nanocomposite.

Characterization

Scanning Electron Microscopy (SEM)

Liquid nitrogen was used for freezing the CS-PANI, and CS-PANI/MMT sheets and then snapped quickly, and then the fractured surface was sputtered with gold and investigated with a Scanning electron microscope instrument (JSM-5900LV) using an acceleration voltage of 20 kV.

X-ray Diffraction (XRD)

D8 Advance Diffractometer (Bruker, U.S.A.) equipped with a CuKα radiation source (λ = 0.154 nm) was utilized for X-ray diffraction. The diffraction data were obtained from 2θ = 1°−80°.

Mechanical Properties

The tensile properties of the sample were incorporated on a universal testing machine (CMT4104, Shenzhen SANS Test Machine Co. Ltd, CIPET, and Odisha) with a tensile rate of 5 mm/min.

Swelling Studies

The swelling study is calculating the extent of water uptake or degree of dehydration. It is using at the time of fabrication of polymer film. It has been shown that most of the mucoadhesive polymers undergo some degree of swelling after hydration, which is necessary to initiate intimate contact of the nanocomposites with the mucosal surface. These studies for monitoring of swelling index of

Table 2: Physical properties of CS-PANI/MMT composite.

Sample	Thickness	Drug content	Weight variation	Moisture content %	Moisture absorption (%)	Folding endurance	Surface pH
CS-PANI-1	0.16±0.11	76.81±0.13	0.28±0.14	2.78±0.13	6.78±0.13	251±1.13	7.4
CS-PANI /MMT-2	0.13±0.16	91.22±0.22	0.32±0.26	3.62±0.23	7.71±0.22	257±1.12	7.4
CS-PANI /MMT-3	0.23±0.38	94.43±0.16	0.43±0.18	2.33±0.17	11.34±0.18	261±1.16	7.4
CS-PANI /MMT-4	0.34±0.43	95.61±0.24	0.51±0.23	2.41±0.22	9.48±0.21	272±1.23	7.4

Table 3: Tensile strength and extensibility of CS-PANI/MMT films.

Sample	Tensile strength N/mm2	Extensibility %
CS	22.12±1.22	20.20±1.76
CS-PANI-1	25.12±1.22	21.43±1.76
CS-PANI /MMT-2	37.23±2.12	13.01±1.40
CS-PANI /MMT-3	28.09±1.04	14.12 ± 0.46
CS-PANI /MMT-4	53.11±1.21	11.22 ± 2.03

the nanocomposites were regulated in the phosphate buffer of pH 7.4. The CS-PANI/MMT nanocomposites (surface area: 1.75 cm^2) was weighed and put in a pre-weighed stainless steel wire sieve of approximately 900 µm mesh. The mesh containing the required quantity of nanocomposites was then submerged in 15 ml of the phosphate buffer medium contained in a porcelain dish. At appropriated time intervals, the stainless steel mesh was removed from the dish, and the excess moisture was removed by carefully clean it off with absorbent tissue, then it was reweighed. The enhancement of weight of the polymer matrix was calculated at each time interval until a constant weight was calculated. The degree of the swelling index of the matrix was analyzed using the following formulation:

$S.I = (w_t - w_0) / w_0$

Where S.I: Swelling Index,

w_t: weight of film at time't'

w_0 : weight of the film at time 0 .

RESULT AND DISCUSSION

SEM

Figure 2 shows SEM of CS-PANI-1 and CS-PANI/MMT-4 at 5000X magnifications. The nanocomposites CS-PANI/MMT-4 were showed a homogenous morphology in comparison with the blending polymer CS-PANI without clay. The CS-PANI/MMT-4 was identified the best nanocomposite polymer. The microstructure obtained smooth homogenous for CS-PANI/MMT-4 comparing with pure CS and CS-PANI. Additionally, the surface morphology of CS-PANI/MMT-4 was demonstrated more exfoliated with homogenous form comparing with other percentages of C30B. This might boost the surface modification of blending polymer nanocomposites[12].

XRD

Figure 3a the X-ray diffraction pattern of the MMT with intense peak appearing near 2θ = 4.85º. In figure 3b the diffraction peaks of CS are located around 10.5°, 19.2°. They are very weak, indicating low crystallinity. Additionally, the diffraction peaks of DOX are located at 2θ =9.12°, 10.34°, 21.13°, 28.13°, 31.10°, 37.23°, 47.12° and 56.10°. This was the high crystalline structure with the strong peak for DOX. However, in the CS-PANI-1 polymer shows two diffraction peaks at 2θ =8.45°, 16.11°, 18.12°, 20.08°, 22.35 and 27.45°. Additionally, when MMT was added with CS-PANI polymer, the diffraction peaks of CS-PANI/MMT-4 is located around $2\theta = 4.67^0$, 8.49°, 16.56°, 20.48°, 22.34°, 28.55° and 37.13°[12,13]. There is negligible difference between in comparison with CS-PANI/MMT-2, CS-PANI/MMT-3. In other hand DOX was added with CS-PANI/MMT-4, three new peaks were observed at 12.11°, 37.21°, 49.32°, indicating crystalline nature.

Mechanical properties

The mechanical strength of a CS-PANI/MMT film was described in terms its tensile strength and percentage of elongation to break that is, extensibility in table 3. A significant influence of the MMT concentration on the mechanical properties of the films was observed. Tensile strength was observed to increase with increase in the MMT content in the films. It was reported in Figure 4 that the tensile strength ranged from 22.12 ± 1.22 N/mm^2 in pure CS film) to 53.11±1.21N/ mm^2 (CS-PANI /MMT-4 film). The enhancement in tensile strength of the nanocomposites might be attributed to the high-aspect ratio and rigidity which results from the strong affinity between the biopolymer and nanoclay. As reported evident from literature, tensile strength values of CS-PANI/MMT nanocomposites increased significantly with increasing nanoclay concentration due to a possible strain-induced alignment of the nanoclay particle layers in the nanocomposites[12] .

The extensibility [Figure 5] ranged from 11.22 ± 2.03 (CS-PANI /MMT-4 film) to 14.12 ± 0.46% (CS-PANI /MMT-3 film), whereas the pure CS film recorded an extensibility of 20.20±1.76%. The effect of MMT on the extensibility shows a significant decrease in elongation to break as the MMT concentration increases. The results were found to be in good agreement with the findings reported by Svoboda *et al*., Hasegawa *et al*. and Bangyekan *et al*.[14-16]

Physical properties

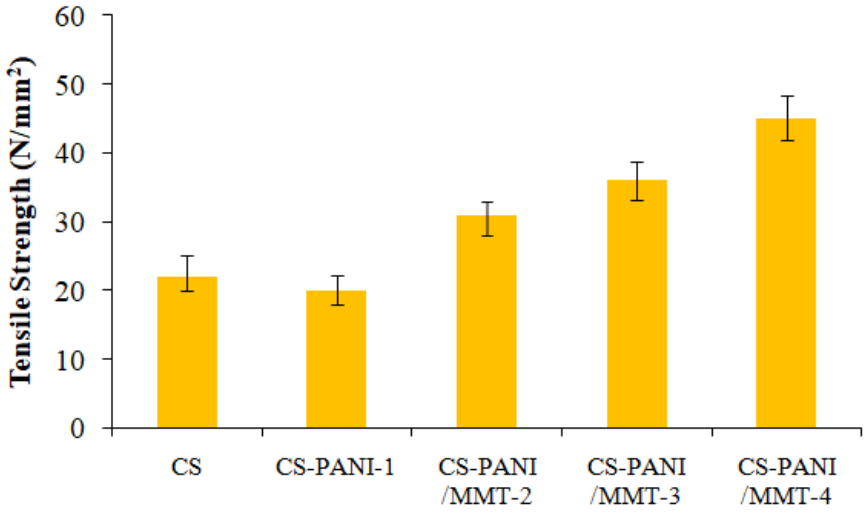

Figure 4: tensile strength of CS, CS-PANI, CS-PANI/MMT-2, CS-PANI/MMT-3, CS-PANI/MMT-4.

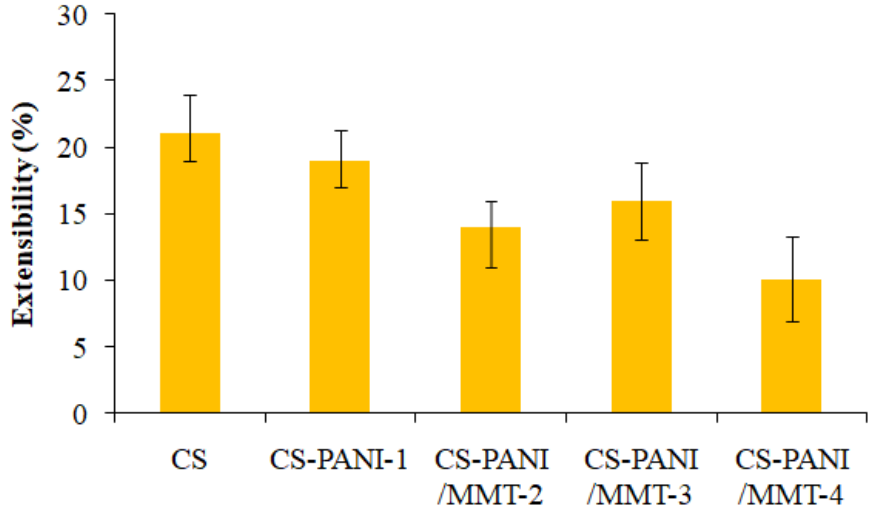

Figure 5: Extensibility of CS-PANI/MMT films.

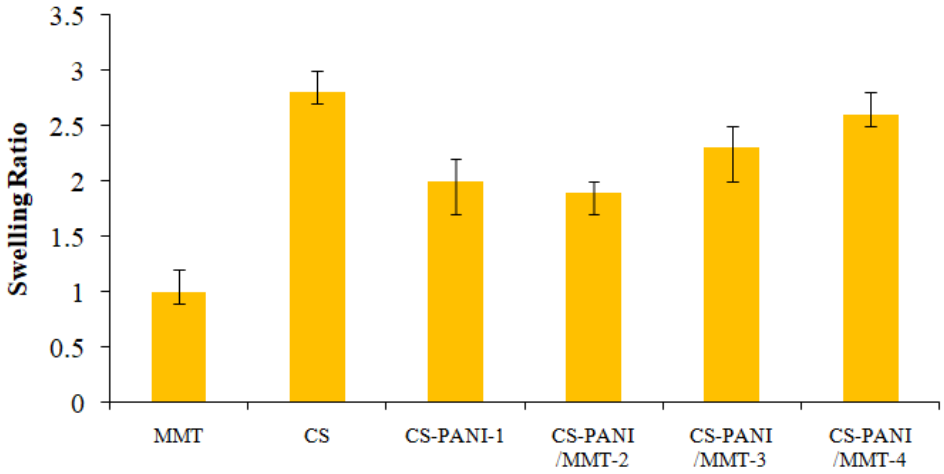

Figure 6: swelling ratio of CS, CS-PANI, CS-PANI/MMT-2, CS-PANI/MMT-3, CS-PANI/MMT-4.

The prepared CS-PANI/MMT were compact, smooth and without pores or imperfections. A synergistic effect of MMT for improving thermal stability, mechanical, and barrier properties of CS has been proposed. The evaluation of CS-PANI/MMT composite films in terms of various physicochemical properties, characterization FTIR and DSC studies and stability testing is discussed here under.

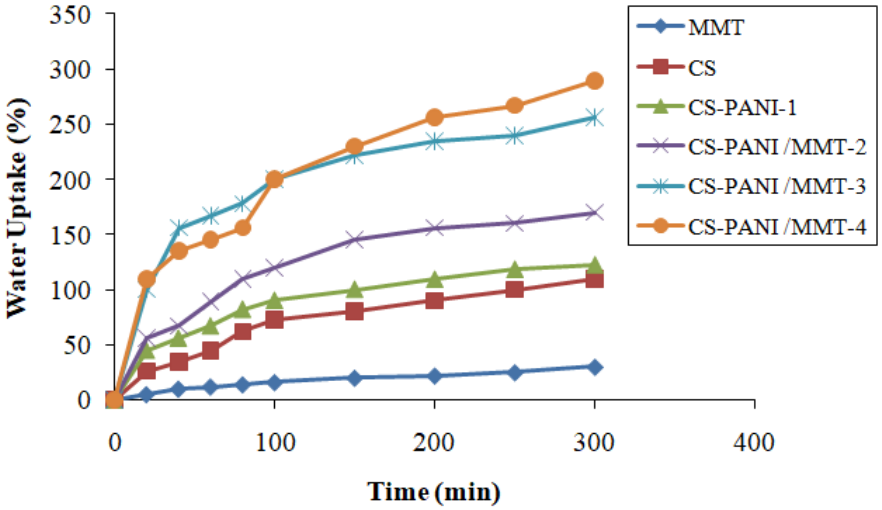

Figure 7: water uptake CS, CS-PANI, CS-PANI/MMT-2, CS-PANI/MMT-3, CS-PANI/MMT-4.

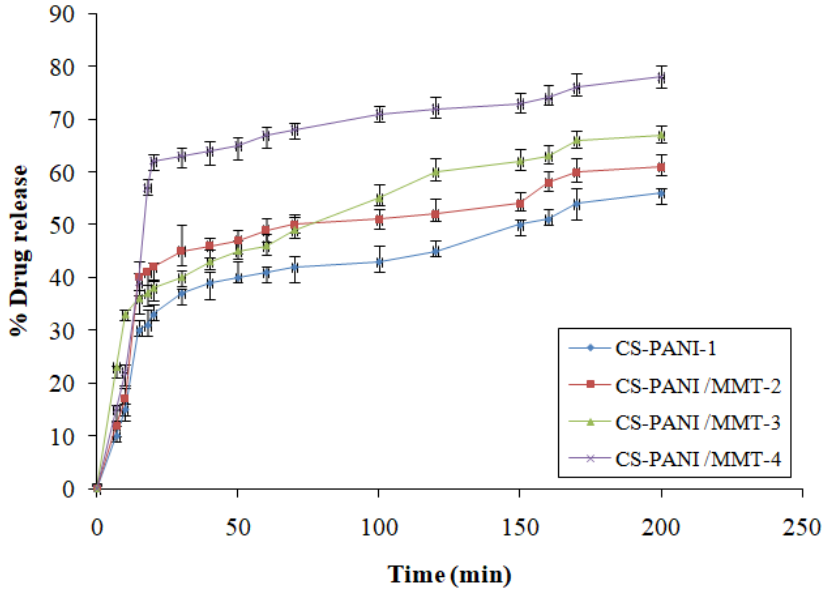

Figure 8: In vitro drug release of CS-PANI/MMT films.

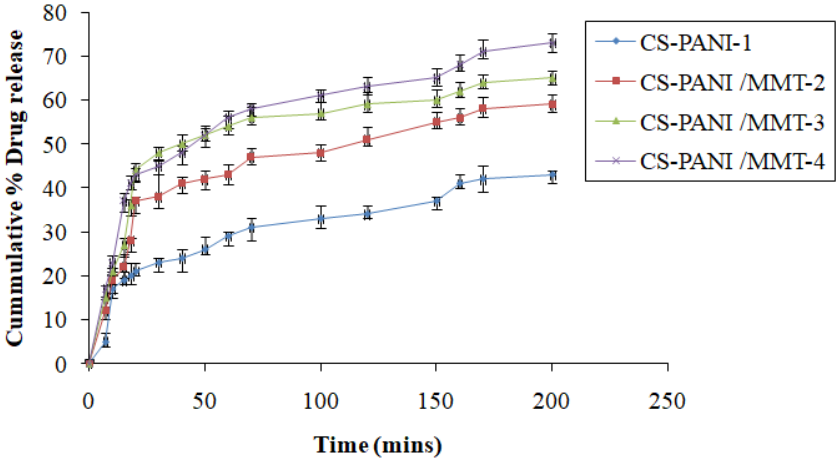

Figure 9: In vitro permeation study of the CS-PANI/MMT films.

Table 2 depicts various physicochemical properties of the prepared CS-PANI/MMT films. The polymeric combination of CS-PANI with MMT exhibited good film forming properties and the method of casting of films was found to produce good films. The thickness of the films vary from 0.16 ± 0.11 to 0.34 ± 0.43 mm while the drug content in the films range from 76.81 ± 0.13% to 95.61 ± 0.24%. The weight of the films ranged from 0.28 ± 0.14 to 0.51 ± 0.23 g. The results indicated that the method selected for the preparation of films was capable of producing films with uniform weight, content and minimal film variability. The folding endurance was found to be ranging between 251 ± 1.13 and 272 ± 1.23 indicating that the formulated films would maintain their integrity when applied to the skin. The pH of the films near about 7.4. Low moisture content is useful for the long term stability of the films. It also reduces the brittleness and protects the formulation from microbial contamination. Moisture content and moisture uptake studies indicated that the increase in the concentration of hydrophilic polymer was directly proportional to the increase in moisture content and moisture uptake of the films, whereas increase in the concentration of hydrophobic polymer lead to the decrease in moisture content and moisture uptake of the films.

Swelling ratio

The swelling ratio studies performed on the films are shown in Figure 6. Similar to the water uptake studies (figure-7), the swelling studies also showed a decrease in the swelling of the films with a decrease in the CS concentration. The swelling ratio was reported to be in the order CS-PANI/MMT 4> CS-PANI/MMT-2> CS-PANI/MMT-3>CS-PANI/MMT-1. The behavior could be explained in terms that the MMT clay particles occupy the free space volume in the CS polymeric network thereby decreasing the volume available for swelling[17].

In vitro release study

The results obtained from the *in vitro* dissolution study of the films are presented in Figure 8. The order of increased drug dissolution using the different approaches was as follows; CS-PANI/MMT-4 > CS-PANI/MMT-3> CS-PANI/MMT-2> CS-PANI-1. It was observed that during the first 60 min of the dissolution study, the CS-PANI/MMT-4 film showed just 78.41 ±0.78% drug release as while the CS-PANI-1, CS-PANI/MMT-2 and CS-PANI/MMT-3 films showed a release of 56.92 ± 1.03%, 61.43 ± 1.34% and 67.23 ± 2.44% respectively. Results of various film tests indicate that among the three samples used for the preparation of CS-PANI/ MMT films, CS-PANI/MMT-4 and CS-PANI/MMT-2 were more effective method than CS-PANI-1 and CS-PANI/MMT-3. This is quite evident from the drug release behavior of the CS-PANI/MMT films after 8 h of dissolution study respectively. CS-PANI/MMT-4 and CS-PANI/MMT-2 showed better sustained release effects. This kind of behaviour is a result of the strong electrostatic interaction between the cationic charges of CS and the anionic charges of MMT[18-20].

In vitro permeation study

Figure 9 presents the results obtained from the in vitro permeation study of the films. The order of increased drug dissolution using the different approaches was as follows; CS-PANI-1> CS-PANI/MMT-2 > CS-PANI/MMT-3> CS-PANI/MMT-4. Similar to the in vitro drug release effects, these results also clearly show that the CS-PANI/MMT-4 show the most sustained release effects as the release of the drug after 8 h of permeation study was 43.63% ± 1.04%, 59.45 ± 4.39%, 65.45 ± 1.39% and 73.03 ± 1.05% for the transdermal films CS/MMT 14, CS/MMT 41 and CS/MMT 11 respectively. This phenomenon confirms that an ionic exchange reaction occurred between CS and MMT and that consequently CS was intercalated into the MMT structure.

Determination of release kinetics

The regression coefficient (*r*2) values of Higuchi equation for CS-PANI/MMT films CS-PANI-1, CS-PANI/MMT-2, CS-PANI/MMT-3 and CS-PANI/MMT-4 were found to be 0.9937, 0.9957, 0.9876 and 0.9881 respectively. To further confirm the mechanism of drug release from the CS/MMT films, the *in vitro* dissolution data were subjected to the Korsmeyer's Peppas equation. The values of the release exponent (*n*) were found to be ranging between 0.45 and 0.61 indicating a nonfickian

Table 4: Release kinetic parameters of films of DOX.

Sample	Zero order		First order		Higuchi		Korsmeyer peppas			Hixon crowell	
	r^2	k_0	r^2	k_0	r^2	k_0	r^2	n	k_0	r^2	k_0
CS-PANI-1	0.8645	0.1123	0.9901	0.0007	0.9937	2.7652	0.9952	0.4311	0.7201	0.9762	0.0021
CS-PANI /MMT-2	0.8781	0.1246	0.9911	0.0007	0.9957	2.8863	0.9945	0.4515	0.7321	0.9730	0.0015
CS-PANI /MMT-3	0.9062	0.1241	0.9721	0.0007	0.9836	2.9732	0.9778	0.5291	0.4428	0.9623	0.0035
CS-PANI /MMT-4	0.9241	0.1043	0.9743	0.0008	0.9844	2.5224	0.9323	0.6273	0.0941	0.9663	0.005

(anomalous) drug release behavior. Due to the swelling ability of the polymer, there is an opening of the pore channels in between the polymer matrix which helps with the diffusion of the drug through the polymer matrix chains, thus, releasing the drug from the films. Table 4 depicts the values of various release kinetics parameters for the films[12,21,23].

CONCLUSION

The prepared nanocomposites were formulated using solvent casting method. CS-PANI/MMT polymer composite were evaluated for physical parametric tests, tensile strength, moisture content, swelling, *in vitro* dissolution studies. The effect of MMT for enhancing mechanical and barrier properties of CS-PANI matrix has been noticed. This could be understood as due to a formation of filler network of MMT within the CS-PANI polymeric chains. The values of tensile strength of CS-PANI/MMT film increased significantly with increasing MMT concentration, while the values of extensibility decreased for high values of MMT concentration. Additionally, doxorubicin loaded CS-PANI/MMT nanocomposite can be of immense importance in the drug delivery. The combination of biodegradable polymeric chains and clay reinforcement can be applied to achieve the desired combination of properties (mechanical, swelling and controlled release) of materials used as a biosensor for diverse biomedical applications.

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