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RESEARCH ARTICLE

Carbohydrate-based gold nanoparticles as colorimetric sensor for cysteine

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ABSTRACT

Background: Gold nanoparticles have been studied extensively for their potential application in the detection of important analytes. Their relative ease of synthesis through numerous procedures makes possible their implementation in a variety of assays. Cysteine (cys), a thiol-containing amino acid implicated in numerous pathologies such as obstructive sleep apnea (OSA), has been routinely detected through expensive fluorometric assay kits.

Objectives: As such, this study aimed to develop a carbohydrate-based gold nanoparticle colorimetric assay for the convenient and straightforward detection of cys.

Methodology: Carbohydrate-based gold nanoparticles (*c*-AuNPs) were synthesized following a microwave-assisted procedure. The as-prepared *c*-AuNPs were used to detect cys by plotting the ratio of the absorbances of the aggregated and dispersed gold nanoparticles against the concentration of cys.

Results: The *c*-AuNP solutions were able to detect cys in the micromolar range, with the glucose-based AuNPs (*glc*-AuNPs) showing the widest linear range (16.7 µm to 167 µm), and the fructose-based gold nanoparticles (*frc*-AuNPs) exhibiting the lowest detection limit (9.0 µm) for cys. Aside from being able to detect cys, the *c*-AuNPs were also responsive to tyr and lys.

Conclusion: This study demonstrates that carbohydrate-based gold nanoparticles prepared following a microwave-assisted procedure using sugars as reducing agents and capping agents can be used successfully in the detection of cysteine.

Keywords: *cysteine, gold nanoparticles, carbohydrates, reducing sugars, starch*

Introduction

Gold nanoparticles (AuNPs) exhibit optoelectronic properties that can be exploited in preparing sensors with high selectivity and sensitivity [1]. Their ability to exhibit surface plasmon resonance makes them a good colorimetric sensor [2]. Preparation of AuNPs involves the reduction of a gold precursor using either an organic or an inorganic reducing agent and the passivation of the resulting nanoparticles with various molecules for stabilization. One of the most common methods for the synthesis of AuNPs is the Brust-Schiffrin method which involves the use of sodium borohydride, toluene, and tetraoctylammonium bromide. While the use of these chemicals can produce gold nanoparticles of good quality (*i.e.*, stable and monodisperse), their toxicity raises environmental concerns. Not surprisingly, other methods for AuNP synthesis have been developed to address these issues [3].

Carbohydrates, aside from acting as reducing agents, can also stabilize AuNPs via their hydroxyl and carbonyl groups. Previous work on AuNP synthesis using carbohydrates as reducing agent, capping agent, or both presented promising results as regards the viability of the gold nanoparticles for practical applications such as in the spectrophotometric detection of biomolecules [3-5].

Cysteine (cys), one of the 20 common amino acids, plays important roles in biosynthesis, physiological regulation, and metabolism. Its roles in diseases such as HIV, rheumatoid arthritis, and obstructive sleep apnea (OSA) make it a potential biomarker for clinical applications and molecular diagnostics [6,7]. Numerous studies have indicated that cys react strongly with AuNPs via its thiol group. In fact, adding thiol-containing molecules into an AuNP solution will either make the solution

more stable or cause it to aggregate depending on its exact structure, concentration, and affinity to the AuNPs [8].

In this contribution, AuNPs were synthesized using monosaccharides and disaccharides as reducing agents and starch, a polymer of glucose, as a capping agent. The AuNPs were characterized using Dynamic Light Scattering analysis and absorption spectroscopy. Cys detection was performed using the synthesized AuNPs through ratiometric absorbance measurements. This study aimed to present a green (*i.e.*, environmentally benign) method for the synthesis of AuNPs and a procedure for rapid detection of cys.

Methodology

Reagents and Apparatus

Tetrachloroauric acid, $HAuCl_4$, (analytical grade) was obtained from Sigma-Aldrich. Deionized water was purchased from Golden Bat Enterprises. All sugars (D-glucose, D-fructose, L-maltose, L-lactose) as well as other reagents used were of analytical grade and were obtained from the Chemistry Laboratory Stockroom of the Department of Physical Sciences and Mathematics, College of Arts and Sciences, University of the Philippines Manila. Commercial corn starch was purchased from a local grocery and was used without further purification. Perkin Elmer Lambda 25 UV/Vis spectrophotometer was used for UV/Vis experiments.

Preparation of Solutions

Stock solutions of sugars with a concentration of 50 millimolar (mM) were prepared. A 10-mM phosphate buffer (PB) with a final pH of 7.1 and a solution of 2 mM $HAuCl_4$ were also prepared. Different concentrations of cys ranging from 0.01 mM to 1 mM were made by serial dilutions of 50 mM cys solution. One millimolar solution of different amino acids were prepared for selectivity studies.

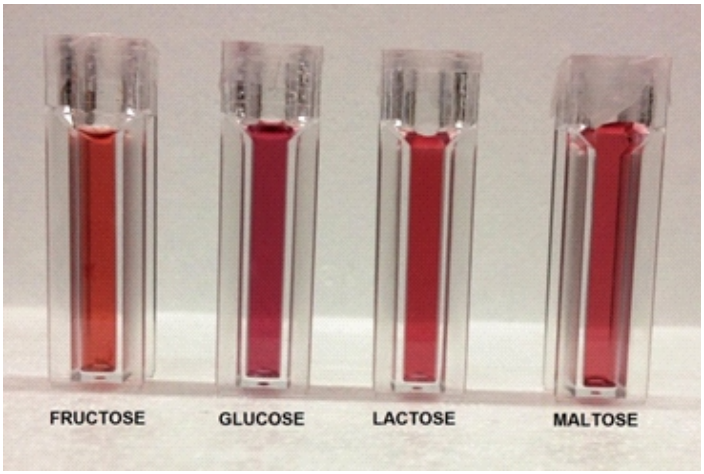

Figure 1. *Various c-AuNP solutions obtained using different sugars as reducing agents. (From left to right: frc, glc, lac, mal)*

Synthesis of Carbohydrate-based Gold Nanoparticles

A constant amount (ca. 4.3 mg) of starch was mixed with 10 mL of 50 mM sugar, 17 mL of PB, and 3 mL of 2 mM $HAuCl_4$. The final solutions were mixed thoroughly before exposing them to 1100-watt microwave for 1 minute in 20-sec intervals, for rigorous mixing. The resulting solutions were left to cool to room temperature for 1 hour.

Detection of Cysteine using Carbohydrate-based Gold Nanoparticles

In a plastic cuvette, 0.5 mL of the *c*-AuNPS solution was mixed with 1 mL of different concentrations of cys, ranging from 0.01 mM to 1 mM, and left to stand for 30 minutes. The final solution was 16.7 mM in *c*-AuNPs. A wavelength scan was performed, and the corresponding UV/Vis spectrum was recorded. The absorbances at wavelengths corresponding to the aggregated and dispersed *c*-AuNPs were measured and the ratio $A_{\lambda,aggregated}/A_{\lambda,dispersed}$ plotted against the concentration of cysteine to generate a straight line.

Testing the Selectivity of Carbohydrate-based Gold Nanoparticles for Cysteine

The selectivity of the *c*-AuNPs for cysteine was tested by adding 0.5 mL of 1 mM solutions of amino acids phenylalanine, glycine, glutamic acid, aspartic acid, histidine, lysine, tryptophan, tyrosine, leucine, and alanine to 0.5 mL of the *c*-AuNPs solution, measuring absorbances at wavelengths corresponding to the aggregated and dispersed *c*-AuNPs, obtaining the ratio $A_{\lambda,aggregated}/A_{\lambda,dispersed}$ and comparing them against that of cys and pure water.

Characterization of Carbohydrate-based Gold Nanoparticles

The Perkin Elmer Lambda 25 UV/Vis spectrophotometer was used in determining the absorbances and wavelengths of maximum absorbances of the nanoparticles. The Malvern Instruments Zetasizer Nano ZS90 (90° scattering detector angle; 4-mW, 632.8-nm red laser) was used to determine the size distribution and hydrodynamic diameter of the *c*-AuNP solutions through Dynamic Light Scattering (DLS) analysis.

Results and Discussions

Gold nanoparticle (AuNP) solutions were synthesized using glucose (glc), fructose (frc), maltose (mal), and lactose (lac) as reducing agents and starch as a capping agent (Figure 1). Monosaccharides such as glc and frc were able to reduce the gold precursor $HAuCl_4$ into elemental gold. Glc, an aldose, is a known reducing sugar while the observed ability of frc to reduce $HAuCl_4$ into AuNPs can be attributed to its isomerization into an aldose sugar [9]. The disaccharides mal and lac, through their reducing ends, were able to reduce $HAuCl_4$ [10,11]. The mechanism of stabilization of carbohydrate-based gold nanoparticles (*c*-AuNPs) by starch was not investigated. However, it is conceivable that the gold atoms on the surface of the AuNPs interact with the hydroxyl groups and carbonyl groups present in starch molecules, leading to the formation of a stabilizing molecular layer of starch on the AuNPs' surfaces.

Table 1. *Wavelengths of Maximum Absorption of Various c-AuNPs Synthesised*

Reducing Sugar	Maximum Wavelength, λ_{max} (nm)
Glucose (glc)	524
Fructose (frc)	519
Maltose (mal)	525
Lactose (lac)	526

The wavelengths of maximum absorbances of the *c*-AuNPs obtained are consistent with the reported spectrophotometric data on AuNPs [12]. Among the solutions prepared, frc gave the shortest λ_{max} while lac gave the longest λ_{max} (Table 1), though the observed differences in their wavelengths were relatively small. The differences in the λ_{max} obtained are due to the different mean sizes of the AuNPs formed which may be attributed to the different reduction potentials of the sugars used; the ease with which a sugar acts as a reducing agent depends on its structure.

The resulting *c*-AuNPs were tested as sensors for the detection of cys. The absorbance ratio A_{620}/A_{520} (*i.e.*, $A_{\lambda,aggregated}/A_{\lambda,dispersed}$) of *c*-AuNPs solutions was a linear function of cys concentration, demonstrating their potential utility in the quantification of cys. Table 2 summarizes the figures of merit for cys detection using c-AuNPs as sensors. The lowest limit of detection was observed with frc-AuNP solutions, having a limit of detection of 9 µM—although this is good for a non-optimized assay, it is inferior compared to the limits of detection of other established assays such as the cys assay kit by Caynam Chemicals, which can detect cys in nanomolar concentrations. Regardless, the assay is still useful for the analysis of cys in biological samples because the concentration of cys in the plasma is in the micromolar range [7]. The relatively poorer limits of detection may be attributable to the *c*-AuNPs being coated with too much starch polymer, to the extent that small amounts of cys were not able to induce

Table 2. *Figures of Merit for Cys Detection using Various c-AuNPs*

Carbohydrate-based Gold Nanoparticles	Reducing Sugar Used	Limit of Detection, µM	Sensitivity (Slope)	Linear Range, µM	Linearity Coefficient (r^2)
***glc*-AuNPs**	glc	16.6	1.76	16.7-167	0.9923
***frc*-AuNPs**	frc	9.0	1.34	33.3-167	0.9980
***lac*-AuNPs**	lac	15.0	3.01	26.7-100	0.9848
***mal*-AuNPs**	mal	18.6	0.78	30-100	0.9977

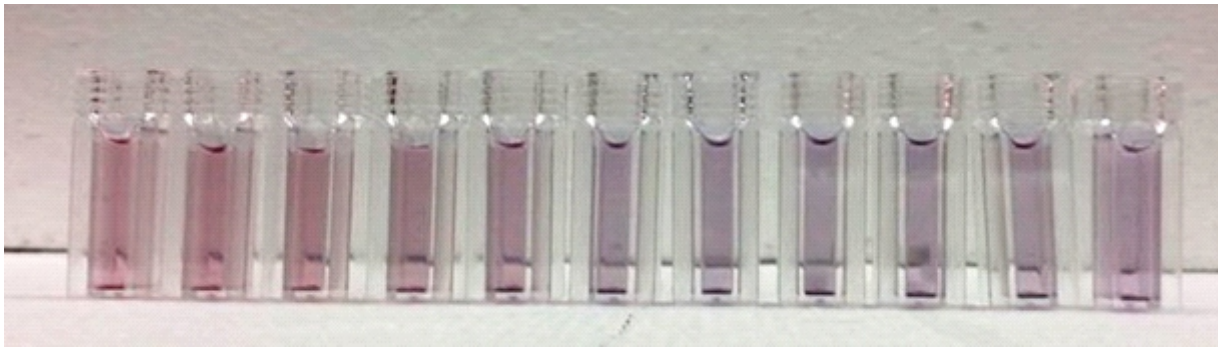

Figure 2. *Visual changes in glc-AuNP solutions in the presence of increasing concentrations of cys. (From left to right: 0.05 mM, 0.06 mM, 0.07 mM, 0.08 mM, 0.09 mM, 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM)*

AuNP aggregation. Although this stability may limit the detection efficiency of the *c*-AuNPs for cys, it is an important factor for long-term storage of the assay reagents. Among the *c*-AuNPs, *lac*-AuNPs displayed the greatest sensitivity. Sensitivity is related to the slope of the linear regression plot. A higher slope means that a small change in the concentration of cys can cause a significant change in the absorbance ratio.

Figure 2 shows the visually observable changes in *glc*-AuNPs solutions upon addition of cys. This corresponds to the appearance of a shoulder in the absorbance spectra at around 620-640 nm upon addition of cys to the solution of *glc*-AuNPs (Figure 3). This observation can be rationalized based on the changes on the surface plasmon resonances of the AuNPs that accompany their aggregation, leading to the enhancement of the plasmon absorption at around 620-640 nm. The colorimetric changes clearly show that the *glc*-AuNPs can be used for the quantitative detection of cys (Figure 4).

The aggregation of citrate-capped AuNPs in the presence of cys has been reported in the literature. It is believed that the thiol group of cys reacts with the AuNPs, leading to the covalent immobilization of cys molecules on the AuNP surface. The ionic interactions between the carboxylate groups and ammonium groups of cys molecules on different AuNPs then lead to nanoparticle cross-linking and aggregation [13]. We believe that the aggregation of the *c*-AuNPs reported in this study occurs through a similar, if not the same, mechanism.

Gold nanoparticles have been used in the detection of cysteine in the past. For instance, Li and Li reported the use of AuNPs in detecting cys; however, their method required the presence of Cu^{2+} ions as it relied on the formation of a Cu^{2+}-cys complex [14]. Wang *et al.* employed AuNPs capped with the surfactant cetyltrimethylammonium bromide (CTAB) in detecting cys in solutions with high salt concentration [15]. The cytotoxicity of CTAB, however, raises concerns about the use of CTAB-containing sensors [16]. Chen and co-workers prepared AuNPs capped with single-stranded DNA (ssDNA) and used the nanoparticles for detecting cys [17]. Compared with the aforementioned reports, the mechanism of detection of the method reported in this contribution is more straightforward and less complicated as it does not rely on complex formation or on specifically tailored molecules (ssDNA) for detection. Moreover, the reagents and the capping agents used in the preparation of the *c*-AuNPs are innocuous (*e.g.*, sugars).

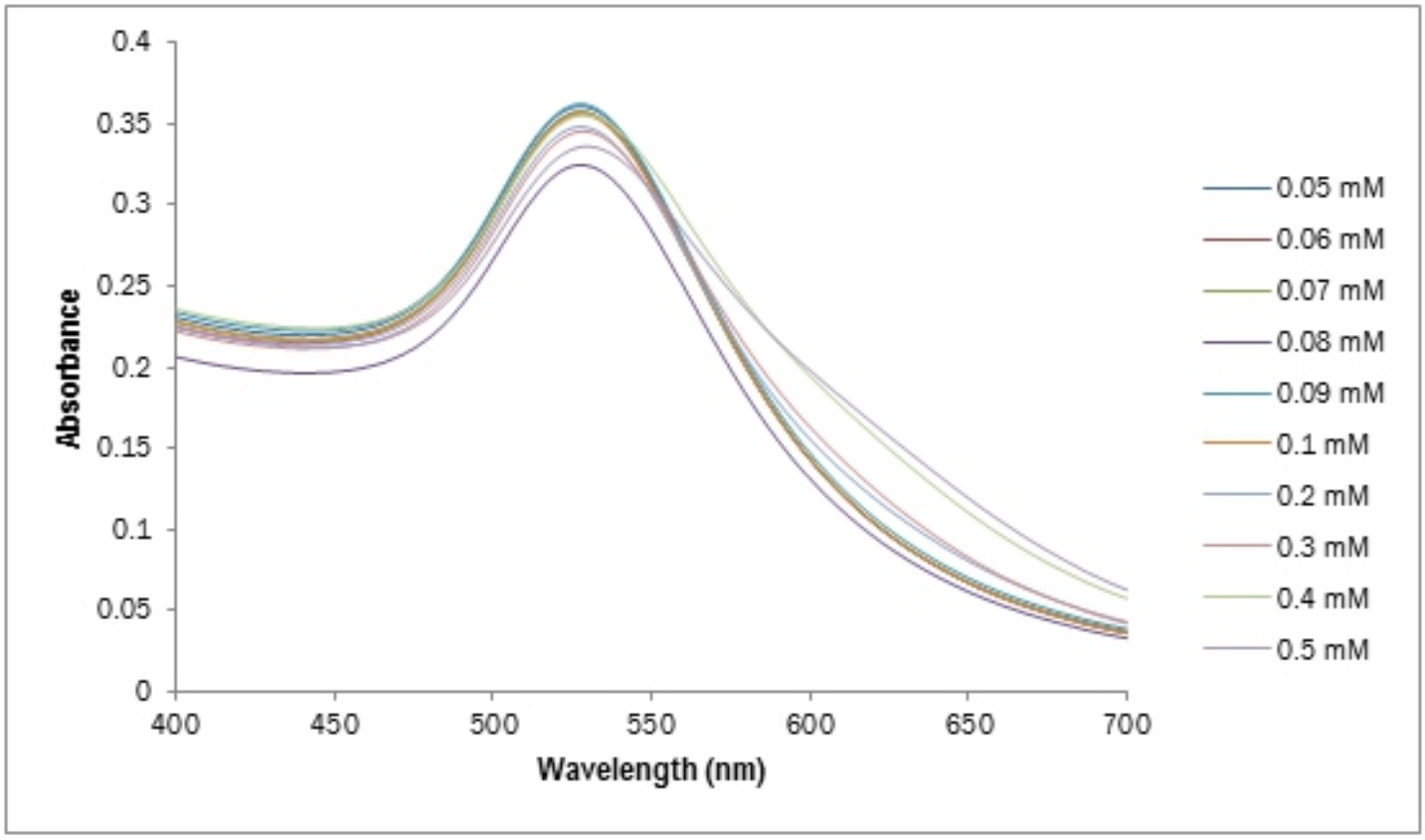

Figure 3. *Visible spectra of glc-AuNPs in the presence of varying concentrations of cys.*

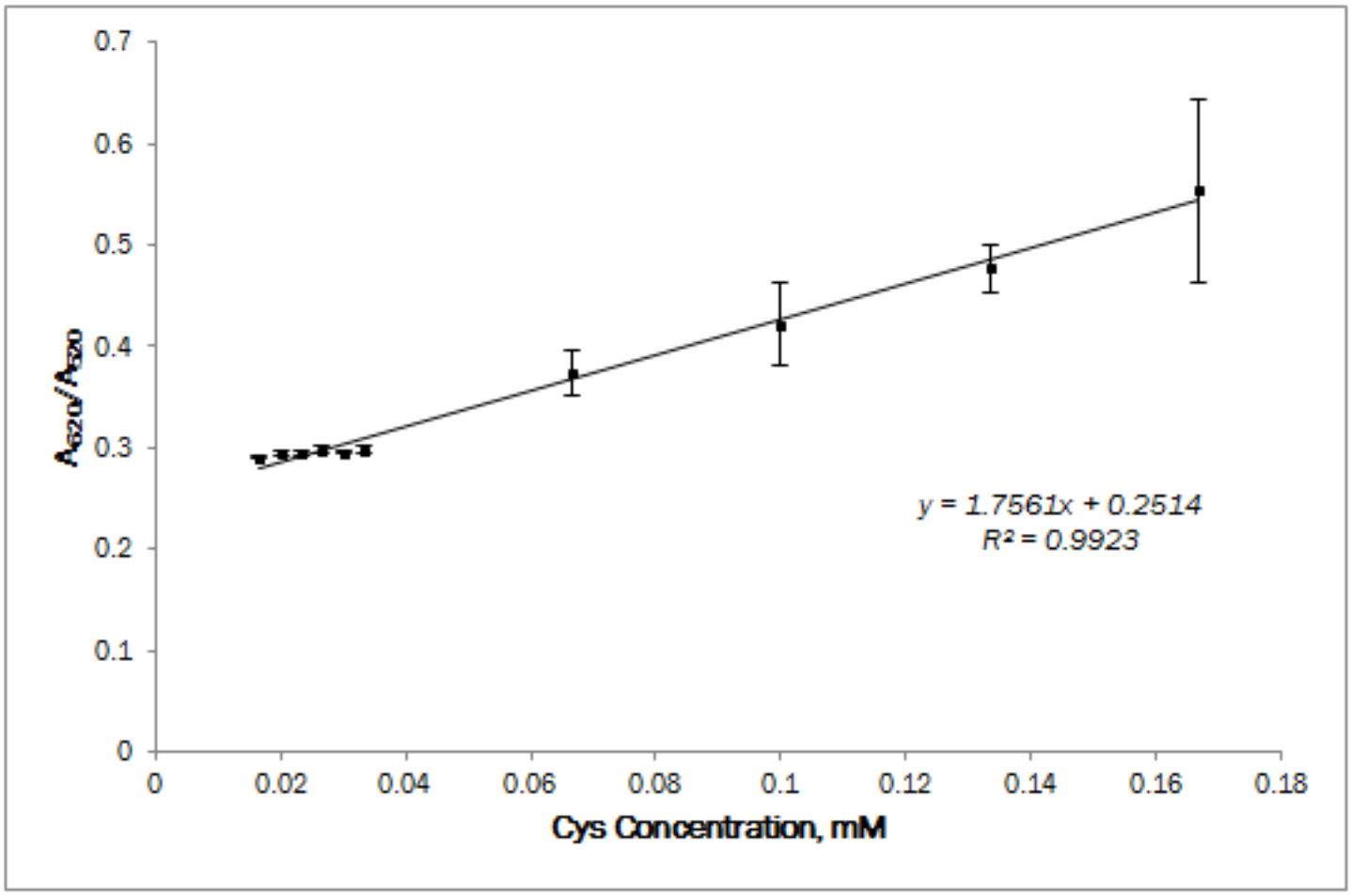

Figure 4. *Linear plot of A_{620}/A_{520} of glc-AuNPs against cys concentration.*

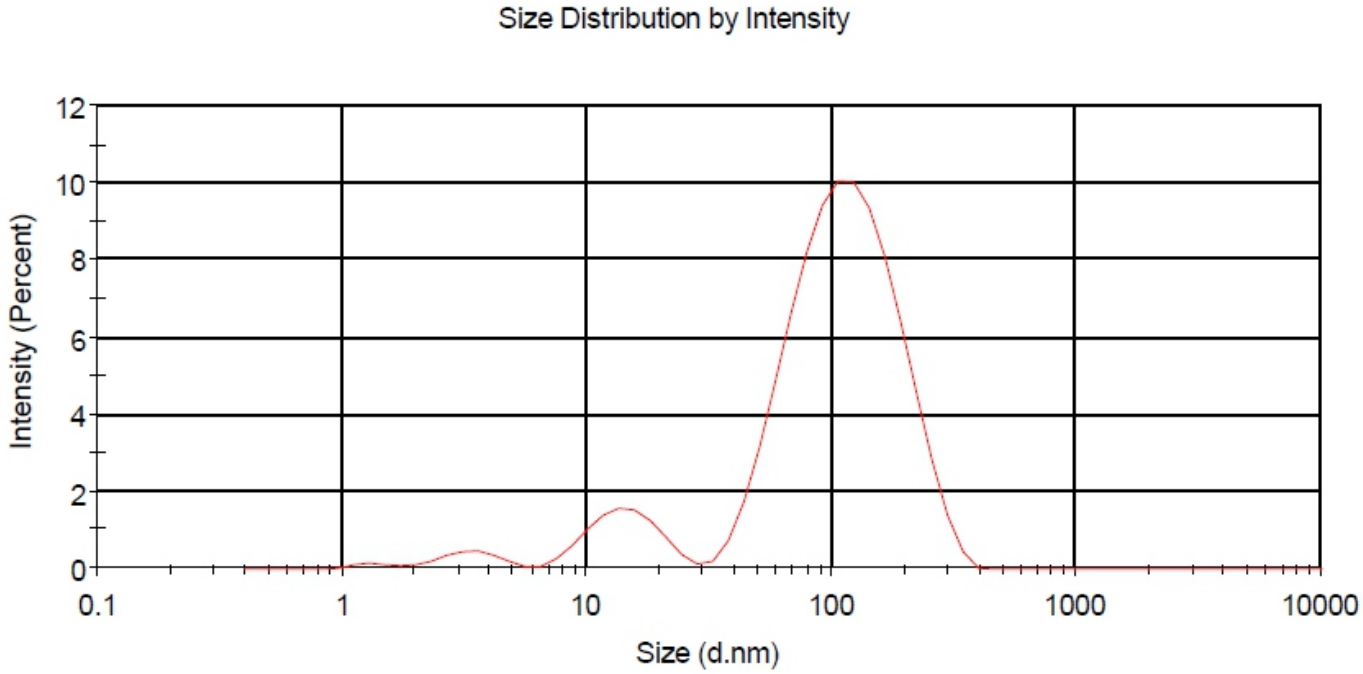

Figure 5. *Dynamic Light Scattering (DLS) profile of glc-AuNPs.*

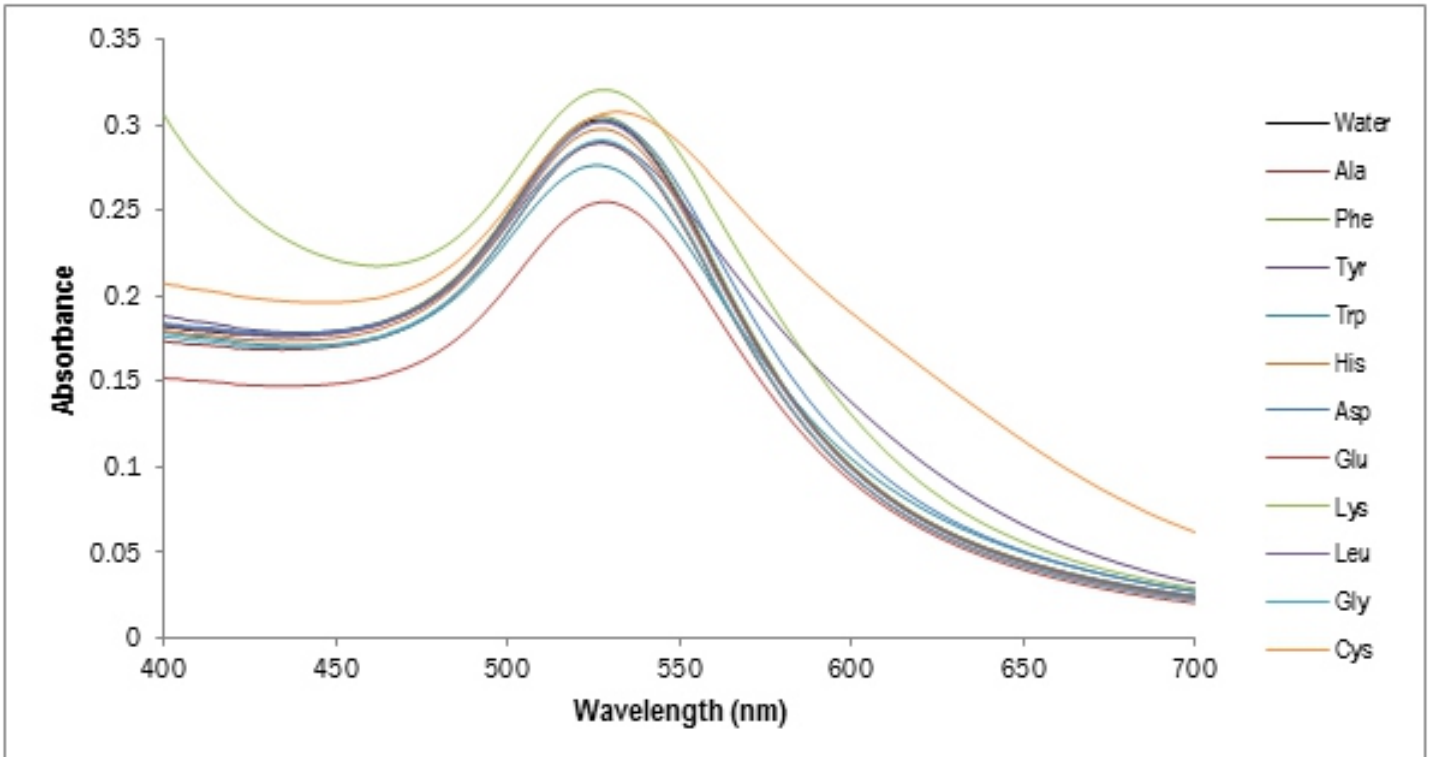

Figure 6. *Visible spectra of glc-AuNPs upon addition of different amino acids.*

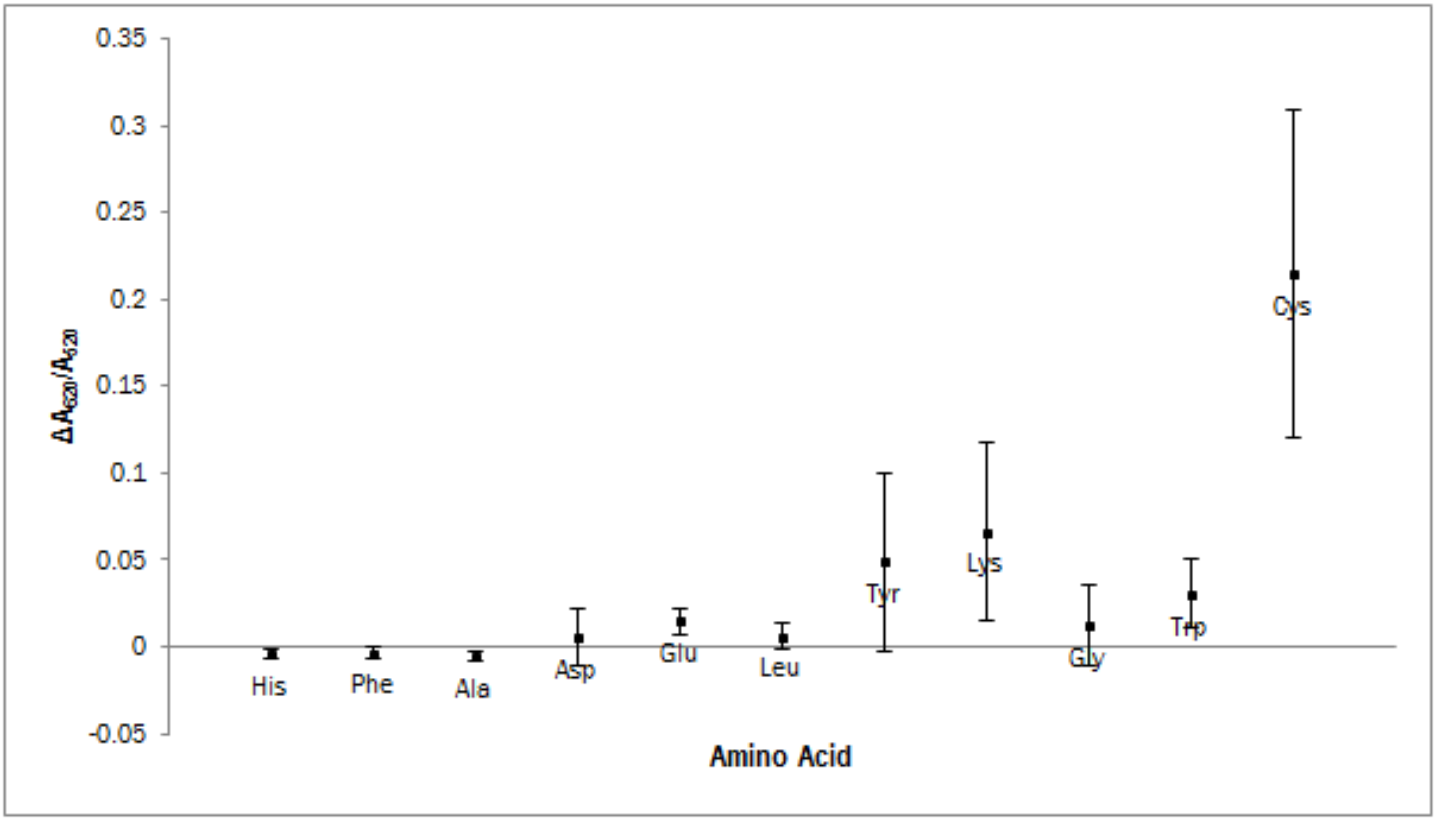

Figure 7. *Change in A_{620}/A_{520} of glc-AuNPs upon addition of different amino acids.*

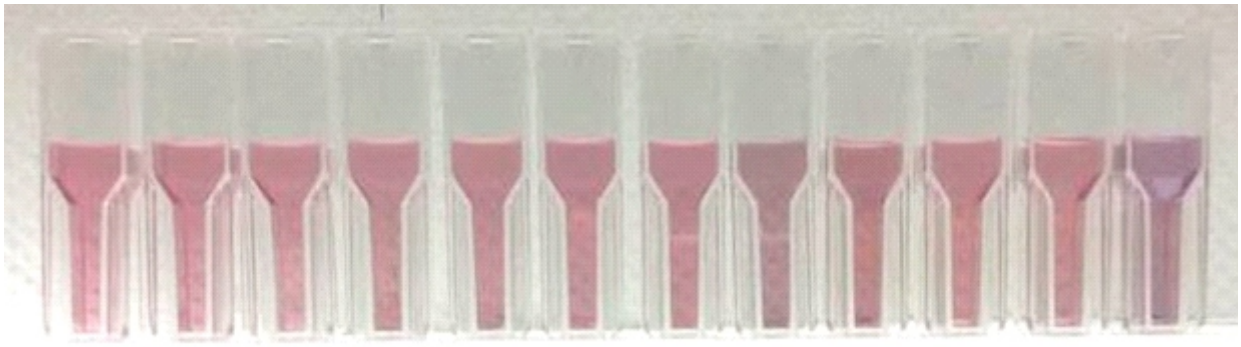

Figure 8. *Visual changes in glc-AuNP solutions upon addition of different amino acids. From left to right: water, ala, phe, his, glu, asp, trp, tyr, lys, leu, gly, cys.*

Figure 5 shows that the *glc*-AuNPs are polydisperse. This polydispersity may be due to the heterogeneity in sizes of the commercial starch molecules used for capping and stabilizing the synthesized AuNPs. A representative hydrodynamic diameter obtained from Dynamic Light Scattering (DLS) experiment is 100 nm. This result is consistent with the successful coating of the AuNPs with the starch polymer. Thus, the hydrodynamic diameters of the nanoparticles obtained were significantly greater than gold core diameters typically obtained from $HAuCl_4$ reduction procedures (~13 nm).

Excess amounts of different amino acids were added to *glc*-AuNPs to evaluate their selectivity for cys. Among the different amino acids, cys gave the highest absorbance at 620-640 nm region (Figure 6) as well as the highest change in the ratio of A_{620}/A_{520} upon addition of the amino acid (Figure 7). Other amino acids including tyrosine (tyr), lysine (lys), and tryptophan (trp), however, gave also noticeable shifts in A_{620}/A_{520}. Tyr possibly caused a shift in absorbance via interaction of its phenolic sidechain with the *glc*-AuNPs [18]. Lys, on the other hand, could have interacted with the *glc*-AuNPs through its basic amino sidechain [19]. Lastly, the indole ring nitrogen of tryptophan could have interacted with the *glc*-AuNPs, causing their aggregation [20].

Visually, only tyr and cys were able to cause the *glc*-AuNPs solutions to change in color from red to purple at the concentration of cys used (Figure 8). However, both tyr and lys caused significant changes in the absorbance ratios of *glc*-AuNPs. Thus, the proposed detection procedure may give false positive results when applied to samples containing tyr and lys. This suggests that a masking procedure/treatment is required prior to the analysis of cys content in a sample using the *c*-AuNPs.

Conclusion

Stable AuNPs were synthesized using different sugars as reducing agents and starch as a capping agent. The *glc*-AuNPs obtained had a representative hydrodynamic diameter of 100 nm. The polydispersity of the *c*-AuNPs produced can be attributed to the size heterogeneity of starch molecules capping the AuNPs. The detection of cys using the synthesized *c*-AuNPs displayed micromolar limits of detection, acceptable sensitivity and linear ranges, and excellent linear correlation coefficients. Cys caused the appearance of a surface plasmon resonance shoulder band at 620-640 nm region through the formation of nanoparticle aggregates. Although cys gave the most pronounced change in A_{620}/A_{520}, the *glc*-AuNPs were observed to respond to tyr and lys as well.

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