Development of a non-enzymatic urea sensor based on a Ni/Au electrode

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Abstract. Measurement of urea concentration in urine is a very important parameter for determining the state of kidney health. With this aim, a non-enzymatic urea sensor comprising Ni metal deposited on an Au electrode has been developed. Ni was first deposited on an Au electrode by varying the potential and time, and then the deposited Ni was activated in KOH to generate NiOOH, which can oxidize urea to CO\(_2\), N\(_2\), and H\(_2\) to allow it to be detected electrochemically. The results show that the Ni/Au electrode can be used to detect urea with a limit of detection (LOD) value of 3.35 × 10\(^{-7}\) mM, a sensitivity of 52.20 mV/μA cm\(^{-2}\), and a linearity of r = 0.997 at a deposition potential of −0.45 V vs. Ag/AgCl after a deposition time of 180 seconds. The Ni/Au electrode has good repeatability and reproducibility (%RSD value) of 0.12% (n = 12) and shows good stability with a %RSD value of 1.60% after 9 days. The Ni/Au electrode performance is not disturbed by the presence of any interfering compounds, such as ascorbic acid, glucose, NaCl, and KCl. The Ni/Au electrode can be used to measure urea levels in urine samples, detecting concentrations of as high as 9.615 mM.

Keywords: Au electrode, electrodeposition, nickel, urea, urine

1. Introduction
Urea is the major residual waste substance produced by protein metabolism processes in the body and is an important parameter for testing kidney and liver function. Urea is toxic to humans and is thus removed from the body along with urine. Normal urea levels in the human body are about 10–50 mg/dL [1]. Excessive urea in the urine may indicate that too much protein is being consumed or that the processing of proteins in the body is excessive. Meanwhile, low levels of urea in urine may indicate malnutrition in the body due to consuming too little protein in food, and as a result, kidney disorder has arisen. If the kidney does not function properly, the levels of urea in the body increase and poison the bodily cells.

Urea biosensors have been developed based on enzymatic electrochemical reactions and are widely used due to enzymes having specific binding capabilities and good catalytic activity [1]. However, biosensors have disadvantages, which include limited life times and cost, because they contain expensive enzymes. Due to the high cost of biosensors, non-enzymatic biosensors using metals and metal oxides to replace the enzymes have been developed. Besides bringing about cost savings, metals and metal oxides are good candidates for use in non-enzymatic biosensors because they have high sensitivity and stability [1].

Metals, alloys, and metal oxides, such as Pt, Rh, Pt–Rh, Cu/Cu oxide, Zn/Zn oxide, Ni/Ni oxide, and Co/Co oxide have been widely used to detect a range of molecules [2]. Ni and Ni oxide catalysts are widely used because they are cheap to produce in comparison with other metal catalysts and have better catalytic properties than other metals/metal oxides [2]. For this reason, they have been extensively used for the electrocatalytic oxidation of biochemicals, such as urea and glucose.
A previous study showed that a non-enzymatic urea sensor, prepared by depositing nickel oxide on carbon nanotube (CNT) cellulose, has an excellent sensitivity of 371 μA mM−1 cm−1, with a 4 second response time. The electrode also exhibited high stability with only a 3.6 % reduction in its sensitivity after being stored for 2 months [3].

In this study, we focused upon the development of a urea sensor comprising Ni electrodeposited on an Au electrode. The electrodeposition was carried out at a predetermined reduction potential using a chronoamperometric method in the presence of a strong base as an electrolyte. Sensing tests were conducted using the resulting Ni/Au electrode to measure the urea concentration over a certain concentration range and stability tests were conducted using a chronoamperometric method. Furthermore, tests were also carried out on real urine samples to determine their levels of urea.

2. Experimental

2.1. Chemical reagents
All chemical reagents were purchased from Merck. NiSO₄ and (NH₄)₂SO₄ were used to deposit the Ni on the Au foil. KOH, urea, ascorbic acid, glucose, NaCl, and KCl were used in electrochemical measurements and validation tests, to test for repeatability, stability, and interference. p-Dimethylamino benzaldehyde, HCl and ethanol (96 %) were used in a spectrophotometric method to determine urea in urine samples.

2.2. Electrode fabrication
The working electrode used was Au foil with a size of 0.5 × 0.5 cm. To this electrode surface, nickel was electrodeposited by varying the nickel reduction potential and deposition time to find the optimum conditions. Pt foil was used as the counter electrode and Ag/AgCl as the reference electrode.

2.3. Electrochemical measurements
A typical three-electrode cell was used for the electrochemical measurements. The electrochemical activities of the electrode were examined in 0.1 M KOH solution in the absence (baseline) and presence of 0.33 mM urea using cyclic voltammetry and chronoamperometric techniques. The chronoamperograms were measured at a constant potential of urea at 0.5 V vs. Ag/AgCl.

3. Results and discussion

3.1. The electrodeposition of Ni on Au
The electrodeposition of Ni on Au was performed using an amperometric technique using 0.1 M NiSO₄ solution in (NH₄)₂SO₄. During the electrodeposition, Ni⁺ was reduced to Ni, as follows:

\[
\text{Ni}^{2+} \text{(aq)} + 2e^{-} \rightarrow \text{Ni} \text{(s)}
\]

The negative potential brought about by this reduction caused the electrode surface to become negatively charged and attract the positively charged Ni, resulting in Ni being deposited on the working electrode surface [4].

In table 1, it can be seen that at 180 seconds, the amount of deposited Ni was \(6.677 \times 10^{-2}\) g/cm², whereas at 60 seconds, the amount of deposited Ni was \(2.861 \times 10^{-1}\) g/cm². This indicates that the longer the time, the greater the mass of Ni deposited on the Au electrode. Therefore, the longer the deposition time, the more Ni will be deposited on the surface of the Au electrode.

| Deposition conditions | Loading (g/cm²) |
|-----------------------|-----------------|
| Ni/Au, -0.35 V for 120 s | 2.428 × 10⁻² |
| Ni/Au, -0.40 V for 120 s | 2.948 × 10⁻² |
| Ni/Au, -0.45 V for 60 s | 2.861 × 10⁻² |
| Ni/Au, -0.45 V for 120 s | 4.769 × 10⁻² |
| Ni/Au, -0.45 V for 180 s | 6.677 × 10⁻² |
Figure 1. Cyclic voltammogram of the Ni/Au electrode response to varying concentrations of urea: (a) 0 (b) 0.06 and (c) 0.11 M.

3.2. Determination of the oxidation potential of urea
Figure 1 shows the increase and shift in the oxidation peak upon adding urea to the solution. The greater the concentration of urea in the solution, the greater the increase in the current, which indicates that the Ni/Au electrode can be used to test for urea.

The shift in the urea oxidation peak at 0.5 V vs. Ag/AgCl indicates that KOH has oxidized the urea. The electrooxidation of urea can be described using the following reaction [5]:

\[
\text{Ni(OH)}_2(s) + \text{OH}^- \rightarrow \text{NiOOH} \quad (s) + \text{H}_2\text{O} \quad (l) + e^-
\]

\[
\text{NiOOH} + \text{CO(NH}_2)_2 \quad (\text{aq}) + \text{H}_2\text{O} \quad (l) \rightarrow \text{Ni(OH)}_2 \quad (s) + \text{N}_2 \quad (g) + 3\text{H}_2 \quad (\text{aq}) + \text{CO}_2 \quad (g).
\]

3.3. The detection of urea
A chronamperometric technique was used to detect urea by measuring how the current changes with each concentration of urea. A test was carried out for 1200 seconds in a 0.1 M KOH solution, under a potential of 0.5 V, which corresponds to the oxidation potential of urea (figure 2). The solution was stirred to ensure homogeneity. After the stirring was stopped, the urea solution diffused to the surface of the working electrode, therefore, continuous stirring was not used during the measurements as it causes convection that affects the detection of urea.

In table 2, it can be seen that after 180 seconds at a potential of −0.45 V, the resulting limit of detection (LOD) is 3.35 × 10⁻² mM, with the greatest sensitivity value among those measured of 52.20 μA⁻¹ cm⁻². From the LOD and the sensitivity, the optimum Ni/Au electrode can be determined as the one produced using a potential of −0.45 V for 180 seconds.

3.4. Stability, repeatability, and interference
Repeatability tests were performed to measure the stability of the Ni/Au electrode to determine whether it can be used repeatedly for urea detection. From the repeatability tests, the RSD% value was found to be 0.12 %, which indicates that the Ni/Au electrode can be used repeatedly for measurements. Stability tests were performed to determine whether the Ni/Au electrode is stable enough to be reused within a specified time range. The measurements show that the stability of the electrode does not undergo much change over a period of 9 days, with a RSD% value of 1.60 %, indicating that the electrode is stable enough to be used over this time period.

Interference tests were carried out to determine the susceptibility of the Ni/Au electrode to possible interfering substances that are also present in urine. In this study, selectivity for urea over ascorbic acid, NaCl, KCl, and glucose was tested (figure 3).

3.5. Tests on real urine samples
The urea content in real urine samples was measured using the optimum Ni/Au electrode. An initial 5 mL urine sample was taken, which was then diluted to 7.5 mL with 0.1 M KOH.
Table 2. Comparison of linearity, LOD, and sensitivity to different Ni/Au deposition conditions.

| Deposition conditions | LOD (mM)   | $r^2$    | Sensitivity ($\mu$AmM$^{-1}$cm$^{-2}$) |
|-----------------------|------------|----------|----------------------------------------|
| Ni/Au, $-0.45$ V for 180 s | $3.35 \times 10^{-2}$ | 0.997 | 52.20 |
| Ni/Au, $-0.45$ V for 120 s | $3.43 \times 10^{-2}$ | 0.9931 | 49.7 |
| Ni/Au, $-0.45$ V for 60 s | $3.62 \times 10^{-2}$ | 0.9863 | 47.4 |
| Ni/Au, $-0.40$ V for 120 s | $3.84 \times 10^{-2}$ | 0.9757 | 51 |
| Ni/Au, $-0.35$ V for 60 s | $4.83 \times 10^{-2}$ | 0.939 | 14.2 |

Figure 2. Amperogram of urea in 0.1 M KOH at a potential of 0.5 V vs. Ag/AgCl using the Ni/Au electrode

Figure 3. Amperogram of a KOH solution upon adding urea, ascorbic acid, NaCl, KCl, and glucose at a potential of 0.5 V using the Ni/Au electrode
Figure 4. Amperogram of the urea content of a urine sample in KOH at a potential of 0.50 V vs. Ag/AgCl over 120 s using the Ni/Au electrode

Figure 5. UV-Vis absorption spectra of urea solutions with different concentrations of (a) 0.006 (b) 0.009 (c) 0.012 (d) 0.015 and (e) 0.018 M.

Figure 4 shows a fairly stable current value at a current value of $7.40 \times 10^{-5}$ A. From this value, the concentration of the urine sample can be determined using the linear equation obtained from the calibration curve generated using the optimum Ni/Au electrode. The urea concentration in the urine was calculated to be 9.615 mM.

To determine whether the urea content measured using the amperometric method is accurate or not, a spectrophotometric method was also used for comparison [6]. This method uses a complexing solution of p-dimethylamino benzaldehyde, which is used to provide color to the solution so that absorption measurements can be carried out using ultraviolet/visible light [7, 8].

In figure 5, it can be seen that the absorbance values obtained from the absorption spectra can then be used to derive the concentration, using the linear equation $y = 1.4667x + 0.0262$ in accordance with the Beer–Lambert law. An absorbance value of the sample of 0.039 was used in the equation to determine the urea content in the urine sample, giving a value of 10.44 mM.

The results between the two methods that were used to measure the urea content in the urine did not differ much, i.e. 9.615 mM and 10.44 mM, with a %RSD value of 7.902%. This suggests that the Ni/Au electrode can be used to determine the levels of urea in urine.
4. Conclusions
A Ni/Au electrode was used to detect urea with a LOD value of $3.35 \times 10^{-2}$ mM, a sensitivity of 52.20 mMA cm$^{-2}$ and a linearity of $r^2 = 0.997$ at a deposition potential $-0.45$ V vs. Ag/AgCl for a deposition time of 180 seconds. The Ni/Au electrode showed good repeatability with a %RSD value of 0.12% (n = 12). The electrode was found to be stable and exhibited no significant loss in activity after 9 days. It was also shown the performance of the electrode was not disturbed by the presence of interferences, such as ascorbic acid, glucose, NaCl, and KCl. The Ni/Au electrode was used to measure the concentration of urea in urine samples with a determined concentration of 9.615 mM.

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