Adsorption of Dyes onto Gold Nanoparticles

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ABSTRACT

This paper involves the synthesis in water of nanoparticles and their interaction with dye molecules. The nanoparticles were synthesized by the citrate reduction method. Factors which affect the synthesis of the nanoparticles and their interaction with various photodynamic therapy dyes have been examined. It was found that when a solution of thiazin dye is titrated against gold hydrosol, at a critical concentration (ca. 3×10^{-6} M) there is an enhanced maximum absorption in the dye. The enhancement on a UV spectrum is approximately 0.65. The extinction coefficient is increased by a factor of 10. This is observed for all the family of thiazin dyes, of which methylene blue and toluidine blue are photodynamic therapy agents. The same enhancement was not observed for anionic, acidic dyes such as Tin Chlorin e6.

Keywords: gold nanoparticles, dyes, adsorption, surface plasmon

1 INTRODUCTION

The first useful method for synthesis of gold nanoparticles was reported by Turkevich et al. in 1951[1]. Their study focused on the process of nucleation and growth in the synthesis of nanoparticles. Turkevich's citrate reduction was described as of 'great importance'. This is remarkable since he had no knowledge the impact this novel synthesis would have. The synthesis required the use of inexpensive sodium citrate as the reducing agent, in a solution of chloroauric acid. This method produced nanoparticles of about 20 nm with and 12.5% standard deviation.

Turkevich's paper would have gone largely unnoticed had Fern's work not been published in 1973[2]. By controlling the amount of citrate added to the chloroauric acid solution, the size of nanoparticles obtained could be varied. Even though mono-dispersity over 30 nm was poor, this was the first dependable attempt to regulate the size of nanoparticles obtained. Fern's paper has become one of the most cited papers in colloidal science, noted for is simplicity in synthesis of colloidal gold and its potential applications from other particle science to cell biology.

1.1 Thiazin Dyes

Thiazin dyes absorb light wavelengths between 550-700 nm, hence give a characteristic blue colour. Toluidine blue is used in staining of various cells, and in conjunction with malachite green for the staining of lipids. Both toluidine blue and methylene blue are used in the diagnosis of cancerous tissue. It was found by Bellin et al.[3] that tumor cells were succeptible to killing by light, when treated with methylene blue.

These light activated antimicrobial agents are promising alternatives for the treatment of topical infections. The use of these antimicrobial agents in conjunction with nanoparticles and a bacterial recognition moiety is advantageous. They are more effective at killing bacteria, since the oxygen produced is concentrated at the bacterial cell wall. Also, there is selective killing, since binding of the photosensitiser is in close proximity to the target organism, and this reduces the collateral damage to neighbouring healthy cells[4].

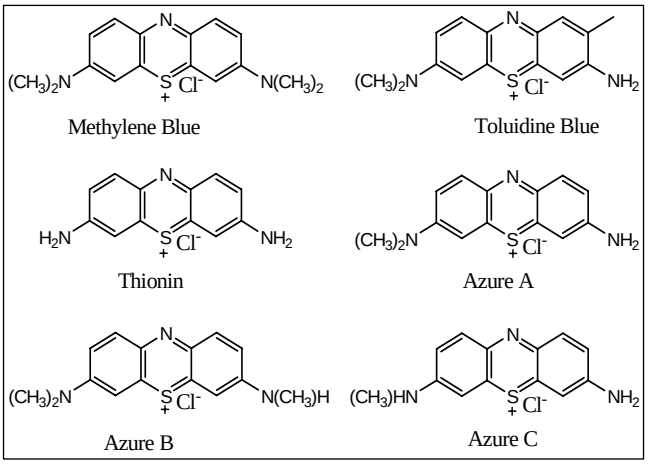

Figure 1. Structure of all the thiazin dyes

1.2 Adsorption of Dyes

Gold nanoparticles have nonspecific adsorption properties, which have allowed their use as tracers for proteins. However, full characteristics at a molecular level of the gold particle suspension and the adsorption of proteins need to be known[5].

Factors affecting adsorption of dyes onto gold nanoparticles

- Particle Size. When proteins, such as Bovine Serum Albumin, are adsorbed onto the surface of gold nanoparticles, the maximum number of molecules that can be associated with a single particle is linearly related to the average projection area of the particle[5]. This would therefore apply to the adsorption of dyes onto a surface, since gold has nonspecific adsorption properties.

- Molecular Weight of Dyes. If an analogy is drawn with protein adsorption, it is assumed that the area occupied by the dye will be equal to its projection onto the gold particle surface. This would give a monomolecular shell of dye covering the nanoparticle. Therefore, the larger the dye molecule, the fewer there will be in contact with the gold nanoparticle surface.

- The Effect of pH. This has not been investigated thoroughly in protein adsorption. However, since nonstabilised nanoparticles aggregate when electrolytes are added, the small increase in ionic strength causes aggregation of particles. Therefore at lower pH values total binding of dyes should decrease.

2 RESULTS AND DISUSSION

2.1 Adsorption of Dyes on the Surface of Gold Nanoparticles

The optical response of gold metallic colloids arises from the ease at which electromagnetic fields excite electrons at the surface of particles. This photo-excitation induces charge density oscillations, a collective movement of electrons, the surface plasmon, gives rise to the intense colour of the solution, and absorption at around 530 nm.

This electronic excitation leads to electronic field redistribution near the metal surface. Therefore molecules which are near the vicinity of this surface are going to experience this intense electric field, and there has been an enhancement in the maximum absorption of these dyes in the visible region.

2.2 Adsorption of Thiazine Dyes

Dyes absorb light in the visible region and have a characteristic spectrum. This spectrum changes when nanoparticles are present and the dye is adsorbed onto the surface of the gold nanoparticle. If there was no communication between the dye and the nanoparticle the shape of the dye absorption spectrum would not change.

The titration experiments were carried out on a number of dyes. The first two dyes were toluidine blue and methylene blue as these were the PDT dyes, that gold nanoparticles enhanced bacterial kill.

Toluidine blue (5 µM) was added in 1 ml aliquots to gold nanoparticles. After each addition a UV spectra was taken. This was done for all the dyes tested.

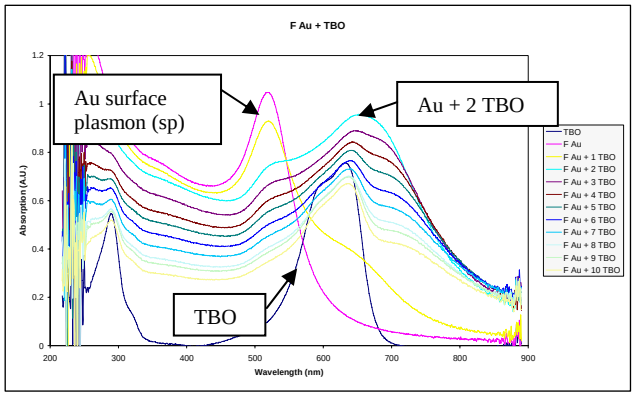

Figure 2. Effect of increasing the amount of toluidine blue (TBO) on a gold nanoparticle solution. FAu + 1 TBO is the addition of 1 ml of toluidine blue (5 µM) to 16 ml of gold nanoparticle solution. FAu + 2 TBO is the addition of 2 ml of toluidine blue (5 µM) to 16 ml of gold nanoparticle solution, etc

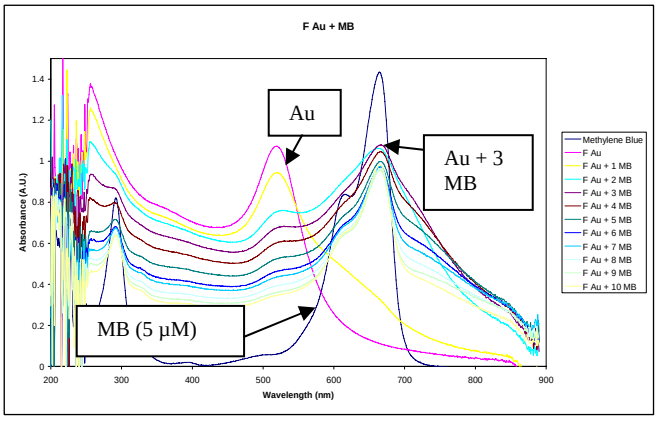

Figure 3. Effect of increasing the amount of methylene blue (MB) on a gold nanoparticle solution. FAu + 1 MB is the addition of 1 ml of methylene blue (5 µM) to 16 ml of gold nanoparticle solution. FAu + 2 MB is the addition of 2 ml of methylene blue (5 µM) to 16 ml of gold nanoparticle solution, etc.

There is a maximum enhancement of the dye absorption after 2 additions of toluidine blue (5 µM), giving a total concentration of 5.56×10^{-7} M in the reaction mixture. The maximum enhanced absorption is observed at 652 nm to 655 nm. There is a gradual decrease in the nanoparticle absorption, which can be attributed to the increase in total volume, hence decrease in gold nanoparticle concentration.

After the initial 2 additions of toluidine blue there is a gradual decrease in the max absorption of the gold nanoparticles and dye (Figure 2).

There is an enhancement of the dye absorption after 2 additions of methylene blue (5 µM) giving a total concentration of 7.89×10^{-7} M in the reaction mixture. The maximum enhanced absorption is observed at 667 nm. There is a gradual decrease in the nanoparticle absorption, which can be attributed to the increase in total volume. After the initial 2 additions of methylene blue there is a gradual decrease in the max absorption of the gold nanoparticles and dye (Figure 3).

All of these thiazin dyes have a similar structure (Figure 1) and show the same enhancement after the dye is added. There is a gradual decrease in maximum nanoparticle absorption, where as the dye peak reaches a maximum. Then this peak begins to decrease. This means that large loadings of dye molecules on gold nanoparticles have detrimental effects on maximum absorption. The surface of the nanoparticle may be covered by the maximum number of dye molecules possible and the additional dye molecules may not be affected by the charge on the nanoparticles. As the inter chromophore distance decreases it may cause flocculation of the nanoparticles.

2.3 Effect of Other Dyes

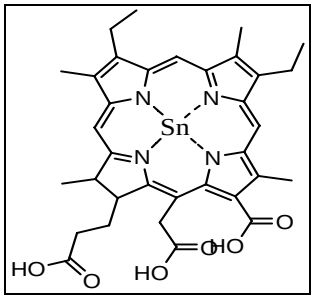

Figure 4. Structure of tin (IV) chlorine e6

Tin chlorin e6 has also been investigated as a possible PDT agent[6], hence the same titration experiments were carried out with this dye and gold nanoparticles. Tin chlorin e6 is an acidic dye, which has higher yields of singlet oxygen when compared to other related chlorines. It also has, reactive carboxylic groups on side chains outside the polycyclic core, where antibody targeting moieties can be conjugated (Figure 4).

Unlike the thiazin dyes there was no enhancement of the dye peak (Figure 5). There was a linear increase in the dye peaks of the absorption at approximately 400 nm and 625 nm, which corresponds to the increase in concentration. This is shown in the same titration experiment with no nanoparticle solution present. There is a familiar decrease in the nanoparticle peak which is a dilution effect.

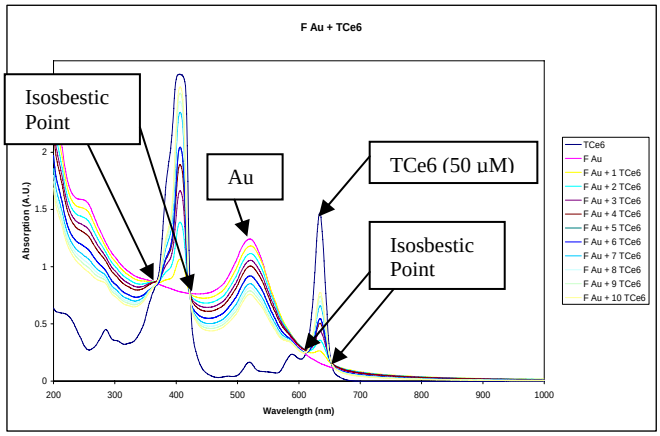

Figure 5. UV absorption spectra of the effect of increasing the amount of tin chlorin e6 on a gold nanoparticle solution. FAu + 1 TCe6 is the addition of 1 ml of tin chlorin e6 (50 µM) to 16 ml of gold nanoparticle solution. FAu + 2 TCe6 is the addition of 2 ml of tin chlorin e6 (50 µM) to 16 ml of gold nanoparticle solution, etc.

There are four isosbestic points on the UV spectra (372 nm, 422 nm, 612nm, 655 nm), which correspond to the wavelength at which the dye and the gold nanoparticle have the same extinction coefficients. The isosbestic points occur because the nanoparticle solution and the dye solution absorb light of that specific wavelength to the same extent regardless of the relative concentrations. There is no communication between the dye and nanoparticle, hence isosbestic points are observed on the UV spectra. Where there is electronic communication between the dye and nanoparticle there is an enhanced absorption, therefore no isosbestic points appear.

2.4 Effect of Adding a Buffer to the Titration Expertiment

The enhanced maximum observed in the UV absorption spectrum also resembles that of particle aggregation which was reported by Turkevich in the 700 nm region[7]. Therefore a control titration experiment was carried out. This involved the same titration experiment with the gold nanoparticle solution with increasing amounts of methylene blue. The added component of this titration was KCl which acts as a flocculating agent.

Adsorbed anionic citrate on the gold nanoparticle forms a layer of electrostatic repulsion, which prevents the aggregation of the nanoparticles. Electrolytes screen the repulsive electrostatic forces of the citrate layer because the positive charge of the electrolyte associate

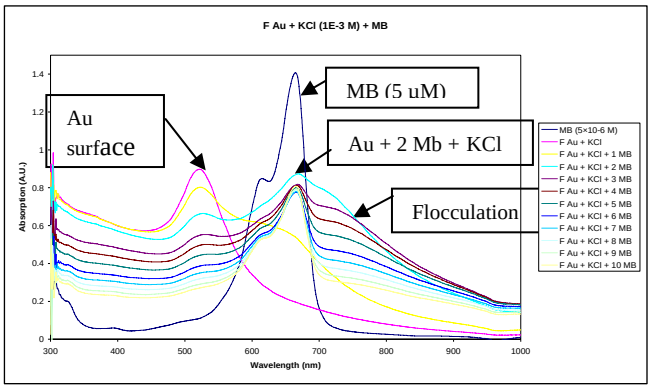

Figure 6. UV Spectrum of gold nanoparticles with increasing amounts of methylene blue in the presence of KCl (1×10^{-3}). FAu + KCl + 1 MB is the addition of 1 ml of methylene blue (5 µM) to 16 ml of gold nanoparticle solution with KCl. FAu + KCl + 2 MB is the addition of 2 ml of methylene blue (5 µM) to 16 ml of gold nanoparticle solution with KCl, etc.

(Figure 6), at first seems no different that the first titration with methylene blue only (Figure 3). However there is an additional absorbance broad peak just below 800 nm which is not present in the methylene blue titration alone. This new peak is probably due to the flocculation induced by KCl. The enhanced absorbance peak of the methylene blue is present as well as the new flocculation peak. Therefore there is adsorption of dye molecules onto the surface which is the cause of this enhanced absorbance as the flocculation peak occurs at a higher absorbance in this case.

5. Conclusion

Synthesis of gold nanoparticles was investigated using a modified Turkevich citrate reduction. By varying the rate of addition different sized nanoparticles were obtained. Fast addition of the citrate produced smaller nanoparticles in the range of 12 nm. Adding the citrate over 50 seconds yielded nanoparticles of approximately 16 nm.

The adsorption of dye molecules onto the surface of the nanoparticle was investigated. The collective movement of electrons, the surface plasmon, gives rise to the intense colour of gold nanoparticles. This electronic redistribution near the surface of the gold nanoparticle effects molecules in close proximity to the surface. An enhanced absorbtion has been observed for the dyes in the thiazin group when the dye is titrated against the nanoparticle solution.

Generally in the UV spectrum of the thiazin dyes, there is a gradual decrease in maximum nanoparticle absorption, where as the dye peak reaches a maximum. The decrease in the nanoparticle intensity in the UV spectrum corresponds to the increase in volume, hence decrease in concentration. The absorption peak for the thiazin dyes generally increase until a critical concentration is reached. This critical concentration gives the maximum enhancement. The maximum absorption then gradually declines, indicating that large loadings of dye molecules on gold nanoparticles have detrimental effects on maximum absorption. The surface of the nanoparticle may be covered by the maximum number of dye molecules possible and the additional dye molecules may not be affected by the charge on the nanoparticles. As the inter chromophore distance decreases it may cause flocculation of the nanoparticles.

An enhancement of approximately 10 in the extinction coefficient was displayed in the UV absorption spectra for all of the thiazin dyes, in the presence of gold nanoparticles (12 nm) at a dye concentration of approximately 3×10^{-6} M. There is an average enhancement of an average of 0.65 in the maximum absorption of the dye, which indicates a direct communication between the nanoparticle and the dye.

This enhancement was not observed for acidic, anionic dyes. This implies that a cationic dye with a nitrogen atom in the conjugated system is vital for enhancement of the absorption of the dye. Acidic, anionic dyes display isosbestic points, which suggest that there is no communication between the dye and nanoparticle.

Flocculation is observed when KCl is added to the gold nanoparticles and dyes. However an enhancement is still observed. The nanoparticles are not very stable and degradation starts within the first ten minutes.

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