Formation and Collapse of Fluorescent Gels by Gold Nanoparticles

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In this article the formation and collapse of fluorescent gels made using bovine serum albumin (BSA) and chloroauric acid (HAuCl₄) is demonstrated. The formation of gels depends upon the concentration of BSA. The formation of the gels is associated with electrostatic interaction between chloroauric acid and BSA. The collapse of the gel is attributed to the reduction of gold as nanoparticles. The gels collapse without any external agents. The gels were characterized using SEM, FTIR, Rheometer, and XRD. The effect of aging upon the gels showed that in time all the gels turned into dark viscous liquid. Particle size distributions showed that higher concentrations of proteins showed inhomogeneous particle sizes indicating that the rate of reduction of gold depended upon the concentration of the proteins. The gels were tested for their abilities to be used for encapsulation of drugs using Rhodamine. Gels of cylindrical shapes of ∼2.5 cm in length and 2 cm in diameter were made using this technique. A model is proposed for the formation and the collapse of the gel.

KEYWORDS: BSA, Gold Nanoparticles, Gel, Fluorescent.

1. INTRODUCTION

Polymer gels are three dimensional networks swollen by a large amount of solvent. They have storage modulus and loss modulus. The value of the loss modulus is smaller than the storage modulus.¹ Gels have liquid like behavior on molecular scale and they have solid like behavior at macroscopic scales.² Gels are classified as physical gels and chemical gels.³–⁵ Physical gels result due to formation of aggregates while chemical gels are formed due to covalent bonds. Gels have several applications that include biotechnology, micro fluidics and lab on a chip.⁵–¹⁴ Gels have wide application in nanoscience that include synthesis of materials for antimicrobial nanomaterials,¹⁵ treatment of cancer,¹⁶ drug release,¹⁷ luminescent materials,¹⁸,¹⁹ thermo-sensitive materials,²⁰ nanocrystals,²¹ porous materials,²² batteries,²³ for catalysis,²⁴,²⁵ capacitors²⁶ and solar cells.²⁷ Synthesis of fluorescent materials has gained a lot of scientific interest recently. Fluorescence occurs due to emission of light by a material that has absorbed light or other electromagnetic radiation. Several fluorescence microscopy and spectroscopy techniques have been developed based on the characteristics of fluorescent probes like life time and intensity has been developed.²⁸ Fluorescence has many applications that include chemical sensors, fluorescent labeling, dyes and as biological detectors.²⁹–³⁸ Recently Xie et al have synthesized stable fluorescent gold nanoclusters in solution using BSA.³⁹ These BSA-Au nanoclusters are very small (as it has 25 atoms of Au and therefore ∼1–2 nm).³⁹ Synthesis of fluorescent gels using proteins opens up possibilities to combine the beneficial aspects of fluorescent materials and gels. In this article, synthesis and characterization of fluorescent gels using BSA and gold is investigated. Potential applications of these fluorescent gels may be in the fields of sensors, biotechnology, lab on a chip and microfluidics.

2. EXPERIMENTAL DETAILS

2.1. Preparation of Gels

Appropriate quantities of BSA was added to 5 mM HAuCl₄ solution and stirred vigorously for 5 minutes. Then 1 M NaOH was added drop by drop while stirring at room temperature to have a final concentration of 250 mM NaOH.

2.2. Characterization

Photoluminescence spectra was recorded at room temperature using HORIBA JOBIN-YVON Fluoromax 4 spectrophotometer. The slit width used was 5 nm. UV/Vis
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spectra were measured with a Perkin Elmer Lambda 25 instrument in the range 200–1100 nm. The instrument Rheoplus was used to measure the rheological properties of the gel. The spacing between the discs was 0.3 mm. SEM images were obtained using the instrument Quanta 200 FEG scanning electron microscope. The voltage used was 30 kV. The instrument Bruker D2 phaser was used for obtaining XRD data. The voltage used was 30 kV and 10 mA current was used. The counts were measured as a function of 2θ. The wavelength used was 1.54184 Å. FTIR measurements were made using Perkin Elmer Spectrum One FT-IR spectrometer. The spectral resolution was 4 cm⁻¹. The sample was placed in between two potassium bromide discs. The particles sizes were also measured using light scattering (Malvern instruments). The refractive index used was 1.33. The duration used was 60 s. Water was used as dispersant.

3. RESULTS AND DISCUSSION

3.1. Formation of Gels

Figure 1 shows the formation of gels as a function of time. Here 25 μL of 1 M HAuCl₄ was added drop wise to glass bottles (10 ml) containing 5 ml of varying concentrations of aqueous solutions of BSA (A–25 mg/ml, B–50 mg/ml, C–75 mg/ml and D–100 mg/ml) to have a final concentration of 5 mM HAuCl₄. The solution was stirred and appropriate amount of NaOH was added to have a final concentration of 250 mM NaOH. The solutions were left undisturbed at 25 °C. It was observed that all the solutions became fluorescent in 24 hr as can be observed in the UV light image. In time the solutions that had lower concentrations of proteins (25 mg/ml) looked darker, indicating the formation of nanoparticles in it. It was observed that gels formed for proteins of concentrations greater than or equal to 75 mg/ml in less than 2 hr. Interestingly, it was also observed that as time progressed, the gels with BSA concentrations 75 mg/ml collapsed. The gel with BSA concentration 100 mg/ml collapsed only after 3 months. This shows that the stability of the gel is dependent upon the concentration of the protein. Proteins of concentrations 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml to which HAuCl₄ (5 mM final concentration) and NaOH (250 mM final concentration) are added will hereafter be referred to as 25 mg/ml BSA-Au, 50 mg/ml BSA-Au, 75 mg/ml BSA-Au and 100 mg/ml BSA-Au.

3.2. Photoluminescence and UV-Vis Measurements

Figures 2(A) and (B) shows the photoluminescence characteristics and the UV/Vis characteristics of the gels. It is observed that 25 mg/ml BSA-Au (solution) has excitation...
and emission maxima at 530 and 720 nm. 50 mg/ml BSA-Au (gel) has excitation maxima at 378 nm and 520 nm and emission maxima at 710 nm. 75 mg/ml BSA-Au (gel) has excitation maxima at 371 nm and 520 nm and emission maxima at 700 nm. 100 mg/ml BSA-Au (gel) has excitation maxima at 420 nm and 520 nm and emission maxima at 710 nm. The difference in the positions of the excitation and emission maxima for different concentrations of BSA is attributed to the change in the surface plasmon characteristics for BSA-Au nanocomposites induced due to the different amounts of Au reduced upon BSA due to variation in concentration of BSA. Figure 2(B) shows that all the samples have an absorption peak at 540 nm (2.29 eV) indicative of formation of fluorescent quantum clusters. The peak at 540 nm gets flattened as the concentration of BSA increases. This is probably due to the reduction in the intensity of fluorescence due to quenching because of excess protein.

3.3. Rheological Properties

Initially, it was observed that the viscosity of the gels reached a steady value in $\sim$ 30 minutes for all the samples. The viscosity increased with increase in concentration of the proteins. The rheological properties were then observed for longer time durations. Figures 3(A)–(C) show the variation of the viscosity, storage modulus and loss modulus of the BSA-Au gels for 48 hr. It is observed that 50 mg/ml, 75 mg/ml and 100 mg/ml BSA-Au have high viscosity. At the beginning ($\sim$ 1 hr), the difference in the storage modulus and the loss modulus for 100 mg/ml and 75 mg/ml is high due to gelling while it is not so for 50 mg/ml and 25 mg/ml BSA-Au as they are solutions. It is observed that for 50 mg/ml BSA-Au and 100 mg/ml BSA-Au their viscosity, storage modulus and loss modulus attain values similar to that of 25 mg/ml BSA-Au solution in 24 hr and 48 hr respectively. At those times, those gels had collapsed. It is also observed that 100 mg/ml BSA-Au gels has its viscosity, storage modulus and loss modulus remain nearly the same as the time varies. Even after 15 days, its viscosity, storage modulus and loss modulus was nearly the same as its initial value. This shows that the gels get harder as the concentration of proteins increases.

3.4. SEM Measurements

Figure 4 shows the SEM images for (A) 100 mg/ml BSA-Au (B) 75 mg/ml BSA-Au (C) 50 mg/ml BSA-Au and (D) 25 mg/ml BSA-Au. In order to obtain SEM images, the gels were dried in a oven at 60 °C for 48 hr. In imaging
these samples, the samples were not coated with gold since the samples already has gold in it. It was observed that 100 mg/ml BSA-Au and 75 mg/ml BSA-Au showed sponge like shapes while 50 mg/ml BSA-Au and 25 mg/ml BSA-Au showed films like surfaces. It was observed that in the case of 100, 75 and 50 mg/ml BSA-Au, the samples tend to get charged easily and therefore it was hard to image at higher magnifications. However for 25 mg/ml BSA-Au, the samples did not get charged that fast. This indicates that 25 mg/ml BSA-Au is more conducting than samples with higher concentration of BSA and therefore has higher reduced gold nanoparticles in it. It was observed that only 25 mg/ml BSA-Au showed nanoparticles of particle size 97.7±18.6 nm (Fig. 4(D)). It was also observed that along with particles of sizes 97.7±18.6 nm several small nanoparticles (≈15–20 nm) seemed to be present and it covered the surface uniformly. The EDAX showed that the gold content was nearly the same at both the regions; ≈10.94% upon the particles of sizes 97.7±18.6 nm and ≈9.73% upon the smaller particles of sizes ≈15–20 nm (Figs. 5(D) and (E)). Interestingly, it was also observed that the particles coalesced as the electron beam exposure time increased. It was observed that the brightness and the contrast remained same with increase in exposure time, indicating coalescence and not material damage. This is expected as gold quantum clusters are unstable under electron beam and has been shown to grow in their sizes with exposure to electron beam. All the samples showed presence of gold and their concentrations increased with the decrease in the concentration of the proteins (Fig. 5).

3.5. XRD Measurements

As during SEM imaging of samples containing higher concentrations of proteins, the samples got charged quickly; it was difficult to affirmatively confirm the presence of nanoparticles. XRD measurements were used to overcome this difficulty as presence of nanoparticles would show peaks in XRD pattern. Figure 6 shows the XRD for (A) 100 mg/ml BSA-Au (B) 75 mg/ml BSA-Au (C) 50 mg/ml BSA-Au and (D) 25 mg/ml BSA-Au gel. It was observed that XRD for 100 mg/ml BSA-Au and 75 mg/ml BSA-Au gels had peaks at 32° which is characteristic of NaCl (PCPDFWIN-01-0993). However no peaks characteristic of Au was observed. This indicates that though gold clusters could have formed (as these gels showed fluorescence that is indicative of formation of gold clusters) the size of the particles were not significantly

![Fig. 4. SEM images for (A) 100 mg/ml BSA-Au (B) 75 mg/ml BSA-Au (C) 50 mg/ml BSA-Au and (D) 25 mg/ml BSA-Au.](image)
large enough for diffraction peaks to be observed. It was observed that XRD for 50 mg/ml BSA-Au showed peaks corresponding to gold at 38.2° (PCPDFWIN-01-1172), NaCl at 32° (PCPDFWIN-01-0993) and NaOH (at 46° and 58°) (PCPDFWIN-75-0642). The prominent peak characteristic of gold at two theta equal to 38.2° was highest for 25 mg/ml BSA-Au. This indicates that gold nanoparticles grow more when the concentration of the proteins is less.

3.6. Secondary Structural Modifications

Figure 7 shows the absorbance and second derivative of absorbance of FTIR spectra for BSA and BSA-Au gels.
Fig. 6. XRD for (A) (1) BSA (2) 100 mg/ml BSA with 5 Mm HAuCl₄ and (3) 100 mg/ml BSA-Au (B) 75 mg/ml BSA-Au (C) 50 mg/ml BSA-Au and (D) 25 mg/ml BSA-Au.

Fig. 7. (A) Absorbance and (B) second derivative of absorbance of FTIR spectra for BSA and BSA-Au gels.
The secondary structure was studied with FTIR by examining the second derivatives of the absorbance spectra in the amide I spectral region (1600–1700 cm\(^{-1}\)). For BSA, the second derivative peak was at 1652 cm\(^{-1}\). This corresponds to \(\alpha\)-helical secondary structure\(^{39}\). It was observed that as the concentration of the proteins decreased, the second derivative peak shifted to position for random coil (1640 cm\(^{-1}\)). The change in the secondary structure of the proteins can be explained as follows. When the concentration of protein is high, the reduced gold nanoparticles are not large enough to alter the \(\alpha\)-helical structure to random coil structure.

### 3.7. For Encapsulation in Drug Delivery

To check if the gels can be used for encapsulation of drugs, 100 \(\mu\)L of Rhodamine of concentration 2000 ppm was injected into the gels made using 75 mg/ml BSA and
100 mg/ml BSA. The gel (10.75 gm) was placed in a container containing 20 ml of water. Using UV-Vis measurements, the concentration of rhodamine that diffused from the gel was measured after 24, 48 and 72 hr by observing the absorbance peak at ∼525 nm. It was observed that in the case of gels made using 75 mg/ml BSA, the amount of rhodamine trapped in the gels after 24, 48 and 72 hr were 99%, 92.5% and 82% respectively. While for gels made using 100 mg/ml BSA it was observed that after 72 hr it was 94%. This indicates that the gels have the potential to be used as drug delivery tools. The encapsulation of drugs and the changes in fluorescence of the gels due to drug–gel interactions is presently being investigated. This can lead to using these gels as sensors for drug detection.

3.8. Aging Effect on the Gels

In time even gels made using 100 mg/ml BSA collapsed. It was observed that nanoparticles formed for all the gels and the solutions became dark brown in color. After four months from the time of preparation of the samples, the sizes of the nanoparticles were measured using DLS (Fig. 8). A single particle size distribution was observed for 25 mg/ml BSA-Au (∼92 nm) and for 50 mg/ml BSA-Au (∼60 nm). The increase in protein concentration from 25 mg/ml to 50 mg/ml can lead to greater availability of nucleation sites for formation of nanoparticles of gold. Thereby more particles with lesser sizes (∼60 nm) are formed for 50 mg/ml BSA solution. However three particle size distributions were observed for 75 mg/ml BSA-Au (∼7, 40 and 165 nm) and 100 mg/ml BSA-Au (∼10, 29 and 208 nm). Interestingly even particles of sizes ∼2 nm were observed in the case of 100 mg/ml BSA-Au. The width of the distribution was also larger for 100 and 75 mg/ml BSA-Au when compared to 50 and 25 mg/ml BSA-Au. This is attributable to the effect on particles sizes due to formation of gels. The initial formation of the gels in the case of 75 and 100 mg/ml BSA-Au can possibly result in slow movement of the Au ions. This could lead to reduction of gold upon various regions of the networks formed during gelling. As a result different particle size distributions are obtained (Fig. 8(E)).

3.9. Model for Formation of Gels

The reduction of gold on proteins of low concentrations results in the formation of fluorescent protein-gold nanocomposites. The reduction of gold on proteins of high concentrations might enhance protein–protein interactions leading to formation of protein–gold–protein networks, resulting in the formation of fluorescent gels. In time, as more gold gets reduced on the protein, the protein–gold–protein networks weakens leading to independent protein–gold nanoparticles. This leads to weakening of the gels and therefore its collapse from gel state to liquid state. The slow reduction of gold on the gel leads to formation of heterogeneous particles. The formation and collapse of the fluorescent gels is shown as schematic in Figure 9.

4. CONCLUSIONS

The formation of gel was made possible by mixing chloroauric acid, NaOH and high concentrations of...
BSA. The gels were found to be fluorescent under UV light. The gels were also observed to collapse in time. Higher concentrations of BSA gave more stable gels. Rheological measurements showed that in time the gels became viscous liquids. Photoluminescence characteristics of the gels depended upon the concentration of the protein possibly due to formation of different BSA-Au clusters/nanoparticles. SEM and XRD measurements of the gels showed that gold nanoparticles grow more when the concentration of the proteins is less. FTIR measurements showed that the concentration of the proteins decreased the second derivative peak shifted to position for random coil indicating that the structure of the protein changes from helical to random coil as the nanoparticles form. The gels were examined for encapsulation of rhodamine and it was observed that the gels gradually released rhodamine. This indicates that the gels have the potential to be used as drug delivery tools. Also the effect of aging upon the gels showed that in time all the gels turned into dark viscous liquid. Also their particle sizes showed that higher concentrations of proteins showed inhomogeneous particle sizes indicating that the rate of reduction of gold depended upon the concentration of the proteins.

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References and Notes