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Original Research Article

Application and evaluation of layered silicate–chitosan composites for site specific delivery of diclofenac

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ABSTRACT

The present study focuses on the *in situ* intercalation of anionic drug (diclofenac sodium, DS) and cationic polymer, Chitosan (CS) in montmorillonite (MMT) for drug release applications. The prepared DS/CS-MMT composites were further compounded with alginate (AL) to form beads to modify release response in gastric juice. The DS/CS-MMT composites were characterized by UV spectroscopy, XRD, FT-IR, TGA and DSC. Antibacterial assay of drug loaded composites was investigated and *in vitro* cell viability assay results point out the drug encapsulated in clay plates are less toxic to the cell than pristine drug. The *in vitro* release experiments revealed that the DS was released from DS/CS-MMT/AL in a controlled and pH dependent manner.

1. Introduction

Nowadays, the interdisciplinary nature of the biopolymer composite materials and its application in the medical science arena brings together scientists, technologies and medical specialists from fundamental science, applied chemistry, biology, physics, materials and biomedical engineering. Biopolymer–clay composites have potential to develop critical formulation that can be extended for biomedical applications, varying from diagnostic tools and medical devices, tissue engineering and controlled drug delivery matrixes to numerous biomedical technologies inspired by fundamental biology and applied biomedical applications [1].

Recently, preparation and application of biopolymer/layered silicate material composites as controlled drug delivery vehicles and biomedical engineering have been attracting much attention owing to their unique structure and functional properties. Layered silicate materials, *e.g.* Smectite clays (laponite, saponite and montmorillonite) have been used for preparing for this class of composites. The synergistic effect of biopolymer and layered silicate material as well as the strong interfacial interactions between them by electrostatic interaction and hydrogen bonding could improve

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the mechanical properties, swelling behavior, drug loading efficiency and controlled release behavior of the pristine biopolymer matrices. In summation, these properties could be further tailored by changing the character and capacity of layered silicate materials. The chitosan/montmorillonite composites were demonstrated to exhibit excellent anti-fatigue behavior and better pulsatile drug release compared with neat chitosan [2]. Wang et al., studied pH-sensitive chitosan-g-poly (acrylic acid)/vermiculite/sodium alginate (CTS-g-PAA/VMT/SA) hydrogel beads. The authors reported that the release rate of drug from the composite hydrogel beads was remarkably slowed down due to presence of vermiculite [2,3]. The construction of hybrid poly(lactic-co-glycolic acid)/montmorillonite could significantly cut the initial burst release of paclitaxel [4,5].

Montmorillonite (MMT) is an ideal material for the formulation of drug delivery vehicle because of its excellent properties, such as the ability to adsorb dietary toxins, bacterial toxins associated with gastrointestinal disturbances, hydrogen ions in acidosis and metabolic toxins such as steroid metabolites associated with pregnancy [4]. Nevertheless, the release of drugs from MMT has been tested to be initially very fast, owing to the weak interaction between the drugs and the MMT particles [6,7]. The compounding of polymer and MMT seems to be a viable means to sustain the release of drugs and to make polymer/MMT composites applications as long-term controlled drug release carriers [8–10]. Diclofenac sodium (DS), [2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid] is a non-steroidal anti-inflammatory drug and one of the best commonly used NSAIDS and its short half-life of 1–2 h demands preparation of a controlled release formulation. In order to prolong the circulation time of DS and increase its efficacy, numerous researchers have attempted to modify its delivery by use of polymer conjugates or by incorporation of the DS into particulate carriers [11–13]. The ultimate aim of these strategies is to reduce DS associated side effects and thereby improve its therapeutic index.

Herein we focused on the layered aluminosilicate clay, montmorillonite (MMT)/chitosan (CS) composites modified with alginate (AL) as delivery systems of diclofenac sodium. CS-MMT and DS/CS-MMT composite hydrogels were prepared under optimal reaction conditions by ion-exchange and gelation techniques and characterized. The drug loaded composites were evaluated for *in vitro* release characteristics in simulated gastric juice and phosphate buffer. In present study, experiments were designed to assess the effect of DS/CS-MMT on viability of A549 (human lung adenocarcinoma epithelial cell line) along with antibacterial activities.

2. Materials and methods

2.1. Materials

Diclofenac sodium salt, alginic acid sodium salt (Viscosity: 20.0–40.0 cP in 1% water, Molecular weight: 7334 Da, according to manufacturer), chitosan, medium molecular weight (Viscosity: 200 cPs in 1% glacial acetic acid, deacetylation degree (DD) 80%, Avg. Molecular weight: 8401 Da, according to manufacturer) and cellulose acetate dialysis tube (Cutoff molecular weight at 7000 Da) were acquired from Sigma–Aldrich, USA. RPMI-1640 (Roswell Park Memorial Institute 1640), Trypan blue, MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide), 0.25% trypsin and 0.02% EDTA mixture, streptomycin, penicillin, amphotericin and DMSO were procured from Himedia laboratory, Mumbai, India. FBS (fetal bovine serum)? were procured from Invitrogen, UK. All other reagents were of analytical grade and used as received. The MMT rich bentonite clay was collected from Akli mines, Barmer district, Rajasthan, India and was purified by reported procedure [6,14]. The bacterial culture of *Staphylococcus aureas* NCIM 2079 was obtained from the National Collection of Industrial Microorganisms, NCL, Pune, India.

2.2. Preparation of the chitosan/layered silicate composites

The 2% (w/v) MMT suspension was prepared by dispersing MMT in Milli-Q water for 24 h followed by 1 h sonication. The 0.5% (w/v) CS was obtained by dispersing CS in 1% (v/v) glacial acetic acid with deionized water under constant 6 h stirring for homogeneous solution. Then, pH of the CS, DS and MMT solutions was adjusted by 1N NaOH to 4. Finally, appropriate quantity of DS, CS solution and MMT suspension were mixed and stirred for 48 h. The drug loaded composites were obtained by centrifuging the suspension at 10,000 RPM for 30 min, 20 °C (Kubota-6500, Kubota Corporation, Japan) and the composite pellets were dispersed in Milli-Q water. This procedure was repeated till the composite pellets were free from non-intercalated CS and DS. The pellet was dried at 60 °C to collect the DS/CS-MMT composites by grinding and subsequent 200 mesh filtering. The DS concentrations were determined by UV-Visible spectroscopy (Shimadzu. UV-2550, Japan) at λ_{max} = 274 nm equipped with a quartz cell having a path length of 1 cm. The CS:MMT weight ratio of 0.5:1, 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1 and 4:1 were examined. Finally, CS:MMT weight ratio of 3:1 was selected for further studies for drug loading based on UV absorbance, XRD analysis, thermal analysis and FT-IR. All intercalation studies were performed in triplicates and the average values were utilized for data analysis.

2.3. Influence of physico-chemical parameters on drug intercalation

2.3.1. Influence of pH

30 ml of CS (0.5%, w/v) from stock solution was gradually added to 100 ml conical flasks containing solutions of DS (50 mg). The DS/CS solutions were treated with 2.5 ml (2%, w/v) of MMT (50 mg) suspension while being sonicated. The pH was adjusted from 2 to 5.5 by HCl and NaOH solutions and final volume was adjusted to 50 ml with milli-Q water. All experiments were performed with continuous shaking (Julabo shaking water bath, SW23) at 50 °C for 48 h. The washing procedure was followed as previously described. The remaining concentrations of DS in the filtrates were measured by UV absorbance.

2.3.2. Initial drug loading concentration

30 ml of CS (0.5%, w/v, 150 mg) from stock solution was gradually added in 100 ml conical flasks containing different concentrations of DS (5–200 mg). The DS/CS solutions were

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treated with 2.5 ml (2%, w/v) of MMT (50 mg) suspension while being sonicated and final volume was adjusted to 50 ml with Milli-Q water. The pH of the suspension was kept at 4 and all experiments were performed with continuous shaking (Julabo shaking water bath, SW23) at 50 °C for 48 h. The washing procedure was followed as described previously. The remaining concentrations of DS in the filtrates were measured by UV absorbance.

2.3.3. *Preparation of DS/CS-MMT/AL composite beads*

The DS/CS–MMT composite was further compounded with AL in bead form. The appropriate amount of AL (1.0 g) was dissolved in deionized water (50 ml) and stirred for 8 h to obtain a homogeneous solution. The required quantity of calcium chloride dihydrate was dissolved in deionized water to prepare 100 mmol solutions. DS/CS-MMT/AL beads were prepared by the means of gelation technique as per our previous reports [6,7]. Appropriate amount of DS loaded CS-MMT (0.7 g) was added to the AL solution and stirred for 5 h to obtain a homogeneous suspension. The resulting solution was then slowly added to the 200 ml calcium chloride solution by dropping it from the tip of a 20-gauge hypodermic needle (falling distance 2 cm, pumping rate 2.5 ml/min) attached to a peristaltic pump (Master flex L/S 7518-00, Cole-Parmer, USA). In this approach the spherical shape of the drop was retained by the gelled suspension. The beads were allowed to cure in calcium chloride solution for 20 min and then separated by filtration. The prepared beads were washed thrice with deionized water, and dried at room temperature. The DS/CS-MMT composites: alginate ratio (1:1.4%, w/w) were optimized to obtain stable beads, having less amount of alginate and controlled release profile.

2.4. *Characterization*

X-ray diffraction (XRD) analysis was carried out on Phillips powder diffractometer X' Pert MPD using PW3123/00 curved Ni-filtered Cu Kα radiation with a scanning of 0.3°/min 2θ range of 2–10°. Fourier transform infrared (FT-IR) spectra were recorded on Perkin-Elmer, GX-FTIR as KBr pellet over the wavelength range 4000–400 cm^{-1}. The particle size distribution and zeta potential were measured by zeta seizer (Zeta Sizer-Nano-ZS90, Nano Series, Malvern instruments Ltd., Malvern, UK), based on the dynamic light scattering technique (DLS). Thermo gravimetric analysis was carried out within 50–800 °C at the heating rate 10 °C/min under nitrogen flow (20 ml/min) using TGA/SDTA 851e, Mettler-Toledo, Switzerland. The differential scanning calorimetric (DSC) was measured in the range of 30–400 °C at the rate 10 °C/min under nitrogen flow (10 ml/min) using Mettler-Toledo, DSC-822e, Switzerland. UV–vis absorbance of DS solutions were measured at λ_{max} = 274 nm using UV–vis spectrophotometer, UV 2550 (Shimadzu, Japan), equipped with a quartz cell having a path length of 1 cm.

2.5. *Antibacterial activity of drug/biopolymer/clay composites*

The antimicrobial activity of MMT, CS-MMT and DS/CS-MMT composites were tested qualitatively by measuring zone of inhibition on agar plates. The bacteria, *S. aureus* was subcultured on nutrient agar and incubated overnight at 37 °C. Then, the cells were dispersed in the same medium to reach the cell density of 10^6 CFU/ml. The bacterial suspensions were spread on agar plates with a sterile glass spreader. The MMT, CS-MMT and DS/CS-MMT (15 mg) were suspended in sterile water and loaded into wells (12 mm diameter) on agar plates and incubated. All the test plates were incubated overnight at 37 °C. The inhibitory response of the bacterial cells to CS-MMT was determined by the size (diameter in mm) of the zone of inhibition. When the materials have an excellent antibacterial activity, the inhibitory zones are large.

2.6. *Cell cultures*

A549 (Human lung adenocarcinoma epithelial cell line) were obtained from the National Repository of Animal Cell Culture, National Centre for Cell Sciences (NCCS), Pune, India. A549 cell line was cultured in 25 cm^2 tissue culture flasks maintained at 37 °C in a humidified environment of 5% CO_2 and were grown in RPMI-1640 with 10% FBS, Streptomycin (1000 U/ml)–penicillin (100 μg/ml)–amphotericin (0.25 μg/ml) mixture replenished every three days.

2.6.1. *In vitro cytotoxicity assay*

The viability of cancer cells upon treatment with DS, DS/CS-MMT and DS/CS-MMT/AL composites was evaluated by the MTT assay. 150 μl of A549 cells was seeded in 96-well plates (Becton Dickinson (BD), USA) at the density of 1.1×10^4 viable cells/well and incubated 24 h to allow cell attachment. Following attachment, the medium was replaced with complete medium (150 μl/well) containing DS, DS/CS-MMT and DS/CS-MMT/AL composites at equivalent drug concentrations ranging from 0.1 to 100 μg/ml for 72 h. Following treatment, the cells were washed with PBS and incubated with 100 μl/well fresh medium containing 0.5 mg/ml MTT. The MTT containing medium was removed after 3 h incubation in dark condition. The MTT formazan was dissolved in 100 μl/well DMSO and the optical density was determined at 570 nm using an ELISA plate reader (Bio-Tek, USA). Cell viability was calculated by the following equation:

$$\text{Cell viability}\ (\%) = \left(\frac{A_s}{A_{control}}\right) \times 100 \quad (1)$$

where A_s was the absorbance of the cells incubated with the DS, DS/CS-MMT and DS/CS-MMT/AL composites and $A_{control}$ was absorbance of the cells incubated with culture medium alone. IC_{50}, the drug concentration at which inhibition of 50% cell growth was observed compared to control was calculated by fitting of the cell viability curve.

2.7. In vitro *drug release*

In vitro release of DS was carried out in USP eight stage dissolution rate test apparatus (Veego, India) using the dialysis bag technique [6,7,14]. Buffer solutions of pH 1.2, pH 6.8 and pH 7.4 were used as dissolution medium. In brief, precisely weighed amounts of DS/CS-MMT and DS/CS-MMT/AL beads dispersed in 5 ml release medium were placed in a standard grade activated cellulose dialysis tubes. Then, the closed tubes

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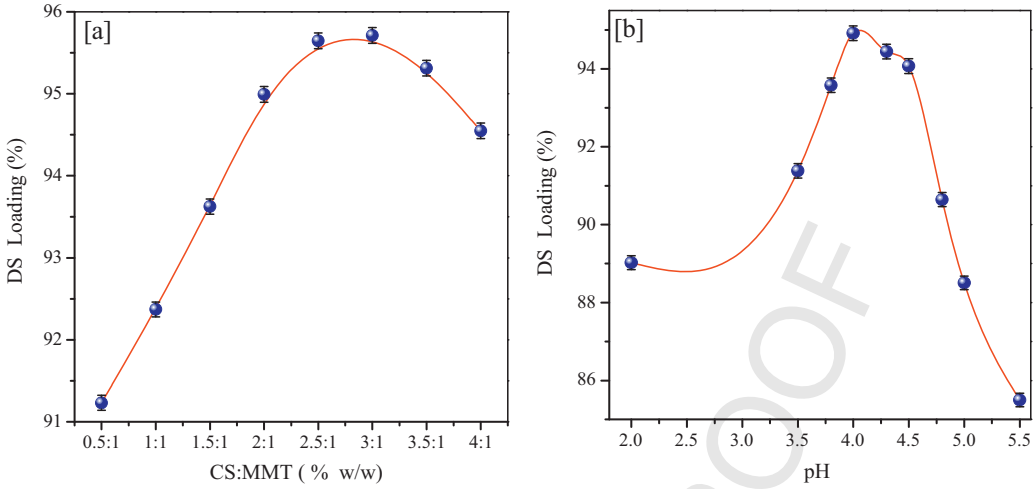

Fig. 1 – [a] Percent drug loading at different CS: MMT (% w/w) ratio: [b] Influence of pH on the intercalation of CS/DS in the gallery of MMT.

were set into a basket and immersed into 500 ml release medium. The temperature was maintained at 37 ± 0.5 °C and rotation frequency kept at 100 rpm. Aliquots (5 ml) were withdrawn at the predetermined time and were replenished immediately with the same volume of the fresh medium. The aliquots, followed by suitable dilution, were assayed spectrophotometrically at λ_{max} = 274 nm. These studies were performed in triplicates for each sample and the average values were used in the data analysis.

3. Result and discussion

3.1. *Intercalation chemistry of DS and CS in interlayer gallery of MMT*

The successful intercalation of DS and CS in MMT was carried out under optimized reaction conditions, *e.g.* biopolymer to clay ratio, pH of the reaction and the initial concentration of drug. The cationic nature of CS and the anionic nature of the DS makes these molecules exceptional candidates for intercalation in MMT by means of ion exchange and chemical interactions between DS/CS and MMT. Depending on the CS concentration added to the MMT suspension, CS chains arrange in monolayer or bilayer configurations between the inorganic layers. In this bilayer arrangement, the excess protonated amino groups that do not interact electrostatically with the MMT layer and is available as anion-exchange sites for DS. Therefore, the cation exchange behavior of the clay was turned into anion-exchange ability [15]. The maximum loading of DS in CS-MMT bilayer were achieved by optimized CS to MMT 3:1 (w/w %) ratio (Fig. 1a). In our results, the characteristic XRD peak of pristine MMT (0 0 1) was shifted toward lower 2ø angle suggesting more space available in the interlayer of CS-MMT for drug loading into composites (Fig. 3).

Fig. 1b illustrates the effect of pH on the intercalation of DS/CS in MMT. A significant increase of DS intercalation was observed when the pH value was in the range 3.5–4. The intercalation attained a plateau at pH = 4 with the highest *d*-spacing of interlayer of CS-MMT, which is reported to be high especially under acidic conditions [9]. The ultimate pH values of CS and DS solutions were adjusted to pH = 4 before adding it to the MMT suspension in order to attain maximum DS intercalation. This pH value is essential to provide $-NH_3^+$ groups in the CS structure to interact with DS. It is specified that the pK_a of the primary amine groups in the CS structure is 6.3 [9,16] and 4.0 in DS [17], with most of the amine groups protonated at final pH value (pH ~ 4.0) of the CS. In such conditions, DS adsorption process was mainly controlled by an ion-exchange mechanism due to the Coulombic interactions between the positive-NH_3^+ groups of the biopolymer and the negative sites in the clay and drug structure [9].

Fig. 2 shows the amount of DS/CS intercalated into the interlayer gallery of MMT at different concentrations of DS. The increasing concentration of DS in the solution increased

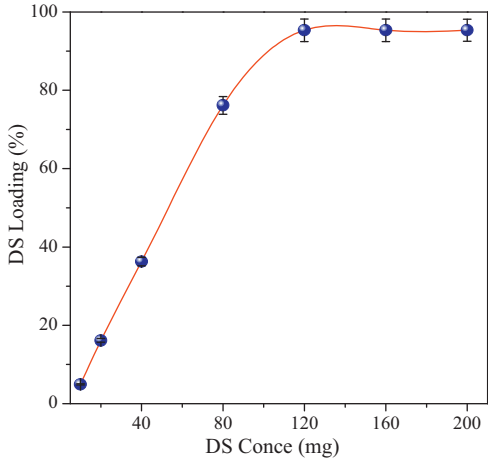

Fig. 2 – Effect of drug concentration gradients on intercalation in the gallery of MMT (Optimal reaction conditions; CS:MMT (% w/w) 3:1, pH = 4, temperature = 50 °C, time = 48 h).

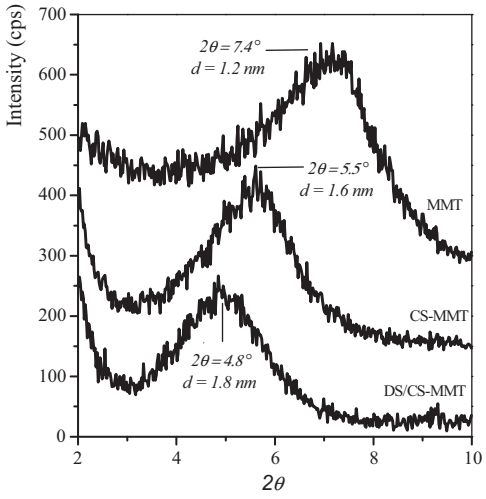

Fig. 3 – XRD pattern of MMT, CS-MMT and DS/CS-MMT composites.

the intercalation rate of DS in the initial phase. However, it reached equilibrium after intercalation of ~95 mg of DS/100 mg of MMT. This functional composite possessed excellent biochemical properties that facilitated its application in the construction of a controlled delivery system for DS.

3.2. *Characterization of DS loaded CS-MMT composites*

3.2.1. *XRD analysis of DS and CS in MMT*

Fig. 3 shows the XRD patterns of pristine MMT, CS-MMT and DS/CS-MMT composites. The intercalation of the DS and biopolymer in the silicate galleries was confirmed by the decrease of 2θ values while the level of intercalation increased. Pristine MMT exhibited $2\theta = 7.4^{\circ}$ and the d_L value (the interlayer distance) was 1.20 nm. In comparison with pristine MMT, the $d_{0\,0\,1}$ peak of CS-MMT shifted toward the lower angle ($2\theta = 5.5^{\circ}$) and the d_L value was 1.60 nm. This observation confirmed that CS had intercalated into the interlayer of unmodified MMT. In case of DS/CS-MMT composites, d_L values of 1.80 nm ($2\theta = 4.8^{\circ}$) nm were observed. The increase in the basal spacing could be explained as uptake of two layers of CS chains and a monolayer of DS molecules by the MMT (sandwich cargo of CS/DS/CS formation). This was further confirmed by tacking vertical dimensions of the DS and CS chain unit which were ~0.71 nm and ~0.43 nm, respectively (Accelreys MS Modeling 3.2, Supplementary data, Fig. S1a and b). By subtracting the assumed thickness of the elemental layers of the silicate (0.96 nm), the CS/DS/CS cargo expanded the interlayer space by ~0.84 nm and ~0.64 nm, respectively, which corresponded to the vertical orientation of the DS molecule and CS chain. The ratio of CS:MMT ratios probably controlled the intercalation of DS as a monolayer or CS as a bilayer in the sandwich cargo formation (Supplementary data, Fig. S2).

3.2.2. *Thermal analysis of composites*

Fig. 4 illustrates the TGA and DTA pattern of dried MMT, DS, CS-MMT and DS/CS-MMT composites. From 50 °C to 800 °C, the plain DS and the CS exhibited about 47.11% (temperature range between, 300 and 730 °C) and 68% (290 and 720 °C) weight loss, respectively. In comparison, only 15% weight loss was observed for dried MMT in the same temperature range. The major weight loss patterns were observed in the temperature range of 80–100 °C and 600–750 °C [6,7,14]. In CS-MMT composites weight loss between 160 °C and ~650 °C was related to adsorbed water molecules and the losses were slightly higher than MMT (28.8%) which indicated higher water-retention capacity of CS. The high thermal stability of CS-MMT composites was evidenced by the elevated temperature required to eliminate the organic matter associated with MMT. This event occurred between 290 °C and 720 °C, corresponding to combustion of the intercalated CS. The content of CS in the CS-MMT was ~14 mass%. The total 42.6% weight loss of DS/CS-MMT composites were observed in three steps at 160 °C, 310 °C and 490 °C. The maximum weight loss percentages for DS/CS-MMT were observed at 310 °C (weight loss 30%) due to DS loading. These results were in agreement with XRD data, revealing intercalation of DS and CS in the MMT

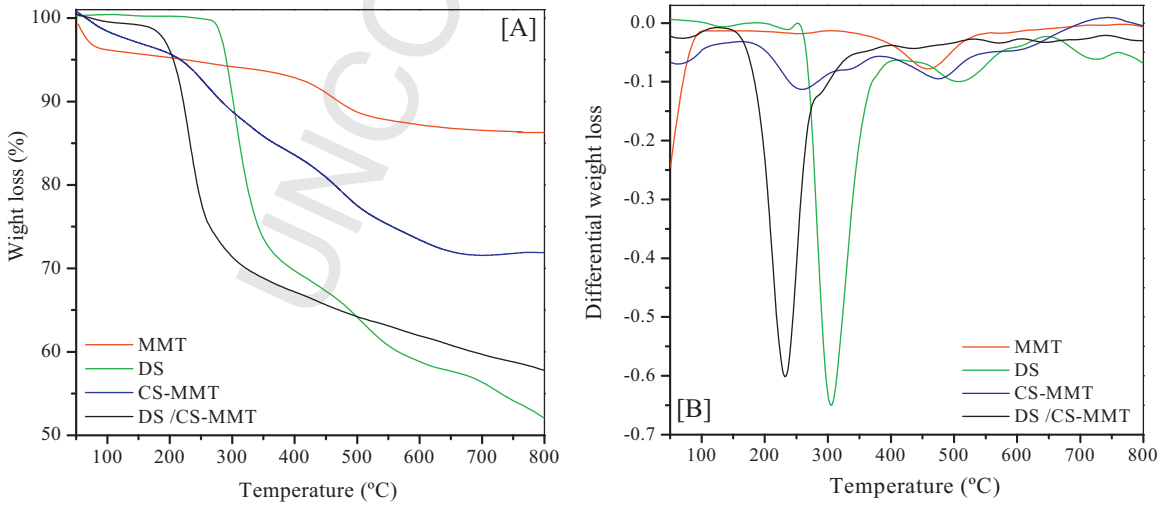

Fig. 4 – [A] TGA and [B] DTA pattern of the formulations and its components.

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interlayer gallery (Supplementary data, FT-IR, Particle size and DSC analysis).

3.3. *Antibacterial assays*

The results of the antibacterial assay of MMT, CS-MMT and the DS/CS-MMT are presented in Fig. 5. The well loaded with MMT had marginal inhibitory zone, indicating lack of significant antibacterial activity against *S. aureus*. However, DS/CS-MMT composites had clear inhibitory zones around the wells. The diameter of the zone of inhibition and the amount of diffusion from the edge of each hole in the agar plate were measured in mm. The zone of inhibition of DS/CS-MMT against *S. aureus* was ~10 mm. The results indicated that DS/CS-MMT had stronger antibacterial activity against the Gram-positive test bacteria. The antibacterial assays indicated that all treated MMTs inhibited growth of test bacteria. This was a sign of diffusion of the DS or CS from treated MMT into the agar. The mechanism of the antibacterial activity of DS or CS could be: (1) adsorption onto the bacterial cell surface; (2) diffusion through the cell wall; (3) binding to the cytoplasmic membrane; (4) disruption of the cytoplasmic membrane; (5) release of the cytoplasmic constituents; and (6) death of the cell.

3.4. In vitro *cell viability*

Fig. 6 was shown *in vitro* viability of A549 cells treated with DS, DS/CS-MMT and DS/CS-MMT/AL at the different concentrations after 72 h culture incubation. The DS showed concentration dependent reduction in cell viability, the degree of cell viability decreased with elevated concentration, which was significantly higher than the cells treated with formulating composites. *In vitro* therapeutic effect of formulating composites was quantitatively evaluated by IC_{50} at a given time period of cell culture, which was defined as the drug concentration at which 50% cells were killed in a given time period. The IC_{50} values of DS, DS/CS-MMT and DS/CS-MMT/AL for A549 cells were ~7.3 μg/ml, ~13.5 μg/ml and 255.5 μg/ml, respectively, which were obtained by interpretation of the data shown in Fig. 6 which shows that DS/CS-MMT hybrid has a smaller amount of IC_{50} compare to DS/CS-MMT/AL. It was due to higher entrapment of DS in CS-MMT and formation of free clay clusters on the cell surface while, DS/CS-MMT/AL was less toxic compared to pristine DS. Therefore, A549 cells were considered to be moderately sensitive to DS/CS-MMT. However, results advocated that CS-MMT/AL could avoid toxic effects of drug.

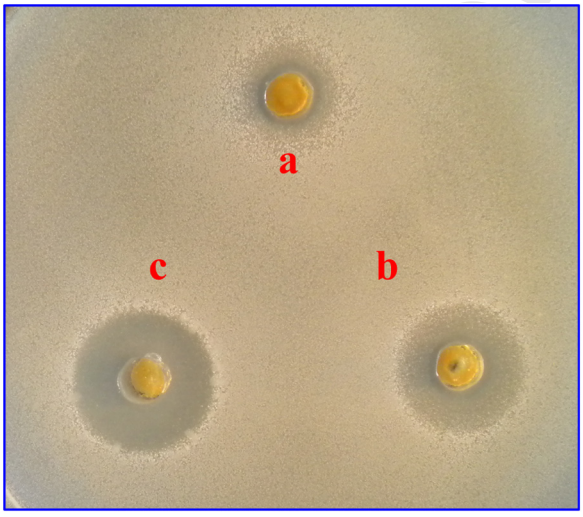

Fig. 5 – Antimicrobial assay of (a) MMT, (b) CS-MMT, and (c) DS/CS-MMT against *S. aureus*.

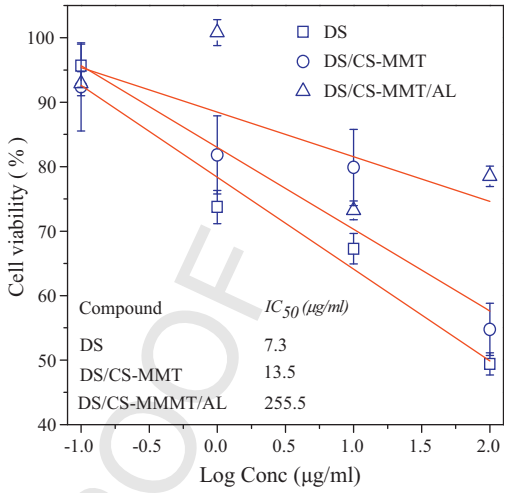

Fig. 6 – [A] *In vitro* viability of A549 (human lung adenocarcinoma epithelial cell line) cancer cells after 72 h treatment with DS, DS/CS-MMT and DS/CS-MMT/AL, at 0.1–100 μg/ml of DS, respectively, Linear regression fitting cell viability assay data with IC_{50} values; data represent mean ± SD (n = 6).

3.5. In vitro *drug release*

In vitro drug release profiles of DS/CS-MMT and DS/CS-MMT/AL composites in buffer solutions at three different pH values of 1.2, 6.8 and 7.4 was obtained at physiological temperature of 37 ± 0.5 °C, by dialysis bag technique (Fig. 7). Approximately 3.3% of the intercalated DS was released within 30 h from DS/CS-MMT composites, while formulations modified using AL significantly reduced DS release in the gastric environment, Fig. 7[A]. 2.2% DS was released from DS/CS-MMT/AL composite beads in 30 h. The negligible DS release from AL composites as compared to DS/CS-MMT composites in the gastric fluids was due to the fact that acidic medium rapidly changes AL to water insoluble alginic acid, consequently blockeing DS release in the media [4,5].

When DS intercalated between layers of MMT is surrounded by an intestinal environment at pH 6.8 and 7.4, the interlayer region of this lamellar host may be considered a micro vessel from which an anionic drug, previously immobilized, is released as a consequence of a de-intercalation process. This CS-MMT composites could be used as a matrix for a new controlled release formulation. In pH 6.8 buffer, release of DS from DS/CS-MMT composite was ~15% in 10 h, ~27% in the first 30 h and ~55% within 60 h, which remained constant up to 72 h. While, in the case of DS/CS-MMT/AL

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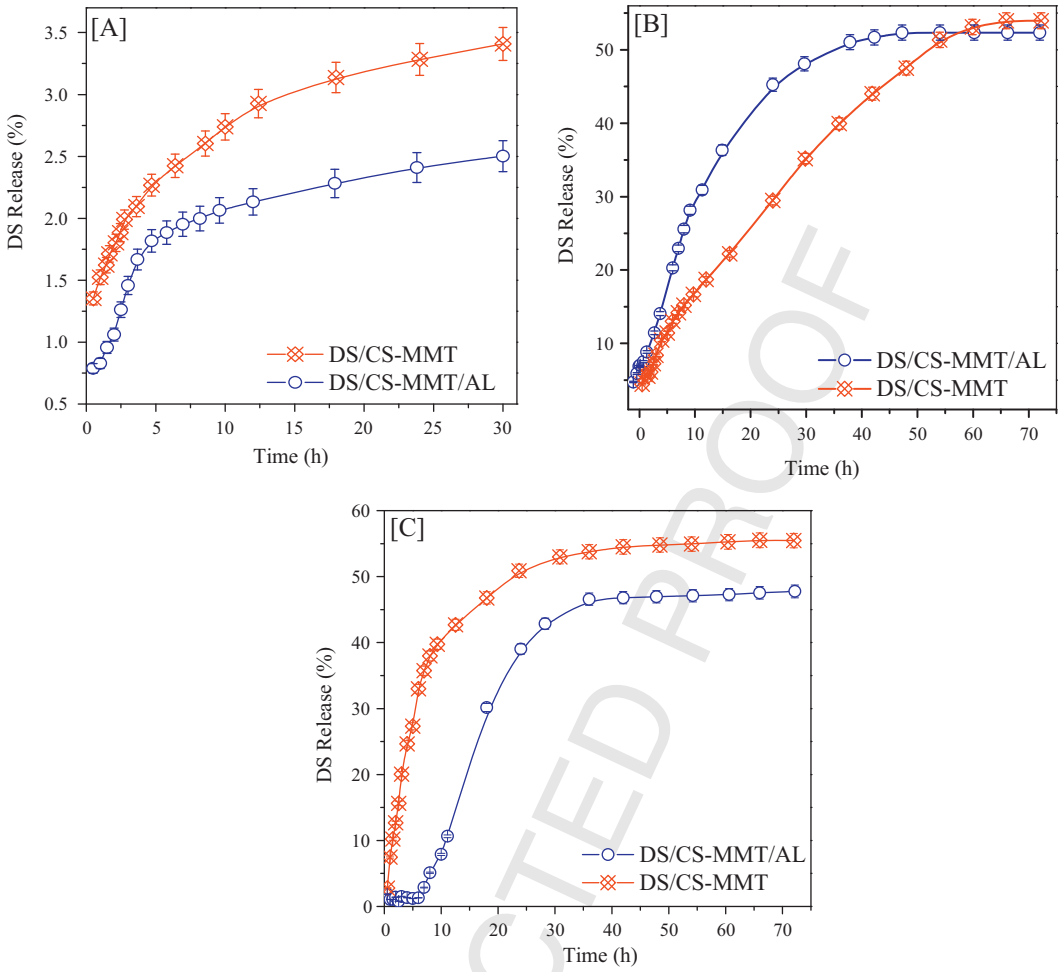

Fig. 7 – *In vitro* release profiles of DS at pH 1.2 [A], pH 6.8 [B], and pH 7.4 [C] at 37 ± 0.5 °C.

composite beads, ~22% and ~46% of DS was released in 10 h and 30 h, respectively. The maximum amount of DS released was ~52% up to 72 h, Fig. 7[B].

In pH 7.4 buffer, release of DS from DS/CS-MMT composite was ~39% in 10 h and ~55% within 30 h which remained steady up to 72 h. While, in the case of DS/CS-MMT/AL composite beads, release of DS was ~6% in 10 h and ~37% in 30 h. The highest amount of DS released was ~42% up to 72 h Fig. 7[B]. The percentage cumulative drug release followed the sequence CS-MMT > CS-MMT/AL at examined pH values. These results indicated that the release rate was slightly faster in DS/CS-MMT composites as compared to DS/CS-MMT/AL composite beads. The release pattern of DS reached plateau more rapidly in the case of DS/CS-MMT composites compared to the DS/CS-MMT/AL composites. The degree of swelling and disintegration of AL increased in the pH 7.4, resulting in slow and controlled release of DS from DS/CS-MMT/AL composites. Moreover, AL was able to retard DS release from DS/CS-MMT composite. Therefore, compounding of DS/CS-MMT composite with AL seemed to have desirable effect to achieve site-specific delivery of the DS. The presence of CS/AL in the composite carrier stabilized the attractive force between DS and the MMT. This was confirmed by slow release of the drug due to presence of CS/AL in the composite carriers. Thus, release of DS from the CS-MMT/AL carrier was slower than CS-MMT at all pH values.

Moreover, the presence of CS/AL in the composite may result in mucoadhesion promoting bioavailability of the drug by interacting with the gastric and intestinal mucosa. Thus, increasing the CS/AL content of the CS-MMT composites could reduce the release rate. The release of drug from the composites could be tuned by controlling the amount of CS/AL in the composites or beads. This implies that a higher cumulative amount of DS would be released at pH 6.8 and pH 7.4 compared with pH 1.2. Furthermore, the continued and higher release of drug for >72 h at pH 6.8 and pH 7.4 from the composite carriers could be an advantage for colon-specific drug release where controlled and extended release is preferred.

4. Conclusions

We have successfully intercalated CS into MMT galleries which was further entrapped in AL matrix to form composites hydrogel beads for oral drug delivery system by ion exchange and gelation methods. The molecular arrangement of the drug

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molecules in the basal spacing of CS-MMT composites were confirmed by XRD, FT-IR, TGA and DSC.

The antibacterial activity of MMT, CS-MMT and DS/CS-MMT was evaluated by well-diffusion on agar. The results showed that DS/CS-MMT composites had stronger antibacterial property. *In vitro* cell viability assay in cancer cells revealed that the drug loaded composites were less toxic than pristine drug. The release of DS was retarded in DS/CS-MMT/AL composites in the gastric environment compared to DS/CS-MMT composite and the site-specific delivery of DS was effectively achieved using AL. Thus, our formulation offer controlled release of anionic drug from the composites made up from clay mineral and biopolymers which are essential requirement for treating inflammatory disease. An additional imminent principle area where drug loaded non-toxic composites can be considered is in preparation of tissue engineering scaffolds and other biomedical applications. Using the approach of composite synthesis described here one can prepare an implant capable of controlled drug release. In summary, our study may be fruitful in the area of biomedical application by formulating carriers of therapeutic molecules using composites as support.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.bbe.2014.08.004.

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