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Microemulsion Assisted Sol-Gel Method as Approach to Load a Model Anticancer Drug inside Silica Nanoparticles for Controlled Release Applications

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ABSTRACT

Silica nanoparticles are attractive carriers due to their improved safety and effectiveness in drug delivery. Silica nanoparticles were synthesized by using microemulsion assisted sol-gel method, and a model anticancer drug 5-fluorouracil (5-FU) was added to the silica precursor before hydrolysis and condensation reactions start. The obtained materials were characterized by Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). Drug encapsulation within silica nanoparticles causes an increase in particle size. However, particle morphology is not affected. The drug release profile was obtained through high performance liquid chromatography (HPLC). The encapsulation approach showed to be effective for sustaining a continuous and increasing release during testing time (98 h). Further studies were performed to evaluate the cytotoxic effects of silica nanoparticles with loaded 5-FU on Chinese hamster ovary cells (CHO-K1). Materials are non-cytotoxic for all concentration tested (5–200 μg/mL).

1. Introduction

Anticancer drug administration faced many challenges such as inadequate biological half-life of drugs and side effects, among others. New technological tools are intended to direct drugs to their site of action, maintaining their concentrations over time and over the necessary therapeutic levels [1–3]. In this vein, encapsulation of pharmacologically active ingredients in nanosized particles is a topic of increasing interest for research [4–10]. Nanosized encapsulation not only provides decreasing lethal side effects, it also offers prolonged activity and protection toward enzyme attacks or acidic degradation [11–14]. By encapsulating a drug into nanosized particles, the drug can be positioned in specific areas of the body, reducing their unspecific distribution and providing the required dosage to the desired effect. The drug is not administered directly as it is included inside nanoparticles that release it in small amounts continuously.

Silica nanoparticles are a good choice for encapsulating drugs due to its high thermal and chemical stability in aqueous suspensions, large surface area, and chemical inertness [15–17]. Moreover, they have attractive physical and chemical properties such as transparency and surface functionalization capability [15]. Due to the electrostatic stabilization, silica surface promotes nanoparticles dispersion in aqueous solution which makes it suitable for testing at a biological level. Traditionally, there are two main approaches for loading organic molecules (e.g., dyes) into silica nanoparticles that could be extended to the case of drug encapsulation: the first one involves covalent binding of the molecule to the silica network, this concept might sometimes increase considerably the difficulty of preparation steps [18]. Another approach is based on non-bonding process such as electrostatic interactions or entrapment within siloxane matrix, this approach represents a promising way and more attention should be paid to its investigation since it has low-cost and does not emphasize the limitation of functionalization [18]. According to the traditional sol-gel method, the incorporation of molecules into silica nanoparticles under non-covalent

Abbreviations: XRD, X-ray Diffraction; FTIR, Fourier Transform Infrared Spectroscopy; TEM, Transmission Electron Microscopy; 5-FU, 5 Fluorouracil; PBS, phosphate buffer; HPLC, high performance liquid chromatography; TEOS, Tetraethylorthosilicate

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bonding is poor and dependent of the absorption force between the molecule itself and the silica precursor [18]. However, a microemulsion process is a plausible route to avoid these drawbacks, since reverse micelles can control the quantity of incorporated molecules into silica nanoparticles.

5-FU is a highly effective anticancer drug which, in combination with others, has been widely used in the clinical treatment of many solid tumors such as breast and colorectal, among others [19]. Usually, drugs work by damaging the RNA or DNA that tells the cell how to copy itself in division. Unfortunately, the biological half-life of 5-FU is rather short, lying in the range of 10–20 min [20,21]. In order to maintain the concentration level for a sufficient time, the drug is often administered repeatedly, which could induce serious side effects [20,21]. Considering the possibility of future contributions to the development of more suitable anticancer drug release, we explore in this work the feasibility of microemulsion assisted sol-gel method as a strategy to load a model anticancer drug (5-FU) inside silica nanoparticles. Specifically, 5-FU is added to silica precursor before hydrolysis and condensation reactions start and, the as-prepared silica nanoparticles with loaded 5-FU are then studied by using different characterization techniques. Details are revealed on how the drug is encapsulated and released to a physiological media. Cytotoxicity data for silica nanoparticles are also presented.

2. Materials and Methods

2.1. Synthesis of Silica Nanoparticles and Drug Encapsulation

The synthesis of silica nanoparticles was carried out using sol-gel method assisted by reverse micelle microemulsion, using tetraethylorthosilicate (TEOS, Merck) as silica precursor, Triton X-100 (Sigma-Aldrich) as surfactant, Methanol (Merck, 98%) as co-surfactant, and cyclohexane (Carlo Erba, 99.5%) as oil phase. Preparation of silica nanoparticles initially involves the formation of a water in oil (w/O) microemulsion (Fig. 1). The molar ratio Triton x-100/Ciclohexano/Methanol used was 1/7.6/24.7.

The next step in encapsulation procedure was the addition of 5-Fluorouracil (5-FU) to the previously formed w/O microemulsion (Fig. 1). Right after the drug is incorporated, the silica precursor (TEOS) was added and hydrolysis and condensation reactions leading to drug encapsulated silica particles were performed for 2 h. This step is critical for nanoparticle shape and sizes so that some molar ratios such as water to TEOS (h), methanol to triton X-100 (ρ), and water to triton X-100 (R) must be controlled carefully in the colloidal solution as was explained in a previous work [22]. Briefly, by adjusting h, ρ and R to 59.1, 4.5 and 9.2 respectively, spherical silica particles in the nanometer range are suitably obtained. Finally, the microemulsion was broken with ethanol, washed and centrifuged in order to remove surfactant traces.

The characterization of silica particles loaded with 5- FU was performed by using FTIR (Shimadzu IRTracer-100) and TEM techniques (Tecnai G2 F20 FEI instrument). The average size of nanoparticles is reported in terms of statistical analysis by digital image analysis of TEM microscopy. Controlled experiments were carried out to obtain the corresponding drug release profiles. Tests were conducted in phosphate buffer solution (PBS) thermostated at 37 °C ± 0.1. Nanoparticles were initially dispersed in PBS (pH = 7.4) to 0.49 mg/mL (in terms of drug content). Small aliquots were taken from the PBS media each 24 h for 96 h, and the samples were analyzed by using HPLC analysis (Shimadzu Nexera UHPLC System).

2.2. Cell Culture and Treatment

CHO-K1 cells were propagated in RPMI 1640 medium (GIBCO) supplemented with 5% FBS (GIBCO). Cells were cultured in T25 culture flasks (BD Falcon) and incubated at 37 °C in a humid atmosphere (> 95%) and 5% CO_2 (standard conditions). After CHO-K1 cells grew to the expected concentration, they were harvested by trypsinizing the cell with 0.025% trypsin/EDTA to exponential growth between 36 and 48 h post seeding, and incubated at 37 °C for 5 min to obtain complete cell detachment. Cell suspension was centrifuged at 4000 rpm for 10 min, cell pellets were resuspended with complete medium. Cell suspensions were treated with different concentration of nanoparticles (5, 10, 15, 20, 25, 50, 75, 100, 200 μg/mL) and added separately into a 96-well plate. Finally, treated cells were incubated for 48 h with 5% CO_2 at 37 °C for cytotoxicity evaluation.

2.3. Cytotoxicity

Cytotoxic effects of silica nanoparticles loaded with 5-FU were assessed using MTT assay. For each exposure concentration described in the section above, four replicates were examined twice per plate. Four hours before the setting time of 48 h compliance, 20 μL of MTT reagent (5 mg/mL) were added maintaining incubation conditions described in the section above. At the end of the incubation period, 100 μL of

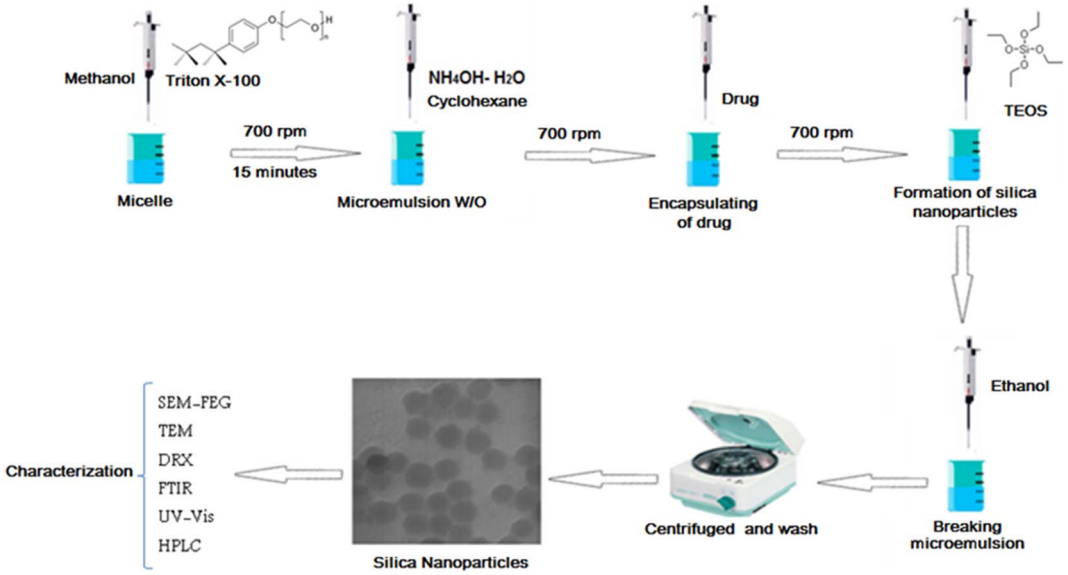

Fig. 1. Encapsulation procedure scheme of 5-fluorouracil in silica nanoparticles.