Chemical Engineering Journal 266 (2015) 171–178

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Facile preparation of pH-sensitive and self-fluorescent mesoporous silica nanoparticles modified with PAMAM dendrimers for label-free imaging and drug delivery

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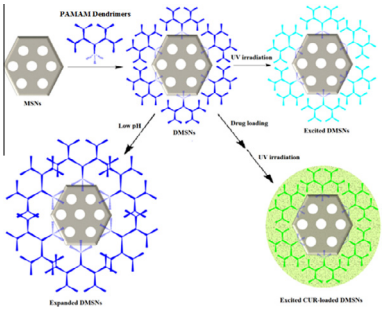
HIGHLIGHTS

- A facile method is used to prepare pH-sensitive and self-fluorescent drug carrier.
- The carrier integrated multifunction into one system.
- The carrier can improve the performance and facilitate the application of CUR.
- PAMAM dendrimers is used as pH-sensitive capping and self-fluorescent agents.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form 9 December 2014
Accepted 18 December 2014
Available online 30 December 2014

Keywords:
Mesoporous silica nanoparticle
Drug delivery
Self-fluorescence
pH-sensitive
PAMAM dendrimers

ABSTRACT

A facile method is described to prepare pH-sensitive and self-fluorescent drug delivery systems using mesoporous silica nanoparticles and PAMAM dendrimers, providing a general route to develop multifunctional and biocompatible platforms, which integrated increasing the number of reaction sites, retaining drug molecules, controlling drug delivery behaviour and fluorescent imaging into one carrier system, for biomedical imaging diagnosis and simultaneous therapy for lesion sites such as cancers.

1. Introduction

Among available nanoparticle-based drug delivery systems, mesoporous silica nanoparticles (MSNs) have been well-demonstrated as one of the most promising and excellent carriers for drug delivery [1–4] because of their interesting properties, such as large surface area and pore volume, high drug loading capacity, easy surface modification, and intrinsic biocompatibility [5]. However, for these carriers, a key challenge is whether they can be designed for biological use through autonomously controlled drug release inside targeted cells in response to external stimuli [6]. To achieve such stimuli–responsive drug release, the outer surface of the MSNs was functioned by sheddable coatings or coatings which can change its conformation upon environmental changes [7]. That basing on the coatings, which can change its conformation upon pH changes, to construct the pH-responsive MSNs can avoid label-missing, premature shedding and drug release. For that, pH-sensitive is used because pH values in different tissues and cellular compartments vary tremendously.

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http://dx.doi.org/10.1016/j.cej.2014.12.075
1385-8947/

Moreover, much effort has focused on developing multifunctional MSNs for in vivo tumour imaging, labelling, and therapeutic use [8]. In order to achieve tumour bioimaging, one of the main approaches is generally achieved by conjugation with fluorescent labels, such as dyes and quantum dots [9]. However, the introduction of fluorescent labels may potentially change the biochemical properties of the molecules of interest and cause potential toxicity to cells [10,11]. Thus, label-free drug carriers are of particular interest [9]. To our best knowledge, the polymer-coating nanoparticle drug carriers, which possess both fluorescence and biocompatibility, are still scarcely reported [12]. Accordingly, it has been a research hotspot to develop stimuli–responsive, label-free and biocompatible mesoporous silica nanoparticles as multifunctional drug carriers in the forefront of materials science.

Thus, here we choose polyamidoamine dendrimers (PAMAM) as the MSNs coating materials to achieve dual-functional carriers with stimuli–responsive and label-free fluorescent behaviour. In recent years, PAMAM dendrimers have been confirmed to possess fluorescence properties [13–16]. Although some reports have studied the ability of PAMAM dendrimers as capping agents of MSNs [17–19], however, as we know, few of researches focus on the pH-sensitive and simultaneous photoluminescent property of the PAMAM dendrimers which can be acted as both smart gatekeepers and safe imaging probes for constructing fluorescent and intelligent MSNs. Furthermore, PAMAM dendrimers have shown promise for biomedical applications because the fact that they can be conjugated with targeting molecules, imaging agents, drugs, and have high water solubility, well-defined chemical structures, and biocompatible [20].

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS) was purchased from Shanghai Kefeng chemical Reagent Co., Inc. (China). Cetyltrimethylammonium bromide (CTAB) and ethylenediamine were purchased from Tianjin Guangfu Fine Chemical Institute (China). 3-Aminopropyltriethoxysilane (APTES) was purchased from Aladdin Chemistry Co., Ltd. (China). Methyl acrylate (MA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Curcumin (CUR) was purchased from Sangon Biotech Co., Ltd. (China). All chemicals were used as received.

2.2. Characterization

FTIR spectroscopy was performed on a Nicolet 670 (USA) IR spectrometer. LR-TEM images were taken using a JEM-1200EX/S (Hitachi, Japan) transmission electron microscope (TEM) operating at an accelerating voltage of 200 kV. HR-TEM images were obtained on a TECNAI G2 TF20 apparatus (FEI, USA). Fluorescence imaging was carried out with Zeiss Leica DM 4000B microscope. The surface area, pore volume and pore size were determined by using a TriStar II 3020 surface area and pore size analyzer (Micromeritics). The elemental analyses were performed on a Vario EL element analyzer (Elementar, Germany).

2.3. Preparation of mesoporous silica nanoparticles (MSNs)

MSNs were prepared as described previously [21]. CTAB (1.00 g) was dissolved in 480 mL of distilled water. Sodium hydroxide (0.28 g) was introduced into the CTAB solution and the temperature of the mixture was adjusted to 80 °C. TEOS (5.3 mL) was added dropwise to the solution under vigorous stirring. The mixture was reacted for 2 h to give rise to a white precipitate. This crude solid product was filtered, washed with pure water and methanol, and dried in air to yield the as-synthesized MSNs. To remove the surfactant template (CTAB), the as-synthesized MSNs was refluxed in ethanol with HCl for 24 h.

2.4. Preparation of triethoxysilane dendrimers

Triethoxysilane polyamidoamine (PAMAM) dendrimers, up to the third generation (G3) were prepared using a modified multistep procedure based on literature [22]. The starting APTES was designated as G0. The synthesis of G0.5 was as follows. APTES and excess MA were added into dry methanol and then sonicated in an ultrasonic water bath at 30 °C under nitrogen atmosphere for 7 h. The excess MA was removed under reduced pressure by repeatedly adding methanol to obtain G0.5 generation. Ethylenediamine methanol solution was added, and then sonicated under nitrogen atmosphere for 7 h. The excess EA was removed under reduced pressure by repeatedly adding methanol to obtain G1. The stepwise growth of dendrimers was repeated until the preferred number of generation (G0, G1, G2 and G3) was achieved using the methyl acrylate and ethylenediamine.

2.5. Preparation of Gn-PAMAM-modified mesoporous silica nanoparticles (n = 0, 1, 2 and 3)

MSNs were suspended in anhydrous N,N-dimethylformamide (DMF) by ultrasonic water bath. G*n*-PAMAM solution in DMF was added into the suspension. The mixture was refluxed for 12 h under nitrogen atmosphere. G*n*-PAMAM-modified MSNs were collected by centrifugation (12,000 rpm, 6 min), washed with distilled water and methanol, and finally dried under vacuum.

2.6. Drug loading and release experiments

PAMAM-modified MSNs samples (100 mg) were added to an anhydrous ethanol solution (10 mL) containing CUR (7.0 mg/mL), and sonicated to maximize the nanoparticle dispersion. After magnetic stirring for 24 h in the dark at room temperature, CUR-loaded PAMAM-modified MSNs were obtained by centrifugation, washed extensively with ethanol, and dried under vacuum. The loading percentage of CUR was estimated through fluorescent measurements by subtracting the amount of CUR in the collected supernatant from the total amount CUR added.

To measure in vitro CUR release, 10 mg of samples was suspended in 5 mL PBS (pH = 5.0 or 7.4) in the dialysis membrane bag (MW cut-off of 3500) and the bag was immersed in 15 mL PBS containing 10% ethanol (v/v) and shaken at a speed of 100 rpm at 37 °C. The amount of CUR release was determined by fluorescence intensity at different time intervals over a period of 24 h.

2.7. Cytotoxicity assays

The in vitro cytotoxicity of the G3-MSNs was assessed on HeLa cells by using the MTT assay. HeLa cells were seeded in a 96-well plate with a density of 10,000 cells/well. After culturing 12 h, samples at the indicated concentrations were added and the cells were further incubated for 48 h. Then, MTT solution was added to the cell cultures and maintained at 37 °C for additional 4 h. Subsequently, the cells were lysed by the addition of DMSO (100 μL). Absorbance values of each well were measured by using a microplate reader (Tecan, Mannedorf, Switzerland) at 490 nm.