Detection of a Model System Using Gold Nanoparticles

Stephen Szajek and Dr. Julia Bingham Wiester

Department of Chemistry, Saint Xavier University, Chicago, IL 60655

Introduction

Cancer is the second most common cause of death in the United States, second only to heart disease.1 Despite global efforts to combat this disease, it remains a major health issue. One way that researchers have attempted to decrease the mortality rate of this disease is by early detection through screening tests.2 Biomarkers are measurable substances whose presence can be indicative of diseases. However, a quick and easy method for detecting biomarkers in medical laboratories is needed.3

Nanoparticles are used in detection assays due to structure-defining traits such as size, shape, high surface area, and unique optical properties.4 Plasmonic nanoparticles, such as gold and silver, are commonly used due to their size-dependent absorption in the ultraviolet-visible range from surface plasmon resonance.5 Plasmonic nanoparticles have been used to improve the limit of detection of the standard ELISA, but they are also used on their own due to their localized surface plasmon resonance (LSPR). In 1998, the first work where LSPR was used for detection was done by Englebienne et al., who showed that neither agglutination nor aggregation were needed for an SPR shift to occur.6 LSPR sensing is based on the change in local refractive index surrounding the nanoparticle, causing a change in the nanoparticle absorption. Due to the high refractive index of proteins and the short electromagnetic field decay length of localized surface plasmons on the same scale as proteins, nanoparticles and biomolecules are ideal for this detection method.7 Not only has this method been used in the detection of biomarkers, but it’s detection capabilities have also been tested using model system such as it is used in this work.

Material And Methods

Materials

The 50nm biotinylated gold nanoparticles were purchased from Cytodiagnostics (Burlington, Canada) and were diluted 50x for all experiments. The streptavidin was purchased from Sigma-Aldrich. The 1000 mL PBS buffer was made using 3.9063g of sodium dihydrogen phosphate monobasic and 20.2109g of sodium phosphate dibasic. A Nalgene disposable filter was used to filter out any microorganisms and the buffer was stored in the refrigerator. An Agilent 8453 UV-Vis spectrometer was used for spectral analysis. The streptavidin was prepared by making a 0.1mg/mL stock solution and 1 mL aliquots were stored frozen.

Streptavidin Detection

Multiple trials were run varying the concentration of streptavidin and buffer conditions. The first run was done using DI water for dilution. For this run, 40µL of biotinylated GNP’s was added to 1.960mL of DI water (50 x dilution), mixed, and the absorbance was measured at 533nm. After this, 10µL of 0.1 mg/mL were mixed in and the absorbance was measured at 533nm. The absorption was then measured every 5 minutes. After 100 min, another 10µL of streptavidin was added by the same method and the absorption was measured every 5 minutes for a total of 15 minutes. This process was repeated three more times for a total of 50µL of streptavidin added. The measured absorbance values were then graphed against the total time of the reaction. (Figure 4) A similar procedure was followed for additional trials. Two trials were done where the pH of the buffer was changed in order to determine if pH affected the system. Once the pH was adjusted, the procedure was repeated as before with the dilution of 40µL of biotinylated GNP’s with 1.960mL of each adjusted buffer.

Purpose

In this work, biotinylated gold nanoparticles were used. The streptavidin binds with the biotin and could cause aggregation (Figure 2) or potentially a change in the local refractive index, causing a change in the absorption spectrum. Here we examine the concentration needed to change the absorption spectrum.

Results

As seen in Figure 6, there was a decrease in absorbance for each trial over time. Each trial showed a different change in absorbance where the runs involving a pH showed the most severe drop in immediate absorbance once the streptavidin was added while the runs with neutral pH buffer and DI water showed the least amount of change. These drops in absorbance are likely due to aggregation of the gold nanoparticles in solution due to the added streptavidin binding with the biotin (Figure 2).

One control consisted only the biotinylated nanoparticles diluted in buffer and absorbance measured vs. time. This run also showed a slight change in absorbance as seen by the top line on the graph starting above 0.94. Although there has been no streptavidin added to cause aggregation, some aggregation of nanoparticles may occur due to electrostatic interactions with the salts in the buffer. The other control used was non-functionalized gold nanoparticles with additions of streptavidin for a total of 1,000µL added. This control also showed a drop in absorbance after each addition as seen in the second line in Figure 6 starting just below 0.84. This control features a greater drop in initial absorbance than the diluted biotinylated control. This greater drop is due to the streptavidin non-specifically binding directly with the surface of the nanoparticles.

We do not observe the desired wavelength shift as initially expected due to streptavidin binding to the biotinylated nanoparticles. After contacting the manufacturers of the nanoparticles, we discovered that the linker molecules were approximately 20-25 nm in length. This long distance between the nanoparticle and the biotin results in a large decrease in sensitivity, thus rendering our UV-Vis unable to detect the shift caused by the biotin-streptavidin binding because it is too far away from the signal enhancing gold nanoparticle.

Due to the results obtained in this experiment, the detection method of change in absorbance is reliable for the more minor change in the wavelength shift method. In the future I hope to continue this work using gold nanoparticles with smaller linker lengths in order to determine if the absorbance method still remains the more reliable method. I would also like to possibly combine this with biomarkers for diseases such as cancer to see if the viability remains.

Discussion and Conclusion

References


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Figure 3: Sample absorption spectrum of gold nanoparticles. Inset: solution of biotinylated gold nanoparticles.

Figure 4: Trial 1 where 10µL of streptavidin was added over time for a total of 50µL. Drops in absorbance are when additional streptavidin was added.

Figure 5: (Trial 4) Change in absorbance over time for the trial run at low concentration of streptavidin (16 x 10⁻⁷ M).

Figure 6: Change in absorbance over time for each trial. Diluted GNP-Control (biotinylated GNP, no streptavidin) and Control (non-functionalized GNP with streptavidin). The sharp drops are where additional streptavidin was added.

Table 1: Trials with varying streptavidin concentrations and buffer conditions.

Table 2: Change in absorbance for each trial as the different amounts of streptavidin were added.