Journal of the Taiwan Institute of Chemical Engineers 60 (2016) 525–531

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Loading kinetics of 5-fluorouracil onto hydrotalcite and *in vitro* drug delivery

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ARTICLE INFO

Article history:
Received 8 April 2015
Revised 28 June 2015
Accepted 30 June 2015
Available online 21 July 2015

Keywords:
Hydrotalcites
5-Fluorouracil
Adsorption kinetics
Sustained release
Acid-pretreatment

ABSTRACT

The Mg-Al-NO_3 hydrotalcite (LDH) was pretreated in weak acid solution in order to improve the stability in acid environment. The pretreated LDH (pt-LDH) were loaded with the anticancer drug 5-fluorouracil (5-FU). The loading process followed the pseudo-first-order kinetics, where both film and intra-particle diffusions were participating in ruling the adsorption diffusion of 5-FU anions. The loaded pt-LDH hybrid (FU/pt-LDH) was characterized by XRD, EDS, FT-IR and TG/DSC, indicating that 5-FU anions were partially intercalated into the interlayer space in the pt-LDH by anion-exchange and the thermal stability of 5-FU in the FU/pt-LDH was enhanced. The release behavior of 5-FU from the FU/pt-LDH hybrid at pH 4.6 and 7.5 was studied. It was found that 5-FU was released faster at pH 4.6 than at pH 7.5, and the released amount was higher, where the acid stability of the pt-LDH carrier increased. The results showed that pt-LDH carrier was capable of slow and controlled release of 5-FU.

1. Introduction

5-Fluorouracil (5-FU), a fluorinated pyrimidine analog, is an anti-neoplastic drug, extensively used in the clinical chemotherapy for the treatment of metastatic carcinomas of breast, gastrointestinal tract, pancreas, head, neck, and ovary [1–3]. However, it shortens the half-lives of drugs in the body and has toxic side effects. One way to increase the therapeutic efficacy of drugs and reduce their toxicity is to encapsulate them in sustained delivery systems by drug carriers [4,5]. For this reason, preparation of drug carriers such as chitosan complexes [6], clays [7], zeolitic imidazolate frameworks [8] and polymer nanoparticles [9] have been investigated.

Layered double hydroxides (LDHs) with exchangeable anions in the positive brucite-like interlayer have been attracting much attention in the field of cellular delivery of anionic drugs, due to their low toxicity, biocompatibility, high stability and enhanced cellular uptake behavior compared with the conventional drug carriers [10–12]. It has been reported that the drug intercalation into Zn/Al-LDH matrices could reduce stomach irritation [13]. Mg/Al LDH could act as biomolecule reservoirs and drugs or genes carriers, and the encapsulated biomolecules are released inside the cell with the carrier dissolving slowly in the cell [14]. The use of these matrices aims to improve the release of drugs in order to maintain a high, constant level of the drug. At present, a great deal of research work has been concerned about the controlled release of 5-FU by layered double hydroxides [15–18].

However, despite the numerous advantages that the intercalation of drugs into the LDHs exhibited, it is difficult to be used as matrices for the controlled release in oral administration, as they are very sensitive to the acidic medium, unless they are surface modified to preserve them from being acidically dissolved. Our previous work reported that Mg_3Al-LDH-CO_3 materials were unstable and dissoluble in acid solutions, especially partial dissolving in the aqueous solution in the range of pH 4.3∼9.7 [19]. In weak acid environment, reprecipitation of amorphous $Al(OH)_3$ [20] on the LDHs could block their further dissolvement in the aqueous environment, and thereby increase their stability in the acid solution.

In this work, Mg_3Al-LDH-NO_3 was pretreated in a weak acid solution to obtain a stable hydrotalcite carrier material in acid environment, and the anti-cancer drug 5-FU was intercalated in the carrier via anion-exchange. The optimal conditions for 5-FU to be adsorbed on the acid-pretreated hydrotalcite carrier were carried out in order to obtain high loading, for which the parameters include the mass ratio of 5-FU to carrier, pH and temperature. The pt-LDH hybrids loaded with 5-FU were characterized by XRD, FT-IR, TG-DSC and UV–vis. In addition, the mechanism of 5-FU adsorption by pt-LDH was clarified based on the comprehensive dynamic analysis. Our aim is to study the slow release of drugs, which are orally administrated in the body.

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http://dx.doi.org/10.1016/j.jtice.2015.06.040
1876-1070/

2. Experimental

2.1. Materials

5-Fluorouracil (5-FU) was purchased from Sigma Aldrich (Shanghai, China). All other chemicals were of analytical grade and used without further purifications, which was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared with decarbonated ultrapure water.

2.2. Preparation of Mg–Al hydrotalcites

Precursor Mg_3Al-LDH-NO_3 with Mg/Al molar ratio of 3.0 (LDH) was synthesized by coprecipitation. The $Mg(NO_3)_2{\cdot}6H_2O$ and $Al(NO_3)_3{\cdot}9H_2O$ were dissolved to give the solution a total metal cations concentration of 0.6 mol/L. The metal salt solution was at 40 °C with vigorous stirring under a N_2 atmosphere for 4 h, and pH in the solution was maintained at about 10.0 by dripping a 1.2 mol/L NaOH solution, and then aged statically at 80 °C for 18 h. After cooling, the resulting slurry was filtered, washed with the decarbonated water and dried at 80 °C. The resulting carrier was designated as LDH. Part of the LDH was pretreated in the weak acid $NaNO_3$–HNO_3 buffer solution at room temperature under a N_2 atmosphere for 4 h. The pH was kept constant at about 5.0 in the adsorption solution. The pretreated product was filtered, washed with decarbonated water and dried, which was designated as pt-LDH.

2.3. Preparation of 5-FU/hydrotalcite hybrids

5-FU is a neutral weak acid, and is difficult to intercalate into the chosen host at a neutral condition. But under alkaline condition, 5-FU existed in the form of anion [21], and could be intercalated into the interlayer of hydrotalcites. The 5-FU solution with a concentration of 15 mg/mL was prepared by dissolving in the NaOH solutions with different pH of 8.0∼11.0. Under a N_2 atmosphere with constant magnetic stirring at 100 rpm, the pt-LDH carrier with different mass ratio of 5-FU to pt-LDH was dispersed in the 5-FU solution under different temperatures for a certain period of time. 5-FU amounts were monitored by UV-visible spectrophotometer (Hitachi U-2910, 265 nm) at regular intervals. After the reaction, the mixture was cooled to room temperature, centrifuged at 7000 rpm for 20 min, and residuum was washed three times with deionized water. The supernatant and solutions produced from the washing steps were stored for 5-FU determination. The residuum was dried at 70 °C, and designated as FU/pt-LDH. The tests were made in triplicate and the results were recorded as an average. Loading amount of 5-FU (mg/g) was the mass ratio of 5-FU to FU/pt-LDH.

2.4. In vitro *drug release study*

The release of 5-FU from the FU/pt-LDH hybrid in the phosphate buffered solution (PBS) was performed at initial pH 4.6 and 7.5, by adding about 20 mg FU/pt-LDH hybrid into 600 mL PBS buffer under magnetic stirrer (100 rpm) at 37 °C for 180 min. A 10 mL dissolution solution was withdrawn at defined time intervals in order to gain the amount of released 5-FU, meanwhile, a same volume of fresh medium was added. After the release experiment, the spent hybrid in the reaction solution with initial pH 7.5 was drying at 70 °C, and designated as spent-pt-LDH for XRD analysis. The accumulated amount of 5-FU released into the PBS buffer was measured momentarily using UV-visible spectrophotometer at 265 nm. The tests were made in triplicate and the results were recorded as an average. The 5-FU release rate (%) was the mass percentage of the released amount of drug to the loading amount of drug before release.

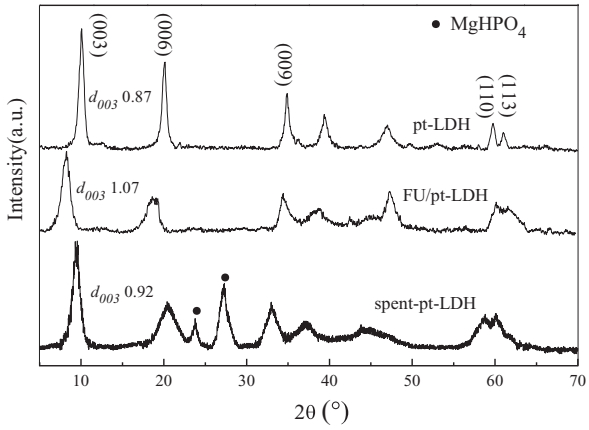

Fig. 1. XRD patterns of the pt-LDH, FU/pt-LDH and spent-pt-LDH samples.

2.5. Characterization

Powder X-ray diffraction (XRD) pattern was obtained in the 2θ range 5∼70° using a Rigaku D/MAX-3C instrument with CuKα source (λ = 0.1541 nm). Fourier transform infrared (FT-IR) spectrum was recorded on Perkin-Elmer Spectrum One B instrument using KBr pellet technique. Simultaneous thermogravimetry differential scanning calorimetry (TG-DSC) was carried out using Mettler Toledo/1-1600HT series equipment. 15.0 mg sample was heated at 10 °C/min up to 800 °C under a N_2 flow at 60 mL/min. The UV absorbance spectrum of the sample was recorded on a Shimadzu UV-2550 spectrophotometer. The energy dispersive X-ray spectroscopy (EDS) analysis was performed by a Noran SystemSix instrument. The EDS was used to determine the contents of C, N, F, Mg and Al elements in the samples. The low-temperature N_2 adsorption–desorption experiments were carried out at −196 °C using a NOVA-e1000 system (Quantachrome, USA). Samples were outgassed at 120 °C in vacuum (1×10^{-5} Torr) for 8 h. The pore size distribution was calculated from desorption isotherm by the Barrett–Joyner–Hallender (BJH) method, and the specific surface area was calculated using the Brunauer–Emmett–Teller (BET) method based on the N_2 adsorption isotherm.

3. Results and discussion

3.1. Characterizations of the carriers

3.1.1. XRD analyses

The XRD patterns of the pt-LDH and FU/pt-LDH are shown in Fig. 1. All samples had a layered double hydroxide crystal structure and no other crystalline phases were observed. Further analyses of the XRD patterns revealed some differences in the cell parameters between the samples. The parameter *a*, average cation–cation distance in the brucite sheet, was calculated from the (110) reflection, of which the *a* value for the pt-LDH was 0.316 nm and 0.308 nm for the FU/pt-LDH. The similarity in *a* value among them indicated that the intercalation of 5-FU anions did not change the structure of the brucite sheets. Comparing to the FU/pt-LDH, the pt-LDH exhibited a more stable baseline with sharper and higher diffraction peaks indicating the better-crystallized layered structure [22], which implied that the crystallinity of the FU/pt-LDH declined. The interlayer space (d_{003} about 0.87 nm) of pt-LDH was typical of a NO_3^- intercalated hydrotalcite [23], whereas the interlayer space of the FU/pt-LDH was 1.07 nm, possibly due to the intercalated 5-FU anions. Since the thickness of brucite sheets was about 0.48 nm and thus, the gallery height in the interlayer space of the FU/pt-LDH could be estimated to be 0.59 nm. Taking the molecular dimension (0.54 nm) of 5-FU into account, it could be speculated that the 5-FU molecules were