Non-Enzymatic Electrochemical Detection of Urea on Silver Nanoparticles Anchored Nitrogen-Doped Single-Walled Carbon Nanotube Modified Electrode

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Herein, we demonstrated synthesis and application of silver nanoparticles (Ag-NPs) decorated nitrogen doped single-walled carbon nanotube through a one-step thermal-reduction method using melamine as the nitrogen source. Field-emission scanning electron microscopy (FE-SEM), Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffraction data confirmed the successful synthesis of Ag-NPs functionalized nitrogen doped single-walled carbon nanotubes (Ag-N-SWCNTs). The nitrogen-doping notably modified the properties of the SWCNT and it showed stronger affinity for the attachment of Ag-NPs. By integrating the high surface area and electrical properties of N-SWCNTs with Ag-NPs, the obtained Ag-N-SWCNTs nanocomposite showed high catalytic activity than N-SWCNTs and pristine-SWCNTs. The enzyme-based methods have some disadvantages. For example, high fabrication cost and poor stability, due to these intrinsic disadvantages, non-enzymatic sensors have received more interest in fabrication of sensors. A non-enzymatic electrochemical urea sensor was developed by modifying glassy carbon electrode (GCE) with Ag-N-SWCNTs and a layer of Nafion (Nf). Thus, the fabricated sensor exhibited lower limit of detection (4.7 nM), with an enhanced sensitivity of 141 μA·mM⁻¹·cm⁻² for urea detection in the range of 66 nM to 20.6 mM (R² = 0.996). The reliability of the as-fabricated sensor was successfully investigated by using it to detect urea in tap water and milk samples. The NF/Ag-N-SWCNTs based urea sensor offers several advantages such as simple fabrication procedure, non-enzymatic and low-cost, so this sensor can be applied to detect urea in various samples from food, fertilizer industries and environmental fields. Moreover, the modified electrode showed phenomenal stability with no loss in activity of storage under ambient conditions. In addition, the novel hybrid NF/Ag-N-SWCNTs/GCE showed high selectivity toward urea with good repeatability and reproducibility further confirmed that this method can be utilized for detection of urea.

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Urea has great importance in the agriculture, food, plastic and drug industry.\textsuperscript{1} 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 6.0 mM, whereas the acceptable level of urea in milk is 11.6 mM as per the Food Safety Standards Authority of India.\textsuperscript{2} 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 milk). 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.\textsuperscript{3} 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.\textsuperscript{4}

The urea concentration is in the range of 2.6 to 6.5 mM in human blood,\textsuperscript{1} and 342 ± 67 mM in 490 to 2690 mL of human urine.\textsuperscript{5} 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.).\textsuperscript{6}

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.\textsuperscript{7} Recently, electrochemical-based methods have attracted great interest because of its remarkable advantages such as low fabrication cost, small size, high sensitivity with selectivity, lower detection limit and have a potential ability for real-time and on-site analysis. All current urea detection methods employ the enzyme-based sensor technology. The most common and serious concern among them is their poor stability due to the intrinsic property of the enzymes, which is difficult to overcome.\textsuperscript{8} Therefore, few methods were reported for the detection of urea by a non-enzymatic approach using Ni/NiO materials.\textsuperscript{9–11} Ni based metal is noted to present superior performance in the non-enzymatic urea sensor.\textsuperscript{11,12} However, Ni based materials easily get degraded and expanded during the urea oxidation.\textsuperscript{11} Moreover, nickel easily react with alkaline solution to form Ni(OH)\textsubscript{2} and NiOOH. Therefore, Ni based electrode efficiency reduced due to OH\textsuperscript{−} anions ascendant activity.\textsuperscript{12–14}

Internet of things (IoT) is emerging as a promising way to obtain fast, reliable, and precise information on the safety of our food products. Smart sensors are key in developing IoT applications for manufacturing as they help modernize and streamline analytics and connectivity.\textsuperscript{15,16} The integration of one-dimensional (1-D) nanomaterials into IoT sensing platforms as labels or transducer modifiers offers substantial advantages for the detection of chemical analytes.\textsuperscript{17–21} Engineered nanomaterials, such as magnetic nanoparticles (MNPs),\textsuperscript{22–24} carbon nanotubes (CNTs),\textsuperscript{25–28} nanowires (NWs),\textsuperscript{29} nanorods (NRs)\textsuperscript{30} and quantum dots (QDs)\textsuperscript{31} have been used in electrochemical sensor research for improved sensitivity. Amongst the many nanomaterials, SWCNTs have been intensively studied due to their extraordinary electrical, mechanical and structural properties. Now it is becoming a key focus in IoT sensor research.\textsuperscript{32–40}

SWCNTs have become an intrinsic building block for carbon-based electrical circuits.\textsuperscript{31} Carbon atoms present on the SWCNTs are in direct contact with the environments so it could provide maximum interaction with the adjacent molecules in the solution as well as in
Figure 1. (A) The schematic representation of Ag-N-SWCNT’s synthesis. (B) Non-enzymatic electrocatalytic oxidation of urea at NF/Ag-N-SWCNTs/GCE.

solid state. So, by incorporating metal nanoparticles (catalysts) with SWCNTs, it could allow us to detect even single target molecule present in the sample. Therefore, SWCNTs can be useful transducer for sensor applications.36,42–44

Recently, nitrogen doped SWCNTs (N-SWCNTs) has beckoned an especial attention by virtue of its magnificent properties in the field of supercapacitors, field effect transistors and fuel cells.45–47 For example, Liu and Gong et al. designed a giant electrocatalytic activity of N-SWCNTs for oxygen reduction in fuel cells.46,48 When compared to the pristine SWCNTs, nitrogen doped nanotubes decreased the reduction potential by 50 mV and doubled the reduction current.46 All these results indicated that N-SWCNTs have been suitable to develop the non-enzymatic electrochemical sensor.

In this work, Ag NPs decorated N-SWCNTs were prepared through a one-step thermal treatment route. As-synthesized Ag-N-SWCNTs were characterized using FE-SEM, XRD, FT-IR spectroscopy and electrochemical measurements. The Nafion (NF)/Ag-N-SWCNTs composite film was first assembled on the GCE surface to produce the non-enzymatic urea sensor (Figs. 1A and 1B). FE-SEM confirmed that thermodynamically reduced Ag-NPs- were well coated on N-SWCNTs, while Ag-N-SWCNTs were tightly bound to the NF. The synergetic effect between the Ag- and N-SWCNTs led to an enhanced electro-catalytic activity for the oxidation of urea, and offers high sensitivity, selectivity, stability and lower limit of detection (LOD).

Experimental

Chemical and reagents.—AP-SWCNTs were purchased from Carbon Solutions, Inc. (CA, USA). Urea, uric acid (UA), ascorbic acid (AA), glucose (Glu, d-(+)-99.5%), sodium hydroxide (NaOH), dimethylformamide (DMF), sodium dodecyl sulfate (SDS), sodium cholate (SC) and Nafion (NF) were purchased from Sigma-Aldrich, India and used without further purification. Prior to the experiments, alkaline electrolyte was freshly prepared by mixing solution of NaOH in milli-Q water.

Preparation of Ag-N-SWCNTs and characterizations.—The Ag-N-SWCNTs were produced through one-step thermal treatment...
method. Briefly, SWCNTs were mixed with silver nitrate and melamine in the ratio of 1:4:8 (SWCNT: silver nitrate: melamine) in 20 mL aqueous solution (10 mL water: 5 mL of 1% SDS: 5 mL of 1% SC). The solution mixture was sonicated for 30 min and then poured into an alumina boat. The temperature of the solution mixture was raised at a rate of 6°C per min from room temperature up to 500°C under nitrogen atmosphere and maintained for 2 h in the high-temperature split-tube furnace. Then, the final product was collected from the alumina boat directly. For comparison studies, similarly, Ag-SWCNT and N-SWCNT were prepared without adding melamine or silver nitrate, respectively.

The structural morphology of the as-synthesized material was characterized by field-emission scanning microscope (FE-SEM; FEI-Quanta-200FEG). The crystallinity and crystal phases of the synthesized materials were examined by X-ray diffraction (XRD; PAN analytical XpertPro.), measured with Cu-Kα radiation, with Cu-Kα radiation (λ = 1.54178 Å) in the range of 10–90° with a scan speed of 8° min⁻¹. The information about the functional groups was obtained by acquiring FT-IR spectra using Alpha-T&E, Brucker’s FTIR spectrophotometer.

**Fabrication of NF/Ag-N-SWCNTs based non-enzymatic sensor.—**2 mg of Ag-N-SWCNTs were dispersed in 5 mL of DMF and then ultrasonicated for 15 min to obtain a uniform suspension. The GCE’s surface was cleaned with alumina powder up to a mirror-like finish and then washed using milli-Q water and dried under N₂ gas before being used. Then, the suspension (6 μL) was dropped on the surface of the GCE using a drop-casting method, followed by drying at room temperature. In the next step, NF solution (0.125%, Wt/V) was prepared by diluting 5% of NF with ethanol and milli-Q water (V/V, 1/1). Finally, 6 μL NF was used to cover the modified electrode, which helps to protect coated electrode surface and reduce the effect of interfering species on sensor response. The modified electrode system has been denoted as NF/Ag/N-SWCNTs/GCE. Similarly, Ag-SWCNTs/GCE, N-SWCNTs/GCE and SWCNTs/GCE were also prepared in the aforementioned method.

All electrochemical measurements were carried out using an electrochemical workstation (CHI Instrument; CHI-760E) connected to a computer with a modified NF/Ag-N-SWCNTs/GCE as the working electrode, an Ag/AgCl (1 M KCl) reference electrode and a platinum (Pt) wire as counter electrode. The cyclic voltammograms (CVs) were recorded in 100 mM NaOH (pH 10) in the potential range of −0.2 to +0.8 V.

**Results and Discussion**

**Characterizations of Ag-N-SWCNTs, Ag-SWCNTs, N-SWCNTs and SWCNTs.—**The surface morphologies of the as-synthesized Ag-N-SWCNTs and Ag-SWCNTs samples were analyzed by FE-SEM. After the nitrogen doping on the SWCNTs, the electron-density of the nanotubes and their surface properties were significantly altered therefore it’s changing their surface properties. 46 We studied the adsorption of Ag⁺ ion and growth rate of Ag-NPs on the N-SWCNTs and pristine SWCNTs under a nitrogen atmosphere at 500°C for 2 h in the high-temperature split-tube furnace. It was found that highly dense and uniform nanoparticles deposited on the N-doped SWCNTs than pristine SWCNTs as shown in Figs. 2a-2d. It further corroborated that N-doped SWCNTs have a higher attachment rate with Ag-NPs than the pristine SWCNTs. 46 Which suggest that N-SWCNTs was attributed to the strong electron withdrawing ability of the chemically bonded nitrogen atoms on adjacent conjugated carbon sides to facilitate the chemical adsorption of Ag⁺ ions. 46

X-ray diffraction data also confirms the nano-crystalline structure of Ag-N-SWCNTs and indicates that the Ag nanoparticles exhibit a face-centered cubic structure (FCC) (Fig. 3d). Two peaks at about 26.5° and 44.5° were from SWCNTs which can be indexed as the (002) and (101) reflections of graphite (JCPDS No. 75-1621), respectively (Fig. 3a). 49 Furthermore, some other characteristic peaks at 38.2°, 44.1°, 64.5°, 77.4° and 81.5° with high intensity were observed which were corresponding to the (111), (200), (220), (311) and (222) planes of the cubic Ag crystal (JCPDS No. 04-0783). 46,51 Moreover, the three sharp peaks at 31°, 33° and 49° with large intensity can be attributed to the crystalline region of N-doped SWCNTs. 52 From the XRD pattern of the (c) Ag-SWCNTs, (b) N-SWCNTs, (d) Ag-N-SWCNTs it can be observed that the diffraction peaks of the SWCNT have been suppressed due to the coating of the Ag-NPs and N on the SWCNTs surface (Fig. 3). 51 These results confirmed that N-SWCNTs were coated successfully with Ag NPs.
Figure 4. FT-IR spectra of (a) pristine SWCNTs (b) N-SWCNTs, (c) Ag-SWCNTs and (d) Ag-N-SWCNTs hybrid nanocomposite, respectively.

Fig. 4 shows the FT-IR spectra of (a) pristine SWCNTs, (b) N-SWCNTs, (c) Ag-SWCNTs and (d) Ag-N-SWCNTs. Figs. 4a–4d shows the presence of characteristic peaks within a wavenumber in the range of 1037–1190 cm$^{-1}$ which are attributed to C–C stretching band of pristine SWCNTs. The existence of large peaks at 629 cm$^{-1}$ and 1128 cm$^{-1}$ is due to the interaction of SWCNTs with N, Ag and Ag-N groups (Figs. 4b–4d). This results in C–C stretching through the intensity of the small C–C bond of the pristine SWCNTs. The appearance of prominent peaks centered at 1460 cm$^{-1}$, 1620 cm$^{-1}$ and 1750 cm$^{-1}$ are attributed to C=O and C–O bands, respectively, which are arising from the carboxylic functional groups. The other characteristic peaks 458 cm$^{-1}$, 2923 cm$^{-1}$ and 2853 cm$^{-1}$ are associated with the C–H stretching vibration mode of SWCNTs. The above intensities reduced from the N-SWCNTs, Ag-SWCNTs, Ag-N-SWCNTs (Figs. 4b–4d) due to the interaction of SWCNTs with N, Ag and Ag-N groups. The broadband centered at 3422 cm$^{-1}$ could be attributed to the presence of –OH groups in the composite (Fig. 4d).

Electrochemical oxidation of urea.—From the CVs recorded using different modified electrodes, Ag-N-SWCNTs modified GCE showed highly predominant oxidation and reduction peaks compared with those of the N-SWCNTs, SWCNTs and bare-GCE (Fig. 5). This result clearly indicated higher electrochemical activity of the Ag-N-SWCNTs with higher surface area (Fig. 5). To investigate urea sensing and its mechanism, the electrochemical properties of the Ag-N-SWCNTs were evaluated by cyclic voltammetry (CV).
In the cathodic scan, the cathodic peaks corresponding to the electro-formation of a few monolayers of AgOH appeared at +0.32, +0.19 and −0.004 V are corresponding to the electro-reduction of the silver oxides formed during the anodic oxidation as shown in Fig. 6. Such characteristic CV features indicated that the surface of N-SWCNTs was covered with Ag NPs.57,58

Next, the stability study of the Ag-N-SWCNTs/GCE and NF/Ag-N-SWCNTs/GCE was also evaluated by CV measurements. From Figs. 7a–7b, In the case of Ag-N-SWCNTs/GCE, the silver oxide anodic peak current (at +0.33 V) was significantly decreased about 65% after 100 potential cycles between −0.2 to +0.8 V (Fig. 7a). But, NF/Ag-N-SWCNTs/GCE showed stable CVs with current changes up to 42% (Fig. 7b). This data confirmed that Nafion coating was helped to protect the Ag-N-SWCNTs from leaching out of the electrode surface.

After adding 66 nM urea into the 100 mM NaOH, the NF/Ag-N-SWCNTs/GCE showed a new oxidation peak at +0.58 V during the (reverse) cathodic scan (Fig. 6, red curve). The electrocatalytic urea oxidation reaction can be attributed to Ag oxidation/reduction as follows (Equations 1–4).13

Oxidation:

\[
\text{Ag(OH)}_2 + 2\text{OH}^- \rightarrow \text{AgO(OH)}_2 + \text{H}_2\text{O} + 2e^- \quad [1]
\]

\[
\text{AgO(OH)}_2 + \text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \rightarrow \text{Ag(OH)}_2 + \text{CO}_2 + \text{N}_2 \quad [2]
\]

Reduction:

\[
2\text{H}_2\text{O} + 2e^- \rightarrow \text{H}_2 + 2\text{OH}^- \quad [3]
\]

Overall:

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{N}_2 + \text{H}_2 \quad [4]
\]

To study the effect of scan rate on the redox peaks of NF/Ag-N-SWCNTs/GCE in 100 mM NaOH solution, CVs were recorded at various scan rates from 10 to 50 mV/s as shown in Figs. 8a–8c. As the scan rate increases, the oxidation peak current density (μA) increased Figs. 8a–8c. The linear relationship was observed between anodic peak current (Ipa) vs square root of scan rate with correlation coefficient value of (R²) 0.988 (Fig. 8d) in the range of 10 to 50 mV/s which showed a diffusion-controlled process.13

To evaluate sensing performance of the fabricated sensor (NF/Ag-N-SWCNTs/GCE) for urea oxidation, the CVs were recorded with successive addition of different concentrations of urea (Fig. 9a). The obtained linear responses were shown in Fig. 9a, wherein, the current increased linearly with the urea concentration from 66 nM to 20.6 mM (Fig. 9b). The linear calibration curve was obtained with a correlation coefficient (R²) of 0.966. Further, the sensitivity of fabricated electrode was estimated to be 141.44 μAmM⁻¹cm⁻². In addition, the limit of detection (LOD) of urea was estimated to be 4.7 nM using the following Equation 5.18

\[
\text{LOD} = 3\text{SD}/S \quad [5]
\]

Where, S is the slope of the calibration curve (141.44 μAmM⁻¹cm⁻²) and SD is the standard deviation of blank (2.22 × 10⁻⁷ μA cm⁻²). The calculated LOD (4.7 nM) is comparable with the other reported urea sensors as given in Table I. As shown in the Table I, the lower LOD of urea is possible with the present method. It might due to the high electrocatalytic activity with enhanced electroactive surface area of NF/Ag-N-SWCNTs modified GCE and fast charge transfer between the active sites on the electrode surface and urea.18

**Selectivity, stability, reproducibility and repeatability.**—The selectivity of NF/Ag-N-SWCNTs/GCE electrode was determined by measuring CV response. As shown in the Fig. 10, after addition of 10 nM urea the fabricated sensor showed oxidation response at +0.69 V. However, the urea response did not affect in the presence of other interfering compounds such as ascorbic acid (AA), uric acid (UA) and glucose (Glu) (Figs. 10a–10c). Hence, the good selective nature of urea sensor can be attributed to the excellent catalytic effect of Ag(OH)₂ and combination effect of NF coating which are facilitating the electron transfer process from electrolyte to electrode surface.
Figure 7. CV responses of (a) Ag-N-SWCNTs modified GCE (100 cycles) and (b) NF/Ag-N-SWCNTs modified GCE (100 cycles) at a scan rate 50 mV/s in a 100 mM NaOH solution.

Figure 8. NF/Ag-N-SWCNTs modified GCE (a-c) CVs at different scan rates (10–50 mV/s) in a 100 mM NaOH solution and (d) shows plots of the anodic peak currents vs. the square root of scan rates.
and also eliminated the interfering compounds due to its negative charge which repels negatively charged molecules such as AA and UA.59

To investigate the storage stability of the modified electrode, successive CV measurements were carried out using a NF/Ag-N-SWCNTs/GCE electrode in 100 mM NaOH at different time intervals (0 to 150 min) (Fig. 11a). The base peak current at +0.58 V was monitored after recording CV data. It was found that the NF/Ag-N-SWCNTs/GCE’s response did not change up to 150 min. Each CV data was recorded three times and relative standard deviation (RSD) was found to be ~3.13% at +0.58 V (Fig. 11b). This study confirmed that the NF/Ag-N-SWCNTs/GCE electrode can be used for multiple measurements without any electrode stability problem.

Moreover, the reproducibility and repeatability were also evaluated by measuring CV curves in the presence of 10 mM urea in 100 mM NaOH electrolyte using similarly fabricated modified electrodes. CV responses were measured for up to 5 consecutive cycles. The RSD of the current measurement was 2.4% and the electrode retained 97.9% of its initial current response after 5 consecutive cycles which showed that electrode fabrication is highly reproducible. Moreover, the fabricated electrodes were used to measure the spiked urea concentration. As shown in Table II, the reproducible results were obtained using independently fabricated modified electrodes. In addition, the present electrode system offers a wide linear range of urea detection with high sensitivity, selectivity and lower LOD.

**Urea detection in real samples.—Detecting urea in milk samples.—** Milk samples were purchased from near a local shop. Before analysis, the milk samples were pre-treated to remove unnecessary molecules like proteins and fat. 5 mL of milk sample was transferred into a centrifuge tube with 10 ml of acetonitrile. Then the tube was fully shaken by hand for 1 min. Thereafter centrifugation was done at 10,000 rpm for 3 min (to remove the precipitates), the collected supernatant fluid 10 μL was spiked with 100 mM NaOH to obtain the test milksample.60 Now different amount of urea concentration (66 nM–10.6 mM) was measured after known amount of urea spiked into the above milk sample following a standard addition method (Fig. 12). The obtained responses were shown in Fig. 12a, where in, the current increases linearly with increasing urea.

| Electrodes                          | Enzymatic/Non-Enzymatic | Sensitivity       | Linear range  | Detection limit | Response time (s) | Reference |
|------------------------------------|-------------------------|-------------------|---------------|----------------|------------------|-----------|
| Urease/ZnO-chitosan composite/ITO  | Enzymatic               | 0.13 μA nM⁻¹ cm⁻²| 0.8–16.6 mM   | 499 μM         | 10               | 66        |
| Urease/chitosan-Fe₃O₄ composite/ITO| Enzymatic               | 12.50 μA nM⁻¹ cm⁻²| 0.8–16.6 mM   | 333 μM         | 10               | 67        |
| Urease/ZrO₂ thin film/Au           | Enzymatic               | 0.07 μA nM⁻¹ cm⁻²| 0.8–16.6 mM   | 80 μM          | 10               | 65        |
| Urease/poly(glycidyl methacrylate-co-vinyllerrocene)/GCE| Enzymatic| 0.32 nA nM⁻¹| 0.1–1.5 mM | 60 μM         | 3                | 64        |
| Urease/ZnO-MWCNT/ITO               | Enzymatic               | 43.02 μA nM⁻¹ cm⁻²| 1.6–16.6 mM   | 238 μM         | 4                | 63        |
| Urease/nanoporous silicate particles/Au | Enzymatic             | -                 | 0.05–15 nM    | 20 μM          | -                | 62        |
| NF/urease/Yb₂O₃/GCE                | Enzymatic               | 124.84 μA nM⁻¹ cm⁻²| 0.05–19 nM    | 2 μM          | 3                | 5         |
| Graphene-PANI/GCE                  | Non-Enzymatic           | 226.9 μA nM⁻¹ cm⁻²| 10–200 μM     | 5.88 μM        | -                | 61        |
| Ni-MOF/MWCNT                       | Non-Enzymatic           | 685.16 μA nM⁻¹ cm⁻²| 0.01–1.12 μM  | 3 μM          | <10              | 26        |
| Ag-ZnO/GCE                        | Non-Enzymatic           | 0.1622 μA nM⁻¹ cm⁻²| 26.3–427 μM   | 13.98 μM       | -                | 13        |
| NF/Ag-N-SWCNTs/GCE                | Non-Enzymatic           | 141.44 μA nM⁻¹ cm⁻²| 66 nM–20.6 mM | 4.7 nM         | 3                | This work |
Figure 10. CV responses of NF/Ag-N-SWCNTs modified GCE to the addition of urea (10 mM) and different interfering species: (a) UA (0.5 mM), (b) AA (0.5 mM), and (c) Glu (0.125 mM) in 100 mM NaOH buffer solution. Scan rate: 50 mV/s.

Figure 11. Stability of the NF/Ag-N-SWCNTs modified GCE stored at ambient conditions in a 100 mM NaOH solution. (a) CVs were recorded at different time intervals and (b) plot demonstrating changes in peak current intensity with the storage time. The RSD was estimated to be ~3.13% (n = 5).
Table II. Determination of urea in milk and tap water samples.

| Samples            | Urea added (mM) | Total found (mM) (n = 5) | RSD % (n = 5) | Recoveries % |
|--------------------|-----------------|--------------------------|---------------|--------------|
| NaOH buffer        | 10              | 9.79                     | 2.40          | 97.9         |
| Milk sample        | 10              | 9.64                     | 3.30          | 96.4         |
| Tap water sample   | 10              | 9.53                     | 3.49          | 95.3         |

Figure 12. CV responses of (a) NF/Ag-N-SWCNTs modified GCE to the addition of urea (66 nM to 10.6 mM) in 100 mM NaOH solution containing milk sample (Scan rate: 50 mV/s). (b) Corresponding calibration plot of urea concentration vs. current response.

Figure 13. CV responses of (a) NF/Ag-N-SWCNTs modified GCE to the addition of urea (66 nM to 10.6 mM) in 100 mM NaOH solution with tap water (Scan rate: 50 mV/s). (b) Corresponding calibration plot of urea concentration vs. current response.
concentration from 66 nM to 10.6 mM (Fig. 12b). The linear calibration curve was obtained with a correlation coefficient (R²) of 0.991. The recovery was 96.4% with the RSD of 3.3% (Table II).

Water sample analysis.—In order to check the practical applicability of NF/Ag-N-SWCNTs/GCE as a non-enzymatic sensor, 10 μL tap water sample was spiked with 100 mM NaOH to obtain the test water sample. After adding various concentration of urea from 66 nM–10.6 mM, the electrode responses were recorded and calibration graph was made as shown in Fig. 13a. The oxidation current increases linearly with increasing urea concentration from 66 nM to 10.6 mM in tap water (Fig. 13b).

The linear calibration curve was obtained with a correlation coefficient (R²) of 0.961. The recovery was 95.3% with the RSD of ~3.5% (Table II). Although, recovery analysis was satisfactory in milk and tap water samples. In some measurements, the actual urea concentration was decreased which we believed that due to the presence of impurities and some common interfering agents. These results indicated that the proposed method can be used for practical applications in detecting urea. The total assay time for the proposed method was 30 min. In addition, this electrochemical sensor fabrication procedure is fast, simple, highly stable, and relatively low-cost compared with the existing methods, i.e., enzymatic sensors and chromatography.26,61–67

Conclusions

In summary, we have synthesized Ag-N-SWCNTs via a facile thermal treatment method to fabricate a urea non-enzymatic sensor. The structural characterization results confirmed the nitrogen doping and successful deposition of Ag NPs on N-SWCNTs. The NF/Ag-N-SWCNTs/GCE electrode exhibited excellent electrocatalytic performance toward urea with high sensitivity (141.44 μA mM⁻¹ cm⁻²), wide linear range of detection (66 nM–20.6 mM), low LOD (4.7 nM) and fast response time (~3 sec). In addition, this modified electrode had good selectivity, stability, reproducibility, and repeatability for the detection of urea. Additionally, the developed non-enzymatic sensor was successfully demonstrated for urea detection in milk and water samples. Overall, the obtained electrochemical sensor (NF/Ag-N-SWCNTs/GCE) could be utilized to fabricate urea sensing devices for quality monitoring.

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