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## Hepatoprotective and Urease Inhibitory Activities of Garlic Conjugated Gold

# Nanoparticles

Muhammad Ateeq,<sup>a</sup> Muhammad Raza Shah,<sup>\*a</sup> Hamid Ali,<sup>b</sup> Nurul Kabir,<sup>c</sup> Ajmal Khan,<sup>d</sup> Said Nadeem<sup>a</sup>

<sup>a</sup>H.E.J. Research Institute of Chemistry. International Center for Chemical and Biological

Sciences, University of Karachi, Karachi-75270, Pakistan.

<sup>b</sup>Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

<sup>c</sup>Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

<sup>d</sup>Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad-22060, Pakistan.

# **Supplementary Information**

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Figure S1 Comparative FT-IR spectrum of garlic extract and G-AuNPs

#### 1. Stability of G-AuNPs against Heat

The G-AuNPs were subjected to heating for 30 minutes at 80°C. The comparison of UV-Vis spectra of G-AuNPs before and after heating (Figure S2) suggested that G-AuNPs are stable at 80°C. A slight reduction was observed in the absorption maxima of the surface plasmon peak (Figure S2) that can be explained on the basis of dominant electronic dephasing mechanism and increasing disorder of the adsorbed ligands on the nanoparticle surface with increasing temperature. Temperature is also an important parameter that dictates the stability and utility of the G-AuNPs. As the temperature increases, the ligand molecules forming a monolayer at the surface of gold become disordered. Furthermore, dominant electronic dephasing may also be

responsible for the slight reduction in the intensity of the adsorption band. Electron-electron interactions at higher temperatures not only lead to a faster electron-electron scattering rate but also an increase in the electron-surface and electron-defect scattering. Electron's velocity depends on its energy state, and hence higher temperature induces excitation of electrons to higher energy levels. An increase in the electron's velocity leads to a larger damping constant and therefore to a faster dephasing; and hence reduction of absorbance of plasmon band.<sup>1</sup> Precipitation was not observed during the whole process.



Figure S2 UV-Visible spectrum of G-AuNPs before and after heating at 80°C

### 2 Stability of G-AuNPs at different pH

The original pH of G-AuNPs was 3.7 and the effect of pH on the stability of G-AuNPs was monitored through UV-Vis spectroscopy by varying pH (i.e. 2-13). At higher pH (i.e. 10–11) and lower pH (i.e. 1–2), the absorbance decreased and peak became broad. A sharp absorbance band

were observed between pH 4 to 11 (Figure S3). In other words, they are more stable at these pH values. The destabilization of the G-AuNPs may be due to the protonation (at acidic pH) and deprotonation (at basic pH) of secondary metabolites or proteins involved in the stabilization of G-AuNPs.



Figure S3 Effect of pH variability on stability of the G-AuNPs

### 3 Stability of G-AuNPs at different ionic strengths

The effect of ionic strengths was evaluated by carrying out experiments over a range of ionic strengths (NaCl concentrations of 1 mM, 10 mM, 100 mM, 500 mM, 1M and 2M), at a constant pH of 7.0 and temperature of 297.65 K. The effect of change in NaCl concentration on the G-AuNPs was monitored through UV-Vis spectroscopy after 24 h of mixing (Figure S4). The G-AuNPs were stable up to 10 mM (Figure S4) while stability decreased above that concentration. The stability of G-AuNPs towards salt concentrations was very low after 24 hours of mixing.

However, these nanoparticles were stable up to 18 hours of mixing with up to 1 M NaCl concentration, but after that aggregation phenomenon started while at higher concentrations of NaCl, the aggregation phenomena started immediately after mixing.



Figure S4 UV-Visible spectra of the G-AuNPs at different concentrations of NaCl

The effect of NaCl on the behavior of the G-AuNPs at a constant bulk concentration with varying ionic strengths can be seen in Figure S4. It can safely be deduced that NaCl is playing an important role in enhancing the aggregation by increasing the ionic strength and decreasing the electrostatic repulsion between particles. The effect of NaCl concentration on the behavior of the G-AuNPs is not significant when the NaCl concentration is low (i.e. less than 10 mM). It is known that G-AuNPs prepared through reduction develops a negative charge on the surface of the nanoparticle due to presence of anions. This negative charge is partially neutralized by the capping ligand or media; however, the gold nanoparticles can still have a slight negative charge. This negative charge can form an electric layer on the surface of gold nanoparticles which is

partially responsible for the stability of the gold nanoparticles. At very low NaCl concentrations (<10 mM), the ions (Na<sup>+</sup> and Cl<sup>-</sup>) in the bulk aqueous phase are stable and do not disturb the surface of the nanoparticles. As the NaCl concentration is increased, the Na<sup>+</sup> ions interact more closely with the partially negative charge on the gold nanoparticles, and thus, aggregation upon addition of NaCl is presumably due to higher interactions with the gold nanoparticles.<sup>2</sup>

### 4 Stability of G-AuNPs in the Rat Blood

The stability of G-AuNPs were also determined in the rat blood. The G-AuNPs were mixed with the rat blood. They were separated from the rat blood via centrifugation after different time interval and were then subjected to UV-Visible spectroscopy. It was found that G-AuNPs tolerate the blood electrolyte condition up to 18 hours (Figure S5). We believe that 18 hours are sufficient time for the nanoparticles to tolerate the blood electrolytic environment and penetrate through the cells and reach the target cells. The electrostatic interactions between gold atoms and capping agents are very important for the following reason: as they are weak, they will release the capping agents after 18 hours of injection and then the gold will be eliminated straight away which would not have been the case if the nanoparticles were more highly stable, and therefore, the side effects of the gold if any will be significantly reduced.



Figure S5 UV-Visible spectrum of the G-AuNPs in Rat Blood after 18 h

# References

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