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A single step and cost-effective flash nanoprecipitation method to prepare chitosan with high curcumin conjugated gold nanoparticles loading and its antibacterial investigation

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Abstract

A synergistic effect of gold nanoparticles and curcumin and a unique combination of chitosan natural amphiphilic polymer due to their widespread biological and technological applications have gained much attention. Their simpler synthesis for encapsulating curcumin-conjugated gold nanoparticles in particulate carriers assembled by polymer as trapping agent via green chemistry has also become of foremost importance. The current study offers to report a new, simple, single-step, and cost-effective room temperature strategy for the fabrication of curcumin-conjugated spherical gold nanoparticles dispersed on the surface of chitosan by pH adjusting that curcumin acts as the reducing and stabilizing agent based on the flash nanoprecipitation. The prepared samples were characterized with UV-Vis, AFM, and TEM analysis. Regarding the obtained data from the antibacterial test by disc diffusion method, the prepared chitosan with high curcumin conjugated gold nanoparticles compared to the pure chitosan and curcumin encapsulated chitosan nanoparticles with high antibacterial activity will open a unique opportunity for industrial scale-up. The inhibition zone diameter was 25 mm which is more than the inhibition zone diameter for chitosan (7 mm) and chitosan with curcumin loading (12 mm).

Keywords: Flash nanoprecipitation**,** Curcumin conjugated gold nanoparticles, Chitosan, Antibacterial activity

1. Introduction

Flash nanoprecipitation strategy is a scalable simple process for encapsulating hydrophobic drugs or drug-conjugated metal nanoparticles in a polymer-based delivery vehicle [1,2]. In this process, by using rapid micromixing with either a four-stream multi-inlet vortex (MIV) mixer15 or two streams confined impingement jet mixer with subsequent dilution, high supersaturation conditions are obtained that leading to the precipitation and encapsulation of drugs in a polymer as trapping agent [3,4]. Curcumin, also known as diferuloylmethane, is an active component in the golden spice turmeric (Curcuma longa) and in [Curcuma xanthorrhiza oil]. It is a highly pleiotropic molecule that exhibits antibacterial, anti-inflammatory, hypoglycemic, antioxidant, wound-healing, and antimicrobial activities [5,6]. Gold nanoparticles refer to submicrometer-sized particles made of gold metal suspended in a fluid [7,8]. These nanoparticles exhibit localized surface plasmon resonant properties and possess exceptional optical and electronic characteristics, making them suitable as biosensors for imaging and diagnostics in the field of Health Professions. Gold nanoparticles with controlled geometrical and optical properties are the subject of intensive studies and biomedical applications, including genomics, biosensors, immunoassays, clinical chemistry, laser phototherapy of cancer cells and tumors, the targeted delivery of drugs, DNA and antigens, optical bioimaging and the monitoring of cells and tissues with the use of state-of-the-art detection systems [9,10].

In the present research work, our interest lies in the use of a cost-effective alternative as a stabilizer and hydroxyl-rich chitosan as a curcumin drug-trapping agent to prepare curcumin-conjugated gold nanoparticles via flash nanoprecipitation. The prepared chitosan-containing high loading of curcumin-conjugated gold nanoparticles was characterized with UV-Vis, AFM, and TEM analysis and was used as an antibacterial agent. This study provides important insights into the cost-effective, rapid, and scalable preparation of a variety of drug-loaded metal nanoparticles through the flash nanoprecipitation technique.

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2. Experimental

2.1. Materials

Chitosan (low molecular weight), Hydrogen tetrachloroaurate (III) ($HAuCl_4$), Tween 80 surfactant, Curcumin ≥80% (Curcumin) were purchased from Sigma-Aldrich company.

2.2. Chitosan-containing high loading of curcumin conjugated gold nanoparticles fabrication via flash nanoprecipitation

Chitosan-containing high loading of curcumin conjugated gold nanoparticles were produced using the antisolvent principle by flash nanoprecipitation via a multi-inlet vortex mixer that four inlets were connected to four hermetic stainless syringes via Teflon tubing. One of the syringes was (50 mL) an aqueous solution of Tween 80 surfactant (0.5 M), the second was an aqueous solution of HAuCl4 (0.01 M) with yellow color and Tween 80 surfactant (0.5 M), the third one was an aqueous solution of NaOH to adjust the pH at 9.3 and the fourth one was curcumin with chitosan in organic solvent tetrahydrofuran (THF) at the concentration of 5 mg/mL with yellow color. Syringe pumps were used digitally to control the ratio of injection speeds of the streams. After mixing a rapid and progressive change in color from pale yellow to turbid/burgundy red to confirm the formation of gold nanoparticles was observed. A control sample was prepared without an aqueous solution of HAuCl4 and water was replaced with this solution to prepare chitosan-containing high loading of curcumin for comparison study of the antibacterial test. Fig. 1, illustrates the flash nanoprecipitation via a multi-inlet vortex mixer in that four inlets were connected to four hermetic stainless syringes via Teflon tubing.

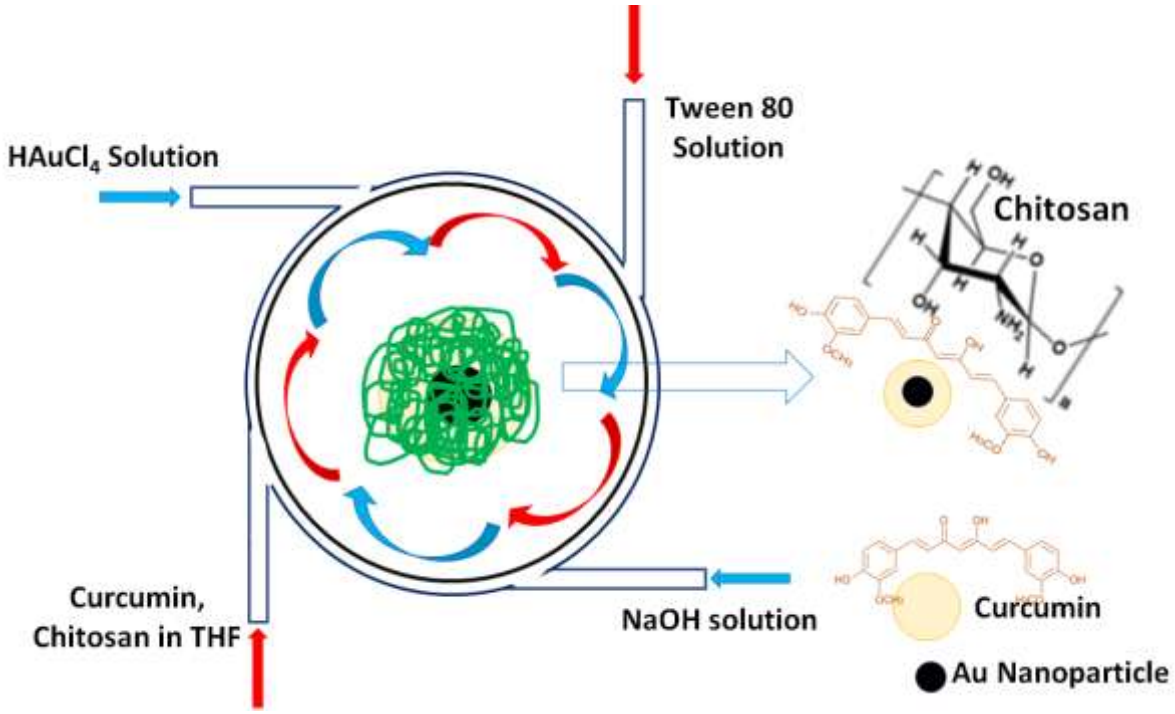

Fig. 1. The flash nanoprecipitation via a multi-inlet vortex mixer with four inlets to prepare chitosan-containing high loading of curcumin conjugated gold nanoparticles

2.3. Characterization

The surface plasmon resonance (SPR) spectra of chitosan-containing high loading of curcumin conjugated gold nanoparticles synthesized was investigated by UV-Vis analysis. TEM image was also used to indicate the particle size for homogenous and spherical in shape of Chitosan-containing high loading of curcumin conjugated gold nanoparticles. The surface topology was characterized with AFM (Nanoscurf), with surface scan of 5μm × 5μm, and speed of 2 Line/S. For AFM analysis a suspension of sample in ethanol was prepared and dropped on the surface a clean glass slide and then dried at room temperature.

2.4. Antibacterial activity study based on the Disk diffusion method

In this method, blank paper disks were put in tubes containing dilutions of all samples and after 5 to 10 minutes samples were absorbed into disks, incubated at a temperature of 37°C and dried completely, and got ready for disks [10-12]. A microbial suspension of 0.5 McFarland (1.5 ×108CFU/ ml) was obtained from the Escherichia coli bacterial strain and then, surface culture was carried out by using a swab on the Mueller-Hinton agar plate. Next, disks containing different samples (0.007 g) were put on the surface of the culture at an appropriate distance from each other and from the edge of the plate. Plates were incubated for 24 hours at a temperature of 37°C and the results of antibacterial effect were calculated by measuring the inhibition zone diameter. To ensure this, the tests were repeated 3 times [13-16].

3. Results and Discussion

3.1. UV-Vis study

Fig. 2. Reveals the UV-Vis spectra of the prepared chitosan-containing high loading of curcumin-conjugated gold nanoparticles via flash nanoprecipitation at pH=9.3. The narrow SPR peak formed by the colloid solution in presence of Tween 80 and also curcumin as a stabilizing agent at 526 nm, confirms the formation of gold nanoparticles capped with curcumin molecules on the surface of chitosan trapping agent and then chitosan-containing high loading of curcumin-conjugated gold nanoparticles [17-19]. It is obvious that in the pH of above 9.0 the intensity and broadens the SPR peak is suitable.

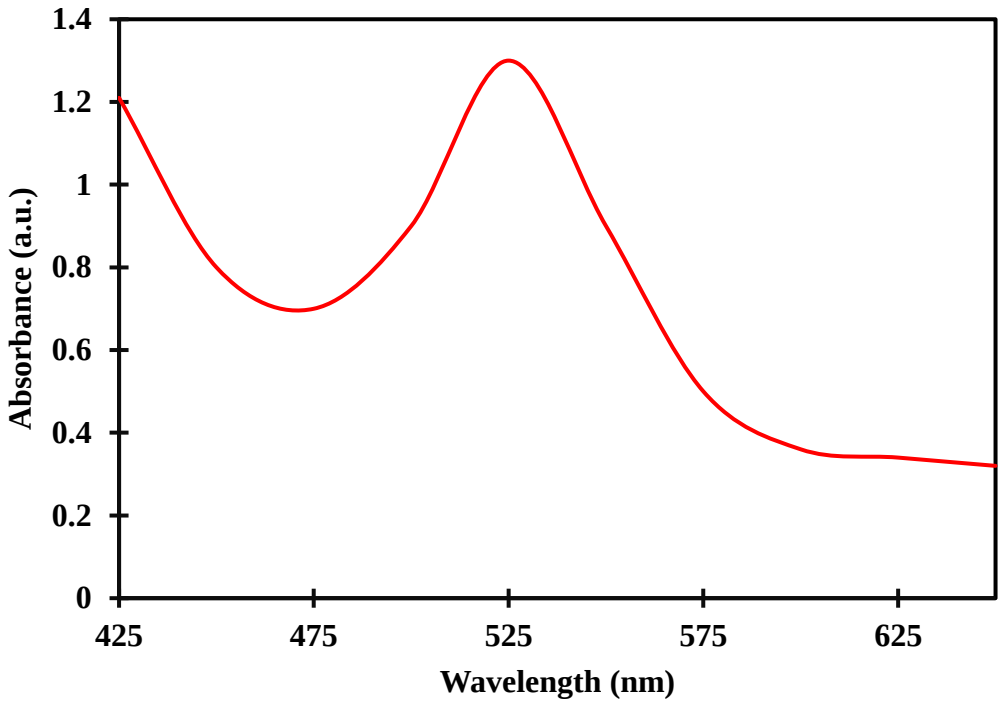

Fig. 2. UV-Vis spectra of chitosan-containing high loading of curcumin conjugated gold nanoparticles via flash nanoprecipitation at pH=9.3

3.2. TEM and AFM images

As can be seen from TEM and AFM images of the chitosan-containing high loading of curcumin-conjugated gold nanoparticles (Fig. 3) is found to be uniformly spherical with an average diameter of 50-70 nm and also indicates that the curcumin-conjugated gold nanoparticles are homogenous and has a good monodispersity due to the presence of the tween 80 surfactant [20,22].

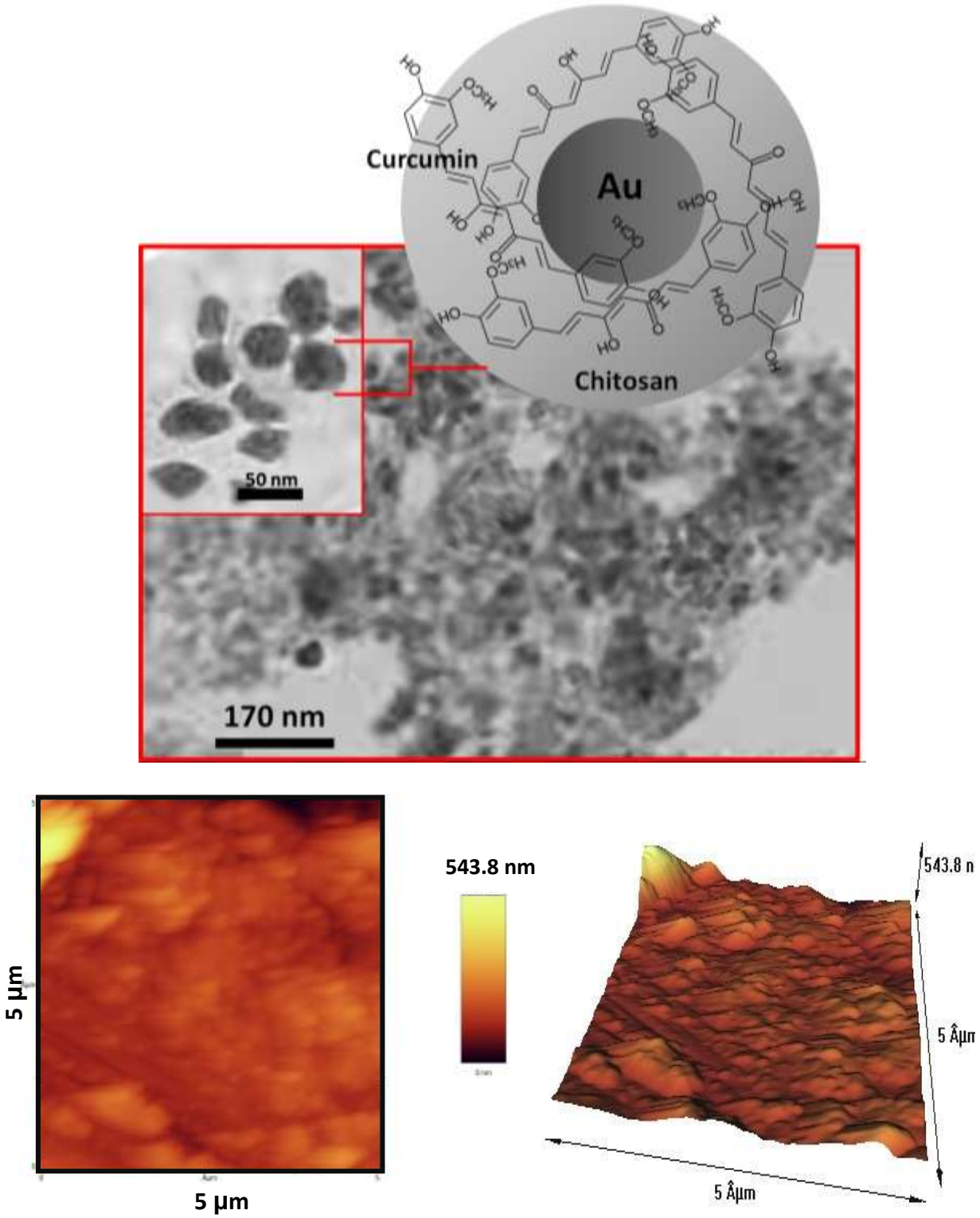

Fig. 3. TEM and AFM images of the chitosan-containing high loading of curcumin conjugated gold nanoparticles via flash nanoprecipitation at pH=9.3 with scale bare of 50 and 170 nm.

3.4. Antibacterial results and suggested mechanism

Considering the antibacterial activity of the examined samples including pure chitosan, chitosan with high loading curcumin, and also chitosan-containing high loading of curcumin conjugated gold nanoparticles. The antimicrobial effects of chitosan-containing high loading of curcumin-conjugated gold nanoparticles were more

than the other samples due to the synergistic effect of chitosan, curcumin, and gold nanoparticles. This sample was effective in 50 mg/ ml concentrations of Escherichia Coli. The inhibition zone diameter for this bacterium in the concentration of 50 mg/ ml is 25 mm in diameter these results were obtained in the method of disk diffusion (Fig. 4) and is more than the inhibition zone diameter for chitosan (7 mm) and chitosan with curcumin loading (12 mm). A schematic mechanism based on the synergistic effect and oxidative stress mediated by chitosan-containing high loading of curcumin-conjugated gold nanoparticles is introduced to prove the antibacterial activity [23-27].

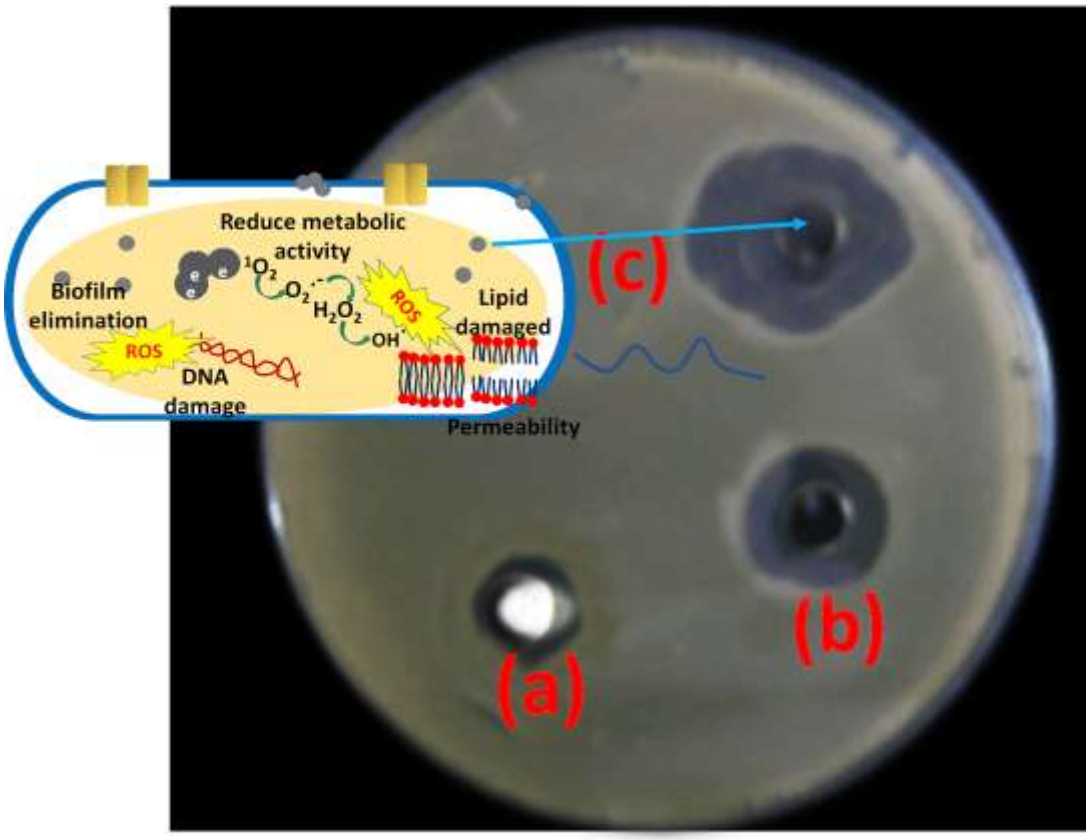

Fig. 4. Antibacterial effect of (a) pure chitosan, (b) chitosan-containing high loading of curcumin and (c) synergistic effect and oxidative stress mediated by chitosan-containing high loading of curcumin conjugated gold nanoparticles .

4. Conclusion

The current study offers to report a new, simple, single-step, and cost-effective room temperature strategy for encapsulating curcumin conjugated spherical gold nanoparticles in particulate carriers assembled by chitosan polymer as a trapping agent by pH adjusting that curcumin acts as the reducing and stabilizing agent based on the flash nanoprecipitation. The prepared samples were characterized with UV-Vis, AFM, and TEM analysis. From TEM and AFM images is found to be uniformly spherical in shape with an average diameter of 50-70 nm for chitosan-containing high loading of curcumin-conjugated gold nanoparticles. Regarding the obtained data from the antibacterial test by disc diffusion method, the prepared chitosan with high curcumin conjugated gold nanoparticles compared to the pure chitosan and curcumin encapsulated chitosan nanoparticles with high antibacterial activity will open a unique opportunity for industrial scale-up. A synergistic effect of gold nanoparticles and curcumin and a unique combination of chitosan natural amphiphilic polymer due to their widespread biological and technological applications have gained much attention.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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