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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