Green Synthesis of Silver Nanoparticles and the Detection of Urea in Milk Sample by Spectrophotometric Method Using Silver Nanoparticles

Sadhan Jyoti Dutta
B.Tech. Food Tech. & Management
Sonepat, Haryana, India.
Sadhanjyotidutta27@gmail.com

Abstract - Synthesis of silver nano particles(AgNPs) and the development of silver nanoparticles(AgNps) has been done using the green synthesis method. Tea extract(Camelia sinensis) has been used as the reducing agent(bioreductor) due to its presence of antioxidant property(Catechin compounds) which will help to reduce silver nitrate(AgNO3) to silver nanoparticles. The Ferrous Reducing Antioxidant Power(FRAP) assay was used to determine the antioxidant power of the plant extract(tea). Accordingly, the plant extract(tea) was added to the silver nitrate(AgNO3) solution to form silver nanoparticles (AgNps) and also citrate-capped AgNps. These two types of AgNps were then characterized with the help of UV-Vis Spectroscopy. The silver nanoparticles that were developed were then used for the detection of urea present in milk which is used as an adulterant to increase the protein content in milk. For this purpose, whey protein was prepared from milk using centrifugation and filtration, thus separating whey protein from casein. Stock samples were prepared by adding AgNps and citrate-capped AgNps to the whey protein with and without urea as contaminant. Visual changes were observed in the contaminated samples and also the change in absorbance was observed with the help of UV-Vis Spectrophotometer.

Keywords- Green synthesis, Silver nanoparticles, Ferrous Reducing Antioxidant Power assay, Urea detection.

I. INTRODUCTION
Nano science and nanotechnology have created incredible new possibilities and novel applications in the food industry. Food safety and quality are the most important aspects and became the attention of public health, as food is an essential part of human life. Therefore foods must be free from contaminants. In this context, nano technology(i.e nanosensors) in the food industry can be used in detecting contaminants on packaging or during food processes, distribution and storage.

Today, several nanoparticles have been used for colorimetric sensor. Among these is silver nano particles(AgNps). Silver nanoparticles has unique physicochemical properties as a size, shape and special optical properties like the localized surface plasmon resonance (LSPR). LSPR is the most important property of metal NPs like Au and Ag that are used for visual detection of analytes. When the frequency of the incident electromagnetic radiation matches with the collective oscillation of the conducting electrons of metal NPs, it is referred as LSPR. Increased scattering intensity of the radiation or a strong absorption band occurs at certain wavelengths for the metal NPs as a result of this property. This property of metal NPs is strongly dependent on the inter-particle distance between NPs. If there is a decrease in the inter-particle distance due to aggregation, overlap between the plasmon fields of the nearby particles occurs, causing a red shift in the LSPR band(s). Along with this, an increase in intensity and change in colour solution is observed. The interaction between the analyte and NPs lead to the aggregation (decrease in the inter-particle distance) of NPs and hence, the colour change. LSPR of silver nanoparticles can be charged by tuning their shape, size and aggregation state when have interaction with analytes.

Silver nano particles has allowed the analysis development with the highly sensitive and selective detection method that is expected to overcome deficiencies in convention methods. With these advantages, silver nanoparticles is suitable for development of method analysis especially in colorimetric sensor. In this study, silver nano particles were used as nanosensors to detect the presence of urea in milk. Urea(CH4N2O) has great importance in the agriculture, food, plastic and drug industry. Specifically, the dairy industry has the demand for highly sensitive/selective urea sensors because of real-time and accurate analytical measurement of urea is necessary since urea is considered one of the adulterant in the milk. Milk is considered one of the complete food which is a main source of large quantities of proteins, minerals and vitamins. The average amount of protein in the cow milk is around 3.4%.Naturally occurring urea concentrations in milk are reported in the range of 3.1 to 6.6 mM, whereas the acceptable level of urea in milk is 11.6 mM as per the
Food Safety Standards Authority of India. In order to increase the nitrogen content, more amount of urea is added with the diluted milk to show that it’s full of milk (bogus protein). Thus, there is a need to monitor accurate level of urea adulteration in milk which is regarded as harmful to human's health. At the same time, urea has been extensively used in agriculture as a fertilizer, de-icing agent, stabilizers in soap and detergents. However, their long-term usages would increase urea concentration in land and water but also can lead to soil acidification and eutrophication, which disturb the eco-system, cause death of aquatic life, and acute poisoning in humans and animals.

The urea concentration is in the range of 2.6 to 6.5 mM in human blood. Higher levels of urea in the blood can be linked to kidney failure, urinary tract obstruction, gastrointestinal bleeding, dehydration, burns and shock, while a lower level of urea results in hepatic failure, nephritic syndrome and cachexia. So, a highly sensitive and selective sensor could be a valuable tool for monitoring the urea concentration in food (milk), water and biological fluids (urine, blood etc.).

Different analytical methods (i.e. gas chromatography, fluorometric, colorimetry and chemiluminescence) have been used for urea determination but these methods are relatively expensive, time-consuming, sample pre-treatment required and not suitable for field testing. Recently, nanoparticles, due to their distinctive chemical and physical properties, have been selected to design novel sensors for detecting various analyte. However, gold and silver nanoparticles (AgNPs) are widely used due to less toxicity, conducting properties and their special optical properties like the localized surface plasmon resonance (LSPR).

II. EXPERIMENTAL METHOD

1. Synthesis of Silver Nanoparticles (AgNPs) using green synthesis method:
   1.1 Preparation of antioxidant from plant extract (tea)-4g green tea extract was taken in 50 ml water and boiled until the desired red colour appear. This is then filtered using filter paper to remove the impurities.
   1.2 Ferrous Reducing antioxidant power assay
   Objective -To measure the antioxidant level of the green tea extract.
   Chemical reagents: Phosphate buffer, Potassium ferricyanide, Trichloro acetic acid, Distilled water, Ferric chloride solution.
   Procedure:
   • Take 100µL of plant extract and add with 400µL of the same solvent(water) in 1:4 dilution.
   • Add 1.25mL of Phosphate buffer(0.2M) and 1.25mL of Potassium ferricyanide(1%) to the plant extract and mix well.
   • Incubate the mixture in hot air oven at 50°C for 30 minutes.
   • After incubation, cool the reaction mixture
   • After cooling, add 1.25mL of 10% Trichloroacetic acid and mix well.
   • Transfer the reaction mixture to centrifuge tube and centrifuge the same at 3000 rpm for 10 minutes.
   • After centrifugation, transfer 1.25mL of upper layer of solution from the centrifuge tube to a test tube.
   • Add 1.25mL of distilled water to the test tube and mix well.
   • Add 0.5 mL of 1% freshly prepared Ferric chloride solution to the test tube, mix well and look for the colour change.
   • Measure the absorbance using visible spectrometer ay 700nm.
   • Ascorbic acid can be used as a standard.

1.3 Preparation of AgNPs- AgNPs by green synthesis method: 1M AgNO3 was taken and made converted to 20mM AgNO3. 20mL H2O was added to break down into Ag3+ and NO-. The sample was placed in the magnetic stirrer for 10 minutes. Continue adding 20µL of the tea extract(antioxidant) after every 10 minutes of stirring until pale yellow colour is developed.

1.4 Characterization of AgNPs- The AgNps were characterized using UV-Vis Spectrophotometer in the range of 400-500nm. The peak absorbance was found to be at 420nm.

2. Preparation of Citrate-Capped AgNPs- AgNps with citrate capping: 0.02M of 10mL AgNO3 and 0.02M of 10 mL sodium citrate(Na3C6H5O7) was prepared and mixed in the magnetic stirrer for 10 minutes. Continue adding 20µL of the tea extract(antioxidant) after every 10 minutes of stirring until colour change is observed.

2.1 Characterization of citrate-capped AgNPs- The AgNps were characterized using UV-Vis Spectrophotometer in the range of 400-600nm. The peak absorbance was found to be at 430nm.

2.2 To study the detection of urea in milk(whey protein)- using the prepared AgNps and citrate-capped AgNps
2.2.1 Preparation of whey protein-
   • Take 50 mL of milk sample
   • Continue adding drops of 2M HCl to the sample and check the pH until it reaches 6.
   • Centrifuge and filtrate the sample inorder to separate the whey protein(liquid part) from the casein(solid part).

2.2.2 Preparation of Urea Solution:
   • 0.5M Urea solution of 1% was prepared.
   • From this stock solution 2 each samples of 1mM,2mM,3mM Urea solutions were prepared.

2.2.3 Urea detection in milk(whey protein) using AgNps as probe:
   • 4 test tubes were taken and marked.
   • Each test tubes were filled with 3mL whey protein
   • Test tube 1 was filled with 1mM urea solution and mixed.
• Test tube 2 was filled with 2mM urea solution and mixed.
• Test tube 3 was filled with 3mM urea solution and mixed.
• Test tube 4 was not filled with urea solution.
• AgNps of 2 drops each was filled in each test tubes and mixed.
• Colour change was observed in each test tubes.
• All the samples in each test tubes were checked for the shift in absorbance in the UV-Vis Spectophotometer in the range of 340-600nm.

2.2.4 Urea detection in milk (whey protein) using Citrate-capped AgNps as probe:
• 4 test tubes were taken and marked.
• Each test tube was filled with 3mL whey protein.
• Test tube 1 was filled with 1mM urea solution and mixed.
• Test tube 2 was filled with 2mM urea solution and mixed.
• Test tube 3 was filled with 3mM urea solution and mixed.
• Test tube 4 was not filled with urea solution.
• Citrate-capped AgNps of 2 drops each was filled in each test tubes and mixed.
• Colour change was observed in each test tubes.
• All the samples in each test tubes were checked for the shift in absorbance in the UV-Vis Spectophotometer in the range of 340-600nm.

III. RESULT AND DISCUSSION
1. Green synthesis of AgNps and Citrate-capped AgNps - The synthesis of silver nanoparticles (AgNps) carried out by green synthesis method using plant extract (tea extract). Silver nitrate are a source of Ag+ and antioxidant present in tea extract as reducing agents that produce AgNps.

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\text{AgNO}_3 + \text{Tea extract} \rightarrow \text{AgNps}
\]

The formation of silver nanoparticles by green synthesis method is carried out by adding the tea extract sequentially drop by drop into AgNO3. The result of AgNps synthesis produce pale yellow colour. Characterization of AgNps was performed using UV-visible spectroscopy. The maximum absorbance was found to be at 420nm. Similarly, Citrate-capped AgNps were synthesized using the green synthesis method from silver nitrate (AgNO3) and sodium citrate (Na3C6H5O7) with addition of tea extract. The result of Citrate-capped AgNps synthesis produce light yellow colour. Characterization of these Citrate-capped AgNps was performed using UV-visible spectroscopy. The maximum absorbance was found to be at 430nm.

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\text{AgNO}_3 + \text{Na}_3\text{C}_6\text{H}_5\text{O}_7 + \text{Tea extract} \rightarrow \text{Citrate-capped AgNps}
\]

2. Detection of urea in Milk (whey protein) using the prepared AgNps:
Different concentrations of urea were prepared and added to the whey protein sample prepared from milk. AgNps were added to all the samples and the following changes were observed:

- Visual changes:
  - Sample with no urea solution (0mM) does not show any colour change (remains pale yellow).
  - Sample with 1mM urea solution shows slight change in colour to lightish blue.
  - Sample with 2mM urea solution shows change in colour to light blue.
  - Sample with 3mM urea solution shows change in colour to smoky blue.

- Optical changes (using UV-Vis Spectrophotometer):
  - With increase in molarity (or concentration) of urea in the samples the absorbance goes on decreasing.

3. Detection of urea in Milk (whey protein) using the prepared Citrate-capped Ag Nps:
Different concentrations of urea were prepared and added to the
whey protein sample prepared from milk. Citrate-capped AgNps were added to all the samples and the following changes were observed:

Visual changes:
- Sample with no urea solution (0mM) does not show any colour change (remains light yellow).
- Sample with 1mM urea solution shows change in colour to shiny yellow.
- Sample with 2mM urea solution shows change in colour to slight blue.
- Sample with 3mM urea solution shows change in colour to greenish blue.

Optical changes (using UV-Vis Spectrophotometer):
- The peak of absorbance of citrate-capped AgNps shifted from 430nm to 400nm for 1mM of urea in the sample.
- The peak of absorbance of citrate-capped AgNps shifted from 430nm to 350nm for both 2mM and 3mM of urea in the sample.

![Figure 3 UV-Vis Spectrum of Citrate-capped AgNps with different concentrations of urea](image)

Figure 3 UV-Vis Spectrum of Citrate-capped AgNps with different concentrations of urea (a)0mM (b)1mM (c)2mM (d)3mM

**IV. CONCLUSION**

This study shows a new approach to detect the presence of urea in milk. For this purpose, suitable sensing material has to be determined and therefore AgNps and Citrate-capped AgNps were chosen. The result of this test determined that citrate-capped AgNps is better than AgNps for the detection of urea in milk. Both these materials showed visual and optical changes with increase in the molarity or concentration of urea in the samples. The reason for the advantage of Citrate-capped AgNps over AgNps is because the Citrate-capped AgNps are already capped and binded with tri-sodium citrate molecules whereas the AgNps as they are left uncapped, binds with the whey protein of milk. Urea concentration in higher amount is hazardous to health. In smaller amount, it remains unaffected. Thus, this method shows a new approach for the detection of urea in milk. It can be concluded that AgNps and Citrate-capped AgNps can be used as colorimetric sensor with simple, rapid and low cost. Further, the results obtained from this test can be used for future purpose for the development of electronic techniques for urea detection. Portable Electronic set up can be developed using visible light to investigate the urea content in home as a means of providing wellness to the society.

**REFERENCES**


