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Colorimetric Detection Of Lead Ions Using Glutathione Stabilized Silver Nanoparticles

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Abstract - Heavy metal poisoning is one of the common problems that occurs due to changes in food chain, environmental conditions, etc., One of the most commonly occurring toxicities due to metal ions is lead poisoning. It causes many diseases like skin lesions, cancer, diabetes, heart and lung related problems etc., in human beings. It was widely reported that the elevated level of Pb (II) in drinking water is primarily related to human health effects such as damage of the brain and nervous system, behavioural and learning disabilities, blood pressure increase, kidney damage and anemia. Extremely low concentrations of lead in blood and urine causes reproductive disorders especially in children and high concentrations causes seizures, coma and sometimes leading to death. In this work, the silver nanoparticles is stabilized with glutathione - a tripeptide, are synthesized (GSH-AgNPs) using the modified Creighton's method and colorimetric assay of heavy metals with GSH-AgNPs was carried out. The synthesized nanoparticles were characterized by XRD, UV-Vis spectrophotometer, FTIR techniques. The sensitivity and selectivity of different metal ions such as chromium, lead, magnesium, manganese, copper, mercury, ferric and ferrous in water and blood samples were checked. Initially, the synthesized GSH-AgNPs were in pale yellow colour colloidal solution. When mixed with different concentrations of lead from 10^{-3} to 10^{-9}M and pH from 3 to 7, it turns from pale yellow to deep orange and remaining metal ions didn't show any colour change. The optimisation was performed for various parameters such as concentration and pH. From the UV absorbance peak intensity, the concentration of lead ion can be obtained. This assay can be used to detect the lead ion even to the lowest concentration level of ~10^{-9}M. The future work is to develop a lab-on-chip sensor using MEMS technique for more accurate detection of metal toxicities in various biological samples. This diagnostic study can be helpful in identifying lead poisoning at an early stage.

Key words: Glutathione stabilized silver nanoparticles, lead poisoning, pH, MEMS.

1. INTRODUCTION

Lead poisoning is one of the most commonly ocuring toxicity of the heavy metal poisoning. Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Human exposure to lead is estimated to account for 143000 deaths every year and 0.6% of the global burden of disease (2). Lead is a cumulative toxicant that affects multiple body systems, including the neurological, haematological, gastrointestinal, cardiovascular and renal systems. Chronic exposure commonly causes haematological effects, such as anaemia, or neurological disturbances, including headache, irritability, lethargy, convulsions, muscle weakness, ataxia, tremors and paralysis. Acute exposures may cause gastrointestinal disturbances (anorexia, nausea, vomiting, abdominal pain), hepatic and renal damage, hypertension and neurological effects (malaise, drowsiness, encephalopathy) that may lead to convulsions and death.

Children are particularly vulnerable to the neurotoxic effects of lead, and even low levels of exposure can cause serious reproductive problems and, in some cases, irreversible neurological damage. The clinical diagnosis of lead poisoning can be difficult when there is no clear history of exposure, because poisoned individuals can be asymptomatic and when they are present, are relatively nonspecific. Laboratory investigations are the only reliable way to diagnose lead-exposed individuals and therefore play an essential role in the identification and management of lead poisoning and in the assessment of occupational and environmental lead exposure. Today, laboratories primarily assess lead exposure with whole blood. Although a number of other human tissues and fluids, such as hair, teeth, bone and urine, also reflect lead exposure, the concentration of lead in whole blood has gained wide acceptance as the most useful tool for screening and diagnostic testing (1, 3).

Due to the simplicity, rapidity, high sensitivity and ease of measurement, colorimetric sensors are gaining increased attention. Metal nanoparticles particularly silver, gold nanoparticles with well-controlled size have recently been the focus of great interest because the colour changes associated with the surface plasmon absorption band

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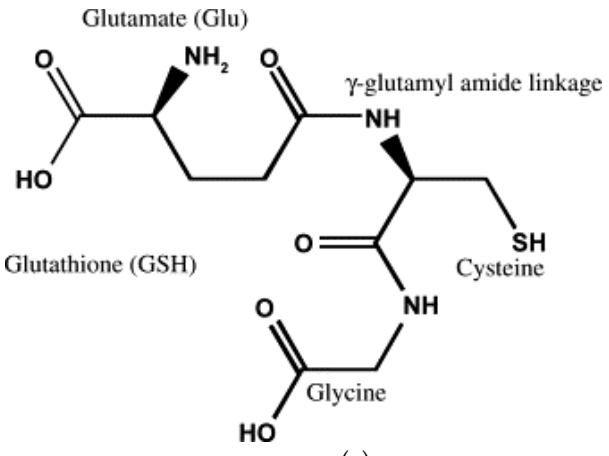
which is dependent on a number of parameters such as the size and shape of the particle, the adsorbed species, the dielectric properties of the medium, and the distance between particles (4). And they have been used with great success for the detection of a range of analytes such as metal ions (5), lectins (6), antibodies, and other analytes (7, 8). In general, the key to develop metal nanoparticles as colorimetric detecting probes is surface modification of metal nanoparticles. The introduction of organic ligands onto the surface of metal nanoparticles provides the stability of these nanoentities in different solvents as well as the desired surface functionality (9-12). Glutathione (GSH) is a tripeptide biomolecule (γ-Glu-Cys-Gly) that contains a –SH group which can be easily adsorbed onto the surface of metal nanoparticles. It is also well known to bind to toxins, such as heavy metals, solvents, and pesticides, and then transform them into a form that can be excreted in urine or bile (13). Meanwhile, there are also many other functional groups such as -NH_2 and –COOH which have strong affinity to the metal ions present in glutathione molecule (14). In view of the importance of this biological molecule, many studies have recently been performed to understand the reactivity of glutathione in the presence of nanoparticles (15-17).

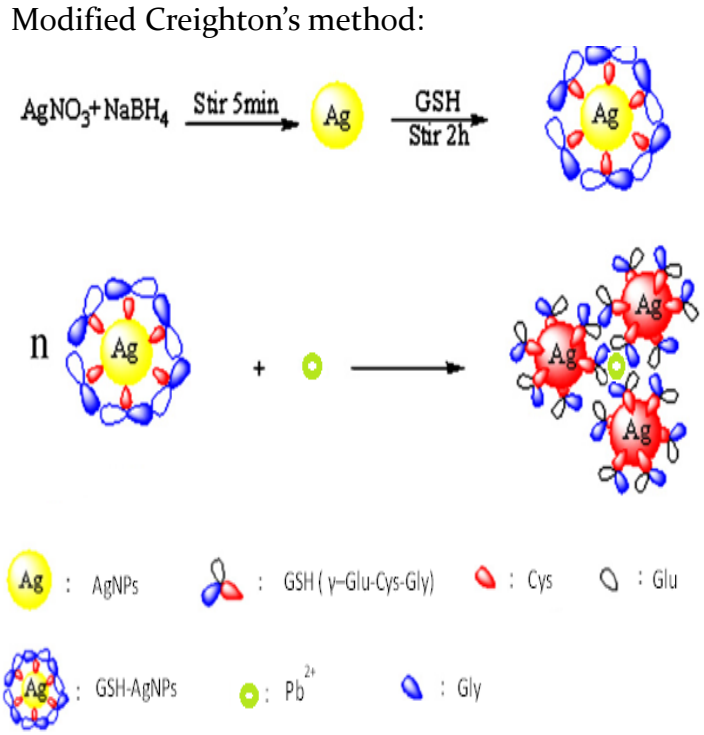
In the present work, glutathione stabilized silver nanoparticles are synthesized (GSH-AgNPs) and colorimetric assay of heavy metals poisoning (lead) detection with GSH-AgNPs was carried out. Various characterizations based on powder X-Ray diffraction, FT-IR and UV-Vis spectrophotometric techniques have been carried out. Sensing of lead ion of a wide concentration level (10^{-3} - 10^{-9} M) was reported.

2. MATERIALS AND METHODS

2.1. Synthesis of glutathione-stabilized silver nanoparticles (GSH-Ag NPs)



(a)



(b)

Fig 1: (a) Structure of Glutathione (b)Schematic representation of synthesized GSH-AgNPs and sensing with Pb^{2+}

As shown in Fig 1(b), silver nanoparticles were prepared by the reduction of silver nitrate with sodium borohydride. 0.012 g NaBH4 was added into 100mL 10^{-4} molL−1 concentrated aqueous solution of silver nitrate with vigorous stirring at room temperature, producing a vivid yellow coloured solution. After stirring for about 5min, 2mL 10^{-3} molL−1 concentrated aqueous solution of glutathione was added dropwise to the silver colloidal solution and left to stir for about 2 h in dark to ensure self-assembly of the glutathione onto the surface of silver particles and sensed with metal ions(Pb^{2+}). All chemicals used are of the highest purity available. All solutions were prepared with Millipore water. AgNO3, NaBH4 and Glutathione were purchased from SD Fine Chemicals Ltd, India. NaOH and HCl were used to adjust the pH of the solutions. Salts of the different cations studied ($MgCl_2$, $MnCl_2$, $CuSO_4$, $FeCl_3$, $FeSO_4$, $ZnSO_4$, $PbNO_4$, Hg_2Cl_2) were obtained and used as received without further purification.

2.2 Characterization techniques

The synthesized GSH-AgNPs were subjected to various characterization techniques like XRD, FT-IR, UV-Vis spectrophotometer, Zeta potential measurements.

XRD

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The Ag-NPs sample was subjected to XRD to know the nature of the nanoparticle.

FT-IR Spectroscopy

To know the functional groups of the synthesized surface modified nanoparticles were subjected to FTIR spectroscopic studies. The spectra were recorded from 400 to 4000 cm^{-1}.

UV–Vis spectroscopy

After the formation, the preliminary characterization of particles was done by UV–vis spectroscopic studies. The spectra were recorded in a UV–visible spectrophotometer from 300 to 800 nm for different pH and days. To know the sensitivity and selectivity, various metal ions such as (Pb^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+},Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}) solutions were prepared by mixing a aliquot of salt in millipore water and different concentrations from 10^{-3} to 10^{-9} M were prepared and subjected to UV-Vis spectrophotometer. Millipore water was used as blank for the synthesized nanoparticles samples and for metal ions samples, GSH-AgNPs used as control. The spectra recorded were then replotted using Origin 6.0 software.

Colorimetric detection of Lead ion

Colorimetric detection of lead ion was carried out by first adding 150µL of lead nitrate solutions to 2850 µL of prepared GSH-AgNPs and leaving it for interaction for 10min. Absorption spectra of the mixture were recorded using cells with 1 cm path length. The concentration of lead ion in the solution can be measured from the values of absorption ratio. The similar procedure was followed to detect the lead concentrations in biological samples such as blood (plasma).

3. RESULTS AND DISCUSSION

3.1. Characterization of GSH-Ag NPs

a. XRD

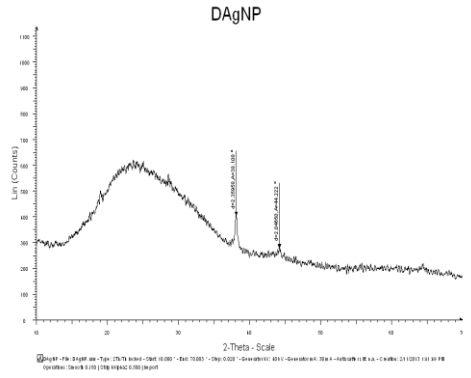


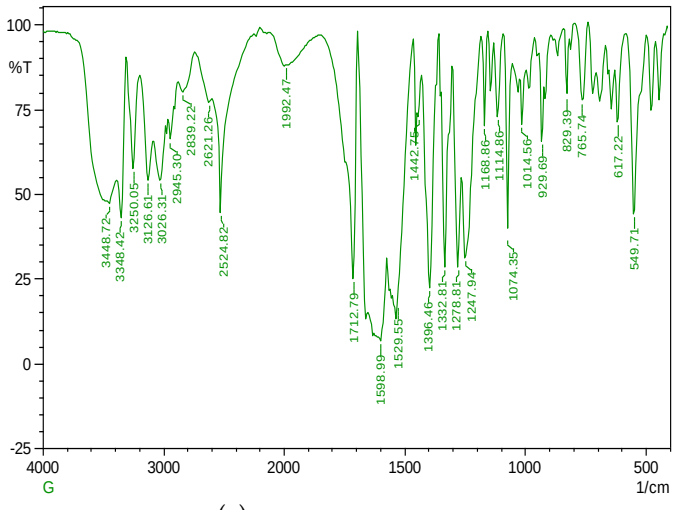
Fig 2: XRD pattern of Ag-NPs

Table 1: Peak indexing from d-spacing

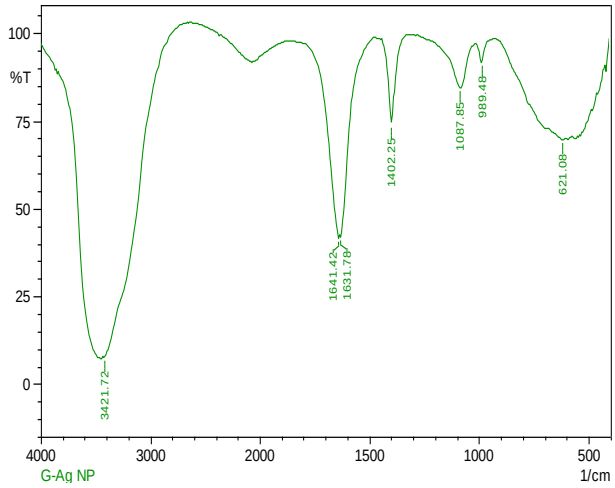
2θ	d	h, k, l
38.108	2.38	1,1,1
44.222	2.04	2,0,0

Fig 2 shows the XRD pattern of the silver nanoparticles. From table 1, the presence of peaks at 2θ values 38.1°, 44.2°corresponds to (111) and (200) planes of silver, respectively. Thus, the XRD spectrum confirmed the crystalline structure of silver nanoparticles. All the peaks in XRD pattern can be readily indexed to a face-centered cubic structure of silver as per available literature (JCPDS File No. 4-0783).

b. FTIR



(a)

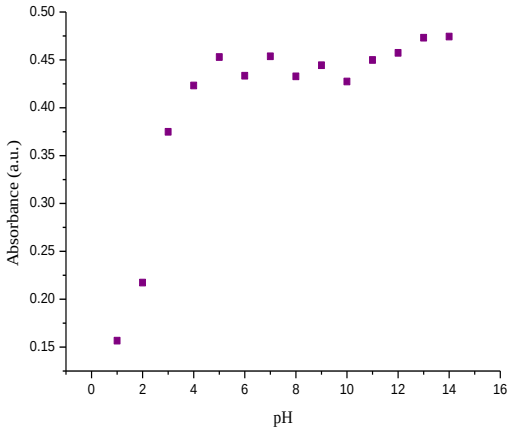


(b)

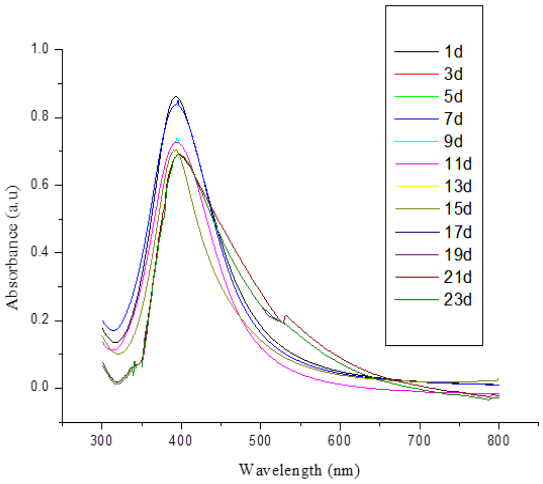
Fig 3: FT-IR of (a) GSH and (b) GSH-AgNPs.

The FT-IR spectra of control GSH and GSH-Ag NPs are shown in Fig 3. Comparing the FT-IR spectra of GSH and GSH-Ag NPs, it was clear through the characteristic absorption peaks for –SH at 2524cm−1 as found in GSH has disappeared in GSH-AgNPs. The dramatic differences between the FT-IR spectra, especially for –SH, suggests that GSH is modified onto the surface of silver nanoparticles via the thiol group from the cysteine moiety of GSH (18, 19).

3.2 Effect of pH and time on the stability of GSH-Ag NPs



(a)



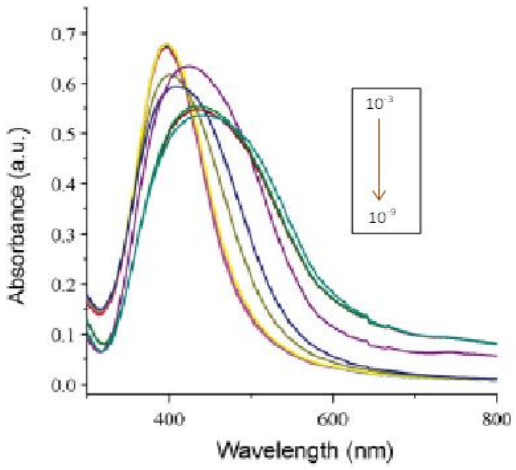
(b)

Fig 4: Effect of (a) pH and (b) time on the stability of GSH-AgNPs.

From the Fig 4(a), it was observed that the synthesized nanoparticles are extremely stable in the pH between 3-14, probably due to strong interparticle electrostatic repulsion between the carboxylate anion of glutathione capping on the nanoparticles surface (20). In Fig 3(b), there was not much change in the shape, position and symmetry of the absorption peak during the initial 23 days, except a little decrease in the absorbance intensity. This result proves that the synthesized GSH-Ag NPs are relatively stable.

3.3 Selectivity and Sensitivity of GSH-AgNPs

(a)



(b)

Fig 5: Sensing of GSH-AgNPs with (a) different metal ions and (b) UV absorbance for different concentrations of lead ions.

Upon interaction, the solutions containing Pb^{2+} changes from pale yellow to deep orange, whereas the remaining metal ions didn't show any colour change (Fig 4(a)). Pb^{2+} is known to bind well with groups or ligands containing lone pair electron such as -NH_2, -COOH etc., via the coordination bond. The structure of glutathione (Fig 1(a)) has both -NH_2, -COOH groups. Both the terminal carboxylate groups of glycine moiety and the free –NH_2 groups from glutamate moiety were believed to be responsible to bind with the Pb^{2+} and helps in cross-linking. With decreasing concentration, the colour change varies from deep orange to mild orange and the absorbance intensity also reduces for different concentrations in the range 10^{-3} to 10^{-9} M of lead ions with GSH-AgNPs (Fig 5(b)). Thus the synthesized GSH-AgNPs were able to detect the lead toxicity even in the lowest concentration of the order 10^{-9}M also.

3.4 Detection in blood samples

Fig 6: Detection of lead ion by GSH-AgNPs in blood samples

Fig 6 shows the response of the GSH – AgNPs to the ten blood samples added. The sample which is poisoned with lead shows a distinct colour change from pale yellow to deep orange. This indicates that the synthesized nanoparticles are able to detect the presence of lead ions in the blood samples.

4. CONCLUSION

In conclusion, GSH-Ag NPs are prepared by reduction of $AgNO_3$ in the presence of GSH. The synthesized GSH-AgNPs tend to aggregate together upon addition of Pb^{2+} due to strong coordination bond between Pb^{2+} and –NH_2, –COOH of glutathione modifier. The aggregation leads to significant shifting in the absorption spectrum with visible colour changes from pale yellow to deep orange and provides a simple and inexpensive means for the determination of lead ion poisoning especially Pb^{2+} toxicities. In future, it can be developed into a lab-on-chip device using MEMS technique for accurate detection of metal toxicities in biological samples which will be helpful in the diagnosing diseases associated with metal poisoning.

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