Research Article

Silver Nanoparticles: Green Synthesis, Characterization, and Their Usage in Determination of Mercury Contamination in Seafoods

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We demonstrate that silver nanoparticles undergo an interaction with Hg^{2+} found in traces. The PEG-PVP-stabilized Ag nanoparticles were successfully synthesized via a reduction approach and characterized with surface plasmon resonance UV/Vis spectroscopy. By utilizing the redox reaction between Ag nanoparticles and Hg^{2+} , and the resulted decrease in UV/Vis signal, we develop a colorimetric method for detection of Hg^{2+} ion. A linear and inversely proportional relationship was found between the absorbance intensity of the Ag nanoparticles and the concentration of Hg^{2+} ion over the range from 10 ppm to 1 ppm at absorption on 411 nm. The detection limit for Hg^{2+} ions in homogeneous aqueous solutions is estimated to be 1 ppm. This system shows excellent selectivity for Hg^{2+} . The results found have potential implications in the development of new colorimetric sensors for easy and selective detection and monitoring of mercuric ions in aqueous solutions. The proposed method was successfully applied to quantify the amount of mercury in seafood.

1. Introduction

Pollution is the introduction of contaminants into a natural environment resulting in instability, disorder, harm, or discomfort to the ecosystem including physical systems and living organisms. Among the pollutants and particularly among heavy metals, mercury is one of the most commonly encountered toxic pollutant in the environment which may be a result of natural processes and emissions from coal burning power plants and gold mining [1]. Similarly, in aqueous solution, bacteria can transform water-soluble mercuric ion (Hg^{2+}) into methylmercury, which is the most common form of mercury in fish, and subsequently bioaccumulates through the food chain [2]. Methylmercury is a potent neurotoxin known to cause health problems such as sensory, motor, and neurological damage. It is particularly dangerous for children, because it can cause developmental delays [3].

Although the traditional instrumental techniques, such as absorption spectroscopy, cold vapor atomic fluorescence

spectrometry, and gas chromatography, give the direct and quantitative detection of Hg^{2+} concentration [4, 5], it is highly desirable to develop facile and quick methods for measuring the level of this detrimental metallic ion in the environment with high sensitivity and selectivity.

To date, several methods providing the optical feedback for the detection of Hg^{2+} based upon fluorophores [6–10] chromogenic redox based fluorescent method [11], chromophores [12], polymer [13], and noble metal-based probes [14–18] have been developed. In this regard, among noble-metal nonmaterials, silver nanoparticles (SNPs) have received considerable attention due to their attractive physicochemical properties. The surface plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labeling [19].

To avoid complicated instrumentation or sample preparation, the objective of this paper is to present experimental

results using silver nanoparticles as source of determination of pollutant in marine food. We hope to obtain systematic results regarding the formation of nanosized silver colloids and their usage to determine mercury contamination in fish food quantitatively. In this paper, we present a method with colorimetric quantitative recognition of Hg^{2+} with excellent selectivity and sensitivity based on an erosion reaction of PVP-stabilized Ag nanoparticles in aqueous media. The easy synthesis and high stability of the PEG-PVP-stabilized Ag nanoparticle allow the method to be very simple and easy to implement.

2. Material and Methods

The present method was designed to be nonhazardous, environment friendly, easy to use, sensitive, rapid, and simple sample preparation for the optimal sensing and detection of polluting metals.

2.1. Chemicals and Materials. All chemicals were of analytical grade and were used as received without further purification. Silver nitrate (AgNO_3) (Merck, Karachi, Pakistan), Stock solution of mercury (Merck, 1000 ppm), and nitric acid (HNO_3) (Merck, Karachi, Pakistan) were of analytical reagent grade used for solution preparation. Polyethyleneglycol (PEG6000) powder 99% and polyvinylpyrrolidone (PVP K30) were purchased from local vendors. All the other solutions were freshly made for all the experimental procedures in this work. Double distilled water was used for all solutions preparation, and all glassware is cleaned with aqua regia and thoroughly rinsed with ultrapure water prior to use.

2.2. Instrumentation. For maintaining temperature requirement, Memmert water bath tub (Japan) was used. A UV-visible Shimadzu 1650PC spectrophotometer with UV Probe software, analytical balance (Sartorius TE2145), Hotplate stirrer (Lab Companion HP 3000L), and Shaker (Labnet Orbit1000) was used in the research work. Throughout the work, only amber glass flasks were used to avoid light effect on the solutions of AgNO_3 , PEG, SNPs, PVP, and Hg^{2+} standards and samples solutions.

2.3. Experimental Conditions. The SNPs development was carried out at 100°C temperature for 60 minutes under temperature maintained water bath tub. The precursor, standard, and sample solutions were all prepared in double distilled water.

3. Analytical Procedure

3.1. Solutions Preparation. A solution of 0.1M AgNO_3 was prepared by dissolving 1.6978 g in 100 mL of double distilled water. The model reducing agent used was PEG-6000, which is an example of a good reducing agent whereas PVP was used as stabilization materials in this study. PEG-6000 and PVP were prepared by separately dissolving in double distilled water at room temperature using a magnetic stirrer and water bath at 80°C . A 20% (w/v) of homogenous PEG-6000 stock

solution was prepared by adding PEG powder to 500 mL reagent bottle containing ultrapure water and agitated with a magnetic stirring at 100 rpm for 30 minutes at room temperature. The PVP 20% (w/v) was prepared by adding PVP powder to 500 mL reagent bottle containing ultrapure water and shaking on water bath shaker at 200 rpm for 30 minutes at temperature of 80°C . After that, the solution of PEG-6000 and PVP was combined at respective amounts to get a solution containing 10% of PEG and PVP each. The mixture was agitated with magnetic stirring until the complete transparent and clear homogenous solution was obtained.

3.2. Preparation of Silver Nanoparticles. For Ag^0 development, 25 mL of 0.1M AgNO_3 was added to same volume of PEG-PVP mixture solution. This solution was kept on water bath at 100°C for a specified duration until a pure yellow characteristic color of silver nanoparticle appeared.

3.3. Standard Hg^{2+} Solution Preparation. For Hg^{2+} standard solutions preparation, 10 mL of 1000 ppm stock solution was diluted in 100 mL volumetric flask and added by distilled water up to the mark, to get 100 ppm Hg^{2+} solution. This stock standard solution was further diluted in distilled water to get 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, and 1 ppm solutions. 1 mL of prepared nanoparticles was reacted with 3 mL of standard mercury solutions of different concentrations and change in absorbance was observed. Observations were made after 10 minutes for the complete amalgamation.

3.4. Sample Preparation. For making sample from seafood, muscle tissues of fish were teased fully and about 5 gm of teased tissues was digested in concentrated HNO_3 . The solution was filtered using Whatman (Schleicher & Schuell) 125 mm filter paper and then diluted to 25 mL volumetric flask by double distilled water. The sample solution was 10 times diluted in 25 mL volumetric flask by taking 2.5 mL of stock sample and diluted by distilled water. 1 mL of prepared nanoparticles was reacted with 3 mL of the final solution of different dilutions and change in absorbance was monitored. Observations were made after 10 minutes for the complete amalgamation.

4. Results and Discussion

4.1. Development and Characterizations of the Ag Nanoparticles. The SNPs were obtained through a reduction reaction of silver nitrate with PEG6000 solution in the presence of PVP as capping agent. The UV-vis absorption spectrum of as-prepared SNPs is given in Figure 1. It shows a characteristic peak centered at 411 nm in the visible light area. Calculated according to Lambert-Beer's law, the extinction coefficients of the SNPs are about $4.46 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ (at 411 nm), which can meet the demand of colorimetric sensing detection.

For developing an efficient method to prepare silver nanoparticles, parameters, such as heating time, precursor's

FIGURE 1: Spectral view for the formation of silver nanoparticles.

concentrations, and ratio, were extensively studied. Uniformity of shape and size has great influence on the experimental work. The formation of uniformly sized nanoparticles requires precise concentrations of PVP, PEG, and AgNO_3 as well as heating time.

In the experiment, AgNO_3 was reacted with PEG which caused the reduction of Ag^+ to Ag^0 . Since atomic form of any specie is very reactive, these newly formed Ag atoms react with each other and form aggregates of larger size, thus increasing their volume and decreasing their surface area. To inhibit their growth, polyvinylpyrrolidone was used as stabilizer cum capping agent. PVP seized the growth of Ag atom and restricted them to nanosized particles.

Since the reaction of AgNO_3 and PEG is quite slow, to achieve high concentration of nanoparticles in short time, the reaction mixture was heated to about 90°C in water bath; for various intervals of time periods as 10 and 20 up to 90 minutes, and on each interval of ten minutes absorbance of SNPs was recorded Figure 1.

This figure shows the characteristic peak of silver nanoparticles at 411 nm due to surface plasmon resonance. The peak grows up as the time of heating was increased, and its intensity also increases due to increased concentration, uniformity of shape, and size of nanoparticles. The different researchers confirmed the presence of nanostructured silver particles having surface plasmon resonance peak around 380 nm to 424 nm by TEM, XRD, and SEM [20, 21], thus confirming the presence of silver nanoparticles in the solution.

Figure 2 shows that the absorbance of nanoparticles increases at 411 nm as the time of heating is increased. These two figures confirm the formation and increase in the concentration of nanoparticles as the heating time increases. Thus, rate of reaction was calculated from the slope of line in

FIGURE 2: Absorbance versus time domain for the formation of nanoparticles at 411 nm.

Figure 2 and was found to be 0.092 abs/min ($y = 0.092x - 0.121$, $R^2 = 0.993$). This confirms that the higher is the heating time the higher will be the production of nanoparticles up to certain limits, until an optimal heating time was achieved, that is, 60 minutes in our experiment, and after that the further heating decreased the SNPs concentration as determined by the absorbance.

Finally, it was found that absorbance is directly related to the concentration of nanoparticles; by Lambert-Beer's Law, absorptivity value was found to be $4.46 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

Further work can be done in this regard on the basis of different temperatures affecting the rate of formation of nanoparticles and different concentrations of PEG and AgNO_3 affecting the rate of nanoparticles formation as well as the effect of different concentrations of PVP on the size, shape and uniformity of nanoparticles.

4.2. Sensing and Detection of Hg^{2+} . After the formation of stabilized nanoparticles, the second stage was to observe the reaction of mercury with these nanoparticles. Since standard electrode potential of Ag^+/Ag is 0.80 V and Hg^{2+}/Hg is 0.85 V, it is expected that a redox reaction can occur between zerovalent silver and Hg^{2+} . Thus, in this work, the sensitivity of silver nanoparticles toward Hg^{2+} was identified by UV-vis absorption spectra.

Reaction time of amalgamation was tested by addition of different concentration of mercury to the constant amount of nanoparticles. Change in absorbance was monitored over a period of time after the addition of mercury; it was apparent that the process of amalgamation was almost complete after 10 minutes. Therefore, further treatment of mercury determination was abandoned after 10 minutes, and absorbance was monitored. As the different concentration of mercury was treated with constant amount of nanoparticles, decrease in the absorbance and change in λ_{max} of the silver nanoparticles were observed; blue shifting of nanoparticles was observed Figures 3 and 5.

Blue shifting was so much small that instrument was unable to formulate any reading that can be used to make any direct or linear relation. However, change in the absorbance at λ_{max} (411 nm) was found to be linearly correlated with the amount of mercury treated with SNPs as shown in Figures 4 and 6 ($y = 0.023x - 0.872$, $R^2 = 0.986$). Detection limit of the method was found to be 0.884 ppm. Linear relation of change in silver nanoparticles concentration and so as the absorbance

FIGURE 3: Effect of different concentration of mercury on the $\lambda_{\max}$ and absorbance of silver nanoparticles.

FIGURE 4: Change in absorbance of silver nanoparticles at 411 nm as mercury increases.

with mercury concentration proves to be vital in the mercury determination.

4.3. Detection of Hg^{2+} in Seafoods. Cautions on the mercury concentration in seafood have sparked the consumers distress about fish utilization. The mercury content in fish has been shown to vary widely depending on factors such as fish species, size, place in the food chain, and location of habitat. The same species of fish tested in separate parts of the world have been shown to contain different levels of mercury [22].

The linear relation as proved above (Figures 4 and 6) was used for the determination of mercury content in seafood fish samples. The digested fish sample was diluted and then reacted with silver nanoparticles and the effect was studied. Mercury in the diluted fish sample was found to be in between 7 and 9 ppm. The results shows very high mercury content of mercury in fish samples, above the EPA criterion of 0.3 mg/Kg (equivalent to 0.3 parts per million, ppm) set to protect public health [23]. Dilution factor was used for reporting the real mercury content in ppm as the standards set by EPA and FDA. These values are far beyond the safe limits of mercury as given by EPA and FDA. These results are alarming for the government to take an immediate action on treating the industrial, agricultural, and domestic wastes in proper

FIGURE 5: Effect of mercury on $\lambda_{\max}$ and absorbance of silver nanoparticles.

FIGURE 6: Effect of mercury addition on absorbance of silver nanoparticles at 411 nm.

manner in order to protect fresh and marine life. This will in return protect the people from having that much high dose of mercury in seafoods and adverse health effects.

5. Conclusion

In conclusion, the PVP-stabilized Ag nanoparticles were obtained via a reduction approach by PEG. Hg^{2+} ions in aqueous media were recognized by these nanoparticles via a colorimetric method with high selectivity and sensitivity. This approach relies on the simple redox reaction between Ag nanoparticles and Hg^{2+} ion solution. The concentration of Hg^{2+} can be determined by the change of the intensity of the silver absorbance peak at room temperature. The easy synthesis, high stability, and high water solubility of the PVP-stabilized Ag nanoparticle probes allow a reliable assay performed in aqueous environments to determine the mercury contamination quantitatively.

Conflict of Interests

It is hereby declared by the authors that there is no conflict of interests and that the research work was not sponsored partially or fully by anyone in any sense.

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