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Green synthesis of nanocomposites consisting of silver and protease alpha chymotrypsin

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ABSTRACT

The synergy of ultrasonication and the exposure to light radiation was found to be necessary in the formation of nanocomposites of silver and a protease alpha chymotrypsin. The reaction was carried out in aqueous medium and the process took just less than 35 min. Ultrasonication alone formed very negligible number of nanoparticles of <100 nm size whereas light alone produced enough number but the size of the particles was >100 nm.

The effects of pH (in the range of 3–5, 9–10), ultrasonication time periods (0–30 min), ultrasonication intensity (33–83 W cm^{-2}), energy of light radiation (short UV, long UV and Fluorescent light) and time period of exposure (5–60 min) to different light radiations were studied.

The formation of nanocomposites under these effects was followed by surface plasmon resonance (SPR) spectra, dynamic light scattering (DLS), transmission electron microscopy (TEM). Ag–chymotrypsin nanocomposites of sizes ranging from 13 to 72 nm were formed using the synergy of ultrasonication and exposure to short UV radiation. Results show that ultrasonication promoted nuclei formation, growth and reduction of polydispersity by Ostwald ripening.

1. Introduction

Nanoparticles which consist of a biological component and an inorganic element have attracted wide attention [1–4]. Two different functional properties often combine in a synergistic fashion in such particles which enhances the range of the kind of applications in diverse areas. As Eby et al. [5] pointed out, largely due to its antimicrobial property, nanosized silver is the most widely used material in the medical area. Their work on the formation of composite nanoparticles of silver and lysozyme shows the synergy of combining the antimicrobial properties of the inorganic component and the biocatalyst (lysozyme hydrolyses the cell membranes of the gram positive bacteria).

Proteases are used as debriding agents (cleaning wounds by digesting foreign matter and adjoining dead tissues) and have proved useful in reduction of inflammation [6]. Adding antimicrobial property to a protease in this context will be obviously valuable. Apart from this, enzymes linked to nanocarriers have been found extremely useful in biocatalysis [3,7,8]. Proteases are useful in catalyzing hydrolysis of proteins and synthesis of esters [9]. Alpha chymotrypsin is a well characterized protease.

In the present work, we show the preparation of Ag–chymotrypsin nanocomposites by a two step process. Step one consists of ultrasonication of a solution of silver ions along with alpha chymotrypsin. This is followed by exposure to UV–visible light for a limited period of time. It was found that without exposure to radiation, negligible amount of particles were formed and no surface plasmon resonance (SPR) spectrum corresponding to formation of Ag nanoparticles could be observed. Exposure to light alone led to the formation of heterogenous population of the nanomaterial of micron sizes. Unlike the earlier work by Eby et al. [5], the reaction medium is aqueous rather than methanol (or any other solvent).

Application of ultrasonication to assist the formation of nanomaterials is well known and has been reviewed recently by Bang and Suslick [10]. Acoustic cavitation generates very high temperature and pressure locally ("hot spots") and is known to facilitate formation of nanosized materials [10,11]. In case of noble metals, sonochemical reduction (without presence of any external reducing agent) due to generation of $H^{\bullet}$ radicals produces small particles [10]. Jiang et al. [12] have described the preparation of silver nanoplates by ultrasonic assistance in DMF (*N*,*N*-dimethylformamide) solution. Microwave assisted formation of silver nanoparticles is another similar green approach [13–15].

Reduction of Ag in the presence of light is fairly well known [16,17]. Again, hydrated electrons or free organic radicals are postulated to mediate this process.

The composite nanomaterials of silver with proteins have been described earlier as well. Silver nanoparticles capped with bovine

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serum albumin can be prepared at large scale by RESOLVE (rapid expansion of supercritical solution into a liquid solvent) process [18,19]. There are many reports of microbes producing nanomaterials. In some cases, the role of proteins in reduction of silver ions has been clearly shown [20]. Similarly, cell free extracts from plant sources have been described with possible involvement of protein/enzymes in formation of silver nanomaterials [21,22]. Mishra and Sardar used a microbial alpha amylase for production of silver nanoparticles [23]. Hendler et al. described formation of silver nanoparticles inside mucin, a protein with "unique dendritic structure" [24].

Recently, we described the formation of BSA conjugated silver nanoparticles [4]. The process, which could take place in aqueous medium involved ultrasonic assistance and required just 15–30 min. Equally important, size and shape of the composite nanoparticles could be controlled by ultrasonication time and Ag^+:BSA molar ratio. While circular dichroism (CD) spectrum showed some changes in secondary structure of BSA, zeta potential measurements indicated that overall protein surface (and hence presumably the tertiary structure of the protein) was not affected significantly upon combination with the silver nanocores [4]. In order to pursue this work further, we attempted to prepare Ag–chymotrypsin hybrid nanocomposites. We believe this to be the first report in which the synergy of ultrasonication and light has been used to prepare a nanocomposite in water.

2. Materials and methods

2.1. Materials

Silver nitrate was purchased from BDH (Mumbai, India) and alpha chymotrypsin was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade. Membrane filtered water obtained from the membrane filteration unit Labconco WaterPro (LabConco Corporation, Kansas City, Missouri) was used throughout the work.

2.2. Synthesis of nanoparticles

Alpha chymotrypsin conjugated silver nanoparticles were synthesized by mixing 1 mg mL^{-1} (40 μM, final concentration) of alpha chymotrypsin solution with 1 mM silver nitrate to obtain a molar ratio ($AgNO_3$/alpha chymotrypsin) of 25, both prepared in water. The pH of the solution was adjusted with 0.1 N NaOH or 0.1 N HCl. The mixture was irradiated with ultrasonic irradiation using a probe sonicator (Sonics Vibra-Cell, 20 kHz, Sonics & Materials, Newtown, CT, USA). Ultrasonic waves were generated using a high grade titanium alloy horn which was directly immersed into the reaction mixture taken in a glass beaker. A stepped microtip of 3 mm diameter was used. The schematic diagram of the experimental set up is shown in Fig. 1. The ultrasonic power used in the present experiments was in the range of 2.3–5.7 W (25–40% acoustic amplitude). The microtip area being 0.7 cm^2, this corresponded to an intensity (power supplied/microtip area) range of 33–83 W cm^{-2}. During ultrasonication, after every 10 min the reaction mixture was cooled in an ice water bath for about 1 min. This maintained the temperature of the reaction mixture at 30 ± 3 °C. After each 10 min of ultrasonication, an aliquot was withdrawn for exposing to short wavelength ultraviolet (SUV) light, or long wavelength ultraviolet (LUV) light or fluorescent light (FL). This exposure to the SUV, LUV or FL was for a specified period of time. This step was carried out using an Ultraviolet Florescence Analysis Cabinet (Khera Instruments Pvt. Ltd., Delhi, India) (Fig. 1). The nanomaterials produced were recovered by centrifugation at 10,000*g* for 30 min, washed with water and finally resuspended in a known volume of water.

2.3. UV–visible spectroscopy

Surface plasmon resonance spectra of the silver nanoparticles were obtained by recording UV–visible absorption spectra in the range of 300–800 nm at 25 °C with a Perkin–Elmer Lambda 35 spectrophotometer at a resolution of 1 nm by using 0.4 mL quartz cuvette with 1 cm path length.

2.4. Dynamic light scattering (DLS)

DLS measurements were performed at 25 °C using the instrument laser spectroscatter 201 by RiNA GmbH (Berlin, Germany). Data analysis was done using PMgr v3.01p17 software supplied with the instrument. The sample (200 μL) was manually injected into a flow cell (1.5 mm path length) and illuminated by a 100 mW, 660 nm laser diode.

2.5. Transmission electron microscopy (TEM)

Transmission electron micrographs were recorded on a Morgagni 268D (Fei Electron Optics) Instrument equipped with digital imaging and 35 mm photography system at 80 kV. A drop of alpha

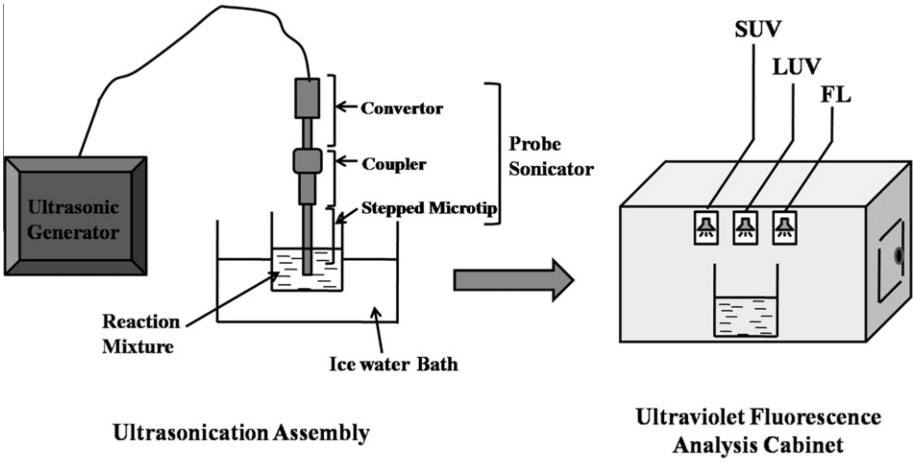

Fig. 1. Schematic diagram of the experimental setup. A stepped microtip with 3 mm diameter was used in the probe sonicator. The SUV has a wavelength of 256 nm and LUV of 365 nm. The power of the SUV, LUV and FL lamps was constant at 9 W.