Modified flower-like nickel oxide on carbon paste electrode (CPE) for analysis cholesterol based on non-enzymatic sensor

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Abstract. The increasing circulation of unhealthy foods in various places threatens the community of malnutrition. One of which is the intake of cholesterol nutrition. When the normal limit is exceeded, it can trigger the spread of various diseases such as coronary heart disease. To anticipate the outbreak of the disease, a practical, stable, simple, and relatively inexpensive, non-enzymatic sensor device for monitoring blood cholesterol levels in some food samples such as milk and meat was developed. In this research, nickel modified catalyst on carbon paste electrodes, NiO/CPE by hydrothermal method and Ni/CPE by electrochemical method were developed. A flower-like morphology for NiO was obtained from hydrothermal method and rock-like morphology was obtained from nickel deposit. Results showed that Ni/CPE worked optimally at pH 14 with sensitivity of 0.8148 μA μM⁻¹ cm⁻² and limit of detection (LoD) of 0.1645 μM, while NiO/CPE worked optimally at pH 12 with a sensitivity of 0.1238 μA μM⁻¹ cm⁻² and LoD of 0.7804 μM. Cholesterol level measurement from the packaged milk sample showed differences of 20.42 % and 47.18 % from the nutrition table information for Ni/CPE and NiO/CPE, respectively.

1. Introduction
Cholesterol is a metabolite compound of mammals and steroid group lipid compound. Cholesterol is found in the cell membrane and circulated in the blood plasma, which is one of the important components of mammalian cell membrane and is a precursor of other biological materials, such as bile acids, steroid hormones and vitamin D [1]. The level of cholesterol in the human body is not only generated from the metabolism of the body but also from the food consumed such as fish, meat, cheese and milk [2]. Hypercholesterolemia is defined as an abnormality of cholesterol level. Hypercholesterolemia resulted from the metabolic changes in cholesterol, is a major cause of cardiovascular disorders, such as atherosclerosis and coronary heart disease [1].

Regular and continuous monitoring of cholesterol levels in the blood helps to prevent from diseases, namely hypercholesterolemia, atherosclerosis and coronary heart disease. Therefore, the development of cholesterol sensor is necessary. Recently, research on metal and metal oxide materials such as nickel, NiO and CuO are developed at a rapid pace in electrochemical systems, one of which is applied as a non-enzymatic based cholesterol sensor which is able to overcome the weaknesses in enzymatic cholesterol sensors [3]. The advantages of non-enzymatic sensors are easy to produce, low cost, high stability and not affected by its operating conditions (pH, temperature, etc.), as well as having a nano-sized structure, hence larger surface area that increases the sensitivity to cholesterol [2]. In the development of non-enzymatic sensors, carbon paste (CPE) electrodes are often used as working electrodes due to their properties like economical manufacturing, wide potential windows, and stable surface [4].

In this study, the non-enzymatic cholesterol sensors NiO/CPE and Ni/CPE were synthesized by hydrothermal and electro-deposition methods. The synthesized sensors were expected to be tested on...
milk samples and compared with the detection results with batch system. In this study, cholesterol detection, calibration, characterization and interference tests were conducted to see the usability and feasibility of the synthesized sensors.

2. Experimental

2.1. Chemicals and materials
Ni(NO$_3$)$_2$, Cholesterol, Cetyltrimethylammonium bromide (CTAB, ≥98%), potassium hydroxide (KOH), 1-butyl-3-methylimidazolium bis(trifluoro methylsulfonyl)imide (BMIM TFSI), graphite were purchased from Sigma Aldrich.

2.1.1. Fabrication NiO with hydrothermal method. About 25 mL of 0.3 M Ni(NO$_3$)$_2$ was poured into a beaker glass, and then 10 mL of CTAB solution was added stepwise under vigorous stirring for 30 minutes. Subsequently, 25 mL of 0.6 M NH$_4$OH was added dropwise under vigorous stirring for 2 hours [5]. The formed suspension was transferred into a 100-mL stainless steel autoclave lined with Teflon, and put in the oven at 185 ºC for 24 hours [6]. The obtained precipitate was calcined at 400 ºC for 2 hours. The NiO powder was characterized using Fourier Transform Infrared (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM-EDX).

2.1.2. Fabrication carbon paste electrode (CPE) and its modified electrode. Graphite powder and Immidazole with ratio 5:1 were mixed to produce a homogenized paste, and then put into a pipette tip as the body electrode. To modify the electrode with NiO, 0.1 g as-synthesized NiO powder was mixed with the homogenized paste. Whereas, for electrode modification with Ni, the Multipulse Amperometry (MPA) technique was used, in which Ni was deposited onto CPE by reduction of Ni$_2^+$ (from Ni(NO$_3$)$_2$ solution) at potential reduction of nickel -0.326V for 90 seconds.

2.1.3. Electrochemistry measurements and sensor test. An amperometric technique was used in each modified electrode to create a calibration curve in a KOH solution with a standard cholesterol solution. Glucose, sucrose and casein were used as interferences into cholesterol solution. Milk samples were used to test the cholesterol detection sensors using a modified electrode.

3. Results and discussion

3.1. Characterization of NiO & deposit of Nickel on CPE
The infrared spectra of the NiO are presented in figure 1a. It can be found the stretching vibration of -OH (3000-3600 cm$^{-1}$), the vibration of the CO$_2$ group function (O=C=O) at 2394 cm$^{-1}$, the OH bending at 1400-1800 cm$^{-1}$ and the specific vibration of the metal oxides functional group at 450-750 cm$^{-1}$. It can be seen that after calcination, the specific peak of metal oxides was increased and the other peak
decreased. This indicates that the NiO became more crystalline and the impurities such as water and organic molecules have been released.

The XRD pattern of synthesized NiO by hydrothermal method before and after calcination is shown in figure 1b, which is in agreement with the diffraction patterns of NiO crystals in JCPDS 47-1049 [7, 8].

Representative SEM images of the NiO powders are presented in figure 2. It can be seen the rosette-like morphology from NiO. This morphology was developed by NiOH surrounding CTAB template, so the OH ions will be attracted to the head of CTAB (~N(CH$_3$)$_3$+). The concentration of alkaline solution used is responsible for the size and porosity of the as-synthesized NiO crystals, because concentration of the alkaline solution affects the diffusion process [4]. From EDX result in figure 2b, the ratio of Ni/O was ~ 2.5. Thus from XRD, infrared spectra and EDX data, it was confirmed that the NiO was successfully formed.

3.1.1. Electrode activation. Prior to use, each modified electrode is required to be activated in an alkaline solution [9]. The modified electrodes showed anodic and cathodic peaks at potential -0.2V–0.8 V. The hydroxyl ions (OH) was involved in redox reaction. The immersion of deposited Ni or NiO in alkaline solution leads to the formation of hydrated αNi(OH)$_2$ and anhydrous βNi(OH)$_2$, where the β species is more stable than α [4]. Ni(OH)$_2$ was oxidized further and formed γ-NiOOH and β-NiOOH. In figure 3, the oxidation peak of Ni/CPE has shifted to a more anodic potential. It indicates that the stable β species has increased in KOH solution and there is a tendency of α species to β [10]. All ongoing redox reaction of Ni could be described in the following equation (1) [3]:

$$NiO + 2OH^- \rightarrow \beta Ni(OH)_{2} + 2e^- - \beta Ni(OH)_{2} + OH^- \rightarrow \gamma NiOOH + H_2O + e^- \quad (1a)$$

$$NiO + 2OH^- \rightarrow \beta Ni(OH)_{2} + \frac{1}{2} O_2 + 2e^- - \beta Ni(OH)_{2} + OH^- \rightarrow \gamma NiOOH + H_2O + e^- \quad (1b)$$
Table 1. Comparison of calibration curve, Linearity, LoD, and Sensitivity of Ni/CPE and NiO/CPE Electrodes on variation of pH

| Electrode | pH | $r^2$ | Sensitivity (µAµM cm$^{-1}$) | LoD (µM) |
|-----------|----|-------|-----------------------------|----------|
| Ni/CPE    | 14 | 0.8309| 0.8148                      | 0.1645   |
| NiO/CPE   | 14 | 0.9585| 0.0534                      | 2.1244   |
| Ni/CPE    | 13 | 0.9305| 0.0795                      | 1.6271   |
| NiO/CPE   | 13 | 0.9925| 0.1238                      | 0.7804   |
| Ni/CPE    | 12 | 0.9156| 0.1559                      | 0.6593   |
| NiO/CPE   | 12 | 0.9312| 0.1449                      | 0.7725   |

Figure 4. Chronoamperometric response of modified electrode (a) NiO/CPE and (b) Ni/CPE toward cholesterol addition in 1.0 M KOH.

3.1.2. Electrochemistry measurements and sensor test. Furthermore, Ni/CPE was being activated faster than NiO/CPE. This suggests that Ni on Ni/CPE is more easily exposed to be activated into Ni$^3+$ (NiOOH) species than Ni$^2+$ on NiO/CPE. Although both have FCC structures [11], when all modified electrodes were activated, Ni species will act as strong oxidizing agents for cholesterol detection sensors at specific oxidation potential (0.58 V). NiOOH was simultaneously reduced to NiO which is represented in the following equation:

$$\text{Ni}^3+ + \text{cholesterol} \rightarrow \text{Ni}^2+ + \text{cholestenone} \quad (2)$$

Figure 4 shows that the modified electrodes responded in a significant increase in peak current after each addition of cholesterol to 1.0 M KOH solution. Ni/CPE (figure 4b) exhibited a higher peak current than NiO/CPE (figure 4a) due to its high electrocatalytic activity and the large active surface of Ni/CPE. Ni/CPE can adsorb and catalyze more reactive substances and resulted in an instantaneous oxidation process [2].

The linear range, sensitivity and detection limit of modified electrode are compared at pH range 12–14 and listed in table 1. The modified electrode shows a linear relationship of electrode response with additional 0.5 µM cholesterol at range volume of 0.5 – 2.0 mL. NiO/CPE has high linearity with $R^2 = 0.9925$ at pH 13 whereas Ni/CPE shows a low detection limit 0.1645 µM of cholesterol sensing and high sensitivity 0.8148 µA µM$^{-1}$ cm$^{-1}$ at pH 14. These results indicated that Ni/CPE and NiO/CPE have a good ability for non-enzymatic detection of cholesterol.

3.1.3. Effect of other glucose and fructose as interferences. Selectivity is an important parameter of the non-enzymatic cholesterol sensor. Other substance in sample such as glucose, sucrose, and casein could interfere the detection of cholesterol. As shown in figure 5 Ni/CPE and NiO/CPE showed identical responses, the presence of glucose and sucrose interfered the cholesterol detection response, while the casein was found to be a non-interfering substance. This is thought to be due to glucose and sucrose having an oxidation potential adjacent to the cholesterol oxidation potential [9].
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To validate the feasibility of modified cholesterol sensor, analysis of cholesterol in milk sample was performed. The test results from milk samples obtained the measured cholesterol levels using non-enzymatic sensors Ni/CPE and NiO/CPE were 86.51 $\mu$M and 37.94 $\mu$M, respectively. Compared to the calculation results of the nutrition table on the packaging milk, the calculated cholesterol level in the sample is 71.84 $\mu$M. The differences in measurement results with non-enzymatic sensors against the nutritional tables are 20.42% and 47.18% for Ni/CPE and NiO/CPE respectively. So, it can be concluded that these non-enzymatic sensors can be applied to measure cholesterol levels in milk.

4. Conclusions
Carbon Paste electrode has been successfully modified with Ni and NiO by electrodeposition and hydrothermal methods. From the above data, when using NiO/CPE does not show a strong enough response to oxidize cholesterol into ketone form because in NiO the presence of Ni bonds with O which makes Ni more difficult to be exposed to activate into Ni$^{3+}$ species, whereas using Ni/CPE gives a higher response, because Ni is easily to be activated and the Ni$^{3+}$ species is rich. The presence of glucose and sucrose interfered the cholesterol detection response while the casein was found to be a non-interfering substance.

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Figure 5. The interference test of modified electrodes in KOH with 0.5 mM cholesterol in the presence of 0.5 mM glucose, sucrose, and casein (a) Ni/CPE and (b) NiO/CPE