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Green synthesis and molecular recognition ability of patuletin coated gold nanoparticles

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ABSTRACT

Patuletin isolated from *Tagetes patula* was used as a capping and reducing agent to synthesize in one pot gold nanoparticles capped with patuletin. Conjugation of gold with patuletin was confirmed by FT-IR and UV–visible spectroscopy and amount of patuletin conjugated to gold nanoparticles was found to be 63.2% by weight. Particle sizes were measured by atomic force microscopy (AFM) and were found to have a mean diameter of about 45 nm. Patuletin-coated gold nanoparticles were found to be highly fluorescent. To examine their potential as chemical sensors, they were contacted with fourteen different drugs. Of these drugs, only one, piroxicam, was found to quench luminescence. Quenching obeyed Beer's law in a concentration range of 20–260 μM. Important for molecular recognition applications, fluorescence quenching by piroxicam was not affected by pH variation, elevated temperatures, addition of other drugs and addition of blood plasma to the colloidal suspensions.

1. Introduction

In the developed world, but also, and in even larger proportion, in developing countries, there is a need of monitoring the amount of pharmaceutical drugs released in the environment. For example, recently we became interested in the occurrence of pharmaceutical drugs in surface waters of Karachi as emerging environmental pollutants. During bioassay directed chemical analyses of the drinking water system of Karachi, Pakistan, a large number of pharmaceuticals drugs were discovered in alarmingly high concentrations in the microgram-per-litre range in different compartments (surface water, drainage, and effluent) (Scheurell et al., 2009; Selke et al., 2010). This prompted us to carry out chemical analysis of water samples thus supplying information about toxic effects and the class of xenobiotics responsible for these effects. One of the drug in these water resources was piroxicam (Fig. S1-I), 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1, 2-benzothiazine-3-carboxyamide-1, and 1-dioxide which belongs to a new class of non-steroidal anti-inflammatory drugs called oxicams. It is recommended for the treatment of rheumatological disorders by clinical practitioners but the adverse effects of these drugs become more prevalent which include kidney and gastrointestinal problems (Ródenas, 1998). Several analytical methods reported for the determination of piroxicam in water and plasma include polarographic, chromatographic, spectrophotometric and fluorimetric techniques (Amin, 2002; Basan et al., 2001; Klopas et al., 1998). Most of them lacks systematic approach and have practical shortcoming such as interference from other drugs, harsh reaction condition (El-Ries, 1998; R.V. et al., 2010) or complicated synthetic route (Kormosha et al., 2011). The unique optical properties of metal nanoparticles due to collective oscillation of conduction electrons after interaction with electromagnetic radiation make them ideal candidate to be explored for chemosensing. The optical response of metal nanoparticles is very much sensitive to the size, shape and local refractive index (RI) near the surface of the metal nanoparticles as well as their interparticle distances. Particularly, nanoparticles of noble metals, such as silver and gold have generated great interest in recent years in scientific community. The noble metal nanoparticles mainly show absorption band in the visible region (380–750 nm). Furthermore, an increase in the RI of the surrounding environment of these metal particles leads to a red shift of the localized surface plasmon resonance band. Thus adsorbate-induced change in local refractive index due to molecular recognition or adsorption at the surface of the Ag or Au nanoparticles induced changes in surface plasmon band which can

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be deduced fairly through scattering or adsorption technique. Based on these specific optical characteristics of noble metal nanoparticles, nanosensors are designed for the detection of piroxicam molecule. Mainly secondary interactions are playing an important role in the chemosensing of nanoparticles such as hydrogen bonding (Boal et al., 2000), ð–ð (Jin et al., 2001), host guest (Liu et al., 1999), van der Waals (Patil et al., 1997), electrostatic (Caruso et al., 1998), charge transfer (Naka et al., 2003) and antigen antibody interactions (Shenton et al., 1999). Gold nanoparticles functionalized with amide group were used as optical sensor for anions. Gold nanoparticles are also used as a chromogenic agent for the spectrophotometric determination of drug (Jana et al., 2012; Ozalp et al., 2011; Tiwari et al., 2011) Nanocarriers are reported in literature based on either synthetic or natural macromolecules (flavonoids, proteins, polysaccharides, and liposomes) (Fang et al., 2011). We used patuletin which is a natural product for reduction and stabilization of nanoparticles. The patuletin (Fig. S1-II) is a natural flavonoid and can serve an ideal candidate for the green synthesis of metallic nanoparticles (Amarnath et al., 2012). Here we present a new, simple, facile and one-pot robust approach for the green synthesis of thermally stable patuletin coated gold nanoparticles using patuletin as a reducing as well as stabilizing agents via the classical Turkevich method (Turkevich et al., 1951). Specifically, we demonstrate the utilization of sequestering ability of phenolic groups of patuletin for stabilization of the gold core. The patuletin coated gold nanoparticles were synthesized from a higher concentration (50 mM) of Au (III) salt and were not susceptible to aggregation up to 100 mM NaCl and in the pH range of 2–13. This improved synthetic approach produces patuletin coated gold nanoparticles with much reduced levels of toxicity of reagents and a facile process, resulting in a suitable analytical protocol for ultra-trace detection of piroxicam.

2. Material and methods

2.1. Materials and instruments

Tetrachloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$) and methanol were purchased from Merck. A digital pH meter model 510 (Oakton, Eutech) equipped with a glass working electrode and a reference Ag/AgCl electrode was used. UV–visible spectra were recorded with a Shimadzu UV-240, Hitachi U-3200 spectrometer with a path length of 1 cm. Fluorescence spectra were recorded using a Perkin-Elmer LS55 spectrophotometer with a path length of 1 cm. The IR spectra were recorded using Shimadzu IR-460 by mixing a 1:1 mixture of patuletin coated gold nanoparticles (after centrifugation) and KBr. This powder mixture is then pressed in a mechanical press to form a translucent pellet and subjected to the beam of a spectrometer. A MALDI-TOF MS spectrum was recorded on Bruker Ultra flex TOF-TOF operated in reflection mode. The matrix used for sample was 4-hydroxy-2-cyanocinnamic acid (HCCA). All solutions were prepared using deionized water. Topographical images were obtained with an Atomic Force Microscope Agilent 5500 operated in tapping mode.

2.2. Isolation of patuletin from tagetes patula flowers

Fresh, undried and uncrushed flowers (9.1 kg) of *T. patula* were extracted with different solvents sequentially with increasing polarity i.e. petroleum ether (PE), dichloromethane (DC), ethyl acetate (EA) and acetone twice at room temperature. The EA extract was evaporated in vacuo giving a thick mass in which a yellow matter was settled down while keeping a few days at room temperature. It was filtered and washed several times with PE followed by DC and EA affording pure patuletin (1, 15.1 g) (Fig. S2) (Faizi et al., 2010a, 2008, 2010b).

2.3. Green synthesis of patuletin coated gold nanoparticles

One mililitre of patuletin (1 mM) in water was added drop wise to 1 mL solution of tetrachloroauric (III) acid (1 mM) in water. Different ratios of patuletin and $HAuCl_4$ were used to optimize the conditions. Highest absorbance with 5:1 (Au:Patuletin) ratio at 530 nm was observed. During addition, the color of the reaction mixture changed from yellow to dark violet. The resulting mixture was stirred for 3 h at room temperature. Patuletin coated gold nanoparticles were collected in the form of precipitate after centrifugation at 11,000 revolutions per minute for 10 min at room temperature.

2.4. Spiking in human blood plasma

Blood sample was collected in heparinized tube from a healthy human volunteer after ethical approval from ethic committee of the center via venous puncture followed by centrifugation at 4000 revolutions per minute for 5 min at room temperature to separate out plasma. Two different stock solutions of 5 mL containing 1 mL of plasma with 2 mL of patuletin coated gold nanoparticles in each solution were prepared in deionized water. One solution was without piroxicam while the other solution was spiked with 50 μL of 500 μM piroxicam.

3. Results and discussion

3.1. Biosynthesis and characterization of patuletin coated gold nanoparticles

The color of reaction mixture rapidly changed from light yellow to dark violet after mixing patuletin with $HAuCl_4$ solution, indicating reduction of the gold ions and formation of patuletin coated gold nanoparticles. UV–visible spectra (Fig. 1a) of the suspensions revealed an absorption maximum at 530 nm. Typically, water suspensions of gold nanoparticles have an absorbance maximum between 520 and 530 nm (Buso et al., 2007). Variations in the absorbance peak were not observed when different ratios of $HAuCl_4$ and patuletin were used for the synthesis of patuletin coated gold nanoparticles. However absorbance intensity gradually increased when gold concentration was increased to 5:1 ratio indicating the complete reduction of gold ions and then suddenly decreased when the gold concentration is further increased which may be due to the decrease in the number of gold nanoparticles due to aggregation (Hameed et al., 2011).

The amount of conjugated patuletin was measured by centrifuging patuletin coated gold nanoparticles out of suspensions, at 11,000 revolutions per minute for 10 min at room temperature. The supernatant was freeze-dried and the residue weighed. These results indicate that conjugates contained about 63.2% by weight of patuletin (Naz et al., 2013).

3.2. Comparative FTIR analysis of patuletin and patuletin coated gold nanoparticles

The formation of patuletin coated gold of nanoparticles was attributed to the interaction of phenolic groups with gold. Conjugation of patuletin to gold was also supported by FT-IR spectroscopy (Fig. 1b), where absorbance bands related to patuletin are observed in the region of 1000–3500 cm^{-1} are 3400, 1658, 1608, 1554, 1497, 1442, 1303, 1210, 1166, 1091 and 1039 cm^{-1}. Among them, the absorbance bands at 3400, 1658, 1608, 1554, 1303 and