Spectroscopy and surface plasmon resonance labreport

Furkan Kaya and Ragni Olsson

October 2017

Abstract

Sprectrophotometry of DNA-PEI popyplexes and gold nanoparticles was performed to measure absorption at different sizes and concentrations. The DNA concentration in the DNA/PEI polyplexes could not be determined due to errors. Three samples of gold nanoparticles at unknown sizes were compared to known standard solutions, to give an estimated size of 5 nm for sample S. Due to the lack of spectra giving sufficient information, no estimate could be done for sample E and N.

1 Introduction

Absorption spectroscopy can be used to measure characteristics such as concentration or size of an unknown sample by comparing with a standard curve. In this report, the goal is to present the results from determining the DNA-concentration in a DNA-PEI polyplex solution, and the size of gold nanoparticles. The experiments were performed using a Nanodrop system at NTNU NanoLab, where the DNA-PEI complexes had been made a few hours before characterization.

2 Theory

2.1 Absorption spectroscopy

Absorption spectroscopy is a group of techniques used to measure absorption of radiation upon interaction with a sample, see figure 1. The absorbed energy is measured to identify and quantify particles in a solution. Among the different techniques and types of set-ups, the most commonly used is to measure the amount of radiation that is transmitted through a sample, to calculate the absorption using the known output from the source.

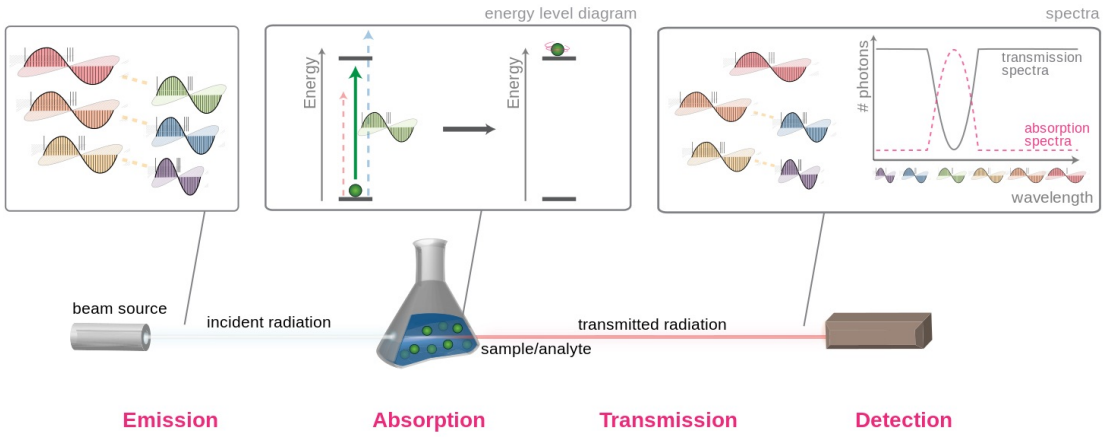

Figure 1: Schematic of absorption spectroscopy.

2.2 Beer-Lamberts law

Beer-Lamberts law is an equation relating the attenuation of light to the properties of the material it is penetrating:

$$A = log\frac{I_0}{I} = \epsilon \times l \times c \tag{1}$$

where A is the absorption, I_0 is the reference intensity, I is the measured intensity, ϵ is a constant, l is the length of the solution the light passes through, and c is the concentration of particles in the solution.

2.3 Localized surface plasmon resonance

Localized surface plasmon resonance (LSPR) is a type of surface plasmon resonance (SPR), where the continuous film of gold is exchanged by metal nanoparticles, often gold or silver. SPR is the resonant oscillation of electrons at the interface between two materials, caused by excitation of the metal nanoparticles by incoming light, see figure 2. In LSPR, the interface is between the metal nanoparticles and the particles in the solution. (Localized) SPR is commonly used to measure the absorption and hence concentration, and is important in applications such as biosensors.

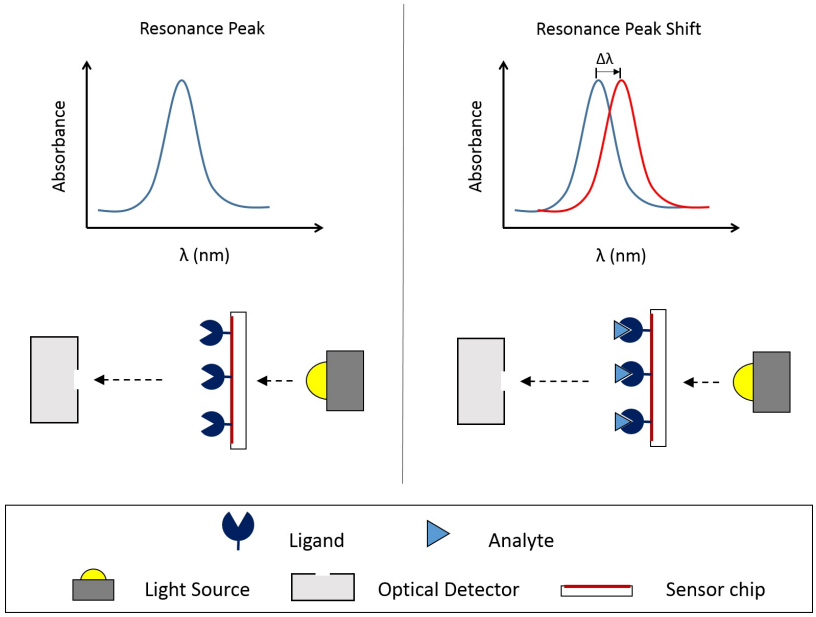

Figure 2: Schematic of surface plasmon resonance.

3 Method

In this report, a user-friendly setup called Nanodrop was used, using ultraviolet light as the radiation source. A drop of the wanted solution was placed on the sample holder using a 10 ul automatic pipette. After performing measurements, the solution was wiped away using an ISO 5 cloth, before wiping with an ISO 5 cloth with DI water. Before measuring the samples, a drop of DI water was measured as a blank. The process was then performed for the following solutions to determine the amount of DNA per polyplex:

- DNA, 1 ug/ml
- DNA, 10 ug/ml
- DNA, 100 ug/ml
- DNA, 1000 ug/ml
- Standard PEI solution
- DNA-PEI polyplex solution

And the following solutions to determine the size of the unknown gold nanoparticles:

- Gold nanoparticles, 5 nm
- Gold nanoparticles, 40 nm
- Gold nanoparticles, 100 nm

- Gold nanoparticles, unknown sample S
- Gold nanoparticles, unknown sample N
- Gold nanoparticles, unknown sample E

4 Results and discussion

4.1 DNA/PEI polyplexes

The aim, as mentioned in section 3, was to perform measurements of DNA solutions at four different concentrations. Unfortunately, we only have three measurements, and they seem to give erroneous spectra. In figure 3, we see the solution of 1 ug/ml DNA in blue, 10 ug/ml DNA in green and 100 ug/ml DNA in red. From available knowledge, it is known that the absorption is supposed to increase upon increasing concentration. From this, it is evident that something has gone wrong, be it confusion of the different solutions, error during measurements or others. Confirming that the curves are in reality correct, only for other concentrations than the ones indicated here, is not possible without performing new measurements, which we unfortunately are not able to accomplish. Without the curve for 100 ug/ml DNA it is also difficult to create a reliable standard curve, and in total this unfortunately means it is not possible to create a standard curve. Nevertheless, it is possible to observe an absorption peak at around 250 nm, and this can be assumed to be correct from the consistency of the three spectra.

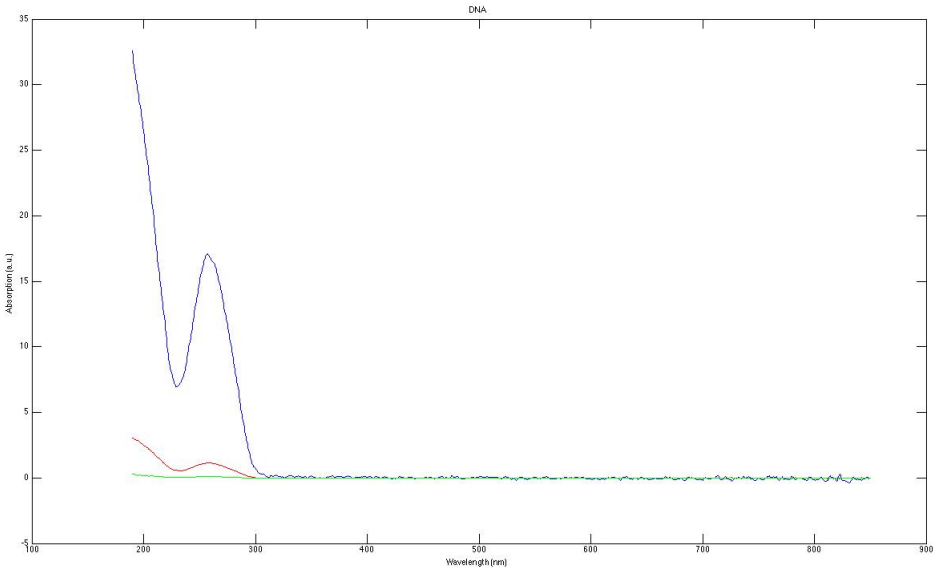

Figure 3: Absorption spectra for DNA solutions in concentrations of 1 (blue), 10 (green) and 100 (red) mg/ml.

The spectra for the DNA/PEI polyplex solution can be seen in figure 4. As can be seen, the absorption peak corresponds with the one observed for the standard solutions, ie around 250 nm.

Due to the lack of a standard curve, unfortunately it is not possible to say anything about the concentration.

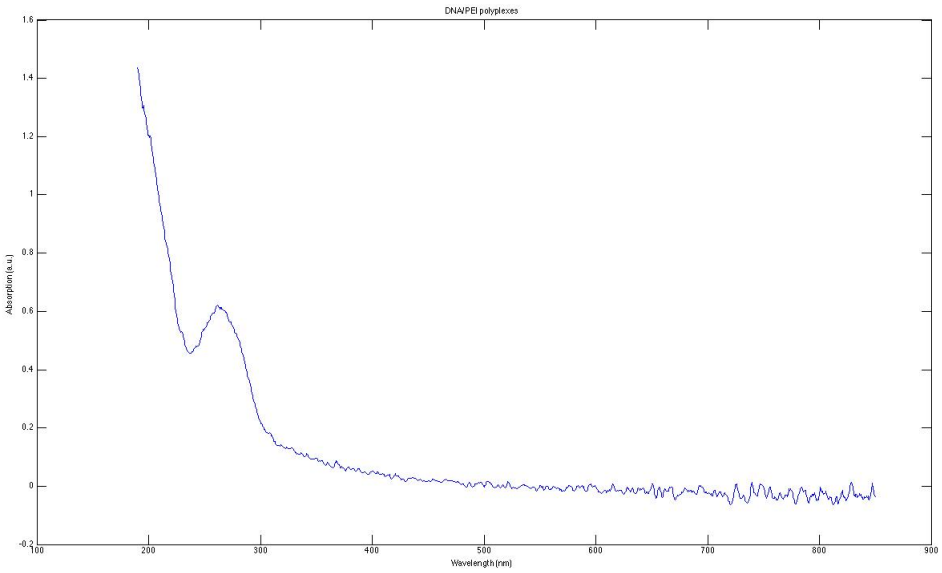

Figure 4: Absorption spectra for the solution of DNA/PEI polyplexes.

PEI did not give any significant absorption for wavelengths in the area of interest, see figure 5, which seems reasonable given the similarity of the spectra for DNA/PEI polyplexes compared with DNA.

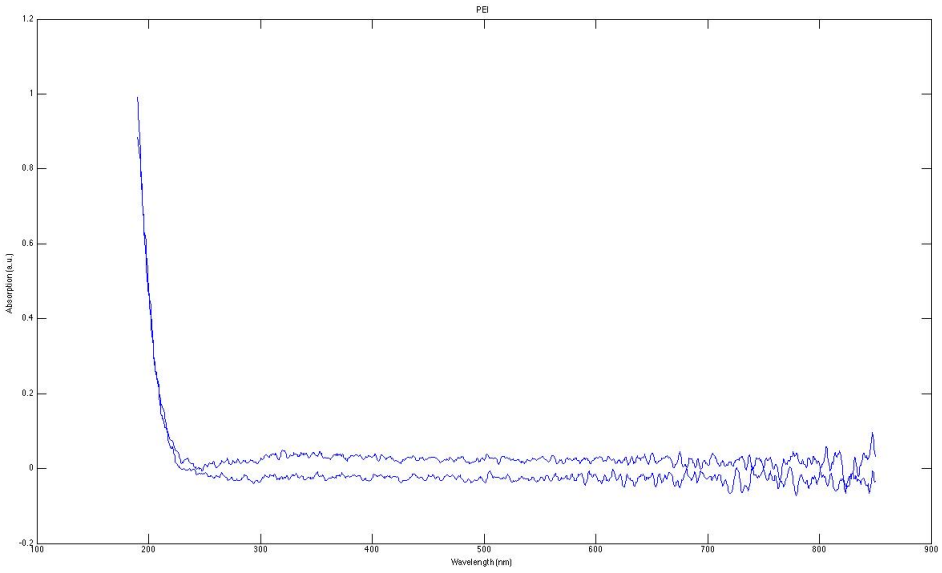

Figure 5: Absorption spectra for PEI solution.

4.2 Gold nanoparticles

Measurements of three samples of gold nanoparticles of different diameter were performed, see figure 6 - 8. Visual inspection of figure 7 leads to the suspicion that there is an error, since the peak is a lot smaller than for the other two graphs. Given that everything else is the same, ie that the particles have the same shape, it should resemble the other two spectra. However, it is possible that the concentration is somehow lower in this sample. By visual inspection the small peak is around 525 nm, very close to the peak of the spectrum for 5 nm but far from the peak in the spectrum for 100 nm, which means this cannot be used in the determination of the three unknown samples.

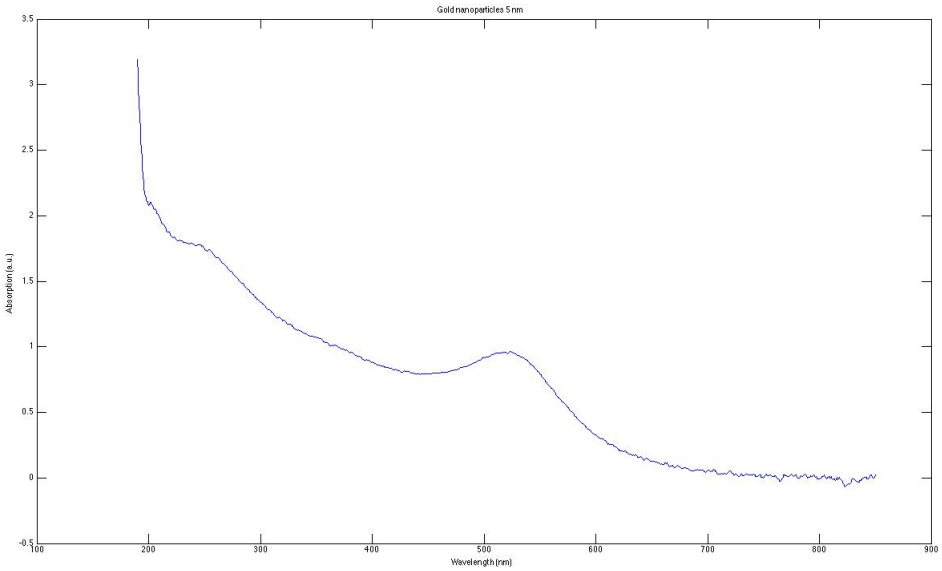

Figure 6: Absorption spectra for gold nanoparticles with 5 nm diameter.

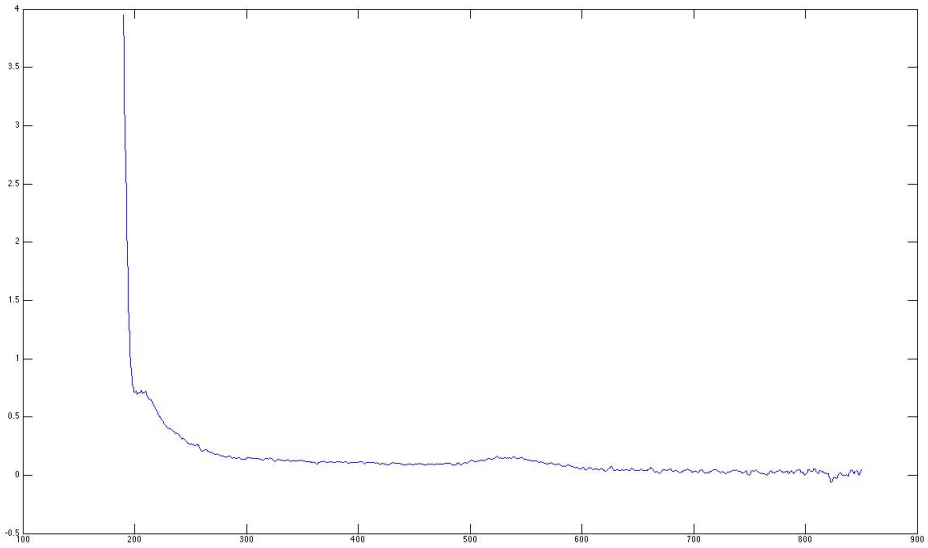

Figure 7: Absorption spectra for gold nanoparticles with 40 nm diameter. There seems to be an error with the spectrum, due to the lack of a distinct peak.

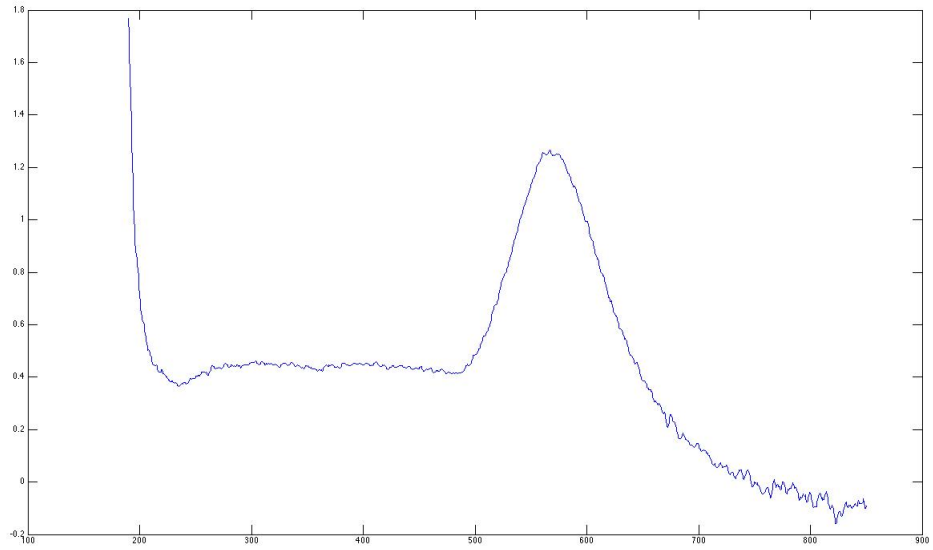

Figure 8: Absorption spectra for gold nanoparticles with 100 nm diameter.

Measurements for the three unknown samples containing gold nanoparticles of unknown size are shown in figure 9 - 11. Unfortunately, there is clear that there is an error in the spectra for sample E (figure 10) an N (figure 11). Thus, an estimated size will only be found for sample S. To accomplish this, a plot of absorption peak versus size will be used, see figure 12. Due to the above mentioned reasons, only the datapoints for 5 and 100 nm will be used.

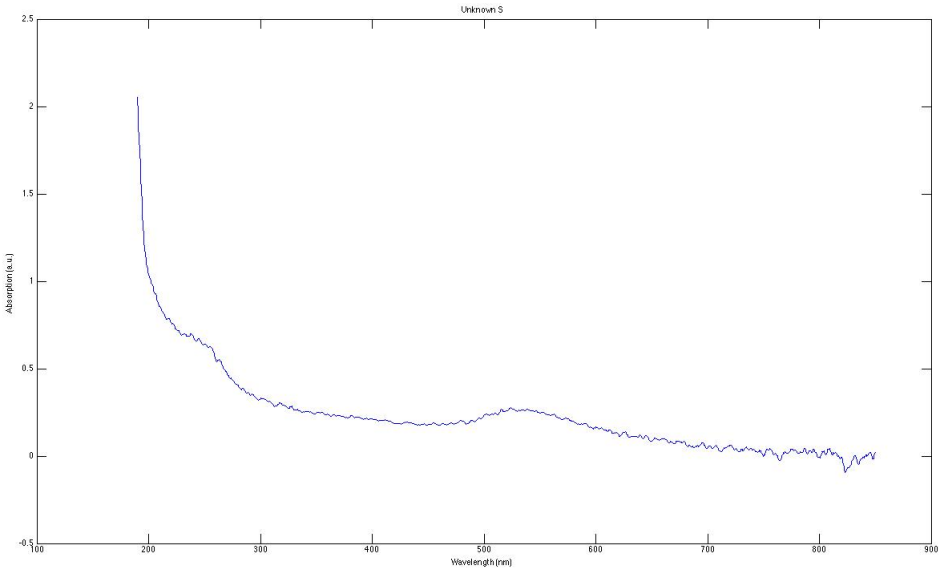

Figure 9: Absorption spectra for gold nanoparticles of unknown diameter, named sample S.

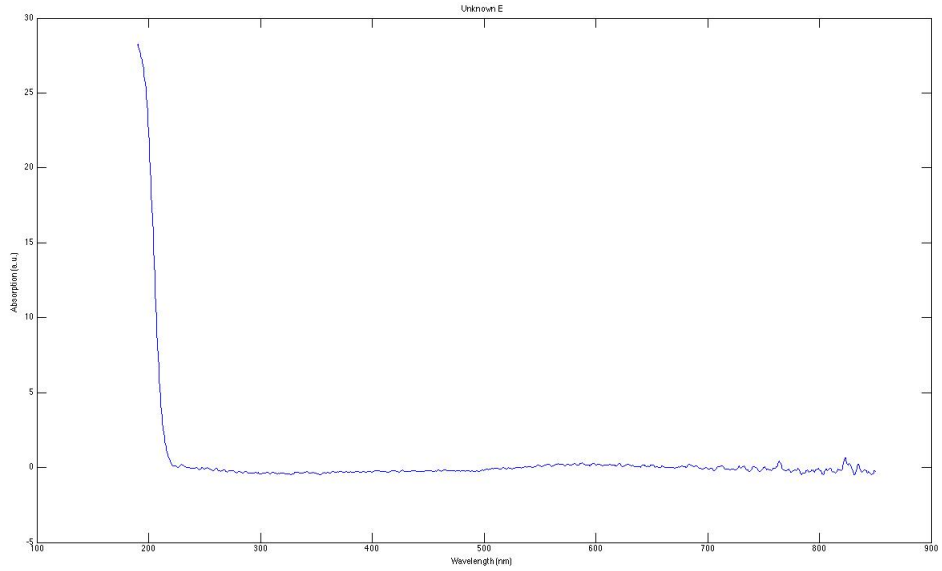

Figure 10: Absorption spectra for gold nanoparticles of unknown diameter, named sample E

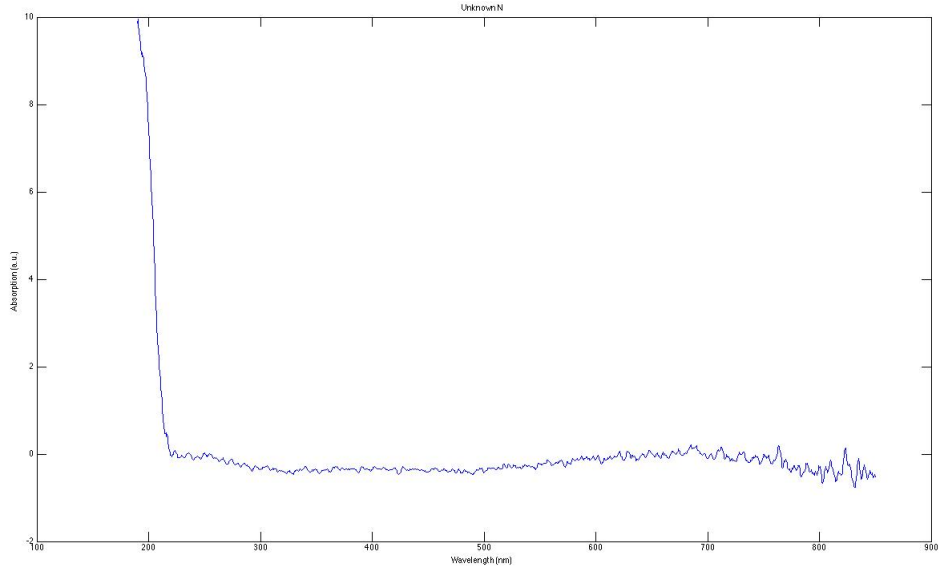

Figure 11: Absorption spectra for gold nanoparticles of unknown diameter, named sample N.

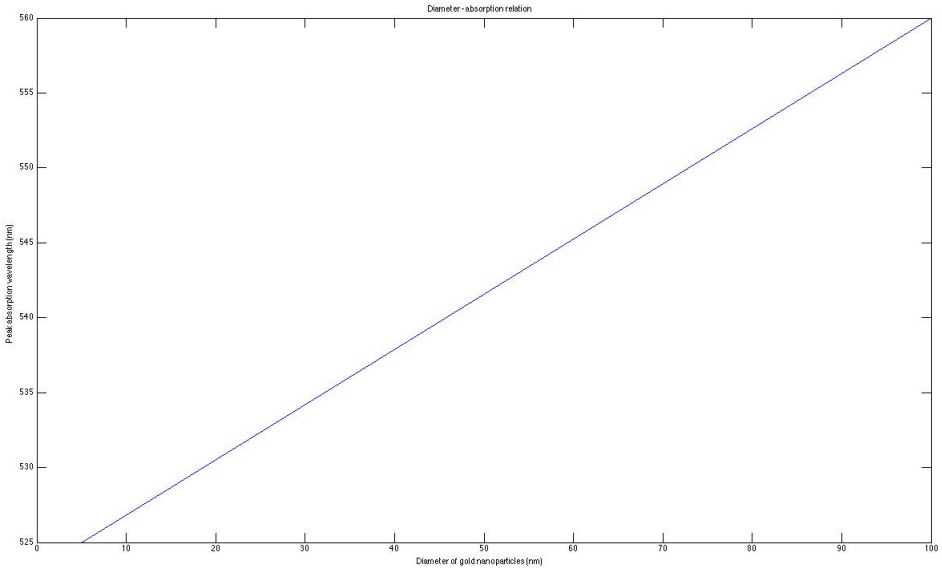

Figure 12: Absorption spectra for gold nanoparticles of unknown diameter, named sample N.

As the peak absorption wavelength is 525 nm for sample S, from the curve in figure 12 the size can

be estimated to be 5 nm. As the two graphs for the standard solution containing gold nanoparticles with a diameter of 5 nm and the unknown sample S are not identically shaped, it seems reasonable to assume that the shape of the particles is somewhat different. It was assumed that the standard solutions contained only spherical gold nanoparticles. They might not, and if they do the particles in sample S may be differently shaped. As seen in figure 13, gold nanoparticles come in varying shapes, and this is known to affect the absorption.

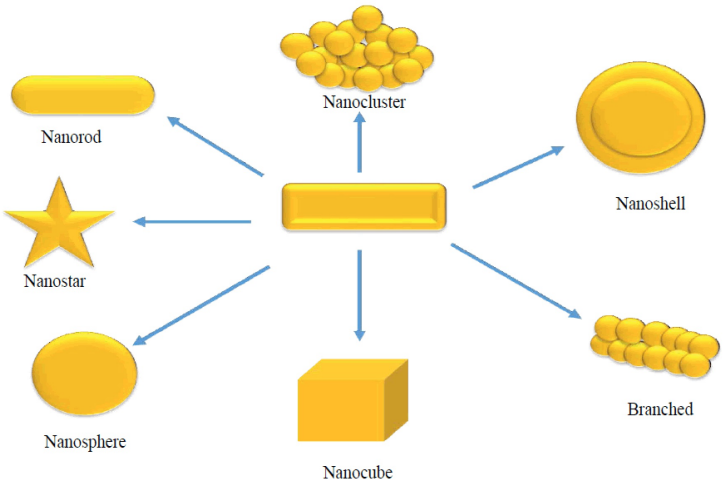

Figure 13: Different shapes of gold nanoparticles.

5 Conclusion

Localized surface plasmon resosnance measurements were performed on different solutions including DNA at different concentrations, PEI, DNA/PEI polyplexes and gold nanoparticles. Determining the concentration of DNA in the polyplexes was not successful, but the unknown gold nanoparticles was estimated to be 5 nm for S, and not found for N and E.