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Biosynthesis of stabilised gold nanoparticle using an aglycone flavonoid, quercetin

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Biosynthesis of gold nanoparticles (AuNPs) was obtained by a simple chemical reduction method using a plant-derived aglycone flavonoid, quercetin, as a reducing agent. The aqueous chloroauric acid when exposed to quercetin was reduced and converted to AuNPs in the size range from 20 to 45 nm. AuNPs were characterised by UV–visual spectroscopy, transmission electron microscopy, atomic force microscopy and dynamic light scattering method. These quercetin-mediated AuNPs have shown excellent stability for more than 30 days at 2–8°C. These quercetin-stabilised AuNPs will have an enormous potential for further conjugation studies since no other external stabilising agent is used.

Keywords: gold nanoparticles; quercetin; chloroauric acid

1. Introduction

Gold nanoparticles (AuNPs) seem more promising in drug designing and various biomedical applications because of its efficient optical, electronic, magnetic and catalytic properties, high zeta potential and better biocompatibility [1–6]. In the recent years of nanorevolution, efficient synthesis of AuNPs is one of the fundamental necessities to nanoresearchers for further application. There are several conventional methods for the synthesis of AuNPs. The conventional method of Turkevitch produces uniformly spherical AuNPs, but the low stability is the drawback of this method [7,8]. AuNPs obtained by borohydride reduction are unsuitable for biomedical application due to its strong reduction potential [9]. Brust–Schiffrin method using organic solvent develops uniform AuNPs and stabilises those utilising thiols as a protective capping agent [8,10]. However, the organic solvents used in this method make them incompatible for solution-based biosensors for detection of biomolecules like proteins, saccharides, etc., and strong thiol–gold interaction makes the particles unsuitable for the development of AuNP-labelled biomolecules for target-specific drug designing [9]. Therefore, biologically benign process of stabilised AuNP synthesis is of great importance to address the growing concern on drug designing and delivery using these nanoparticles. Although synthesis of AuNPs by different phytocompounds such as catechin, catechin gallate, epicatechin gallate and epigallocatechin have been reported, the potential of phytocompounds as biological materials for the synthesis of AuNPs is yet to be fully explored [9]. This approach describes for

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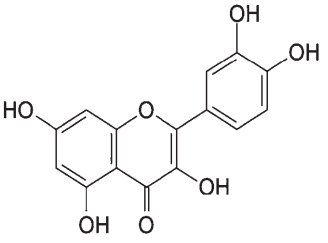

Figure 1. Structure of quercetin.

the first time, quercetin-mediated biosynthesis of stabilised AuNPs without using any external stabiliser. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Figure 1) is a plant-derived aglycone flavonoid which has shown the highest antioxidative potential and different biological effects [11–13]. Due to this high antioxidative property, quercetin imparts many crucial biological properties like antitumor, anti-inflammatory and antimicrobial activities [12,13].

2. Materials and methods

2.1. *Chemicals*

Quercetin ($C_{15}H_{10}O_7$, FW 302.24) (Sigma–Aldrich, St Louis, Germany), HAu(III)Cl_4,3H_2O (s.d.fiNE-CHEM Ltd, Mumbai, India) and dimethyl sulphoxide (DMSO; Merck, India) were purchased commercially.

2.2. *Synthesis of AuNPs*

Qercetin solution (85 mM) was prepared using DMSO as a solvent. Chloroauric acid (1 gm%) solution was prepared using triple distilled water. Stock chloroauric acid solution (200 μL) was mixed with triple distilled water to prepare 0.01 gm% solution. To this, 20 μL of quercetin solution was added drop wise with continuous stirring in an airtight container at a constant temperature of 40°C. The yellow colour of the bulk gold chloride gradually turned into a wine red colour (Figure 2) within 2–3 min. The prepared aqueous quercetin AuNPs (Q-AuNP) were stored properly for its further characterisation.

2.3. *Characterisation of AuNPs*

The surface plasmon resonance (SPR) peak was analysed by UV–visual (UV–vis) spectrophotometer (Hitachi U-4100). The physical natures of AuNPs were characterised by atomic force microscopy (AFM; VEECO Multimode, Nanoscope-iiia, Taping mode) and transmission electron microscopy (TEM; FEI Tecnai 12 Bio-twin). The hydrodynamic diameter and the charge on the surface of the nanoparticles were determined by dynamic light scattering (DLS; Malvern, Zetasizer Ver. 6.00) method. Few drops of AuNP solution was coated on to a mica sheet and air dried for AFM characterisation. Few drops of Q-AuNP solution were coated on a copper grid for TEM analysis.

3. Results and discussion

3.1. *UV–vis spectroscopic analysis*

SPR phenomenon is an obvious characteristic of noble nanoparticles. When AuNPs are exposed to visible light, a plasmon band is created, which has an absorption peak in the visible range 530–540 nm mainly due to the presence of six free electrons in their outer orbit [14]. This absorption peak mainly depends on morphology, particle–particle distance, stabiliser used for

Figure 2. (Colour online) Wine red colour of the prepared Q-AuNPs.

the stabilisation of the nanoparticles and chemical surroundings. According to the Mie theory, SPR phenomenon is absent for AuNPs less than 2 nm and greater than 500 nm. The UV–vis absorption spectrums of aqueous Q-AuNPs are shown in Figure 3. The SPR peak obtained is at 528 nm, which is in visible light range which correlates with the theoretical study.

3.2. *TEM analysis*

The TEM image of the prepared Q-AuNPs is shown in Figure 4. The AuNPs are spherical in nature and show a smooth topology. This study reveals that the diameter of the particles are in the range 20–45 nm which is more biocompatible as larger nanoparticles of around 50 nm have shown highest cellular uptake and no toxicity unlike small AuNPs (1–2 nm) [6,15–21].

3.3. *AFM analysis*

The size and shape of the Q-AuNPs are again confirmed by AFM. The AFM image shown in Figure 5 reveals the smooth and spherical morpholology of the prepared AuNPs. The size of the nanoparticles ranges around 20–50 nm which is in good agreement with TEM analysis.

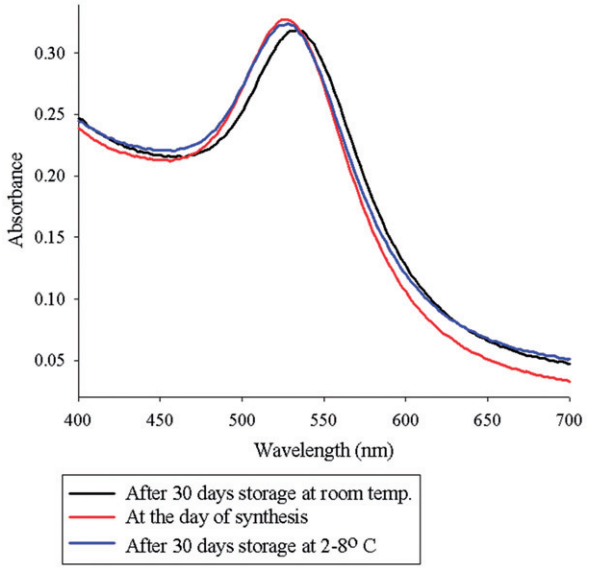

Figure 3. UV–vis spectra of Q-AuNPs.

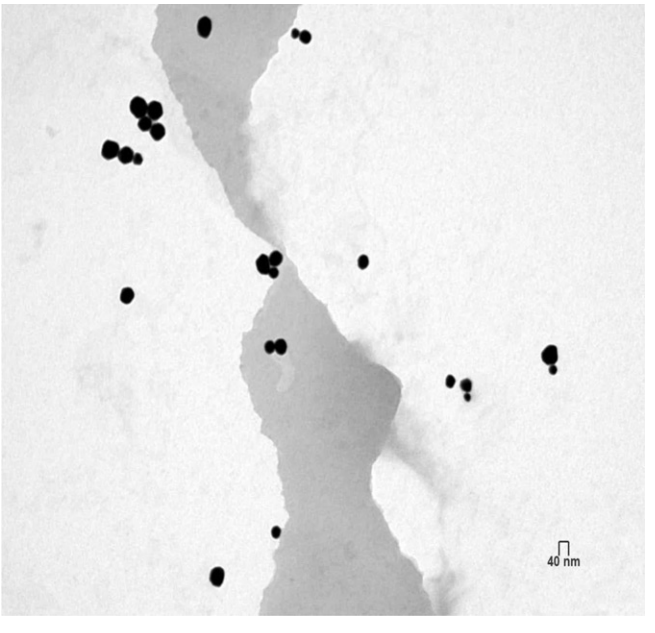

Figure 4. TEM image of Q-AuNPs.

3.4. *DLS study*

The hydrodynamic diameter of AuNPs obtained from the DLS method was 60 nm, suggesting that quercetin makes a coating on the AuNPs which may prevent those from agglomeration. The probable orientation of quercetin on gold nanoaprticles is shown in Figure 6. The charge on

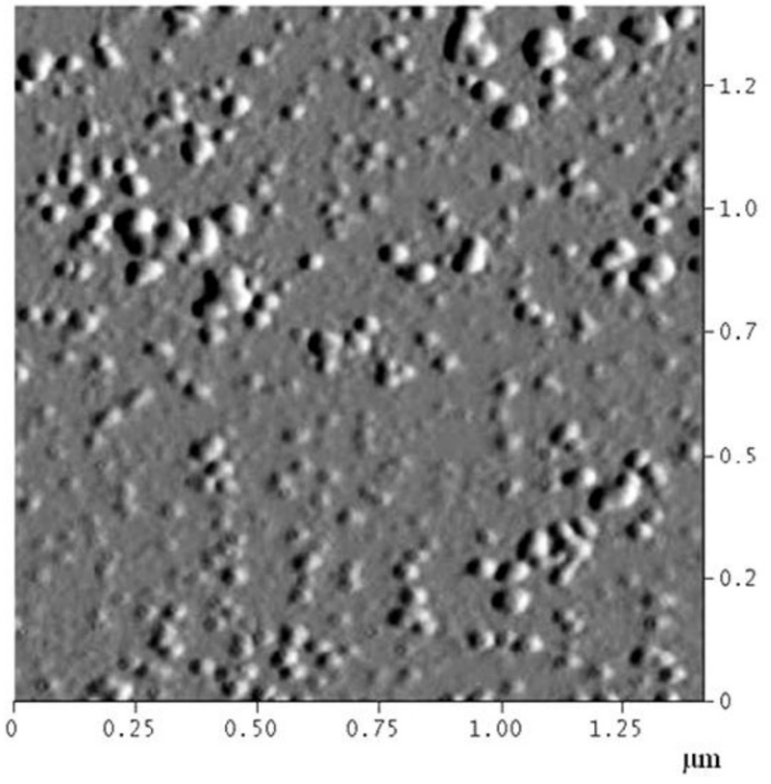

Figure 5. AFM image of Q-AuNPs.

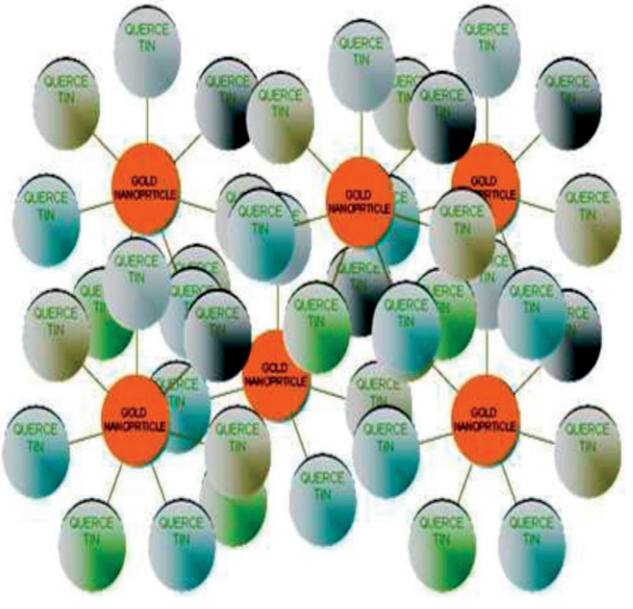

Figure 6. (Colour online) Schematic diagram of the prepared Q-AuNPs.

the surface, zeta potential (ζ), of the nanoparticles was also measured by the DLS method. The negative zeta potential, -24.09 mV of the prepared AuNPs indicates that the particles repeal each other and there was no tendency of particle agglomeration. The measurements of the charge of the particles, zeta potential (ζ), provides crucial information about the stability of the nanodispersion.

3.5. *Stability study of AuNPs*

To determine the *in vitro* stability of the prepared AuNPs, aqueous AuNP solution was kept at room temperature and at 2–8°C separately. After 30 days of storage, the samples were analysed by UV–vis spectroscopy, which is shown in Figure 3. This study shows the SPR peak at 534 and 528 nm for the solution kept at room temperature and at 2–8°C, respectively. As described earlier in Section 3.1, SPR depends on the particle size and shape, so the shifting of SPR peak towards larger wavelength may be due to particle agglomeration after 30 days of storage at room temperature. The stability of nanoparticles might be due to capping of quercetin on AuNPs. The stability of nanopaticles depends upon the attractive and repulsive forces that exist between the nanoparticles. If all the particles have a mutual repulsion, then the dispersion will remain stable. However, little or no repulsion make the particles agglomerate.

4. Conclusion

In summary, quercetin effectively reduced chloroauric acid to synthesise spherical and stable AuNPs in the range 20–45 nm. The rate of reaction is very fast, which is only 2–3 min to synthesize stable nanoparticles. This simple procedure of stable AuNP biosynthesis has several advantages, such as compatibility for wide biomedical and pharmaceutical applications, as there is no other external stabilising agent applied to render the nanoparticles stable. They can be delivered into cells easily and conjugated with different biomolecules like saccharides, proteins (antibodies, etc.) or can itself be used as an efficient drug.

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