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Short communication

Hydrothermal synthesis of anatase titanium dioxide mesoporous microspheres and their antimicrobial activity

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ABSTRACT

We report here the synthesis of high surface area mesoporous microspheres of anatase titanium dioxide via nanoparticle assembly in a simple one pot hydrothermal reaction. The same procedure is applied to synthesize Fe-doped titanium dioxide mesoporous microspheres. The surface areas of the microspheres are as high as 283 $m^2 g^{-1}$. The antimicrobial activity of these microspheres is investigated against a broad range of Gram-negative and Gram-positive bacteria. The Fe-doped titanium dioxide microspheres exhibited improved antimicrobial activity against *Mycobacterium smegmatis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis*.

1. Introduction

Titanium dioxide (TiO_2) is a widely investigated inorganic semiconductor over the decades [1,2]. It emerges as a prospective material for application in two important domains namely energy and environment because of its high chemical stability, photoactivity, biocompatibility, low cost and ease in processing. Several notable and pioneering research groups across the globe have demonstrated the importance of TiO_2 in energy domain such as solar cells, batteries, water splitting, electrochromics and hydrogen storage [2–8]. It has also far greater impact in environment domain as biocidal agent, pollutants remover from air and water [1]. Investigation shows that pristine TiO_2 demonstrates excellent photocatalytic and antibacterial properties [9–21]. Further, these properties can be significantly improved by tuning the surface chemistry of TiO_2. For example, surface modified TiO_2 by Ag, Au, Pt, Cu, chitosan nanoparticles showed better activity than pristine TiO_2 [9–21]. However, these multi component systems though exhibit enhanced activity, complexity involves while integrating the constituent components into a single system. Therefore, the challenge is to design single component systems which yield efficient activity.

An effective approach in achieving single component photocatalytic and antibacterial agents would be via doping the TiO_2 crystallites. It is because the physicochemical properties of TiO_2 change dramatically due to doping [22–27]. Few important examples are mentioned. N doping on the anion sublattice of TiO_2 resulted in band gap narrowing and, therefore, improved photocatalytic activity under visible light [25]. Enhanced photochemical water splitting was observed for C doped TiO_2 which showed lower band-gap energy than rutile (2.32 *vs* 3.00 eV) [26]. Nb and N codoping help achieving the band gap of anatase TiO_2 from 3.2 eV to 2.0 eV [27]. Similarly, S doped TiO_2 exhibits a strong visible light induced antibacterial effect by lowering the band gap [21]. Therefore, impurity doping in the crystal structure plays a vital role in improving the photocatalytic or antibacterial activity.

On the other hand, mesoporous materials of TiO_2 show enhanced antibacterial activity than TiO_2 nanoparticles [28]. The activity improves as a consequence of large surface area of mesoporous materials that help generating more hydroxyl radicals and super oxides [1,28]. These radicals in turn increase the photoactivity of TiO_2. Mesoporous materials have been conventionally synthesized utilizing various structure directing agents [2,29]. Although specific ordered pore structures can be realized by the

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structure directing agents, intricate materials processing steps are involved [2,29]. A facile approach to synthesize certain type of disordered mesoporous materials is to employ in-situ grown amorphous carbon from water soluble carbohydrates as the template and further its removal by calcination at high temperature [30–35]. Moreover, this procedure facilitates formation of micrometer sized spherical particles. It can be termed as mesoporous microspheres (MMS). There are two important advantages of using MMS over nanoparticles as photocatalytic and antibacterial agents. First, these microspheres can overcome the challenge of nanoparticles aggregation. Second, due to larger size, it can be recovered by simple filtration that is not possible with nanoparticle. It renders MMS to be a potential candidate for antibacterial activity application. Hence, opportunities exist to synergistically improve the activity of antibacterial agents via coupling doping and mesoporosity in TiO_2 microsphere.

Therefore, we focused on synthesizing impurity doped TiO_2 mesoporous microspheres to accomplish the dual benefit of doping and mesoporosity. Hydrothermal treatment of carbohydrates at high temperature was utilized to obtain the microspheres. In the present work, we report a systematic study on the synthesis, structural characterization and antibacterial activity of anatase titanium dioxide mesoporous microspheres and Fe-doped TiO_2 mesoporous microspheres.

2. Experimental

2.1. Synthesis of pristine TiO_2 and Fe doped TiO_2 nanoparticle

Titanium tetraisopropoxide and $FeCl_3.6H_2O$ were respectively used as titanium dioxide and iron precursor. For pristine TiO_2 nanoparticles, 2.73 ml of TTIP is dissolved in 100 ml of ethanol and slowly added to 100 ml of distilled water maintained at 80 °C which gives white product. The product was recovered by centrifugation and washed thoroughly with deionized water and ethanol several times to remove any unreacted species or by-products and finally dried in air at 120 °C for 24 h.

5% Fe doped anatase TiO_2 nanoparticles were synthesized by a simple one step method similar to that reported in Ref. [36]. TTIP of 0.19 M concentration is dissolved in 100 ml of ethanol. This transparent solution was slowly added to 0.01 M aqueous solution (100 ml) of $FeCl_3.6H_2O$ maintained at 80 °C and stirred continuously. The reaction was continued for 1 h at 80 °C. The addition of TTIP to the aqueous solution of $FeCl_3$ resulted in an instantaneous formation of a light orange colored gel which became darker with completion of the reaction. The resultant orange product was recovered by centrifugation and washed thoroughly with deionized water and ethanol several times and finally dried in air at 120 °C for 24 h.

2.2. Synthesis of pristine TiO_2 and Fe doped TiO2 microspheres

A desired concentration (0.5 g) of pristine TiO_2 and 5% Fe doped anatase TiO_2 nanoparticles were dispersed in 35 ml of 0.75 M aqueous sucrose solution by ultrasonication. The suspension was transferred to a 50 ml Teflon lined autoclave and heated at 180 °C for 8 h. After cooling, the resulting black product was harvested by centrifugation and washed with deionized water and ethanol at least five times. The product was dried at 120 °C for 24 h. After drying, it was further calcined at 550 °C for 3 h under air.

Pristine TiO_2 nanoparticle, Fe doped TiO_2 nanoparticle, carbon-TiO_2 microspheres, TiO_2 mesoporous microspheres, carbon-Fe doped TiO_2 microspheres and Fe doped TiO_2 mesoporous microspheres are designated as TiO_2 NP, Fe–TiO_2 NP, C–TiO_2 MS, TiO_2 MMS, C–Fe–TiO_2 MS and Fe–TiO_2 MMS respectively.

2.3. Materials characterizations

The crystallographic phase identification of the products was performed using powder x-ray diffraction (XRD; Philips X'Pert Pro diffractometer; Cu-K_α radiation, $\lambda = 1.5418$ Å). The particle size and morphology was observed by scanning electron microscopy (SEM, JEOL JSM-5600LV) and transmission electron microscopy (TEM, FEI Technai T20; accelerating voltage 200 kV). Specific surface area (BET) and pore-size distribution were obtained from nitrogen adsorption–desorption isotherms. Ultraviolet–visible (UV–Vis) and photoluminescence (PL) spectroscopy techniques were utilized respectively for determining the optical absorption and emission properties of various TiO_2. The UV–Vis absorption spectra were recorded with Shimadzu UV 2450 spectrometer. The PL spectra were recorded with Perkin Elmer LS55 spectrometer at excitation wavelength of 260 nm. The spectra were recorded using solid state measurement. Barium sulfate powder was used as reference in UV–Vis measurement. X-ray photoelectron spectra (XPS) of selected samples were recorded in a Thermo Scientific Multilab 2000 instrument. Binding energies reported here are with reference to C(1s) at 284.5 eV and are accurate within ±0.1 eV.

2.4. Antimicrobial activity using agar-well diffusion assay

The antimicrobial activity of all materials was investigated against both Gram-negative and Gram-positive bacteria using the agar-well diffusion assay method. The bacteria are *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium smegmatis* and *Klebsiella pneumoniae*. The bacterial glycerol stock cultures were revived in Luria broth overnight and the cells were centrifuged at 5000 rpm followed by washing twice with phosphate buffer. A 100 μl of the suspended bacterial culture (10^5 CFU ml^{-1}) was used to prepare a uniform lawn on agar plates and the wells were prepared using sterilized well borer. Each well was loaded with a 100 μl samples containing different concentrations (10 mg ml^{-1}, 20 mg ml^{-1}, 40 mg ml^{-1} and 60 mg ml^{-1}) of various TiO_2 materials. Agar well containing ampicillin (5 mg ml^{-1}) was used as standard antibiotic control. Following incubation on a rotary shaker at 37 °C (180 rpm) for 24 h, the agar plates were scored for the antibacterial activity by measuring the diameter zone of clearance.

3. Results and discussion

Initially, pristine TiO_2 and Fe-doped TiO_2 nanoparticles were synthesized as described earlier. The synthesized nanoparticles are approximately 5 nm in size according to TEM micrographs (Fig. 1a, b). XRD patterns (Fig. 1c) of pristine TiO_2 and Fe-doped TiO_2 crystallites showed broad diffraction peaks which can be indexed to anatase crystal phase (JCPDS No. 21–1272). From the full width at half maximum (FWHM) for the (101) peak ($2\theta = 25.50°$), the crystallite size using the Scherrer equation is estimated to be approximately 5.6 nm.

The Fe doping in TiO_2 crystal structure was confirmed by ultraviolet–visible (UV–Vis) absorption and photoluminescence (PL) spectroscopy. The Fe doped TiO_2 clearly shows notable differences compared to pristine TiO_2 in the absorption (Fig. 2a) and emission spectra (Fig. 2b). Excitonic feature of pristine TiO_2 appears at 390 nm. A red shift in absorption spectra is observed for Fe–TiO_2 NP corresponding to an absorption edge at 470 nm. The optical band gap energy of all TiO_2 materials was derived from the Tauc equation. According to Tauc equation, for a given indirect transition the photon energy ($h\upsilon$) can be related to band gap energy (E) by the following relationship: $(\alpha h\upsilon)^{0.5}{=}A(h\upsilon{-}E)$, where α, E and A are absorption coefficient, band gap energy and a constant respectively. Band gap energies were estimated by extrapolating the linear