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Research paper

Preparation and characterization of Lecithin–heparin intercalated in montmorillonite nanocomposite

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ABSTRACT

In this work, a study of Lecithin–heparin intercalated in montmorillonite was performed in order to synthesize an antithrombogenic hybrid. The hybrid of montmorillonite (Mt)/Lecithin–heparin (LEC-HEP) was synthesized by the intercalation method under mechanical stirring. The composite was characterized by X-ray diffraction (XRD) analysis, X-ray fluorescence (XRF) and C, H, and N elemental analyses, zeta potentials, Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA) techniques. The d_{001}-value was increased by LEC-HEP intercalation. Fourier transform infrared spectroscopy also confirmed the intercalation of Lecithin–heparin into Mt interlayer space. In vitro release study of the antithrombogenic drug–Mt intercalates in phosphate buffer saline (pH 7.4) media at 37 °C was investigated. The heparin showed an initial burst effect for 24 h and then continuously released for 30 d. In-vitro blood compatibility of the Mt/LEC-HEP was carried out via hemolysis assay. The Mt/LEC-HEP exhibited hemolysis below 5% which is permissible for biomaterials. These modified materials have the potential for being used as blood contact materials.

1. Introduction

Montmorillonite (Mt) is one of the smectite group minerals, composed of an octahedral sheet sandwiched between 2 tetrahedral sheets (Bergaya and Lagaly, 2013). The imperfection of the crystal lattice and isomorphous substitutions induce a net negative charge that leads to the adsorption of hydrated alkaline earth metal ions in the interlayer space (Chiou et al., 2005). Since the layers are negatively charged, they have large cation-exchange capacities; they are characteristically in the range of 7–10 cmol/100 g. This property renders them suitable for the incorporation of various positively charged organic or inorganic compounds between the layers, generating the so-called intercalation complexes (Cyras et al., 2008; Wang et al., 2008). One of their important fields of application is the pharmaceutical industry. They are utilized not only for coating pharmaceutical preparations but also as active pharmaceutical vehicles as well as for the intercalation and controlled release of active agents (Mohanambe and Vasudevan, 2005; Deshamane et al., 2007; Zheng et al., 2007; Joshi et al., 2009; Meng and Zhou, 2012; Hou et al., 2014).

Heparin (HEP) is a highly sulfated glycosaminoglycan with the highest negative charge density of any known biological molecule. It has been widely used as anticoagulant in clinics for the prevention of thromboembolic diseases as well as for kidney dialysis and cardiac surgery. The anticoagulant activity of heparin is exerted through binding to the antithrombin III (ATIII) protease inhibitor through a specific pentasaccharide sequence. Upon binding, ATIII under-goes a conformational change, resulting in the inhibition of thrombin along with other coagulation cascade proteases (Lever and Page, 2002; Gray et al., 2008). Due to its excellent antithrombogenic function, heparin has been immobilized in many polymeric biomaterials through either physical absorption or chemical binding. Numerous reports demonstrated that immobilization of heparin in polymeric biomaterials can substantially reduce the thrombogenicity (Christensen et al., 2001; Liu et al., 2009).

In previous work, Mt/cetyltrimethylammonium bromide-heparin were prepared (Meng and Zhou, 2014). The literature on modified clays is still dominated by studies on cationic surfactants. Cationic surfactant is used in environmental remediation (Lee and Tiwari, 2012), but they can be considered toxic (Reeve and Fallowfield, 2017) and they might not be applicable for certain large scale environmental and biomedical

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applications. Although there is an amount of literature on the modifications of montmorillonite with surfactants (Ma et al., 2016; Wang et al., 2017; Zhu et al., 2017), there is no specific study with lecithin as amphiphilic surfactant complexed with heparin intercalated in Mt.

The main driving force behind the development of novel surfactants is the search for environmentally friendly products. Lecithin (LEC) is a naturally occurring zwitterionic phospholipid-based liquid surfactant (extracted from egg and soybean) (Park et al., 2008). It has been extensively studied as structuring agent for food, pharmaceutical and cosmetic applications (Dahan et al., 2008; Graf et al., 2008; Pestana et al., 2008). Since it is part of the biomembrane, Phospholipids have a positively charged head group and a hydrocarbon tail that has various levels of unsaturation and the existence of the hydrocarbon groups on the surface was believed to lead to improved blood compatibility (Fricker et al., 2010).

In this work, heparin complexed with Lecithin and the resulting complex. LEC-HEP was then intercalated into Mt to form a composite. The resulting material was characterized by FT-IR, XRD, TG and zeta potential. The release profile of HEP from the resulting material was monitored at 37 ± 0.5 °C using in phosphate buffer saline (pH 7.4) media. The hemocompatibility of the modified-Mt was further assessed by hemolysis test in vitro.

2. Experimental procedure

2.1. Materials

Lecithin, purchased from A.X.B biotechnology Co., Ltd. (Beijing, China) and heparin (sodium salt Sigma, 150 U/mg, St. Louis, MO, USA) were used as received. Mt with a cation exchange capacity (CEC) of 0.090 cmol/g was supplied by ZheJiang Clay Minerals Co. Chemical composition of the Mt was determined by X-ray fluorescence spectrometer (Table 1). Fresh whole blood was collected from consenting donors by Jiangsu blood center with ethics approval. The phosphate buffer saline (PBS) were purchased from Sigma Aldrich (Spain). The toluidine blue was also purchased from Sigma; Deionized water was used for the preparation of all solutions.

2.2. Preparation of Mt/LEC-HEP composites

The desired amount (0.8 g) of the LEC was dissolved in acetic ether. Various amounts (0.1 g, 0.2 g, 0.4 g, 0.6 g) of heparin were added within 1 h. A yellow dispersion was obtained and used in later steps.

1 g of Mt was dispersed in 100 ml of distilled water, then the above yellow dispersion was poured into the Mt mixture under 80 °C for 3 h. The dispersions were mixed and stirred for 30 min then ultracentrifuged at 12000 rpm for 1 h. The precipitate was filtered and washing with ethanol and distilled water for several times to remove residual organic molecules. Ethanol would remove the adsorbed species on the Mt/LEC-HEP external surface (Silva et al., 2014). The amount of LEC and HEP loss during the hybrid formation was measured by UV absorption of the filtrate.

2.3. Characterization

Powder X-ray diffraction (PXRD) patterns of pristine Mt, Mt/LEC and synthesized Mt/LEC-HEP composites were recorded on an X-ray diffractometer (D/max 2500/PC) using a Cu K α line (40 kV and 30 mA) as an X-ray source. Measurements were made between 0 and 10° (2θ) at a step size of 0.02°. 1° was selected for the divergent slit and scatter slit. A small amount of 200 mesh sieve powder sample evenly into the grooved glass plate, pressed with a glass plate so that the powder does not fall off, to ensure that the sample surface to be tested flush with the surface of the sample plate.

Thermogravimetric analyses were carried out on a Perkin-Elmer TG 7 instrument from room temperature to 800 °C with a heating rate of 10 °C/min under a nitrogen flow of 60 ml/min. The specimen weight was in the range of 7–15 mg. TG curves were used to determine the percentage of mass loss.

The chemical structure of the obtained materials was confirmed by recording their IR spectra. The instrument used was a German Nicolet FT-IR Nexus-670 IR spectrophotometer. Measurements were carried out using the KBr tablet technique.

The disks were prepared by pressing a mixture of powdered sample (0.9 mg) and KBr (80 mg). The spectra recorded over the wavenumber range from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and 64 scans were averaged to reduce noise. The instrument's software was used to identify several bands.

The elemental analysis of Mt and Mt/LEC systems were carried out using a Vario EL III elemental analyzer.

This test was performed with the aid of X-rays fluorescence spectrometry.

(XRF) on a XRF PANalytical. The trace elements were detected by using XOS specific high-resolution spectroscopy.

Zeta potentials of solid particles in Mt/water and organoclay/water suspensions from the experiments was determined by electrophoretic light scattering using a zeta potential analyzer (Nano 2890) at the natural pH of sample in aqueous media and 25 °C.

2.4. In vitro release of heparin

To study the release rate of heparin, the 1000 mg Mt/LEC-HEP sample was placed in a dialysis bag and 5 ml of preheated PBS solution as a release medium was subsequently added. The dialysis bag was then sealed and put into a bottle. Another 95 ml of PBS solution was added in the bottle. At specific time intervals, 2 ml of dissolution media were withdrawn and the released amount of heparin was calculated according to the standard curve (Meng and Zhou, 2012).

The amount of heparin released was determined using the colorimetric toluidine blue test (Wang et al., 2016). The above mentioned 2 ml dialysis solution was placed in a test tube. 3 ml of 100 μg/ml toluidine blue aqueous solution was added into the test tube to complex with heparin. Hexane (3 ml) was then added to separate heparin–toluidine blue complex. The test tube was vortexed vigorously to ensure completely extraction. The aqueous layer inside the test tube was taken out for absorbance measurement was measured in the collected filtrate using a Cary50 UV–vis spectrometer (Varian, USA). and a quartz cuvette at λ_{max} = 631 nm, using pH 7.4 phosphate buffer as a solvent. The data were reported as an average of three measurements.

2.5. Blood compatibility analysis (hemolysis test)

This assay was carried out on Mt/LEC-HEP composites. Fresh anticoagulated blood from human volunteers (2 ml) was diluted with 2.5 ml of normal saline solution.

The diluted blood (0.2 ml) was added to Mt/LEC-HEP dispersions with different concentration. The mixture was kept at 37 °C for 60 min and then centrifuged at 1000 rpm for 10 min. The supernatant was

Table 1
Chemical analysis of raw Mt.

Composition	SiO2	Al_2O_3	MgO	Fe_2O_3	CaO	K_2O	Na_2O	P_2O_5	SO_3	Others
Mt	67.437	17.113	6.714	4.314	3.476	0.172	0.163	0.045	0.037	0.529