Journal of Colloid and Interface Science 456 (2015) 59–65

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Organically modified titania nanoparticles for sustained drug release applications

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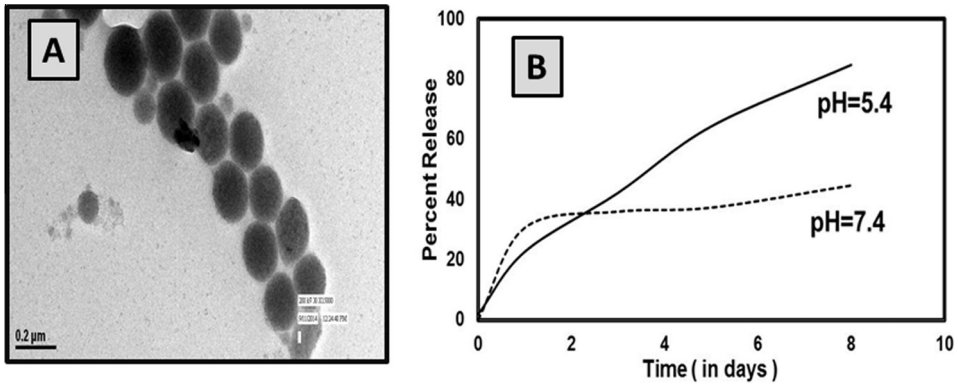
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 April 2015
Accepted 3 June 2015
Available online 15 June 2015

Keywords:
Oil-in-water microemulsion
Organically modified titania nanoparticles
Porosity
Colloidal stability
Drug release

ABSTRACT

In this paper, we report the synthesis, characterization of drug-doped organically modified titania nanoparticles, and their applications in sustained drug release. The drug-doped nanoparticles were synthesized in the hydrophobic core of oil-in-water microemulsion medium. Structural aspects obtained through TEM and FESEM depicted that organically modified titania nanoparticles are monodispersed with spherical morphology, with an average size of around 200 nm. Their polymorphic forms and porosity were determined using powder XRD and BET, respectively, which showed that they are present in the anatase form, with a surface area of 136.5 m^2/g and pore-diameter of 5.23 nm. After synthesis and basic structural characterizations, optical properties were studied for both fluorophore and drug encapsulated nanoparticles. The results showed that though the optical properties of the fluorophore are partially diminished upon nanoencapsulation, it became more stable against chemical quenching. The nanoparticles showed pH-dependent drug release pattern. In vitro studies showed that the nanoparticles were efficiently uptaken by cells. Cell viability assay results showed that though the placebo nanoparticles are non-cytotoxic, the drug-doped nanoparticles show drug-induced toxicity. Therefore, such porous nanoparticles can be used in non-toxic drug delivery applications.

1. Introduction

Inorganic-based nanoparticles have emerged as promising candidates for various biomedical applications. Their structural robustness, resistance to microbial attack, non-immunogenicity,

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http://dx.doi.org/10.1016/j.jcis.2015.06.006
0021-9797/

unique response to external stimulus such as light or magnetic field, etc., makes them very important for biomedical applications, mainly as diagnostic probes [1–3]. However, these materials are often structurally rigid and colloidally unstable, thus being poorly suited for drug-delivery applications. These shortcomings can be overcome with the development of composite or hybrid materials, whereby organic/polymeric components are introduced in the inorganic matrix to generate materials with advanced properties [4,5]. The presence of organic/polymeric constituents in the inorganic matrix imparts flexibility, porosity, as well as their ability to host bioactive molecules and their subsequent release. Such hybrid materials can be designed using two major synthetic approaches. In one approach organic molecules can be just embedded into an inorganic material and *vice versa*. The other approach involves nanoparticle synthesis from hybrid precursors containing both organic/polymeric and inorganic components [3–5].

Among the various inorganic nanoparticles, titanium dioxide, or titania (TiO_2) nanoparticles are popular owing to their structural stability, non-immunogenicity, ease of scaling up, and unique photoresponsive properties [6–8]. Titanium dioxide is mostly explored in the field of photocatalysis, photodegradation, photovoltaics, etc., owing to its efficient optical absorption, low price and chemical stability [9–15]. Several recent studies have shown that these nanomaterials are biocompatible [16] and environment friendly [17,18]. This material can be amorphous, as well as exhibit polymorphism and exist in three different crystalline forms: anatase, brookite, and rutile [19]. Nano-TiO_2 is used in paints and coatings as self-cleaning, antimicrobial, and antifouling agents and in cosmetics as a UV-absorber [20]. In addition to physical applications, some biomedical applications of TiO_2 nanoparticles are also cited in literature. For example, Fujishima et al. first time reported the photoactive killing of HeLa cells by using titanium oxide film electrode [21–23]. Since then, a number of studies related to the use of doped TiO_2 nanoparticles for DNA delivery, diagnostic imaging, and biosensors have been reported in literature [24–29]. Mesoporous TiO_2 whiskers and nanoparticles have also been synthesized for encapsulation and release of drug molecules [30,31]. However, most of the nanoparticles reported for drug delivery large in size (average diameter more than 350 nm). Also, these nanoparticles required surface coating with polymeric agents to maintain stable colloidal dispersion [32].

Fabrication of titania-based nanoparticles with well defined porosity and improved colloidal stability is a synthetic challenge. To address this challenge, we have designed monodispersed nanoparticles of organically-modified titania (ORTM), using the hydrolysis of the organometallic precursor cyclopentadienyl (IV) titanium trichloride, in the oil-in-water microemulsion (AOT/water/butanol) system. The lipophilic fluorophore nile red (NR) or the anticancer drug doxorubicin (Dox) were used as 'guest' molecules in order to probe the encapsulation and release of drugs from these nanoparticles. The nanoparticles were characterized for their physical properties, such as size, porosity, composition, and optical properties. Doped ORTM was found to display pH-dependent controlled release, making it a perfect candidate for drug delivery. Simultaneously it was seen to have significant cellular uptake and appreciable drug-induced toxicity from the released drug.

2. Materials and methods

2.1. Materials

Oil-in-water microemulsion was prepared using AOT (Acros) as surfactant and butanol (Merck) as co-surfactant. Cyclopentadienyl titanium trichloride ($CpTiCl_3$) (Sigma Aldrich) precursor was used for the synthesis of nanoparticles. Solvents like Dimethyl sulphoxide (DMSO) (Merck) and ethanol (Merck) were of AR grade and used without any further purification. Glacial acetic acid was purchased from Rankem. L-Ascorbic acid, sodium bisulphite and sodium hydroxide were procured from Thomas Baker, Sigma Aldrich and Merck, respectively. Organic fluorophore nile red (NR), drug doxorubicin hydrochloride (Dox) and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reagent were obtained from Sigma Aldrich. The lung carcinoma cell line (A549) was purchased from ATCC, VA, and cultured according to instructions supplied by the vendor. Cell culture reagents, phosphate buffer saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Amphotericin-B (antifungal), Penicillin Streptomycin (antibiotic) and Trypsin were obtained from Thermo Fischer scientific. Double distilled water was used to carry out all experiments.

2.2. Microemulsion mediated synthesis of ORTM nanoparticles in oil-in-water microemulsion system

Oil-in-water microemulsion was prepared using AOT/water/BuOH system, where AOT was used as a surfactant and n-BuOH as a co-surfactant. The details of the synthetic procedure are given below:

AOT (0.44 g) was added in 20 mL of double distilled water and magnetically stirred for 20 min. To the resulting turbid aqueous mixture, 800 μL of butanol was added and was magnetically stirred until a clear microemulsion solution was obtained. To the prepared microemulsion, 1 mL of cyclopentadienyl titanium trichloride in DMSO (45.6 mM) was added and the mixture obtained was left for overnight stirring at 30 °C. After overnight stirring, yellowish turbid solution was obtained. The solution obtained was dialysed using 12 kD dialysis membranes obtained from Sigma Aldrich in double distilled water to remove DMSO, AOT, and other unreacted ingredients from the solution. A similar procedure has been used for the synthesis of NR or Dox-encapsulated nanoparticles, except that prior to the addition of precursor, 14 μL of NR solution in DMSO (15.70 mM) or Dox solution in DMSO (1.724 mM) was mixed.

2.3. Characterization of nanoparticles, with and without encapsulated Dox

The physical aspects of the nanoparticles were probed by a number of characterization techniques, such as TEM, FESEM, SAED, powder XRD, and FTIR. A drop of the aqueous dispersion of nanoparticles was added on a formvar coated 200 mesh copper grid and was dried at room temperature. Then nanoparticles were examined using transmission electron microscopy (TEM) using a TECNAI G2 instrument, operating at a 300 kV. The crystalline diffraction pattern (selected area electron diffraction, or SAED) and elemental composition of the sample EDX (Energy Dispersive Spectra), was also obtained using the same instrument. Moreover, a finely powdered sample of nanoparticles were placed over the sample holder for gold plating and then was scanned using MIRA3 TESCAN instrument of 20.0 kV for obtaining field emission scanning electron microscopic (FESEM) images. The hydrodynamic size of the prepared nanoparticles was resolved using dynamic light scattering experiment (DLS) In this dialysed sample was taken in a glass cuvette and was studied using NANO-ZS series MALVERN ZETASIZER instrument. The light source was a He–Ne laser having power of 4 mW and wavelength equal to 633 nm. A graph was plotted as a function of average hydrodynamic size distribution of nanoparticles. Next, high resolution X-ray diffraction (HRXRD) analysis was carried on powdered nanoparticles using a Brukar D8 Discover X-ray spectrometer,