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Optimization of MCM-41 type silica nanoparticles for biological applications: Control of size and absence of aggregation and cell cytotoxicity

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ABSTRACT

Mesoporous silica nanoparticles were synthesized at high pH using CTAB as a template and TEOS as a silica precursor. It was shown that varying the NaOH concentration between 5 and 27.5 mM allows the size, pore and silica structure of mesoporous nanoparticles to be precisely tuned. In particular, monodisperse nanoparticles with the MCM-41 structure with size ranging from 90 nm to 450 nm were obtained by increasing the NaOH concentration from 12.5 to 22.5 mM. It thus demonstrates that NaOH concentration must range between 12.5 and 15 mM in order to prepare MCM-41 silica nanoparticles with optimal size for nanovectorization. We also found that under usual conditions the aggregation of the obtained MSNs was due to the presence of carbonates and ethanol in the reaction mixture. Careful elimination of these species by using carbonate-free NaOH and purging with a N_2 flow allows a highly stable suspension of non-aggregated MSN–MCM-41 to be obtained. After a complete CTAB extraction which was confirmed by Raman spectroscopy, cytotoxicity assays carried out in SW480 cancer cells show that these nanoparticles are not toxic up to 100 μg/mL.

1. Introduction

Over the past decades, numerous nanometer-sized delivery platforms (3–200 nm) have been developed to increase the efficiency of anticancer drugs. Mainly organic systems such as polymeric nanoparticles, micelles, dendrimers or liposomes [1–6] were considered in the literature. These nanovectors are delivered using passive or active targeting strategies and are used to protect the drugs from the biological environment or to overcome cellular drug resistance or to deliver poorly water soluble drugs [7–9]. Much attention has also been paid to mesoporous silica nanoparticles (MSNs) as drug-delivery systems during the last few years [10]. Indeed, these inorganic nanomaterials possess unique physico-chemical properties such as biocompatibility, ordered pore network, tunable pore size (2 nm to 10 nm), high pore volume (~1 cm^3/g), and high surface area (>800 m^2/g) suitable for biomedical applications [11–15]. Moreover, the functionalization inside or outside the pore channels makes these nanomaterials ideal candidates for biomedical applications both in diagnostics and in photodynamic therapy [16,17].

To deliver the drug-loaded nanoparticles in the tumor cells via the enhanced permeability and retention (EPR) effect, the size of the nanovectors should be precisely controlled between 60 and 150 nm to avoid rapid clearance from the bloodstream with subsequent accumulation in non-targeted organs [18–20]. It implies that the nanoparticles must not be aggregated directly after the synthesis to allow an efficient in situ post-functionalization which is essential for rational design of MSNs for biomedical applications.

Grün et al. were the first to report the synthesis of polydispersed silica nanoparticles with a MCM-41 porous structure by combining the sol–gel chemistry of silica in basic conditions and the self-association properties of surfactants [21–23]. Thereafter, Cai et al. reported the synthesis of monodispersed MCM-41 nanospheres with about 100 nm in size by using an extremely dilute cationic surfactant solution and NaOH as the catalyst [24,25]. At the same time, Fowler et al. developed a versatile approach where a stable suspension of nanoparticles with adjustable sizes ranging from 20 to 100 nm was prepared by dilution quenching and subsequent neutralization of the standard reaction mixture at highly basic conditions [26]. However, this high dilution method led to low yields and difficulty in product collection. In order to increase the yield, Möller et al. replaced the usual NaOH base by polyalcohol triethanolamine (TEA) [27]. By varying the synthesis time and temperature, they were able to obtain non-agglomerated particles with size ranging from 50 to 100 nm. However, these particles were not highly ordered that prevented a fine control of the drug loading and release kinetics. More recently, the particle size of MSNs was adjusted in mild pH conditions using different pH buffers that control the hydrolysis and condensation kinetics [28]. Yu et al. also synthesized monodisperse mesoporous silica nanoparticles in the presence of acetate buffer [29].

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The particle size was adjusted by the reaction temperature and the pore size by a heat post-treatment. The particles were redispersible even if calcination was used to remove the template. Unfortunately, these particles did not reveal ordered structure.

Despite all these recent developments, the method proposed by Cai et al. remains the most frequently used strategy due to its ease of implementation. However, the influence of NaOH in a narrow range of concentration and the effect of the synthesis atmosphere on the physicochemical properties of the MSNs were not described in details.

The aim of this paper is to present the best conditions to prepare mesoporous silica nanoparticles suitable for nanovectorization i.e. nanoparticles with a size between 60 and 150 nm and an ordered porosity, stable nanoparticles in water suspension allowing further biofunctionalization, and non-toxic nanoparticles.

2. Experimental

2.1. Materials

N-cetyltrimethylammonium bromide (C_{16}TAB ≥ 99.0%), standard solution of sodium hydroxide (2 M NaOH) and tetraethyl orthosilicate (TEOS ≥ 99.0%) were purchased from Sigma-Aldrich. Ammonium nitrate (NH_4NO_3 ≥ 99.0%) was provided by Fisher Chemicals. All the chemicals were used as received.

2.2. Synthesis of mesoporous silica nanoparticles

Mesoporous silica nanoparticles (MSNs) were synthesized following the procedure reported by Cai et al. [24,25] with slight modifications. In a typical synthesis, 416.6 mg of C_{16}TAB and 200 mL of ultrapure water (18 MΩ·cm) were mixed in a 500 mL four neck round bottom flask. After adding 1.51 mL of 2 M NaOH standard solution to the C_{16}TAB solution, the mixture was heated at 343 K for 2 h and stirred at 700 rpm. TEOS (2.01 mL) was then added dropwise (1 $mL \cdot min^{-1}$) and the mixture was stirred for another 2 h at 343 K. The molar composition of the suspension was TEOS/NaOH/C_{16}TAB/H_2O = 1.0:0.34:0.127:1235. To investigate the effect of NaOH on the size, the morphology and the mesostructure of the particles, the base concentration was varied between 5 mM and 27.5 mM with steps of 2.5 mM. The suspension was diluted twofold with water and washed in deionized water and/or ethanol using an ultrafiltration system (Millipore, 30 kDa). Ethanol was then slowly removed by rotary evaporation at 353 K. The powder was calcined in air (air flow = 0.5 $L \cdot min^{-1}$) at 823 K for 8 h (heating rate = 1 $K \cdot min^{-1}$).

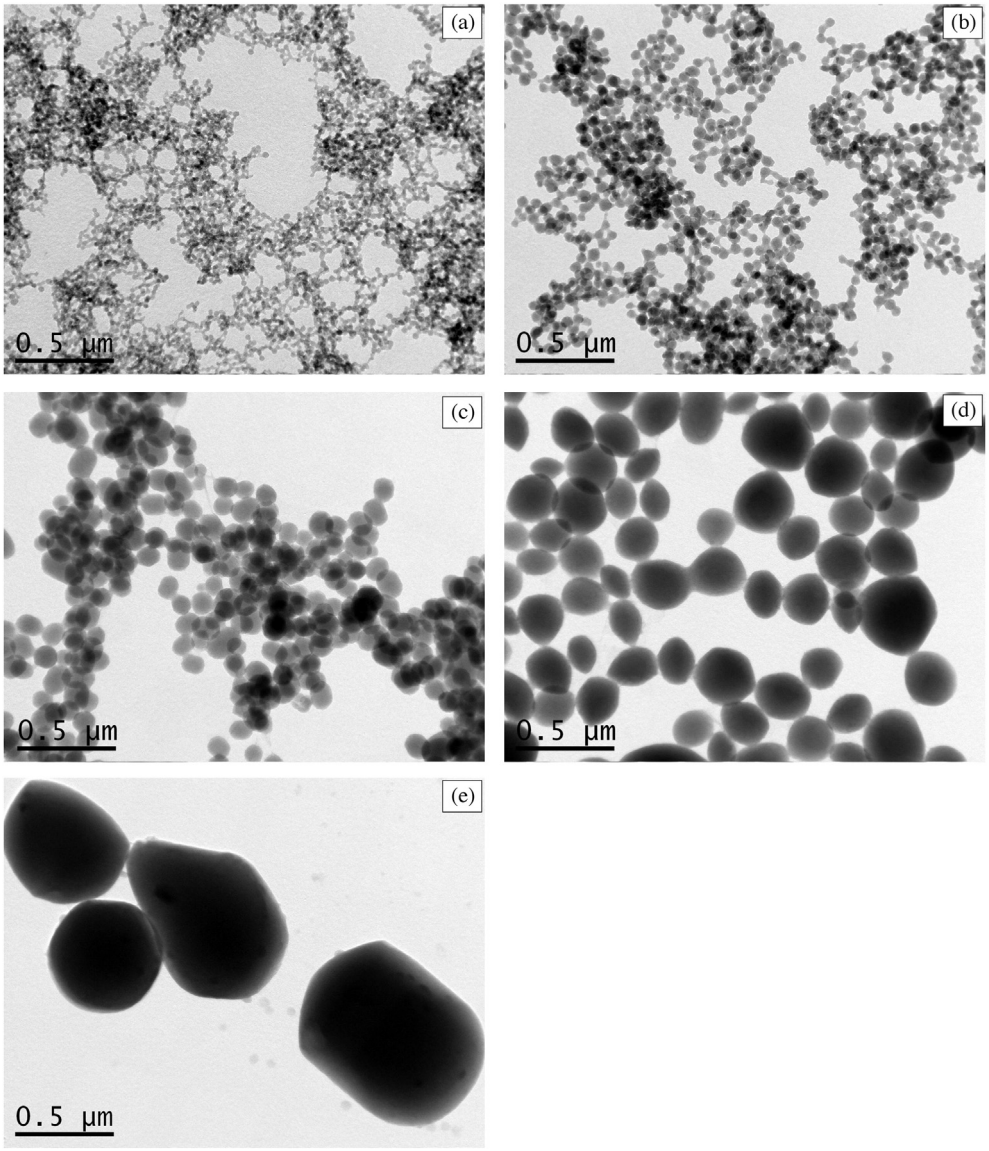

Fig. 1. TEM images of as-prepared MSNs with different NaOH concentrations: a) 5 mM, b) 10 mM, c) 15 mM, d) 20 mM, and e) 25 mM.