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Sonochemically synthesized biocompatible zirconium phosphate nanoparticles for pH sensitive drug delivery application

Himani Kalita [a], B.N. Prashanth Kumar [b], Suraj Konar [a], Sangeeta Tantubay [a], Madhusudan Kr. Mahto [a], Mahitosh Mandal [b], Amita Pathak [a,*]

[a] Department of Chemistry, Indian Institute of Technology Kharagpur, West Bengal 721302, India
[b] School of Medical Science and Technology, Indian Institute of Technology Kharagpur, West Bengal 721302, India

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ABSTRACT

The present work reports the synthesis of biocompatible zirconium phosphate (ZP) nanoparticles as nanocarrier for drug delivery application. The ZP nanoparticles were synthesized via a simple sonochemical method in the presence of cetyltrimethylammonium bromide and their efficacy for the delivery of drugs has been tested through various in-vitro experiments. The particle size and BET surface area of the nanoparticles were found to be ~48 nm and 206.51 m^2/g respectively. The conventional MTT assay and cellular localization studies of the particles, performed on MDA-MB-231 cell lines, demonstrate their excellent biocompatibility and cellular internalization behavior. The loading of curcumin, an antitumor drug, onto the ZP nanoparticles shows the rapid drug uptake ability of the particles, while the drug release study, performed at two different pH values (at 7.4 and 5) depicts pH sensitive release-profile. The MTT assay and cellular localization studies revealed higher cellular inhibition and better bioavailability of the nanoformulated curcumin compared to free curcumin.

1. Introduction

Over the last few decades, the development of nanoparticle assisted drug delivery systems that can circumvent the toxic effects of drug over dose and overcome the limitations of inadequate drug concentration at the affected sites has gained a surge of interest in the area of controlled delivery of drugs. Some of the materials that have gained attention in this area include zirconia [1–2], hydroxyapatite [3–5], titania [6–9], silica [10–13], zirconium phosphate [14–15], magnetite [16–20] etc. Among the materials, zirconium phosphate (ZP) is one of the most promising candidate for drug delivery application owing to its excellent biocompatibility, high thermal stability, wide availability and good chemical as well as biological inertness. Additionally, ZP is reported to dissociate into phosphate ions and non-toxic zirconium salts in the presence of lysosome and peroxisomes in the biological systems [21], which can be easily absorbed or eliminated from the body. In fact, layered ZPs (α-ZP and θ-ZP) in the form of nanoplatelets and nanosheets have already been reported as carriers for the delivery of drugs [14–15,21] and hormones [22]. They are also reported for the immobilization of enzymes [23] and binding of proteins [24]. However, almost no literature report is available on the usage of crystalline nanoparticles of ZP as nanocarrier for the delivery of drug molecules till date, to the best of our knowledge. This is despite the fact that the crystalline nanoparticles of ZP, by virtue of their small size and high surface to volume ratio, are likely to act as an efficient nanocarrier for drug delivery. Moreover, there are only few reports available on the synthesis of ZP nanoparticles (NPs) which includes the solvothermal [25], chemical precipitation [26] and water-in-oil microemulsion routes [27].

The aim of the present study is, therefore, focused on establishing a simple methodology for the preparation of ZP NPs and to test their efficacy as nanocarrier for drug delivery application. The ZP NPs were synthesized via a simple sonochemical approach at ambient temperature (25 ± 1 °C) using zirconium oxychloride and orthophosphoric acid as the precursors for zirconium and phosphate respectively in the presence of cetyltrimethylammonium bromide (CTAB). The suitability of the prepared particles as nanocarrier for the delivery of drugs has been tested by the conventional MTT assay and cellular uptake studies using MDA-MB-231 cell lines. The drug loading and releasing efficiency of the NPs has been studied through in-vitro experiments using curcumin ((1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) as the model drug, which is reported to exhibit anti-inflammatory, antioxidant and anti-tumor properties. The cytotoxicity and cellular internalization behavior of the nanoformulated curcumin was also tested through MTT assay and cellular uptake studies using MDA-MB-231 cell lines.

* Corresponding author.
E-mail addresses: hkalita74@gmail.com (H. Kalita), prasanthkumar999@gmail.com (B.N. Prashanth Kumar), suraj.konar@gmail.com (S. Konar), sang.chem2@gmail.com (S. Tantubay), mahtomk0@gmail.com (M. Kr. Mahto), mahitosh@smst.iitkgp.ernet.in (M. Mandal), ami@chem.iitkgp.ernet.in (A. Pathak).

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2. Experimental

2.1. Materials

Zirconium oxychloride octahydrate was purchased from S.D. Fine-Chem Limited (India), while orthophosphoric acid was obtained from Merck (India). Cetyltrimethylammonium bromide (CTAB) was bought from Spectrochem Private Limited (India). (3-Aminopropyl) triethoxysilane (APTES) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich, while rhodamine B (Rh-B) was purchased from Loba Chemie Private Limited (India). N-Ethyl-N′-(3-Dimethylaminopropyl) N′-ethylcarbodiimide Hydrochloride (EDC), N-Hydroxysuccinimide (NHS) and curcumin (CUR) were procured from SRL (India). Human breast cancer cell lines MDA-MB-231 were obtained from the National Center for Cell Science (NCCS), Pune (India). Milli-Q water and absolute ethanol were used throughout the study.

2.2. Synthesis of zirconium phosphate nanoparticles

For the synthesis of zirconium phosphate nanoparticles (ZP NPs), zirconium oxychloride (1 mmol) was initially added to an aqueous solution of CTAB (0.1 mmol) followed by the addition of o-phosphoric acid (2 mmol). The mixture was then sonicated using a probe sonicator for 2 h with pulse on and off mode for 5 and 1 s respectively. The product was collected, washed three times with milli-Q water and dried at 70 °C for 12 h. Finally, the dried white mass was calcined at 700 °C for 4 h to remove the CTAB molecules.

2.3. Synthesis of amine functionalized and fluorescent-labeled ZP NPs

To optically monitor the cellular internalization process, fluorescent Rh-B moieties were attached to the ZP NPs. The ZP NPs were initially surface functionalized with APTES, thereby rendering amine functionalities on their surface to which Rh-B was covalently attached via amide linkage with the aid of EDC–NHS coupling (Fig. 1).

Briefly, 30 mg ZP NPs were added to 30 mL APTES in ethanol and stirred for 6 h. The residue was collected, washed three times with ethanol to remove excess APTES and finally dried at 50 °C for 4 h to obtain the powder of amine functionalized ZP NPs (ZP–APTES). For the conjugation of fluorescent Rh-B to the amine functionalized ZP NPs, 1 mg Rh-B was dissolved in 5 mL water followed by the addition of required amount of EDC and NHS. The solution was stirred for 6 h and then ZP–APTES was added to it. After 6 h of stirring, the pinkish mass was collected, washed with water and dried at 60 °C for 10 h to obtain the powder of ZP–APTES-RhB.

2.4. Drug loading and releasing studies

For drug (curcumin) loading studies, an ethanolic solution of curcumin (200 μg/mL, pH 7) was prepared and ZP NPs were added to it. The solution was stirred for 2 h in absence of light at 25 °C. The yellowish deposits were collected by centrifugation, washed three times with ethanol to remove unbound curcumin and then dried at 50 °C for 5 h to get the curcumin loaded ZP NPs (ZP-CUR). The drug encapsulation efficiency (EE) of the ZP NPs at different time intervals was determined spectrophotometrically at λ_{max} value of 425 nm, using Eq. (1).

$$\mathrm{EE}(\%) = \frac{\text{Total curcumin} - \text{curcumin in supernatant}}{\text{Total curcumin}} \times 100\%. \quad (1)$$

The in-vitro drug release profile of the curcumin loaded ZP NPs (ZP-CUR) was determined spectrophotometrically at pH values of 7.4 and 5 by dialysis membrane method. Briefly, ZP-CUR was dispersed in distilled water, placed in dialysis bag with a molecular cut off of 14 kDa and then suspended in ethanol (maintained at pH 7.4 and pH 5) as the release medium at 37 °C. Due to the poor solubility of curcumin in water, ethanol was used as the release medium to provide sink conditions [28] and pH was maintained at 7.4 and 5 to mimic the physiological pH of blood and the acidic intracellular environment in tumor cells respectively [29]. To determine the drug release profile, requisite volume of the released medium was taken at appropriate time intervals and spectrophotometrically analyzed at λ_{max} value of 425 nm. The amount of drug released was calculated using Eq. (2).

$$\text{Drug released}(\%) = \frac{\text{Curcumin released}}{\text{Total curcumin}} \times 100\%. \quad (2)$$

2.5. Cytotoxicity studies

The cytotoxic effect of ZP NPs on MDA-MB-231 cells was assessed through MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. For the study, the cells were trypsinized, seeded in 96-well plate and allowed to grow for 24 h. Then the cells were treated with varying concentrations of the ZP NPs (0–250 μg/mL) and incubated for 48 h. After that, the nanoparticle containing medium was replaced with MTT (1 mg/mL) solution and further incubated for 4 h, following

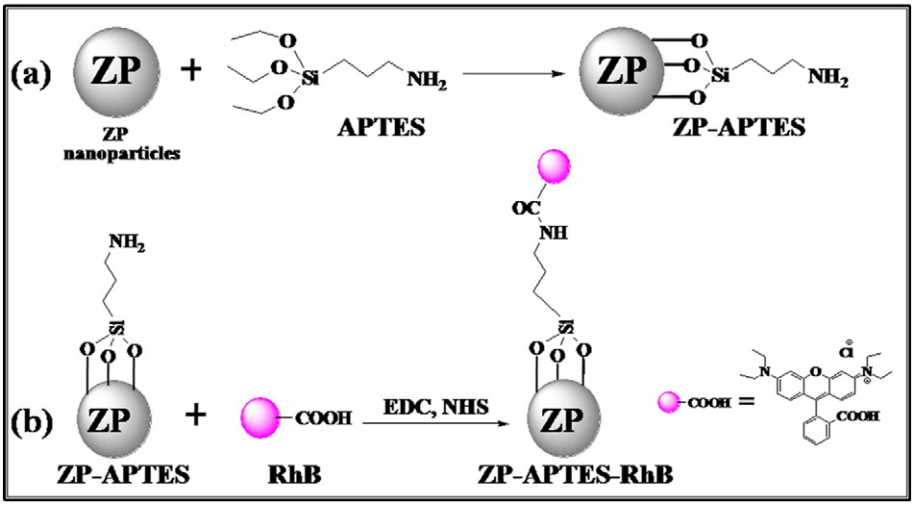

Fig. 1. Schematic representation for the synthesis of amine functionalized ZP NPs (a), and fluorescent ZP NPs (b).