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Research paper

Synthesis and characterization of chlorhexidine acetate drug–montmorillonite intercalates for antibacterial applications

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ABSTRACT

In this study, a drug intercalated montmorillonite (Mt) has been prepared which can be useful in designing novel topical drug delivery system. The drug–Mt intercalates were synthesized by ion exchange route where interlayer cations i.e., K^+, Na^+ etc. of Na^+–Mt exchange with the cation of the drug, chlorhexidine acetate (Ca^{++}). The characterization of drug–Mt intercalates has been done using X-ray diffraction, Fourier transform infrared spectroscopy, thermogravimetric technique and energy dispersive X-ray analysis; all of which indicate successful intercalation of drug into the interlayer space. These drug–Mt intercalates strongly inhibited the growth of a wide range of microorganisms including both *Staphylococcus aureus* and *Escherichia coli*. In vitro release study of the antibacterial drug–Mt intercalates in phosphate buffer saline (pH 7.4) media at 37 °C was investigated. The pattern was found to be initially burst release followed by sustained release. The Ca^{++}–Mt intercalates with a wide range of bioactivity against microbes and controlled release characteristics have the potential for application in the area of topical drug delivery.

1. Introduction

Montmorillonite (Na^+–Mt), a bioinert clay mineral is one of the important members of the smectite family. The interlayer space of Mt is generally occupied by various exchangeable cations such as Na^+, K^+, Ca^{2+}, Mg^{2+} and water molecules, explaining high cation exchange capacity (CEC: 70–120 mequiv/100 g) for this class of clay minerals. Unique crystalline structure of Na^+–Mt allows it to expand and contract the interlayer space while retaining the octahedral and/or tetrahedral two dimensional crystallographic integrity via substitution with various organic and inorganic cations to form the intercalates (Lagaly et al., 2013; Lin et al., 2002). Na^+–Mt also has large specific surface area and colloidal properties, good absorbability, adhesive ability and drug carrying capability (Iliescu et al., 2011; Seema and Datta, 2013; Van Olphen, 1963). Thus, Mt is one of the most extensively used minerals both as the excipient as well as the active substance in pharmaceutical products (Deshamane et al., 2007; Koerner et al., 2006; Wang et al., 2008; Yuan et al., 2008).

Chlorhexidine acetate (Ca^{++}) is a cationic antibiotic acting as a bacteriostatic as well as a bactericidal agent at higher concentrations (Russell, 1986). At low concentrations, the mechanism of action of this biguanide drug is ATPase inactivation whereas at higher bactericidal concentrations, it induces damage of cytoplasmic membrane by precipitating essential protein and nucleic acids. This antibiotic drug has established application for treating the nosocomial transmission (Wang et al., 2008) of infections caused by the bacteria. It is also applicable for topical applications such as antiseptic, pharmaceutical and cosmetic preservatives and also as an antiplaque agent (Hugo and Russell, 1982; Walihauser, 1984).

Nanotechnology is actually focusing on drug delivery through clay minerals (McGinity and Lach, 1976; Wai and Banker, 1966) to give protection of the drug in the systematic circulation, to provide restricted accession of the drug to the affected site and also to deliver the drug entity to the action site at a controlled manner. The current literature reports various types of release profile of modified Mt. For example Na^+–Mt (Zheng et al., 2007) has been shown to act as a sustained release drug carrier after intercalation of ibuprofen (IBU) drug. The release rate is pH dependent and IBU–Mt intercalate is useful for oral administration. Another in vitro release study of Ca^{++} intercalated in Mt (Menga et al., 2009) reveals that this system is useful as an advanced drug delivery carrier with controlled release characteristics. In another study, amido cationic drug, acyclovir–Mt intercalate (Junping et al., 2007) leads to the development of a pH dependent controlled release system which could be used for oral applications.

The present paper focuses on the preparation and characterization of a controlled drug release system based on the intercalation of Ca^{++} drug into the interlayer space of Na^+–Mt via cation exchange route. The drug loaded Mt (Ca^{++}–Mt) was characterized by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric (TGA) technique and energy dispersive X-ray (EDX) analysis. The antimicrobial activity of Ca^{++}–Mt intercalates was determined against both gram-positive and gram-negative bacteria. In vitro release study of the antibacterial intercalates was investigated in phosphate buffer saline (PBS, pH 7.4) media at 37 °C. These bioactive Ca^{++}–Mt

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intercalates have the potential for application as efficient antibacterial agents in the area of medical textiles. They can also act as a precursor for the preparation of clay polymer nanocomposite with potential application for topical drug delivery systems.

2. Experimental details

2.1. Materials

Sodium montmorillonite (Na^+–Mt) with a cation exchange capacity of 120 mequiv/100 g was procured from Southern Clay Products, Inc. (Japan) and used without further treatment.

Chlorhexidine acetate (Ca^{++}; white powder; M.W. 625.55; M.P. 155 °C; solubility: 19 mg/ml of water at 25 °C) was obtained from Sigma-Aldrich Company Ltd. (Dorset, UK) and used as received. The chemical structure ($C_{22}H_{30}Cl_2N_{10}\cdot 2C_2H_4O_2$) of the model drug is shown in Fig. 1.

Orthophosphoric acid (H_3PO_4; liquid; M.W. 98; density: 1.69 g/ml; B.P. 158 °C) was procured from Thermo Fisher Scientific India Pvt. Ltd. (Mumbai, India). Dialysis membranes (molecular weight cut-off ≤12,400) were obtained from Sigma-Aldrich, Bangalore. All other chemicals and solvents were of reagent grade and used without further purification.

2.2. Synthesis of drug loaded clay mineral

The Ca^{++}–Mt intercalates were prepared through cation exchange reaction of Ca^{++} drug with Na^+–Mt. 1.0 g of Na^+–Mt was dispersed in 30 ml of distilled water with vigorous stirring for 0.5 h at room temperature. Fixed amount of Ca^{++} drug was also dispersed in desired amount of distilled water separately. The concentrations of Ca^{++} used were 0.2, 0.5, 1.0 and 2.0 times CEC of Mt, respectively.

These two solutions were mixed together at optimized conditions (pH value adjusted to 4.1 with H_3PO_4) using a magnetic stirrer at 500 rpm for about 3 h at 80 °C. After cooling, the mixture was centrifuged at 5000 rpm for 0.5 h. The supernatant solution was decanted and the residue was collected. All products were washed with water, dried at 90 °C and ground into fine powder form suitable for further characterization. The different product codes (drug–Mt intercalates) are listed in Table 1.

2.3. Characterization of drug loaded clay mineral

XRD studies of Na^+–Mt and Ca^{++}–Mt intercalates were carried out on a PANalytical X-ray Powder Diffractometer using Ni-filtered CuK_α radiation of wavelength 1.5418 Å, working voltage 40 kV and working current 30 mA. Scanning was carried out in the range 2θ values from 2 to 20° at a scanning rate of 2°/min for all the samples.

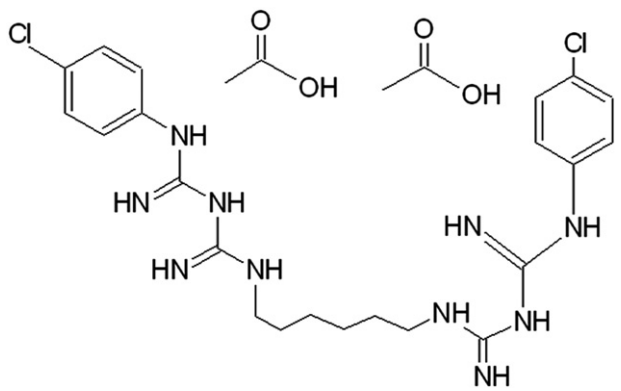

Fig. 1. Chemical structure of chlorhexidine acetate drug.

Table 1
Product codes for drug–montmorillonite intercalates.

Sample codes	Concentration of Ca^{++} used (Fraction of CEC of Na^+–Mt)
0.2Ca^{++}–Mt	0.2
0.5Ca^{++}–Mt	0.5
1.0Ca^{++}–Mt	1.0
2.0Ca^{++}–Mt	2.0

Perkin Elmer, Spectrum BX FT-IR system was used for determining the functional groups present in Na^+–Mt, Ca^{++} drug and Ca^{++}–Mt intercalates. FT— IR samples were prepared by KBr pressed disk technique applying 400 kg/cm^2 pressure. Analyses were performed in the transmission mode between 4000 and 400 cm^{-1}, with a resolution of 2 cm^{-1}.

Perkin Elmer, TGA-7 system was used to determine degradation behavior of Na^+–Mt, Ca^{++} drug and Ca^{++}–Mt intercalates. The amount of drug loading (Ca^{++} in mass%) was determined from the TGA analysis. Testing was carried out at a heating rate of 20 °C/min up to 900 °C in nitrogen atmosphere.

ZEISS EVO 50 Scanning Electron Microscope working with EDX attachment (detection limits typically 0.1–100 mass%) was used to determine the elemental composition of the Na^+–Mt, Ca^{++} drug and various Ca^{++}–Mt intercalates. EDX spectra based on the silicon drift detector (SDD) technology with an energy resolution of 127 eV at MnK_α were recorded. EDX samples were coated with carbon to prevent charging during exposure to the electron beam prior to the experiment.

2.4. Antibacterial activity assay

2.4.1. Colony counting method

Minimum inhibitory concentration (MIC) of the Ca^{++} drug and various Ca^{++}–Mt intercalates was determined by colony counting method (AATCC 100). AATCC stands for American Association of Textile Chemists and Colorists. AATCC 100 is an antimicrobial test method which provides a quantitative evaluation of the antimicrobial activity of the test material. The MIC is defined as the lowest concentration of antimicrobial agent which inhibits a visible growth of bacterial colony formation.

Luria broth solution was freshly prepared by dispersing 2 g of Luria broth in 100 ml of distilled water. This solution was sterilized by autoclaving at 15 lb pressure for 15 min. Neat Na^+–Mt (control), Ca^{++} and Ca^{++}–Mt powders at different concentration levels ranging from 0.1 to 10 ppm were dispersed in conical flask containing Luria broth solution and then inoculated with 10 μl of *Staphylococcus aureus* (10^6 CFU/ml) and *Escherichia coli* (10^6 CFU/ml) bacterial solution and kept at 37 °C for 24 h. Serial dilution of these solutions was made in sterilized DI water. Dilutions of 10^{-4} and 10^{-5} were used for colony counting. 10 μl of diluted solutions was spread on to the agar plate and plates were incubated at 37 °C for 24 h. After incubation bacterial colonies were counted using Yorco digital colony counter on the surface of agar plate. The minimum concentration at which 99% reduction in number of colonies in neat drug takes place as compared to the control clay mineral is indicated as MIC of Ca^{++} drug.

2.4.2. Disk diffusion test

Antibacterial activity of the Ca^{++}–Mt intercalates was tested against both gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli*) by the disk diffusion test (AATCC 90). AATCC 90 is an antimicrobial test method which provides a qualitative assessment of the bacteriostatic activity of the test material that is treated with antimicrobials and is capable of producing a zone of inhibition.

Homogeneous pastes of Na^+–Mt and Ca^{++}–Mt were prepared by mixing 0.1 g of each in 0.2 ml of DI water. Pastes were uniformly applied on the paper disks of about 15 mm diameter. The disks were placed in UV chamber for 30 min for sterilization. Nutrient agar solution was made by suspending 20 g of Luria broth in 1000 ml of DI water and 15 g of