Synthesis of Sheet Like Morphology of NiO for Sensitive and Selective Determination of Urea

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An efficient and simple method has been demonstrated for the synthesis of nickel oxide nanostructures using urea as a capping agent. The nanosheet-like morphology was confirmed by scanning electron microscopy, crystalline nature was studied by using the X-ray diffraction (XRD) and surface area of nanomaterial was investigated by automated sorption analyzer. Then synthesized NiO nanostructures were used to fabricate the surface of glassy carbon electrode (GCE). The electrocatalytic parameters of modified NiO/GCE electrode were investigated by using various techniques such as electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), differential pulse voltammetry (DPV), normal pulse voltammetry (NPV) and cyclic voltammetry (CV) and chronoamperometry. Various working experimental conditions were optimized in order to attain the highest sensitivity for the determination of urea and the highest peak current 1032 μA of response were obtained at 100 μM concentration of urea. A linear calibration plot was obtained for peak current versus concentration of urea in the range of 10 μM urea to 80 μM urea with a good detection limit of 2 μM . The proposed working strategy was successfully employed for the estimation of urea in human urine samples and the obtained results are found satisfactory. The newly functional urea sensor can be exploited at large scale as an alternative analytical device beside to the other reported urea sensors.

Keywords: Urea, Electrochemical Sensor, Nickel Oxide Nanostructures, Cyclic Voltammetry, Amperometry, Urine Samples.

1. INTRODUCTION

Human urine along with fertilizer plants and animal are intended to strengthen the urea in an aqueous environment.¹ There is the severe effect of higher and lower level of urea in human beings and animals such as urinary barricade, renal botching, stomach and intestine hemorrhage of blood, dehydration, shock burns, hepatic and cachexia infection, and nephritic diseases.² Besides its adverse impacts, it is essential gradient used to estimate the urea concentration in different medical and environment samples. The determination of urea in different samples containing amperometry,^{3,4} piezoelectricity,⁵

potentiometry,^{6,7} molecularly imprinted polymer^{8,9} and surface Plasmon resonance have been realized as benchmark mark methods.^{10,11} However, the above-mentioned methods are still susceptible to certain problems to find out the urea concentration in different samples. These problems are the utilization of a number of different chemicals, lower sensitivity, partial selectivity and economic constraints of sensor application. These problems are subsided by metal oxide nanoparticles having effective catalytic characteristics. They provide an electrochemical procedure to metal electrodes surface by protecting them from oxidation of different oxidizable species. Most of the electrochemical sensors use metal oxide nanoparticles to determine numerous species such as melamine,¹²

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hydrazine,^{13,14} glucose,^{15,16} serotonin, dopamine^{17,18} and so on. The less attention is paid to work on utilization the sensing ability of nanoparticles for urea sensing.^{19,20} In earlier work urea was electrochemically detected by metal oxide nanostructures fabricated with graphene oxide without utilizing urease enzyme having a detection limit of 5 μM urea. The reported methods have a complex arrangement; consumption of numerous costly chemicals and extensive synthesis process and up-gradation of electrode makes the whole process very expensive. An additional report about enzyme-less sensors based on TiO_2 used to detect urea is illustrated as sensing material but the lower sensitivity is a questionable.¹⁶ Another report about urea sensing material is demonstrated in which zinc oxide film along with urease are used to detect urea with a detection limit of 13.5 mM in which enzyme use is essential.²¹ Further reports^{21–28} accentuate on harnessing urease as a crucial constituent of the electrochemical sensor was fabricated with metal oxides. Previously reported protocols were made these methods less sensitive, less selective and economically costly.

In the present work, we have ingested the urea as a capping agent for the synthesis NiO nanostructure in the presence of liquid ammonia by hydrothermal method. Furthermore, these NiO nanostructures were used as the first time to account based on simply synthesized material and its stable immobilization on the surface of GCE and highly sensitive to determine the concentration of urea without any enzyme assistance or fabrication as relating to some metal oxide nanostructures previously described. Therefore, these nanostructures used as a sensor in consequence of having enormous characteristics such as extremely sensitive, highly selective, highly stable, environment-friendly

and also cost effective than the earlier reported electrochemical methods for the determination of urea. Successfully this electrochemical sensor is used for the detection of urea from human urine samples according to age factor and obtained reliable recovery response.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Reagents

All reagents and chemicals such as acetic acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, potassium chloride, hydrochloric acid (37%), nickel chloride hexahydrate, ammonia (33%), urea, uric acid, vitamin C (ascorbic acid), glucose (E. Merck, Darmstadt), sodium hydroxide, acetone, ethanol and 2-propanol (Daejung Korea) were used as received. All solutions were prepared in mili-Q water and nafion (1%) solution (Aldrich, Sigma) were prepared in 2-propanol.

All glasswares were washed by soaking in 3 M HNO_3 overnight followed by washing with detergent water. It was then thoroughly washed with tap water and finally rinsed at least 3 times with doubly distilled water. The glassware was then dried in an oven at 110 $^\circ\text{C}$.

2.2. Instrumentation

All voltammetric measurements were carried out using a CHI 760D Electrochemical Workstations. The software (CHI 9.22, Austin, USA) was used in combination with the electrochemical workstation. A conventional assembly of the 3-electrode system was used. Glassy carbon electrode in combination with platinum (Pt) wire as a counter and Ag/AgCl as reference electrodes respectively. NiO modified GCE was used as working electrode.

Scheme 1. Graphical representation of experimental work.

The high-resolution SEM images of NiO nanostructures were obtained from (model.JSM.6380 of Jeol, Japan). For characterization of NiO nanostructures, The surface area of NiO nanostructures was investigated by Automated Gas Sorption Analyzer (Quantachrome® ASiQwin™ version 5, USA) and the crystalline nature of the NiO nanostructure were captured by using XRD powdered diffractometer (D-8 from Bruker, Japan).

2.3. Synthetic Procedure

Synthesis of CuO NSs was carried out through ambient temperature using aqueous media wet chemical method. In this procedure, we prepared 0.1 M copper chloride hexahydrate ($\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$) in reaction vessel, then added 0.5 g P-villain and the final solution was homogenized by adding 5.0 mL of 33% NH_3 solution with dilution to 100 mL. The reaction vessel was compactly covered with aluminum foil to avoid external interference as well as solution spill. The reaction vessel was placed in an electric oven at 95 °C for 4 h. After that, fine particulate of CuO NSs were separated by using centrifugation process. The separated material was washed carefully with (1:1) metrics solution of deionized water and ethanol to remove unwanted impurities and ions. Furthermore, resulting material was calcinated in an electric furnace at 95 °C for about 1 hour to completely convert the copper hydroxide-oxide phase to the pure oxide phase.

2.4. Fabrication of Electrode

The surface of the GCE was polished with 0.1 mg of alumina oxide powder then the electrode was sonicated with acetone and milli-Q water up to 5 minutes. Furthermore, the electrode was dried under the stream of nitrogen gas. (GCE was modified with 5 μL of NiO nanostructures that was prepared in methanol). Than dried the electrode under normal condition and after that added 5 μL of 1% of nafion in order to stabilize the NiO nanostructures at the surface of GCE.)

The modified NiO/GCE used as a working electrode for the analysis of urea in sodium hydroxide used as a supporting electrolyte. Then different modes were applied such as cyclic voltammetry (CV) to check the behavior of analyte, electrode study, mode study, scan rate, reproducibility and also the stability of NiO nanostructures was checked while chronoamperometry was used for stability of modified electrode over 10 days and we obtained good agreements.

2.5. Real Samples Preparations

Specially, urine samples collected from 15 kidney failure patients of Liquat Medical University Diagnostic Laboratory by following sampling manual of international sampling standard for urine. Immediately, Liquid samples were stored at 20 °C before analysis. 0.1 g of active carbon added into 5 mL of sample for decolouring. Then the sample was centrifuged by using vortex-mixture for 20 s. Then

a sample was separated onto the filter paper. In the last sample, filtrate yield was diluted in (0.1 M/100 mL) NaOH solution and the solution was mixed by using sonication procedure until it was completely diluted.

3. RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. XRD Analysis

SEM imaging was carried out for the investigation of morphological features of as-synthesized NiO nanostructures, by mounting a 1 mL of the sample on a glass slide. Both low and high magnification images of NiO nanostructures are enclosed in Figures 1(a), (b) respectively.²³ It can be clearly seen from Figures 1(a) and (b) that the synthesized NiO nanostructures exhibit sheet-like morphology and with porous features which are highly favorable for electro catalytic performance.

3.1.2. XRD Analysis

The X-ray diffraction patterns reveal the synthesized NiO nanostructures totally crystalline in nature. And it is clearly shown in XRD spectrogram these several peaks such as (111), (200), (220), (311) and (322) which confirms the synthesized material is nickel oxide and in additional it illustrate these structures having cubic crystal structure and the obtained results are according to standard (JCPDS card no: 87-0712).

Figure 2 shows more intense two peaks and these peaks are more prominent which further confirms the crystalline nature of synthesized NiO nanostructures. These peaks also illustrate the phase purity of NiO nanostructures. In an additional study, the size of these nanostructures was calculated from these XRD patterns by implying Scherrer formula.

$$D_p = \frac{K\lambda}{\beta_{1/2} \cos \theta}$$

In which lambda (λ) reveals wavelength of XRD, the crystalline nanometer size represents by (D_p), K represents the shape factor having value 0.94 and ($\beta_{1/2}$) represents line

Fig. 1. (a) SEM image at low magnification and (b) SEM image at high magnification.

Fig. 2. Shows the XRD patterns of synthesized NiO nanostructures.

broadening at half maximum intensity. The average size of NiO nanostructures was calculated in nanometer range is equal to 8.82 nm and range from 5.38 nm to 12.01 nm. Furthermore, the structure of prepared nanomaterial was confirmed by using scanning electron microscopy (SEM).

3.1.3. Surface Analysis

The porosity of the synthesized NiO nanostructures was analyzed by using automated sorption analyzer that works under BET theory and the BET data shows that NiO nanostructure contains a high specific surface area which is equal 44.351 m²/g, the pore volume is equal 0.0019 cc/g and average pore diameter is equal 9.976 Å. Herein, small pore size and larger surface area that means larger size to volume ratio and according to this these nanostructures having more active sites which play vital role for the electrochemical determination of urea. In addition, we can imply this nanostructure to examine the level of urea in urine samples and we obtain highly sensitive their response toward the urea.

3.1.4. EIS Analysis

Figure 3(A) prior use of modified electrode we characterized the electrode by Electrochemical impedance spectroscopy (EIS), the EIS electrogram were studied in the presence of 5 mM [Fe(CN)₆]^{3-/4-} (1:1), 0.1 M KCl containing the solution this solution were used as an electrode probe. In this study, we compare the response of the GCE with modified NiO/GCE electrode. The Figure 3 in which modified electrode response possess greater dots as compare to GCE and from this we can say the modified electrode is highly conductive in nature than the GCE. The electron transfer phenomena more prominent at modified electrode.²²

3.2. Electrochemical Detection of Urea

3.2.1. Electrochemical Study

The electrocatalytic oxidation of urea was examined at bare and modified NiO/GCE electrode using cyclic voltammetry

Fig. 3. (A) The electrochemical impedance spectroscopy (EIS) (a) response at GCE and (b) response at modified NiO/GCE in 5 mM [Fe(CN)₆]^{3-/4-} (1:1) solution containing 0.1 M KCl. (B) CV response of blank on (a), (c) and 100 μM urea (b) and (d) on GCE and NiO-GCE respectively.

in the absence and presence of 100 μM urea in 0.1 M NaOH. Here NaOH used as a supporting electrolyte. It can be seen in Figure 3(B(a)) show response bare GCE with blank solution (b) show the response of bare GCE with urea. After that modified NiO/GCE was used, (c) demonstrates the response of modified electrode with low charging current value absence of urea and (d) indicates that presence of micromolar concentration urea the response of peak current has been increased which means it enhances the catalytic activity of GCE. Recent electrochemical study demonstrated that the modified NiO/GCE electrode highly sensitive the electrooxidation of urea.

3.2.2. Influence of Voltammetric Mode

Figure 4 show the comparison of different voltammetric mode including, square wave voltammetry (SWV), cyclic voltammetry (CV), normal pulse voltammetry (NPV) and differential pulse voltammetry (DPV) in order to investigate the highest sensitivity for the electro-oxidation of urea at modified NiO/GCE. Figure 4 illustrates the four voltammograms recorded in NaOH at 100 μM concentration of

Fig. 4. This graph shows that various mode applied to test highest peak current response of modified NiO/GCE electrode (a) CV, (b) DPV, (c) NPV, and (d) SWV.

urea. It can be seen from Figure 4(c) demonstrate that the highest sensitive response was gained using cyclic voltammetry (CV) that was selected for all subsequent studies.

3.2.3. Influence of Supporting Electrolyte

As we know that the supporting electrolyte wherein charge transfer function take place and we select different supporting electrolyte to investigate the influence of it on peak current response. Figure 5 illustrate that influence of supporting electrolyte on the peak current response for the detection of urea was examined by using 5 supporting electrolytes such as Britton-Robinson buffer (4 mM), phosphate buffer (0.1 M), KCl buffer (0.05 M), Acetate buffer each (0.04 M) at pH 7.4 and NaOH (0.1 M). It can be clearly seen from Figure 5 that highest sensitive response in terms of peak current was achieved in sodium hydroxide and it was selected for all experiments.

Fig. 5. CV's response to attain maximum peak current response by using difference supporting electrolyte such as (a) 0.1 M phosphate pH = 7.2, (b) 0.1 M KCl/HCl pH = 4 (c) 0.1 M BRB pH = 7.4 (d) 0.1 M NaOH.

3.2.4. Influence of Varying Scan Rates

Figure 6 shows the effect of variation in scan rate was observed in order to investigate the diffusion and adsorption controlled electron transfer kinetics for the oxidation of urea. The peak currents were found to depend linearly on the square root of scan rates in the range 10 mVs^{-1} – 100 mVs^{-1} as illustrated in bellow Figure 6.

A plot of peak current versus square root of scan rates follows a straight line with the regression equation $y = 65.05x + 591.2$ with $r^2 = 0.99$ as shown in the Figure 6 indicating the oxidation of urea at modified NiO/GCE electrode tracks diffusion controlled process.

3.3. Analytical Protocols

3.3.1. Quantitative Analysis of Urea

In order to examine the relationship of peak current to the concentration of urea, an amperometric technique was used. From this, an amperometric i - t curve was recorded

Fig. 6. CV's response shows that the influence of scan rate on peak current and the corresponding calibration of peak current versus square root of scan rate is analytically describing the diffusion controlled behavior of NiO modified GCE.

Fig. 7. The amperometric $i-t$ curve illustrates the calibration study of urea with successive addition of $10 \mu\text{M}$ once: of urea through continuous stirring at applied potential is $+0.8$ and conce: range is $10-80 \mu\text{M}$.

under optimistic conditions such as the successive addition of $10 \mu\text{M}$ concentrations of urea in the range of $10 \mu\text{M}$ to $80 \mu\text{M}$ with continuous stirring at $120 \text{ rev} \cdot \text{s}^{-1}$ and applied potential was $+0.8 \text{ V}$. A plot of peak current versus concentration follows a linear relationship with the regression coefficient of 0.990 as shown in Figure 7 from this estimated limit of detection was found to be $2 \mu\text{M}$ and limit of quantification was calculated to be $7 \mu\text{M}$.

3.3.2. Importance of Urea Sensor

It is clear that sensors²¹⁻²⁸ as an integral part of most use, depending on the enzyme urease sensing materials. As a method of such a system is extremely complex and makes expensive and hence difficult to be afforded by the poor, especially in the underdeveloped countries.

In addition to the sensor fabrication of a variety of chemicals, because of that, these are expensive and not eco-friendly. The resignation of the sensor makes it somewhat impractical to economic and environmental aspects of spending time. Although the electrochemical sensors but there is a lack of sensitivity as compared to all previous reported, our report urea assisted NiO/GCE amperometric sensor is highly sensitive, stable and profitable. All qualities such reports so far different from the previously reported urea sensors.

Developed NiO/GCE based an amperometric sensor is applied on real samples, for example, ($n = 4$) biological

Table I. This table shows the comparative study of the developed sensor with previous reported electrochemical urea sensors.

Sensing nanomaterial	Electrochemical methods	LOD	Type	Reference
ZnO Nanowire	Amperometry	0.1 mM	Urease-base	[23]
NiO/ITO	Conductometry	$10 \mu\text{M}$	Urease-base	[24]
NiO/Cellulose/CNT	Amperometry	$7 \mu\text{M}$	Enzyme-free	[25]
ZnO NRs	CV	13.5 mM	Urease-base	[21]
TiO/Er ₂ O ₃	EIS	3 mM	Enzyme-base	[26]
NiCo ₂ O ₄ /Graphene	Chronoamperometry	5 mM	Enzyme-free	[27]
Glass/Ag/ZnO NRs	CV	$10 \mu\text{M}$	Urease-base	[28]
NiO/GCE-Nafion	Amperometry	$2 \mu\text{M}$	Enzyme-free	Present study

urine samples of Childs, teenagers, youngsters, and aged patients use to test recovery of urea. Data is presented in Table II.

3.3.3. Interference, Reproducibility, and Stability

The novel approach of selectivity under the same conditions investigation of modified NiO/GCE carried out and used in many common interfering substances, such as uric acid, ascorbic acid, glucose, calcium and magnesium ions with $100 \mu\text{M}$ equimolar concentration. Figure 8(A) clearly shown that these analytes did not interfere with the determination of urea, i.e., the modified NiO/GCE electrode for sensing urea in the presence of interfering compounds was highly selective in nature for the above mentioned.

Figure 8(B) it reveals reproducibility of the modified NiO/GCE electrode is investigated using cyclic voltammetric mode at repeated several runs in the presence of $100 \mu\text{M}$ concentration of urea the relative standard deviation is calculated ($\text{RSD} = 1.05\%$) and stability of modified NiO/GCE electrode is examined under same experimental condition after 10 days and single modified electrode is used to test the stability by chronoamperometry technique ($100 \mu\text{M}$) concentration of urea as shown in Figure 8(C). The relative standard deviation (RSD) was calculated ($\text{RSD} = 2.8\%$) and it confirms the stability and reproducibility of modified NiO/GCE, therefore, it is best for the determination of urea in standard as well as real samples. This study verifies the economic suitability of developed sensor is compared with various other sensors.

Table II. This table shows the determination of urea from human urine samples via recovery protocol ($n = 4$).

Samples	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
Child	50	47.6 ± 0.11	95.2	2.31
Teenage	50	51.8 ± 0.19	103.6	3.66
Younger	50	51.4 ± 0.10	102.0	1.94
Aged	50	51.8 ± 0.08	102.6	0.15

Note: * (Found-found without addition) $\times 100/\text{added}$.

Fig. 8. (A) An interference study with the use of successive additions of equimolar 100 μM concentration urea to each of the following substances uric acid, ascorbic acid, glucose and mixture of (Ca^{2+}/Mg^{2+}) under the conditions of continuous stirring of the solution at +0.80 V in 0.1 M NaOH, (B) chronoamperometric response for stability over 10 days and (C) five repeated CV runs to test reproducibility of modified electrode.

3.4. Practical Application of Developed Sensor

The newly developed electrochemical sensor was applied to various human urine samples according to age factor and estimates the reliability and performance of recovery method.

4. CONCLUSION

The current study focuses on the one step synthesis of the NiO nanostructures capped with urea molecules. The structural study reveals that synthesized NiO nanostructures have cubic sheet-like shape, purely crystalline nature, and possess high specific surface area. Furthermore, the high charge transfer capability of modified NiO/GCE with compare to GCE carried out by electrochemical impedance spectroscopy. The resultant efficient urea based NiO nanostructures demonstrate superior catalytic activity for the electrochemical oxidation of urea when present at the surface of GCE. The modified electrode can be applied to quantify urea in real samples with excellent recoveries. The newly developed urea sensor can be capitalized at large scale as an alternative analytical tool in addition to the existing other urea sensors.

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