Materials Science and Engineering C 68 (2016) 557–564

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

MgAl- Layered Double Hydroxide Nanoparticles for controlled release of Salicylate

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ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form 26 May 2016
Accepted 8 June 2016
Available online 10 June 2016

Keywords:
Mg–Al LDH
Nanocarrier
Salicylate
Drug release

ABSTRACT

Layered double hydroxides (LDHs), have been known for many decades as catalyst and ceramic precursors, traps for anionic pollutants, and additives for polymers. Recently, their successful synthesis on the nanometer scale opened up a whole new field for their application in nanomedicine. Here we report the efficacy of $Mg_{1-x}Al_x(NO_3)_x(OH)_2$ LDH nanoparticles as a carrier and for controlled release of one of the non-steroidal *anti*-inflammatory drugs (NSAID), sodium salicylate. $Mg_{1-x}Al_x(NO_3)_x(OH)_2.nH_2O$ nanoparticles were synthesized using co-precipitation method from an aqueous solution of $Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.9H_2O$. Salicylate was intercalated in the interlayer space of Mg-Al LDH after suspending nanoparticles in 0.0025(M) HNO_3 and 0.75 (M) $NaNO_3$ solution and using anion exchange method under N_2 atmosphere. The shift in the basal planes like (003) and (006) to lower 2θ value in the XRD plot of intercalated sample confirmed the increase in basal spacing in LDH because of intercalation of salicylate into the interlayer space of LDH. FTIR spectroscopy of SA-LDH nano hybrid revealed a red shift in the frequency band of carboxylate group in salicylate indicating an electrostatic interaction between cationic LDH sheet and anionic drug. Differential thermal analysis of LDH-SA nanohybrid indicated higher thermal stability of salicylate in the intercalated form into LDH as compared to its free state. DLS studies showed a particle size distribution between 30-60 nm for pristine LDH whereas salicylate intercalated LDH exhibited a particle size distribution between 40-80 nm which is ideal for its efficacy as a superior carrier for drugs and biomolecules. The cumulative release kinetic of salicylate from MgAl-LDH–SA hybrids in phosphate buffer saline (PBS) at pH 7.4 showed a sustained release of salicylate up to 72 h that closely resembled first order release kinetics through a combination of drug diffusion and dissolution of LDH under physiological conditions. Also the cytotoxicity tests performed revealed the less toxic nature of the nanohybrid as compared to the bare SA drug.

1. Introduction

Layered double hydroxides (LDHs) have been known for many decades as catalyst and ceramic precursors, traps for anionic pollutants, catalysts and additives for polymers, but their successful synthesis on the nanometer scale a few years ago opened up a whole new field for their application in nanomedicine [1]. Layered Double Hydroxides are special type of ceramic with hydrotalcite like clay material that have surface layers formed of positively charged brucite type layer made up of mixed metal hydroxides of divalent and trivalent metals with exchangeable intercalated negatively charged species in between the two surface layers that compensate for the positive charge of the brucitelayer [1,2]. The chemical composition of LDH is generally expressed as $M(II)_{1-x}M(III)_x(OH)_2(A^{n-})_{x/n} \times y\,H_2O$, where M(II) is divalent metal cation, M(III) is trivalent metal cation, 'A_n' is interlayer anionic species, 'n' is charge on interlayer anion, 'x' and 'y' are fraction constants. It can be concluded from the formula that the formal positive charge of the layer depends on the M^{2+}/M^{3+} ratio [2]. Although the most common anion is carbonate, many LDHs have been synthesized with different anions, both organic and inorganic, such as halides, silicates, polyoxometalates, anionic coordination compounds, carboxylates, etc.;. Such inorganic nanomaterials based drug carriers [3,4], show much better properties than organic carriers (hydrogels [5], cellulose [6], polysaccharides [7,8]) including ease of controlled synthesis and environmental friendliness [9]. Thus the recently designed organic-inorganic nanohybrids based drug carriers such as LDH-chitosan [10,11] displays good biocompatibility and avoid the drug leaching Among the organic anions, intercalation of benzoate and terephthalate inclusion has been also widely studied [12,13]. A wide variety of biomolecules such as DNA, RNA, ATP molecules, etc. and drugs can be intercalated into LDH and its efficacy in releasing those biologically important molecules in a controlled and sustained manner has been studied [14,15].

LDH has been used as a carrier of different kind of drugs including cardiovascular, anticancerous, anti-inflammatory drugs. Cardiovascular drug such as captopril (Cpl) intercalated drug-MgAl LDH nanocomposite was

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http://dx.doi.org/10.1016/j.msec.2016.06.029
0928-4931/

developed by Zhang et al. [16] using coprecipitation method. The intercalation of *anti*-cancer drugs in LDHs has been less widely investigated. A nanohybrid of podophyllotoxin–LDH (PPT–LDH) developed by Wang et al. [17] showed long-term suppression effect on tumor growth and also enhanced apoptosis of tumor cells. Intercalation of 5-fluorouracil (FU) into LDH via a coprecipitation was studied by Choy et al. where they found sustained release of anticancerous drug (FU) upto 72 h from drug-LDH nanohybrid [18]. In another study, Choy et al. investigated the efficacy of methotrexate (MTX)-LDH nanohybrid in suppressing the growth of human breast adenocarcinoma MCF-7 cells [19,20].

Non-steroidal anti-inflammatory drug (NSAID), are a class of drugs that provides analgesic (pain-killing) and antipyretic (fever-reducing) effects, and in higher doses for anti-inflammatory effects. NSAID, widely used in rheumatism treatment, very often show adverse secondary effects, such as gastric and duodenal ulcers formation. When intercalated in the interlayer space of properly functionalized LDH, it can provide sustained and controlled release of NSAIDs at specific sites [21,22]. Khan et al. [23] intercalated a series of cardiovascular, anti-inflammatory and analgesic agents like diclofenac, gemfibrozil, ibuprofen, naproxen, 2-propylpentanoic acid, 4-biphenylacetic acid and tolfenamic acid into Li-Al-layered double hydroxide to formulate a novel tunable drug delivery system. Ambrogi et al. [24] observed that ibuprofen anions exchanged all chloride ions of hydrotalcite-like compounds, producing an intercalation compound with a drug loading of 50% in *w*/w. Del Arco et al. [25] carried out a in vivo pharmacological study of the interaction between hydrotalcite and indomethacin showing that intercalation of the drug reduces the ulcerating damage by it. Diclofenac has also been intercalated by several methods, making LDHs act as matrices for non-steroidal anti-inflammatory drugs [26]. In another study, Gordijo et al. [27] have reported the immobilization of ibuprofen and copper-ibuprofen drugs on layered double hydroxides. Mg-Al LDH have already found pharmaceutical applications as drug stabilizers [28,29], ingredients in sustained-release pharmaceuticals containing nifedipine [30], components in adhesives for transdermal delivery [31], for symptomatic treatment of peptic ulcers, [32] for the therapy of digestive disorders and for preparation of aluminum magnesium salts of antipyretic, analgesic and anti-inflammatory drugs. In another syudy, Xu et al. [33] found that ibuprofen (IBU) release rate from dense and oriented Mg_2Al–LDH-IBU sample was much slower as compared to loosely aggregated powders, due to the longer average diffusion path and higher diffusion resistance.

Though a lot of literature reports are available regarding controlled release of anti-inflammatory drugs from MgAl LDH nanocarrier, the mechanism of drug release from the interlayer space of Mg Al LDH nanohybrid has rarely been investigated. Herein, we report synthesis of a MgAl-LDH nanocarrier using simple co-precipitation method. Sodium salicylate was intercalated into the LDH interlayer by an anion exchange technique. The LDH-salicylate composite was fully characterized using powder X-ray diffraction (XRD), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and particle size analysis. Salicylate loading in the formulation was confirmed by carbon hydrogen nitrogen (CHN) elemental analysis. The release behavior of salicylate from the hybrid material was studied using UV Spectrophotometer. Different drug release models and release kinetic equations were compared with the actual salicylate release rate from Mg AlLDH nanopowders and the results were discussed and correlated, which shows the potential of Mg-Al-LDH in controlled drug delivery. Cytotoxicity tests of the LDH-SA nanohybrid was performed on the HEK 293 cells and determined using MTT assay. The results suggest that SA intercalated into LDH was much less cytotoxic than the bare SA on kidney cells.

2. Experimental procedure

2.1. Materials

Magnesium nitrate hexahydrate [$Mg(NO_3)_2.6H_2O$], aluminum nitrate nonahydrate [$Al(NO_3)_3\cdot 9H_2O$], ammonium hydroxide [NH_4OH] were purchased from Sigma-Aldrich, USA. Sodium salicylate [NaOSal] was synthesized from salicylic acid [HOSal] (Merck, India) and sodium hydroxide [NaOH] (Merck, India). Deionized and decarbonated ultra-pure water (Millipore, specific resistivity18 MΩ) was used in all preparations and the chemicals utilized in this study were used as received without further purification.

2.2. Synthesis of MgAl-salicylate LDH hybrid material

0.495 (M) $Mg(NO_3)_2.6H_2O$ and 0.165 (M) $Al(NO_3)_3\cdot 9H_2O$ were dissolved in 250 ml of water to synthesize Mg—Al LDH with Mg: Al ratio of 3:1. To maintain the pH of the mixed precursor solution at 8, ammonia was dropwise added to it with constant stirring for 24 h. The appearance of a white gelatinous precipitate indicated the formation of MgAl-LDH. The precipitate was collected by centrifugation and repeatedly washed by redispersing it in water followed by centrifugation (at 3000 rpm for 5 mins) to remove excess nitrate anions. The washed LDH precipitate was then oven dried at 100C to get MgAl-LDH nanopowder. Subsequently, 2 g sodium salicylate was dissolved in 50 ml water of pH 7.5, and the solution was added to100 ml aqueous suspension in 0.0025(M) HNO_3 and 0.75 (M) $NaNO_3$ containing 1 g LDH. The pH of the LDH-salicylate mixture was raised to 9 by dropwise addition of 0.01 M NaOH. The reaction mixture was agitated for 72 h in a nitrogen atmosphere. The product, MgAl-LDH– salicylate, was centrifuged, washed with deionized water and then dried in a vacuum oven at 50 °C

2.3. Preparation of samples for UV–spectrophotometer analysis

20 mg of MgAl LDH–Salicylate formulation was dispersed in 2 ml of PBS of pH 7.4 in 8 different eppendorf, marked and kept at a constant stirring. After definite time interval, one eppendorf was taken at a time and centrifuged (at 3000 rpm for 5 mins). The solution was then collected in a tube, filtered through 0.2 μm filter paper and the filtrate was kept for analysis using an UV–visible spectrophotometer.

2.4. Characterization

Powder X-ray diffraction (XRD) patterns were obtained for MgAl LDH and MgAl LDH –salicylate powders with X'Pert High Score diffractometer (Rigaku, Japan) using CuKα.

(λ-1.5418° A) radiation at 40 mA, 40 kV. The step size of 0.005° was used in the scan range of 1–60° (2θ). The peak arising from (003) planes of LDH powder was used to calculate the basal spacings in all the samples using Bragg's equation. Fourier transform infrared (FTIR) spectra of the *as*-prepared powders were recorded at room temperature using the KBr pellet method (sample: KBr = 1:100) on a spectrometer (F Varian 3600,USA) in the wavenumber range of 400–4000 cm^{-1} with an average of 50 scans. Thermogravimetric (TG), and differential thermal analysis (DTA) of the as-prepared powders, were studied using a TG/DTA analyzer (Netzsch, Germany) and measurements were recorded from 50 °C to 1000 °C at 10 °C/min heating rate in air. Particle size and morphology of MgAl-LDH and MgAl-LDH–salicylate samples were examined using the transmission electron microscope (TEM) (FEI Tecnai 30 G^2 *S*-Twin, Netherland) operated at 300 kV. The elemental composition of MgAl-LDH and MgAl-LDH–salicylate samples was studied using energy dispersive spectroscopy (EDS) attached to the TEM. The particle size of both MgAl-LDH and MgAl-LDH–salicylate powders were measured using dynamic light scattering technique (Microtrac Zetatrac, PA, USA) on respective aqueous suspensions. The elemental analyzes for carbon, hydrogen and nitrogen (CHN) of MgAl-LDH–salicylate sample was conducted using model-2400, Series II CHN analyzer (Perkin Elmer, USA). UV–visible spectrophotometer (Perkin Elmer, USA) was used to determine the salicylate loading and release from LDH-SA nanopowders in PBS at pH 7.4. The dissolution kinetics of MgAl-LDH nanopowders in phosphate buffer solution of pH 7.4 was