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C. dots Supported Silver Nanoparticles for the Effective Sensing of Cysteine

Roshni V[a*]

[a]Department of Basic Science & Humanities, BharatiVidyapeeth (Deemed to be University) College ofEngineering, Pune 411043, India

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Abstract

Carbon dots (C. dots) are the latest member of the carbon nanomaterial family. They are zero-dimensional carbon nanoparticles with size below 10 nm. They have gained intensive attention from the research community due to their remarkable properties like excellent fluorescence, good biocompatibility, high photostability and other electronic and chemical properties. All these attributes aided them in a number of applications in various fields such as optical sensing, bioimaging, photovoltaic devices, catalysis and drug delivery applications. In this article the C. dots obtained from Sunflower seeds are used as reducing agent for the conversion of Ag(I)to AgNPs. This C. dots- AgNPs are then used for the detection of Cysteine (Cys) and the limit of detection is found to be 6.31µM

Keywords: C. dots, fluorescence, Cysteine, AgNPs, Limit of Detection

1. Introduction

The widespread attention gained by C. dots are mainly attributed to its physical, chemical and optical properties which include its remarkable fluorescence, aqueous solubility, photostability, electron transfer behaviour *etc*. [1-2]. The reducing ability of C. dots can be utilized for the reduction of metal ions. Also, C. dots can act as good structural scaffolds to stabilize metal nanoparticles [3]. The electron donating property of C. dots is due to the presence of electron rich surface functional groups which in turn helps the C. dots to act as a good reducing agent towards metal ions.

White lustrous metal, silver (Ag), is widely distributed in nature mainly in the form of many naturally occurring minerals. Ag, due to the presence of single electron outside the completed *d* shell shows an oxidation state of predominantly +1 in its compounds. Its bright white colour, malleable and ductile properties, resistance to atmospheric oxidation *etc*. makes it a precious metal for coins, ornaments and other jewellery items. Ag compounds are also widely used in electronic devices, photographic, pharmaceutical industries and also as versatile catalyst for various chemical reactions [4-5]. Generally, the concentrations of Ag compounds are not too high in the soil. However, the effluents from the industries where soluble Ag compounds are used and also the Ag residuals from cloud seeding causes an increase in the concentration of Ag in the environment [6]. At higher concentrations, it causes skin irritation, dizziness, headaches and respiratory problems [7]. Ag compounds are hazardous to marine life as Ag(I) binds easily to the active sites of enzymes, causing inhibition of biological

functions [8]. Nevertheless, when they get converted to AgNPs they no longer bind with enzymes rather they can be employed effectively as antibiotics in the medical field or as catalyst in many chemical reactions [9]. The conventional methods for the reduction of Ag compounds to AgNPs include the use of reagents like sodium borohydride, N,Ndimethylformamide*etc*. [10-11].

Recently, the green chemistry pathways are more preferred where natural substances are used over chemicals for the effective conversion of Ag compounds into AgNPs. Iravani*et al*. has synthesized AgNPs using reducing sugar as reducing agent [12]. The reduction of Ag(I) to AgNPs was achieved by different plant extracts [13]. Velez *et al*. used aqueous aloe vera extract for the reduction where they were able to produce AgNPs of size 3-14 nm and assessed its antibacterial activity and mercury removal ability [14]. Recently, the reducing property of C. dots has been explored for the synthesis of AgNPs. Choi *et al.* prepared C. dots by thermal decomposition of alpha-cyclodextrin, followed by surface passivation with polyethylene glycol (PEG). They used C. dots as a reducing agent for the formation of AgNPs and this C. dot supported AgNPs was employed as a template for the fabrication of polymer light emitting diodes and polymer solar cells [15]. Beiraghi*et al.* used citric acid and ethylenediamine for the synthesis of C. dots and along with AgNPs formed from $AgNO_3$ using $NaBH_4$ as reducing agent, was used for the detection of cupric ions [16]. The above mentioned points highlight the possibility for extensive research using electron rich C. dots obtained from natural and easily available sources towards the synthesis and stabilization of AgNPs.

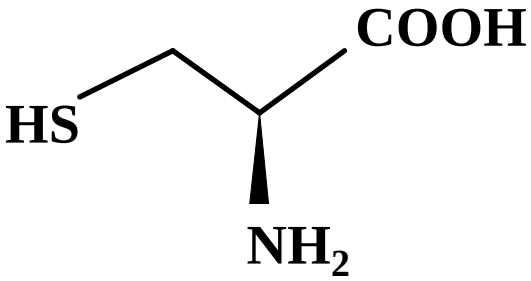

Figure 1: Structure of cysteine

Cysteine is a sulphur containing polar, noncharged and nonessential amino acid synthesized mainly in the liver from homocysteine. The thiol side chain in Cys participates in enzymatic reactions as a nucleophile. The disulphide bonds, which could be formed between two Cys residues, play essential structural roles in many proteins [17]. The structure of Cys is shown in Figure 1. Cys is important in the production of glutathione in the body, protein folding, metabolism, detoxification and many enzymatic reactions. Hence its deficiency as well as its elevated levels in plasma causes many abnormal pathological conditions. Its deficiency causes hair depigmentation, liver damage, skin lesions, muscle weakness and fatigue [18]. At the same time, the higher level of Cys plays a major role in motor neuron, Parkinson and Alzheimer diseases [19]. Hence, it is very important to detect Cys for health monitoring purposes.

In recent years considerable attention was given to the development of suitable methods for the detection of Cys. These detection techniques include high performance liquid chromatography (HPLC), atomic absorption spectrometry, electrochemical sensors, optical

spectroscopy *etc.* [20-23]. However, most of these methods suffer some drawbacks such as complicated and expensive detection system which prevent them from using for routine analysis. In fact, noble metal functionalized nanoparticles for Cys detection based on their distinctive optical properties has gained momentum as these methods are cost effective, less time consuming and are based on green aspects of chemistry. Amjadi *et al.* has studied the interaction of C. dots synthesized from orange juice with AgNPs based on FRET and used it for Cys sensing. They synthesized AgNPs from $AgNO_3$ using $NaBH_4$ as a reducing agent and sodium citrate as stabilizing agent [24]. Easy and efficient C. dots system for the synthesis of AgNPs without any external reducing and stabilizing agent is an area worth looking at. Highly advantageous green strategies for the synthesis of C. dots-AgNPs system were adopted in this work for Cys sensing.

2. Experimental

Synthesis of AgNPs Using C. dots

C. dot sfrom Sunflower seeds were prepared by the previously reported method [25]. AgNPs were synthesized using a simple methodology. 3 mL of 1×10^{-2} M $AgNO_3$ solution was added to 10 mL of C. dots solution (1 mg/mL) and mixed thoroughly. Under mild heating at 50 °C for 5 min., the colour of the solution turned yellow and then to light brown. The obtained solution was then vacuum dried to get the powdered sample. This solid mass as well as the solution obtained by dispersing it in water was used for various analyses. To optimize the reaction conditions, different concentrations of $AgNO_3$ (1M-100 µM) were added to C. dots solution to find the quenching efficiency. At a lower concentration of $AgNO_3$, the quenching efficiency was found to be decreased and at higher concentrations, it showed the precipitation of AgNPs. So for the synthesis of C.dot-AgNPs, the optimized concentration of $AgNO_3$ was kept as 1×10^{-2} M.

Determination of Cys concentration

For the detection of Cys, 1 mL of C. dot-AgNPs (1 mg/mL, 0.01 M PBS buffer) was added with varied concentration of Cys (0.03-0.3 mL, 1 mM) making final volume to 3 mL. Fluorescence intensity was measured at 440 nm on excitation at 360 nm.

3. Results and Discussion

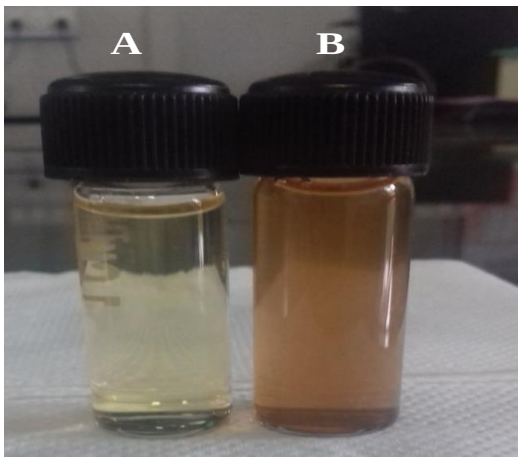

Figure 2: Photograph showing A) C. dots solution B) C.dot-AgNPs

The electron rich functional groups on the C. dots enabled them to act as good reducing agents. Here, the reducing nature of C. dots was utilized for the reduction of $AgNO_3$ to form Ag nanoparticles. The excellent electron donating ability of C. dots helped in the reduction of Ag(I) to Ag(0). The photograph of C. dots and C.dot-AgNPs are shown in Figure 2A and B.

Characterization of C. dot-AgNPs

HRTEM analysis

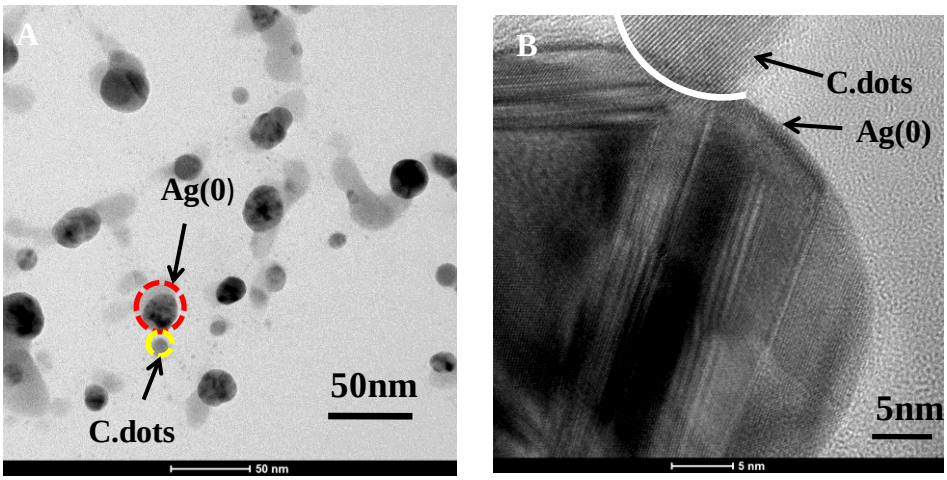

Figure 3: A) TEM and B) HRTEM images of C.dot-AgNPs

Figure 3A represents the TEM image which shows the presence of C. dots and AgNPs. C. dots showed the size distribution in the range of 2-6 nm. AgNPs were found to be with a higher diameter and a size distribution of 16-20 nm. The AgNPs formed were well separated from each other and attached to the surface of C. dots. Generally, AgNPs shows an agglomeration tendency in the absence of any stabilizing agent. However, in this case, the TEM image ruled out the formation of any agglomerated product. The AgNPs formed were monodispersed indicating that C. dots can act as both reducing agent and stabilizing agent. The HRTEM images show two different types of lattice fringes for C. dots and AgNPs (Figure 3B).

UV- Vis. Absorption and Fluorescence Spectroscopy

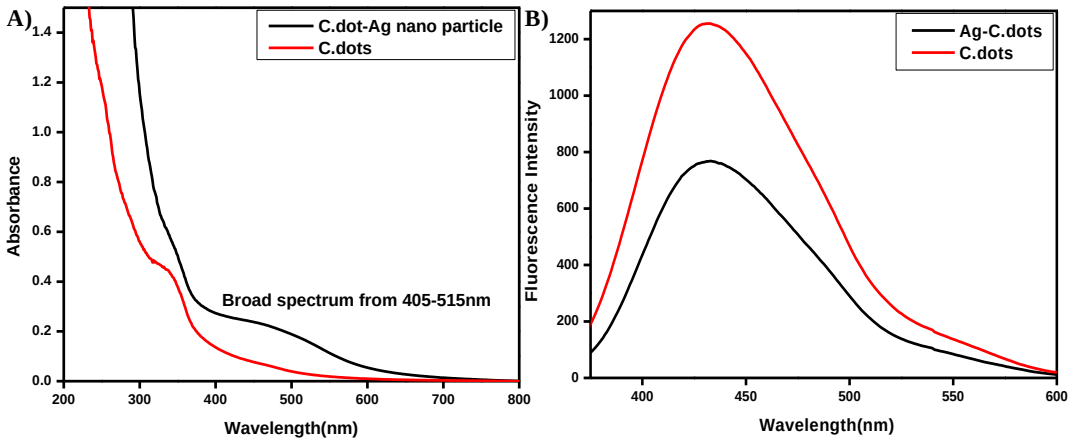

Figure 4: A) UV-Vis. spectra of C. dots and C.dot-AgNPs and B) Effect of AgNPs on the fluorescence intensity of C. dots

The absorbance spectrum of C. dot-Ag showed a broad peak from 405 nm to 515 nm and the distinct peaks obtained for C. dots were found to be absent as shown in Figure 4A. The fluorescence spectrum of C.dot-Ag showed a quenched fluorescence when compared to C. dots (Figure 4B). More than one reason can be presumed for the fluorescence quenching behaviour of C.dot-AgNPs. One reason could be due to the shifting of electron density towards $AgNO_3$ for the formation of AgNPs, which were responsible for the fluorescence of C. dots [26]. Another reason is the possibility of plasmonic coupling [14]. When the light hits on AgNPs surface, the plasmonic resonance occurs which couples with the dielectric field of excitons of C. dots leading to the plasmonic coupling between them. This plasmonic coupling prevents the radiative recombination of electrons and holes thereby causing a quenched fluorescence.

Detection of Cys using C. dot-AgNPs

Selectivity study

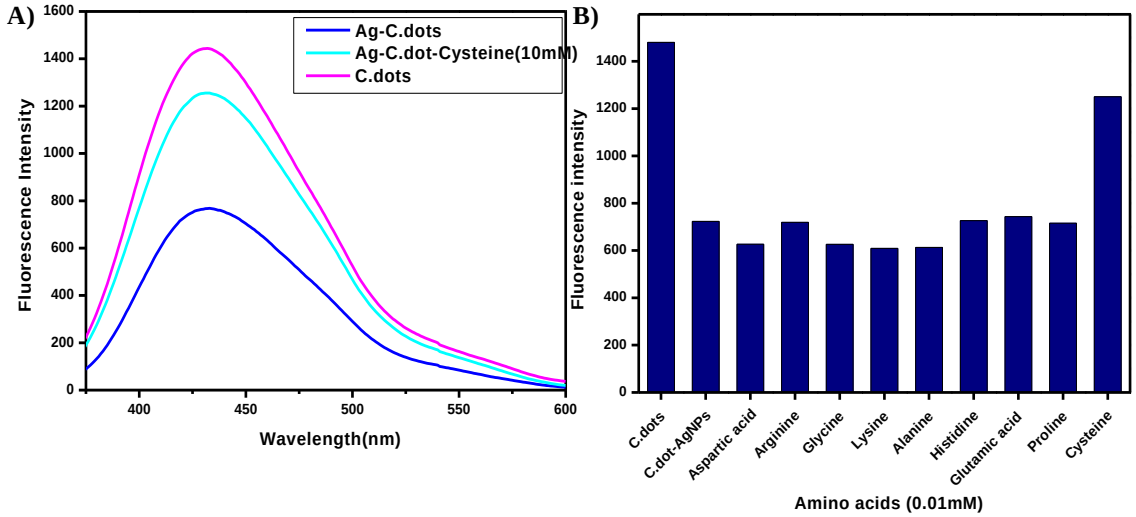

Figure 5: A) Enhancement in the fluorescence intensity with the addition of Cys to C.dot-AgNPs B) Effect of different amino acids on the fluorescence of C.dot-AgNPs

The analytical applicability of the C. dot-AgNPs system was explored by examining its interaction with Cys. Fluorescence spectra of C. dots, C. dot-AgNPs, C. dot-AgNPs-Cys was taken at the same pH in the buffer solution. It was found that the fluorescence of C. dots was not affected by adding Cys to it indicating its non-interaction with C. dots whereas in presence of AgNPs, quenched fluorescence of C. dots were recovered by the addition of Cys as shown in Figure 5A. The explanation for this recovery of fluorescence of C. dot-AgNPs by Cys can be as follows. The quenching of fluorescence of C. dots by AgNPs was due to strong interaction of amino groups on the C. dot surface with AgNPs. The recovery of fluorescence by Cys was due to the stronger complexing ability of Cys towards AgNPs in which a strong Ag-S bond formation takes place between AgNPs and Cys [27]. This resulted in the interruption of interaction between C. dots and AgNPs, leading to the recovery of fluorescence of C. dots.

In order to study the selectivity of the C.dot-AgNPs towards Cys, a series of amino acids were selected. Along with Cys, other amino acids selected for the sensing included aspartic acid, arginine, glycine, lysine, alanine, histidine, glutamic acid and proline. All the possible interfering ions were kept at a concentration of 0.01 mM. It was found that there was a

significant enhancement in the fluorescence of C. dot-AgNPs with Cys as compared to other amino acids thus proving its selectivity (Figure 5B).

Sensitivity Study

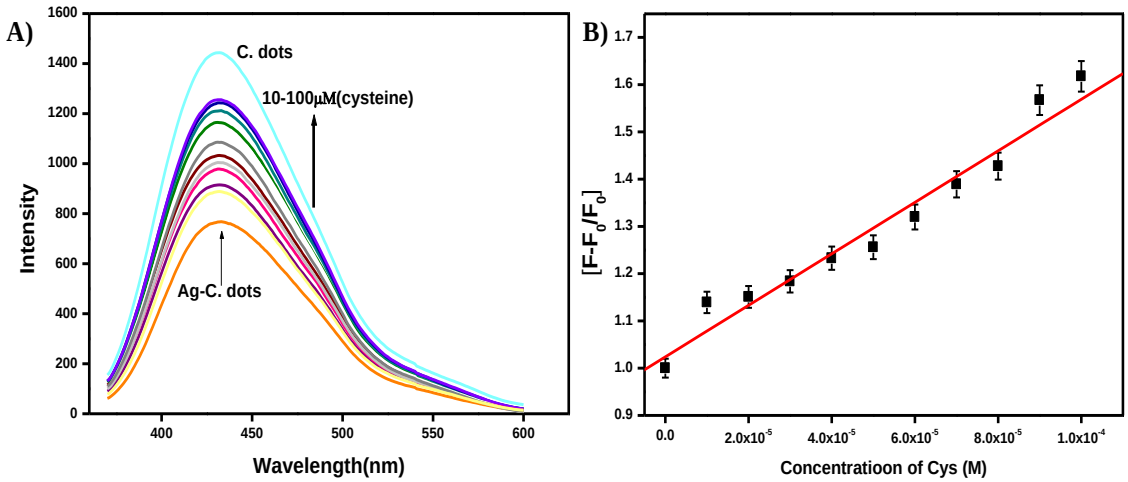

Figure 6: A) Effect of a range of concentration of Cys (10-100 μM) on the fluorescence intensity of C. dot-AgNPs B) Plot showing the fluorescence variation of C. dot-AgNPs with an increase in Cys concentration (linear relationship between $[(F-F_0)/F_0]$ and Cys concentration)

The sensitivity of C.dot-AgNPs towards Cys was calculated by taking a series of concentrations of Cys from 10-100 μM. The fluorescence intensity of C. dots which was quenched by AgNPs showed a concentration dependent recovery as shown in Figure 6A. The C. dot-AgNPs sensor system showed the maximum detection limit in micromolar concentrations for Cys calculated using the standard IUPAC 3σ method. The LOD of Cys using C. dot-AgNPs were obtained as 6.31μM (Figure 6B)

Conclusion

The C. dots obtained from Sunflower seeds were effectively used as a reducing agent for the formation of AgNPs from $AgNO_3$ without using any external reducing or stabilizing agents. The formation of AgNPs from C. dots synthesized from natural sources is rarely reported. This novel C.dot-AgNPs was then effectively used for the selective and sensitive detection of Cys with LOD of 6.31 μM.

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