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Original article

Synthesis pretreatment and characterization of a magnetic layered double hydroxides fluorescent probe

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ABSTRACT

Using magnetic layered double hydroxide (MLDH) as carrier of fluorescein (FLU), a fluorescent composite of MLDH-FLU was prepared *via* intercalation reaction of ion change. The crystal properties of MLDH-FLU were investigated through XRD, IR, TEM and TG-DSC characterization. It is shown that the crystal type of MLDH-FLU composite matched well with R-hexagonal crystal system of MLDH, with crystal cell parameters of *a*, *c* and channel height *h* equal to 0.3199, 2.411 and 0.3267 nm respectively. The superabundant pigment adsorbed outside the composite should be cleared before interference with cells, but excessive wash would decrease stability and cause crystal phase transformation of MLDH.

1. Introduction

Layered double hydroxides (LDHs) have excellent cell transmission and controlled release properties [1–10], which can be used in the biomedical field to deliver certain biological molecules and specific drugs. Magnetic layered double hydroxide (MLDH) is a new targeted drug carrier invented with a coprecipitation reaction of paramagnetic ions [11,12], on which the "dextran-magnetic layered double hydroxide-fluorouracil" (DMF) delivery system was established [13]. The magnetic targeting and slow-release effect of the DMF system have been validated by a series of *in vitro* and *in vivo* experiments [14–18]. To provide further cell biology evidences for the MLDH delivery system, it is necessary to develop a fluorescent marker probe for tracking its cell-transmission.

Since the side groups of fluorescent iso-thiocyanate (FITC) can be connected to biomolecules with sulfydryl and amido groups through nucleophilic reaction, FITC is widely applied to mark proteins and antibodies as fluorescent dyes [19]. As the study of LDH applied in the biomedical domain goes deeper, researchers have done a lot of work in marking LDH of magnesium, zinc, and aluminum matrices with FITC [20–23]. The probe mark function of FITC-LDH is based on intercalation assembly, effective transport, and controlled release of FITC by the electropositive brucite laminate of LDH, but the free FITC molecules uncombined with LDH layers can polymerize to isothiocyanate during nucleophilic substitution, which would lead to a false-positive result. Furthermore, FITC, as a popular immuno-fluorescent marker, is not the best for tracking cell transfer of the LDH system, for its fluorescence is unstable and easy to quench. Fluorescein ($C_{20}H_{12}O_5$, FLU) is very electronegative, highly stable, and strongly fluoresces in reflected or perspective light, which makes it suitable for intercalation assembly with LDH. Anthony *et al.* have investigated the properties of MgAl-LDH intercalated with fluorescein [24]. In this paper, we used the magnetic layered double hydroxides $[Fe^{II}_2Fe^{III}(OH)_6][Cl{\cdot}H_2O]$ [12] as precursor to intercalate fluorescein, synthesizing a magnetic MLDH-FLU probe with ion exchange reaction, and inspected its appropriate pretreatment effects.

2. Experimental

2.1. Synthesis of MLDH precursor

MLDH with chemical formula $[Fe^{II}_2Fe^{III}(OH)_6][Cl{\cdot}H_2O]$ was prepared by coprecipitation of $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ according to the literature [12]. In detail, 61.63 g (0.310 mol) $FeCl_2{\cdot}4H_2O$ and 41.91 g (0.155 mol) $FeCl_3{\cdot}6H_2O$ were dissolved in 300 mL of water to prepare the mixed salt solution, then 500 mL of NaOH solutions in the concentration 2 mol/L was added under N_2 protection and 35 °C constant temperature conditions, and the pH of the reaction terminal point was kept at 6.41. The resulting slurry was aged *in situ* for 45 min, centrifuged with 5000 r/min for 10 min

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at −10 °C to separate the liquid–solid phases, then deposited at low temperature for later use.

2.2. Synthesis of MLDH-FLU fluorescent probe

Fluorescein (6.25 g, 0.0188 mol) was wetted with little water, dissolved by concentrated NaOH, diluted with 400 mL water, and adjusted to pH 7.45. The weakly alkaline solution of FLU was added into a 500 mL reactor assembled with a pH electrode and N_2 pipeline. After the air inside the reactor was empty and the bath temperature was increased to 35 °C, 6.07 g (0.0188 mol) of MLDH solid was added into the reactor under stirring and circulation of nitrogen, and was kept reacting with FLU for 90 min [25]. 10 mL terminal liquid sample was taken for analysis, and the rest of the slurry was centrifuged in 5000 r/min for 15 min at −10 °C to obtain the MLDH-FLU solid product.

2.3. Elution of adsorption pigment on the surface of MLDH-FLU

Small magnon (2× 5 mm) and 8 mL of distilled water were added into the sample tube of MLDH-FLU, and the solid was washed for 10 min at room temperature with a magnetic stirrer in N_2 atmosphere and centrifuged with 7000 r/min for 10 min at −10 °C. To prepare the analysis sample of eluent, the supernatant liquid was filtered through a syringe-driven filter, and 1.0 mL of the filtered solution was diluted to 50.0 mL, and its fluorescence intensity was finally measured at the λ_{max} of 526 nm. The operation of washing and analysis above was repeated 12 times.

2.4. Characterization of MLDH–FLU samples

Powder XRD patterns were obtained by a Rigaku D/Max-rB X-ray powder diffractometer (Cu Kα, λ = 0.1542 nm) with scan rate of 2°/min and scan range in 5–80°. Fourier transform infrared spectra of the samples were recorded over the range of 400–4000 cm^{-1} on a TENEOR27 infrared spectrophotometer (Brooke Company of Germany) with 4 cm^{-1} resolution, using the KBr disk method. The thermal behavior of the samples was examined using thermogravimetric and differential thermogravimetric analyses on a SETSYS-1750CS thermal analyzer (SETARAM of French) in N_2 atmosphere at the temperature range from 16 °C to 650 °C with a heating rate of 8 °C/min. TEM images were obtained with a Hitachi H-7560B transmission electron microscope at an accelerating voltage of 80 kV, amplifying 70,000–120,000 times. Elemental analysis was completed on a JMS-5600 LV low vacuum scanning electron microscopy (Japanese electron optical company) assembled JEOL X-ray energy dispersive spectrometer (Kevex companies in USA), with the resolution of 131.7 eV and scan range in 0–5000 eV.

The magnetic properties of MLDH and MLDH-FLU solid samples were evaluated with Ancient Egypt magnetic balance (CTP-II magnetic scales, NanJing Sangli Electronics Equipment Workshop), using $CuSO_4 \cdot 5H_2O$ as a standard substance to correct magnetic field intensity. The weight-change of an empty sample cell, standard substance cells, and equal altitude samples to be tested in 300–400 mT magnetic field were measured by a Mettler-Toledo analytical balance (AL 104, Mettler Toledo instrument Shanghai Co., LTD.), and magnetic susceptibility calculations were made using the relative comparison method.

3. Results and discussion

3.1. Liquid phase pH variation during synthesis course of MLDH and MLDH-FLU

The liquid pH change along with the coprecipitation of MLDH and the ion exchange reaction of MLDH-FLU are shown in Fig. 1.

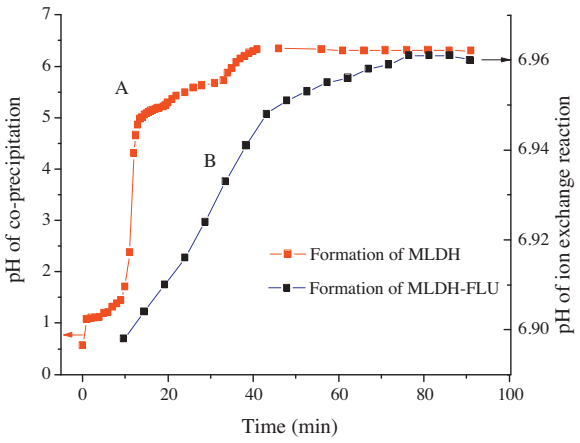

Fig. 1. Liquid pH change with reaction course.

Fig. 1A shows that the formation of MLDH went through two flat pH curves, which increased slowly in the pH range of 1.1–1.7 and 5.0–5.8, respectively. They correspond to the successive precipitation of $Fe(OH)_3$ and $Fe(OH)_2$, similar to the coprecipitation course of MgAl-LDH [26]. Fig. 1 B shows that the liquid phase pH rose along with the ion exchange of MLDH with FLU, implying that the original intercalated ions were replaced gradually. Due to the dissolution of OH^- ions displaced by FLU from interlayer of MLDH, OH^- concentration of the reaction solution increased slowly, which causes the rise of pH.

The fluorescent encapsulating rate of MLDH-FLU approached 98.9% that was calculated by the working curve of fluorescein, which indicated that the ion exchange reaction between MLDH and FLU solution was complete. Fig. 2 shows the fluorescence spectra of MLDH-FLU and fluorescein solution. There was a redshift of 10 nm between the maximum emission wavelength of MLDH-FLU aqueous solution and that of the intercalating agent fluorescein, and a low emission peak of MLDH-FLU presents at 363 nm of wavelength. Overall, the fluorescence properties of the intercalating agent did not change too much.

3.2. Solid phase characterization of MLDH and MLDH-FLU

As the MLDH is composed of paramagnetic iron ions, it should present some magnetic features [11,27,28]. Table 1 shows the mass magnetisability of MLDH and MLDH-FLU samples tested in the range of 300–400 mT. The magnetic susceptibility of MLDH,

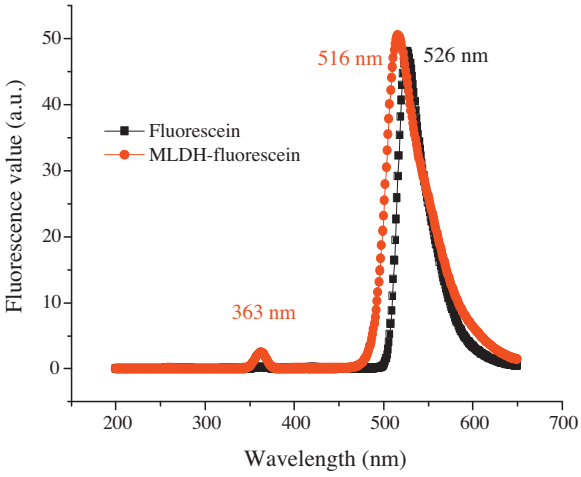

Fig. 2. The fluorescence spectra of MLDH-FLU and fluorescein solution.