Proceeding Paper

Humic Acid Functionalized-Silver Nanoparticles as a Colorimetric Nanosensor for the Rapid Detection of Divalent Nickel Ions in Aqueous Solutions †

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Abstract: Heavy metal contamination in groundwater has become more prevalent due to the leaching of toxic wastes from various anthropogenic sources. When ingested, this can cause serious ill effects detrimental to human health. Hence, there is a need to monitor the levels of heavy metals in various water sources to ensure that they are fit for human consumption. Standard detection methods such as AAS and ICP-MS are typically used for quantifying the concentration of heavy metals. However, these require expensive equipment, not to mention the need for a trained and highly-skilled technician to operate the equipment. Nanosensors offer a low-cost alternative to these methods. By utilizing the localized surface plasmon resonance (LSPR) and the properties of noble metal nanoparticles such as AgNPs, the colorimetric detection of heavy metals is made possible. Herein, we report the synthesis of humic-acid-functionalized silver nanoparticles (HA-AgNPs) using a borohydride reduction approach as a colorimetric nanosensor for Ni (II) detection in aqueous solutions. Humic acid acts as a capping agent that stabilizes the AgNPs in the colloidal mixture while providing functional groups for the detection of heavy metals. The synthesized HA-AgNPs had an average hydrodynamic diameter of 42.9 nm, a polydispersity index of 0.438, and an LSPR peak of 400.6 nm. The nanosensor could be used for the colorimetric detection of Ni (II) ions within the linear range of 0.15–0.40 mM Ni (II) with a limit of detection (LoD) of 2.35 mg L^{-1}. The HA-AgNPs were shown to be selective when detecting Ni (II) ions; common metals in water such as Ca (II), Mg (II), Al (III), Zn (II), Na (I), and K (I) did not interfere with Ni (II) detection. As such, HA-AgNPs can be used as reliable and environmentally friendly colorimetric nanosensors for the rapid and point-of-need detection of Ni (II) ions in aqueous solutions.

Keywords: humic acid; silver nanoparticles; colorimetric detection; heavy metals; localized surface plasmon resonance

1. Introduction

Nickel is used and processed by many industries, such as the mining, electroplating, and metal processing industries. It is used to produce Ni alloys and Ni-plated metals primarily because of its many desirable properties, such as corrosion resistance, heat resistance, hardness, and strength. It is also used in producing coins, electrode materials in Ni-Cd and Ni-metal hydride batteries, and pigments and catalysts [1]. During the processing of nickel, these industries release toxic nickel ions into various bodies of water, which can cause contamination of the groundwater and other drinking water sources. Nickel has been classified by the US Department of Health and Human Services as a carcinogen. It causes genetic damage in the form of DNA strand breaks, mutations, chromosomal damage, cell transformation, and disrupted DNA repair [2]. Aside from this, the oral intake of nickel

may cause cardiovascular complications; gastrointestinal distress; increased blood reticulocytes; increased bilirubin and albumin levels; muscular pains; and ophthalmological, neurological, immunological, and lymphoreticular changes [1].

Because of the health risks associated with nickel ingestion, it is imperative to monitor its concentration in drinking water sources. Currently, the Philippine Department of Health has set a maximum nickel concentration in drinking water of 0.7 mg L^{-1} [3]. Meanwhile, the Philippine Department of Environment and Natural Resources has set a maximum allowable concentration of 0.2 mg L^{-1} in Class C water (fishery and agricultural water), 0.02 mg L^{-1} for Class AA and Class A water (public water supply), and 1.0 mg L^{-1} as a general effluent standard for industries discharging Class C water [4].

Inductively coupled plasma mass spectrometry (ICP-MS) has been the widely accepted testing method for determining the nickel concentration in various matrices [5]. The established methods for nickel detection are highly accurate; the current limits of detection for widely established detection methods are in the range of 0.1 mgL^{-1} by ICP-MS, 0.5 mgL^{-1} by Flame AAS, and 10 mgL^{-1} by ICP-AES [6]. Nevertheless, these require the use of costly equipment that skilled chemists and chemical technicians must operate. Moreover, these methods are not viable when an immediate and point-of-need quantification of the nickel concentration is desired. Hence, other quantitative methods have been investigated by numerous researchers in an attempt to develop low-cost and reliable sensors for the detection of nickel. Electrochemical techniques such as cathodic stripping voltammetry [7,8] have yielded promising results. Still, these typically use one-time-use electrodes and expensive potentiostats, making them costly for commercial applications. Hence, colorimetric methods are still preferable over electrochemical methods because portable colorimeters and spectrophotometers are more readily available and easier to use, even by untrained operators. This creates a need to develop a colorimetric assay for the point-of-need colorimetric detection of Ni (II) ions in water.

Previous researchers involved in the colorimetric detection of various analytes have exploited metal nanoparticles' localized surface plasmon resonance (LSPR). In principle, LSPR, or the collective oscillation of conduction band electrons in noble metal nanoparticles when they interact with light, produces wavelength-selective increases in optical absorption and scattering at the nanoparticle's surface upon interaction with an external analyte [9]. This causes a change in the nanoparticles' optical properties, such as changes in color. These changes in the colors of solutions can be used as signals for the quantitative determination of analyte concentration. This can now be exploited for the colorimetric sensing of target analytes [10].

In particular, silver nanoparticles (AgNPs) have been extensively studied in the past for the detection of heavy metals such as Fe (II) [11], As (III) [12], Hg (II) [13], Mn (II) [14], Cr (III) [15], and Cu (II) [16]. The use of functionalized AgNPs has become an attractive prospect for the colorimetric detection of various analytes because it offers facile and label-free detection, with minimal sample preparation [10] compared to the tedious process required for traditional instrumental techniques such as ICP and AAS. Various functionalizing agents have been used to introduce functionalities on the surfaces of AgNPs to improve their colorimetric performance. Among such functionalizing agents are polymers such as gelatin [17], DNA oligonucleotides [18], cysteine [19], isonicotinic acid [20], and mercaptonicotinic acid [21], among others. Nevertheless, colorimetric sensing is quite complicated because AgNPs tend to agglomerate, which can cause false concentration readings. In this regard, stabilizing agents are typically used in AgNP synthesis regimens. Stabilization is typically carried out using citrate [22] and polymeric capping agents such as polymethylacrylic acid [11], chitosan [23], and CTAB [13], among others. While the use of both functionalizing and stabilizing agents can improve the sensing performance of AgNPs, it could affect the cost-effectiveness of AgNPs for commercial applications. Hence, cheaper alternatives should be sought instead of the previously-used functionalizing and stabilizing agents without sacrificing the detection capability of AgNPs in colorimetric assays.

Along with this, humic acid (HA), a natural organic material (NOM), has been explored to function as a stabilizing and functionalizing agent, thus minimizing the use of chemicals during the synthesis of AgNPs. HA-functionalized AgNPs (HA-AgNPs) have been previously reported for herbicide detection [24]. Herein, HA-AgNPs were synthesized and then used as environment-friendly, point-of-need colorimetric nanosensors for quantitatively detecting Ni (II) in aqueous matrices. The calibration curve was established based on the observed changes in the absorbance spectra of HA-AgNPs as a function of Ni (II) concentration. A selectivity study was also performed against common metal ions found in groundwater sources.

2. Experimental Details

2.1. Materials

Silver nitrate ($\geq$99.9% $AgNO_3$, Loba Chemie, Pvt. Ltd., Mumbai, India) was used as a precursor for the synthesis of HA-AgNPs. Sodium borohydride ($NaBH_4$, $\geq$95%, Ajax FineChem, Pty. Ltd., New South Wales, Australia) was used as a reducing agent, while humic acid (sodium salt, $\geq$99.5%, HiMedia Laboratories, Pvt. Ltd., Maharashtra, India was used as a functionalizing agent. Nickel sulfate ($NiSO_4 \cdot 5H_2O$, $\geq$96%, Ajax Finechem, Pty. Ltd., New South Wales, Australia) was used for the preparation of synthetic aqueous Ni (II) solutions. Zinc (II) chloride ($\geq$98.0% $ZnCl_2$, Ajax FineChem, Ltd.), potassium chloride ($\geq$99.0% KCl, Duksan Pure Chemicals Co., Ltd., Gyeonggi-Do, Republic of Korea), sodium sulfate ($\geq$99.0% Na_2SO_4, Merck & Co., Inc., Rahway, NJ, USA), calcium chloride ($\geq$96.0% $CaCl_2$, Sigma-Aldrich Pte. Ltd., Singapore), aluminum (III) sulfate hydrate chloride ($\geq$98.0% $Al_2(SO_4)_3 \cdot H_2O$, Sigma-Aldrich Pte. Ltd.), and magnesium sulfate heptahydrate ($\geq$98.0% $MgSO_4 \cdot 7H_2O$, Ajax FineChem, Ltd.) were used as precursor salts for the preparation of metal solutions. Ultrapure water ($\leq$18.2 M$\Omega \cdot$cm at 25 °C, Milli-Q, Merck & Co., Inc., Rahway, NJ, USA) was used in all preparations. All reagents were used as received, without further purification.

2.2. Synthesis and Characterization of HA-AgNPs

A wet chemical reduction using $NaBH_4$ was used to synthesize HA-AgNPs, as discussed previously in various studies, with minor modifications [6]. Briefly, 10 mL of 5 mg L^{-1} HA solution was added to 10 mL of 0.001 M $AgNO_3$. Next, 10 mL of freshly-prepared 0.01 M $NaBH_4$ solution was added dropwise at a rate of 0.1 mL s^{-1} to the mixture of $AgNO_3$ and HA under continuous stirring in an ice bath. The resulting mixture was allowed to stabilize completely for 60 min at room temperature before it was used for the characterization and sensing experiments.

The synthesized HA-AgNPs were then characterized for their hydrodynamics using a dynamic light scattering analyzer (Zetasizer Pro, Particulate Systemes, Norcross, GA, USA). Furthermore, their corresponding absorption spectra were obtained using a Vis-NIR spectrophotometer (Go Direct SpectroVis Plus, Vernier, OR, USA) from 390 nm to 900 nm.

2.3. HA-AgNPs as Colorimetric Sensors for Nickel (II) Detection

Freshly prepared HA-AgNPs were used for the colorimetric detection of Ni (II) ions in aqueous solutions. In a typical experiment, 3.0 mL of HA-AgNPs was mixed with 500 μL of Ni (II) solutions of varying concentrations (0.0–0.5 mM). The mixture was allowed to stabilize for 1 min before the corresponding Vis-NIR spectra were determined. Calibration curves were generated by least-squares numerical fitting, and the limit of detection (LoD), the limit of quantification (LoQ), and the limit of blank (LoB) were estimated.

2.4. Selectivity of HA-AgNPs as Colorimetric Sensors for Nickel (II) Detection

A selectivity study was performed on HA-AgNPs against common metal ions found in groundwater using a similar detection protocol to that of Ni (II) sensing. As such, 0.25 mM synthetic metal solutions [Al (III), Zn (II), Na (I), K (I), Ca (II), and Mg (II)] were prepared using their corresponding metal precursors. Briefly, 2.40 mL of freshly-prepared

HA-AgNPs were mixed with 400 μL of the as-prepared metal solutions. The absorbance ratios were then compared against Ni (II), and relative response analyses were performed.

3. Results and Discussion

3.1. Synthesis and Characterization of HA-AgNPs

HA-AgNPs were successfully synthesized by a wet chemical reduction of $AgNO_3$ as an Ag precursor using $NaBH_4$ as a reducing agent, as evidenced by the characteristic golden-yellow color of AgNPs shown in the inset photo in Figure 1a. Based on the obtained optical absorbance spectra of freshly-prepared HA-AgNPs, the maximum peak of the HA-AgNPs was seen at 404.5 nm, which is similar to that of AgNPs, and this was consistent with the value previously reported in the literature [25]. Further analysis of the absorbance spectra revealed that the HA-AgNPs had an FWHM of 45.12 nm, indicating their monodispersity in the colloidal mixture. This was further evidenced by the hydrodynamic diameter distribution of HA-AgNPs, as shown in Figure 1b. The HA-AgNPs were found to have an average hydrodynamic diameter of 42.9 nm, with a polydispersity index of 0.438.
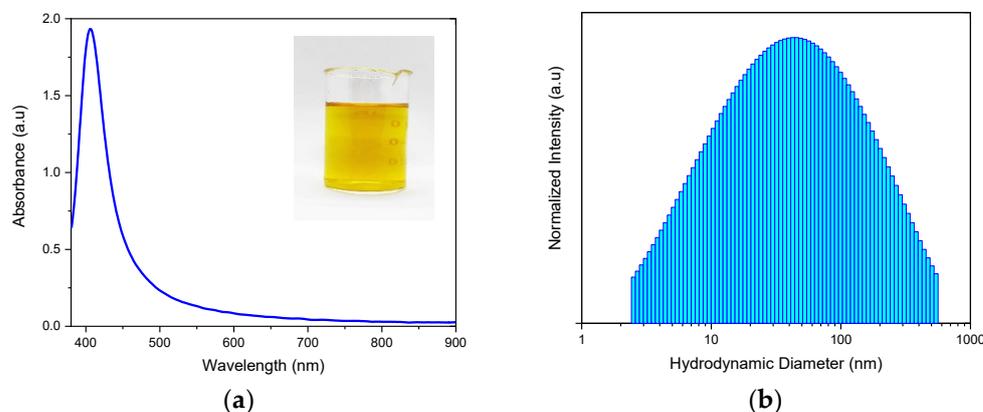

(a)   (b)

Figure 1. (**a**) Vis-NIR optical absorbance spectra and (**b**) hydrodynamic diameter distribution of freshly-prepared HA-AgNPs (HA = 5 mg L^{-1}) prepared by wet chemical reduction using sodium borohydride as reducing agent.

3.2. HA-AgNPs as Colorimetric Sensors for Nickel (II) Detection

Immediate colorimetric changes were seen in the HA-AgNP assay upon the addition of Ni (II) solutions of varying concentrations, as shown in Figure 2a. This is primarily attributed to the aggregation of individual HA-AgNPs resulting in changes in their optical properties. Changes in the absorbance spectra at varying Ni (II) concentrations were recorded and are shown in Figure 2b. As seen from the figure, peak widening occurred upon the addition of Ni (II) ions. This can be attributed to the shift of the LSPR to longer wavelengths due to nanoparticle coupling during aggregation [26,27], which causes changes in the optical absorbance spectra of the HA-AgNPs. Therefore, the changing spectra of the HA-AgNPs according to Ni (II) concentration are varied and can be used to quantify Ni (II) ions in aqueous matrices. Specifically, the changes in the LSPR intensity of HA-AgNPs at 400.6 nm and the changes in intensity at 559.1 nm, attributed to the complexation reaction between HA and Ni (II) ions, can be used to generate calibration curves for Ni (II) detection.

Based on the changes in the absorbance ratio (559.1/404.5 nm) of the HA-AgNPs as the Ni (II) concentration varied, a calibration curve was established as a basis for Ni (II) sensing. This calibration curve, together with the 95% confidence band and 95% prediction band, is shown in Figure 3a. The absorbance ratio was found to be linearly dependent, with the concentration of Ni (II) at a dynamic range of 0.15–0.40 mM and a coefficient of determination of 0.9806. The limit of detection (LoD), the limit of quantification (LoQ), and the limit of blank (LoB) of the HA-AgNPs with respect to Ni (II) sensing were estimated to be 2.3514 ± 0.2131 mg L^{-1}, 2.8035 ± 0.3065 mg L^{-1}, and 2.1579 ± 0.1730 mg L^{-1}, respectively. The predicted Ni (II) concentration based on the generated calibration curve

was compared against the actual Ni (II) concentration of the as-prepared solutions, and this is shown in Figure 3b. At a 95% confidence level, the predicted readings based on the model and actual Ni (II) concentration were not significantly different. This shows that the generated calibration curve can be used reliably for quantifying the amount of Ni (II) in an unknown sample in an aqueous matrix. As such, this can be used for fast and reliable on-site detection of Ni (II) without the need for expensive analytical equipment. This is especially important for the rapid detection of Ni (II) contamination in a particular sample, enabling the people to respond immediately to mitigate such a contamination.
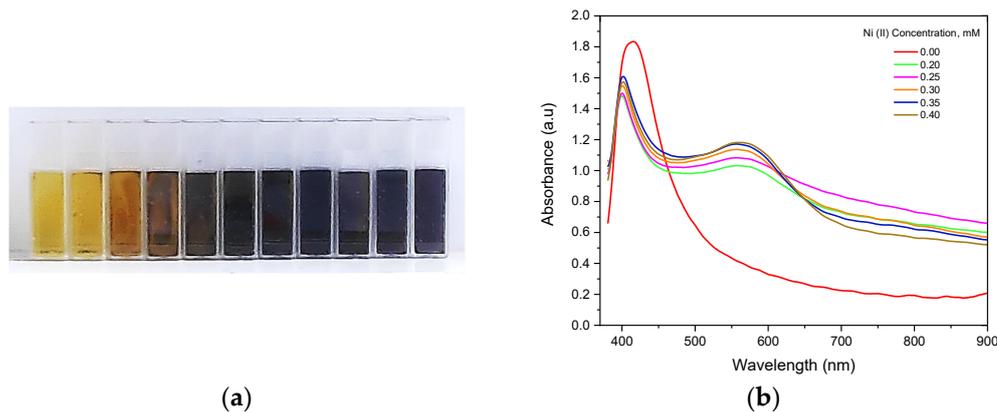

(**a**)   (**b**)

Figure 2. (**a**) Colorimetric changes in HA-AgNPs upon addition of varying Ni (II) concentrations and (**b**) Vis-NIR optical absorption spectra of freshly-prepared HA-AgNPs (HA = 5 mg L^{-1}) at varying Ni (II) concentration.
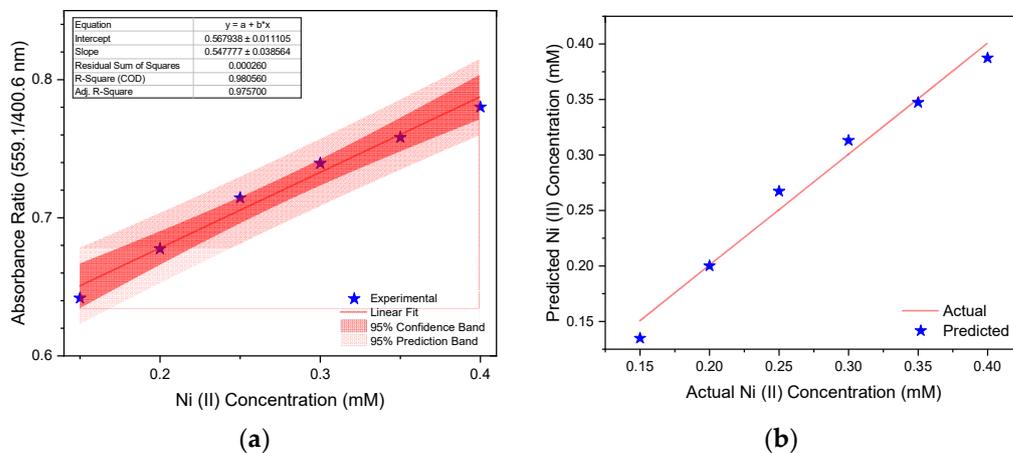

(**a**)   (**b**)

Figure 3. (**a**) Calibration curve showing the linear dependence of absorbance ratio at 559.1/404.5 nm with the Ni (II) concentration and (**b**) the comparison of the predicted Ni (II) concentration based on the generated calibration curve and the actual concentration of as-prepared Ni (II) solutions.

3.3. Selectivity of HA-AgNPs as Colorimetric Sensor for Nickel (II) Detection

A selectivity study involving various metal ions as analytes was conducted using freshly-prepared HA-AgNPs with a similar sensing protocol for Ni (II) detection. The absorbance ratios (559.1 nm/400.6 nm) for various metal analytes were measured, and the relative responses against Ni (II) are shown in Figure 4. The synthesized HA-AgNPs were shown to be selective for Ni (II) ions with respect to all ions tested in this study. The Ca (II) and Mg (II) ions, which account for water hardness, had relative responses between 0.57–0.81 against Ni (II) ions. Na (I), Zn (II), Al (III), and K (I), commonly found in groundwater, had relative responses between 0.10 and 0.22. None of these ions caused significant interferences with respect to Ni (II) detection at a 95% confidence interval. Nevertheless, further improvements to the HA-AgNPs assay can be made to decrease the

relative responses of Ca (II) and Mg (II) and to further enhance its selectivity towards Ni (II) sensing.
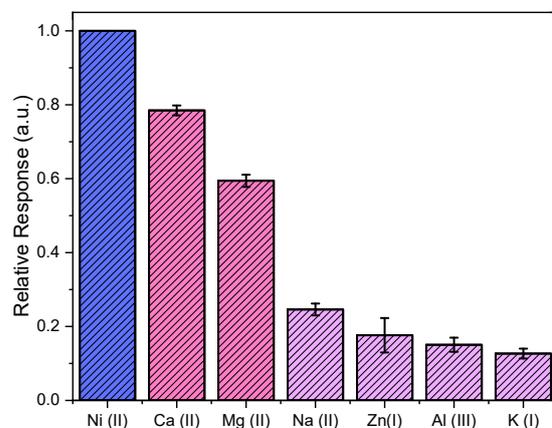

Figure 4. Relative response of various metal ions against Ni (II) ions in aqueous solution showing the selectivity of the synthesized HA-AgNPs assay as a colorimetric nanosensor for Ni (II) detection against other common ions in water.

4. Conclusions

Humic-acid-functionalized silver nanoparticles (HA-AgNPs) were successfully synthesized and used as nanosensors for the colorimetric detection of Ni (II) ions in an aqueous medium. The HA-AgNPs had an average hydrodynamic diameter of 42.9 nm, with a polydispersity index of 0.438. They showed a strong linear response to Ni (II) concentrations at a concentration range of 0.15–0.40 mM, with a limit of detection (LoD) of 2.35 mg L^{-1}, a limit of quantification (LoQ) of 2.80 mg L^{-1}, and a limit of blank (LoB) of 2.16 mg L^{-1}. Nickel readings based on the HA-AgNP colorimetric assay were not significantly different compared to the actual Ni (II) concentrations at a 95% confidence level. Metals common in water, such as Ca (II), Mg (II), Al (III), Zn (II), Na (I), and K (I), did not interfere with Ni (II) detection based on the selectivity study. This shows that HA-AgNPs can be used as reliable and environment-friendly colorimetric nanosensors for the rapid detection of Ni (II) ions in aqueous solutions. Further improvements to the assay can be made to further enhance its selectivity and extend its linear range for Ni (II) sensing.

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Conflicts of Interest: The author declares no conflict of interest.

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