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SHORT COMMUNICATION

Amprometric detection of Glycine, L-Serine, and L-Alanine using glassy carbon electrode modified by NiO nanoparticles

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Abstract Glassy carbon electrode modified with nickel oxide nanoparticles has been used to investigate the electrochemical oxidation of Glycine, L-Serine, and L-Alanine in an alkaloid solution. The electrochemical behavior of the modified electrode was characterized by cyclic voltammetry in detail. The electrocatalytic behavior is further exploited as a sensitive detection scheme for the above amino acids by hydrodynamic amperometry. Under optimized conditions, the calibration curves are linear in the concentration ranges of 1–200 μM for Glycine, 1–400 μM for L-Serine, and 30–200 μM for L-Alanine, respectively. The respective detection limit ($S/N = 3$) and sensitivity are 0.9 μM and 24.3 nA μM^{-1} for Glycine, 0.85 μM and 12.4 nA μM^{-1} for L-Serine, and 29.67 μM and 0.4 nA μM^{-1} for L-Alanine. The prepared electrode exhibits a satisfactory stability and long life-time, while it is stored at ambient conditions.

Keywords Modified glassy carbon electrode · Nickel oxide nanoparticles · Amperometry · Glycine · L-Serine · L-Alanine

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1 Introduction

During recent years, extensive research has been focused on the preparation of novel nanoscale materials [1, 2]. Among various nanostructure compounds, metal oxide nanoparticles such as ZnO [3], MnO_2 [4], NiO [5–9], and CuO [10, 11] have been of increasing interest in electrochemical studies. Until today most of the studies published in the literature have been primarily concerned with reporting the experimental methods of producing these materials, and characterizing the resulting samples.

The literature survey shows that various studies have been devoted to investigate electrochemistry of nickel oxide nanoparticles (NiONPs) for different applications, including the direct electrochemistry of cytochrome C [12], direct electron transfer of horseradish peroxidase and H_2O_2 determination [13], direct electrochemistry and electrocatalytic activity of catalase for nitrite reduction [14], direct voltammetry and electrocatalytic properties of immobilized hemoglobin for hydrogen peroxide reduction [15], direct voltammetry and electrocatalytic properties of immobilized hemoglobin for glucose oxidation [16], electrochemical behavior of immobilized myoglobin for electrocatalytic detection of hydrogen peroxide [17], oxidation of glucose at nickel(II) oxide/multi-walled carbon nanotube modified glassy carbon electrode (GCE) [18, 19], electroanalytical determination of aspirin on NiONPs supported on graphite [20], and electrochemical detection of amino acids at carbon nanotube and nickel-carbon nanotube modified electrodes [21].

In the past, research on the synthesis of nano-sized porous nickel oxide materials and their applications to catalytic reactions, industrial processes, and electrochromic devices have also been reported [22, 23]. Furthermore, due to the excellent electrocatalytic activity and good

antifouling properties of electrodes modified with nickel oxide, these modified electrodes have been used for electrocatalytic oxidation and determination of various analytes such as insulin, thiols, disulfides, mercaptans, and sulfur oxoanions [24–26]. To the best our knowledge, up to now there is no report on the application of NiONPs/GCE to the detection of Glycine (Gly), L-Serine, and L-Alanine. The main aim of this study is electroanalytical detection of these amino acids using the modified NiONPs/GCE.

2 Experimental

2.1 Reagents and solutions

$Ni(NO_3)_2{\cdot}6H_2O$ was purchased from Fluka and used without any further purification. Gly, L-Serine, L-Alanine, CH_3COONa, and other reagents were of analytical grade from Merck or Fluka Companies and used as received. Buffer solutions (0.10 M) were prepared using Na_3PO_4, Na_2HPO_4, NaH_2PO_4, NaOH, and HCl for the pH range of 1–13.

2.2 Apparatus

All electrochemical experiments were performed with a computer controlled μ-Autolab type III modular electrochemical system (Eco Chemie Ultecht, The Netherlands), driven with GPES software (Eco Chemie). A conventional three-electrode cell was used with an Ag/AgCl (sat KCl) reference electrode. A platinum wire counter electrode and a GCE modified with NiONPs (which prepared as follows) were employed for the electrochemical studies. A Metrohm drive shaft to rotate the working electrodes was used in amprometric detection. Furthermore, the atomic force microscopic (model nanosurf Mobile S software version 1.8) at operating mode of dynamic force and non contact scan type was used. The electrochemical measurements were carried out at a constant temperature of 25.0 ± 0.1 °C.

2.3 Preparation of nickel oxide modified glassy carbon electrode (NiONPs/GCE)

The electrode modification was carried out as reported previously [14]. The GCE (2 mm diameter) was carefully polished with alumina on polishing cloth. The electrode was then placed in ethanol and sonicated to remove adsorbed particles. The electrodeposition of metallic nickel was carried out using cyclic potential (40 scans between 0 and −0.8 V at a scan rate of 100 mV s^{-1}) in pH 4 maintained by an acetate buffer solution containing 2 mM of nickel nitrate. Then, the potential was repetitively cycled (40 scans) from 0 to 0.6 V at scan rate of 100 mV s^{-1} in fresh NaOH solution (0.10 M) for electrodissolution and passivation of a nickel oxide layer at a GCE [22, 23]. The effective area of the electrode modified with nickel oxide nanoscale islands was determined as 0.086 cm^2 from cyclic voltammograms of 1 mM $K_4[Fe(CN)_6]$ in buffer solution at pH 7. In order to characterize the structure of these nanoparticles and particularly the smaller ones, utilization of atomic force microscopy (AFM) was considered.

3 Results and discussion

3.1 Characterization of prepared nano-sized nickel oxide

Figure 1 depicts the AFM images of bare GCE and GCE modified with nickel oxide nanoparticles. Correspondingly, an extensive sample examination with the help of the AFM techniques presented the existence of different geometrical shapes of nickel oxide particles. These geometrical structures resulted in a better immobilization of proteins on their surfaces. Based on AFM utilization, the average diameter of the nickel oxide particles found to be about 20–50 nm.

Also, the formation of nickel oxide layer on the electrode surface was confirmed by recording cyclic voltammograms of the modified electrode in a 0.1 M fresh NaOH solution (not shown). The cyclic voltammogram exhibited a broad oxidation peak starting at 0.3 V versus reference electrode, which can be attributed to the dissolution of nickel and formation of nickel oxide. The anodic peak current increased up to the 35th cycle and then a current plateau and stable voltammetric response was observed. After nickel dissolution and oxide formation in alkaline solution, the observed anodic peak at 0.41 V is due to the oxidation of the $Ni(OH)_2$ phase to NiO(OH) while the corresponding cathodic peak at 0.35 V revealed the reduction of NiO(OH) to $Ni(OH)_2$, according to the following reaction [3]:

$$Ni(OH)_2 + OH^- \leftrightarrows NiO(OH) + H_2O + e$$

The effect of potential scan rate on the cyclic voltammograms was examined in the range of 10–100 mV s^{-1} (Fig. 2). As seen, the ratio of cathodic to anodic peak currents is about unity and the cathodic and anodic peak currents are directly proportional to the scan rate of potential. This is obviously indicative of a surface confined redox process corresponding to a rapid conversion of surface film without diffusion or kinetically controlled reaction step. In addition, the shift in peak potential is negligible at scan rates 10–100 mV s^{-1} (Fig. 2), suggesting a facile charge transfer kinetics over this range of scan rate. Meanwhile, at higher sweep rates, the peak potentials were found to be dependent on the scan rate, which is reflective of

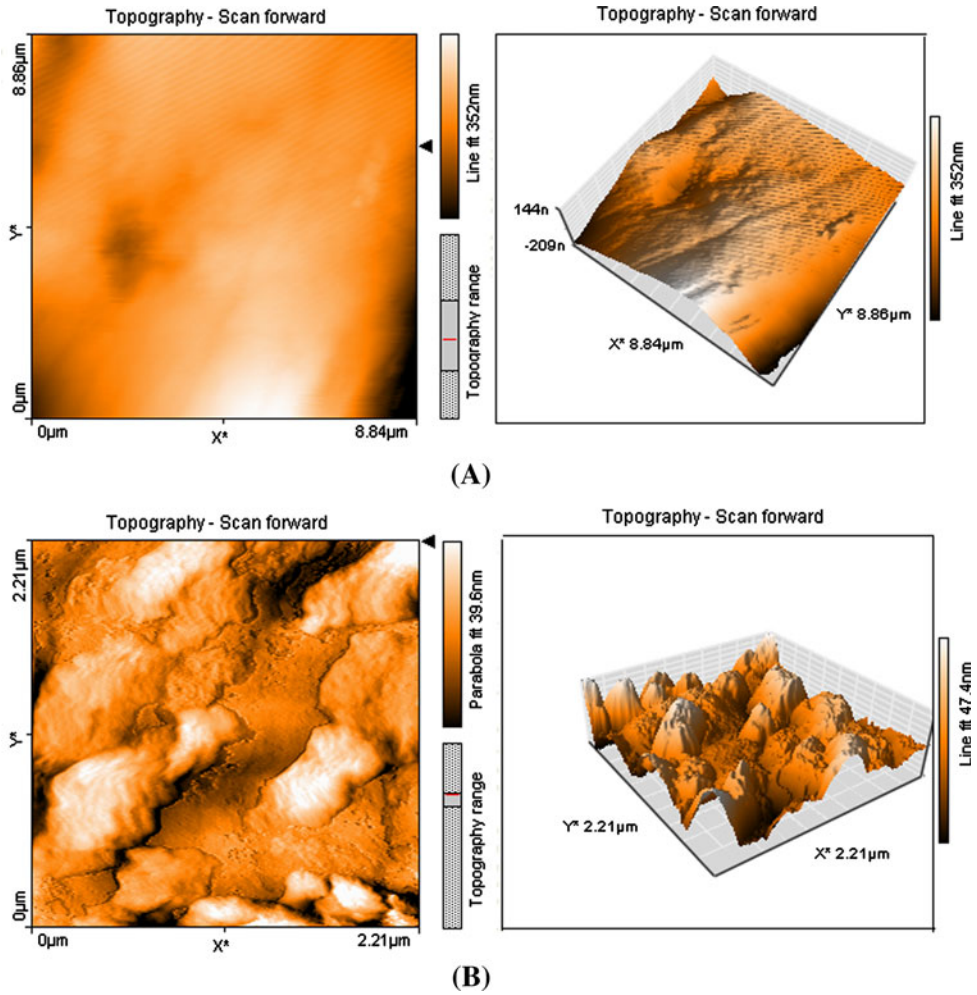

Fig. 1 AFM images for bare (**a**) and modified (**b**) GCE

the relatively slow diffusion of hydroxide ions into the limited wetting section of electrode surfaces.

3.2 Electrocatalytic oxidation of Gly, L-Serine, and L-Alanine at NiONPs/GCE

An objective of the present study was to fabricate a modified electrode capable of electrocatalytic oxidation of amino acids. Thus, to investigate the electrocatalytic activity of the modified electrodes, the cyclic voltammograms were obtained in the presence and the absence of the amino acids at both bare GCE and NiONPs/GCE (Figs. 3, 4, 5). Figure 3 compares the recorded typical CVs for the oxidation of L-Serine at the bare and modified GCEs. At the bare electrode, L-Serine is not electroactive at potential range of 0.0–1 V, suggesting a very slow electron transfer of L-Serine at bare GCE. The oxidation process commencing from 0.4 V was largely facilitated at NiONPs/GCE. Similar behaviors were observed for electro-oxidation of L-Alanine and Gly at the bare and modified GCEs (Figs. 4, 5).

3.3 Amperometric determination of simple amino acids by NiO nanoparticle modified GCE

As discussed in the previous section, NiONPs modified GCE reveals an excellent electrocatalytic behavior, which facilities the amperometric detection of the amino acids of interest at low potentials. Figures 6 and 7 show chronoamperograms for two different concentration of Gly, recorded by a rotating modified GCE with a rotation speed of 1,500 rpm, at a maintained potential of 0.4 V in 0.1 M NaOH solutions of pH 13. As is obvious, during the successive addition of 5 and 1,500 µM of Gly a well-defined response is observed. For each addition of Gly, within a response time of less than 1 s, a sharp rise in the current was observed. A corresponding plot of current versus Gly concentration is shown in the inset of Fig. 7. As seen, the measured currents were increased with increasing concentration of Gly in solution. The calibration plot for determination of Gly was linear in over a wide concentration range of 1–200 µM. Linear least square calibration curves over the range of 5–75 µM (by 12 determinations)

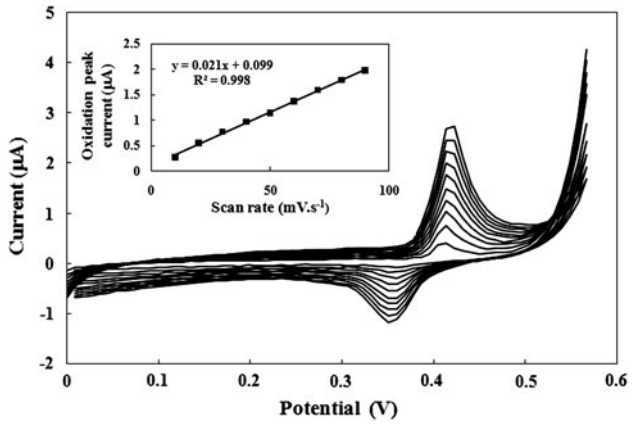

Fig. 2 Cyclic voltammograms of GCE modified by NiONPs in 0.1 M NaOH solution at different scan rates (from *inner* to *outer* 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mV s^{-1}). *Inset* shows a plot of peak currents versus scan rate

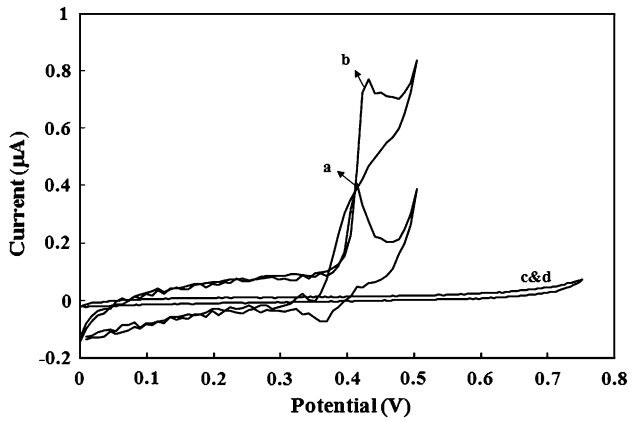

Fig. 3 Cyclic voltammograms in a pH 13 solution at scan rate of 10 mV s^{-1} for GCE modified by NiONPs in the absence (*a*) and the presence (*b*) of 0.5 mM L-Serine solution and for the bare GCE in the absence (*c*) and the presence (*d*) of 0.5 mM L-Serine solution

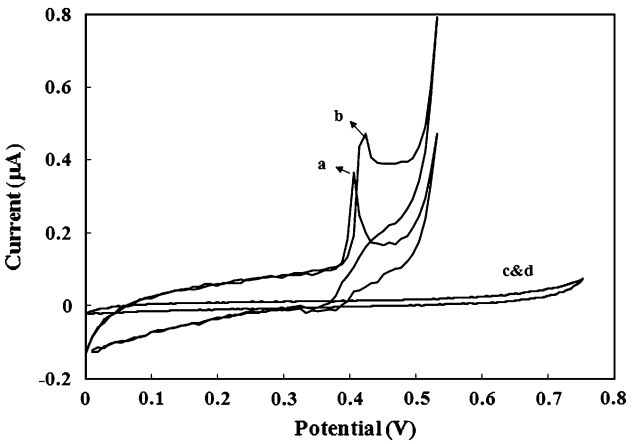

Fig. 4 Cyclic voltammograms in a pH 13 solution at scan rate of 10 mV s^{-1} for GCE modified by NiONPs in the absence (*a*) and the presence (*b*) of 1 mM L-Alanine solution and for the bare GCE in the absence (*c*) and the presence (*d*) of 1 mM L-Alanine solution

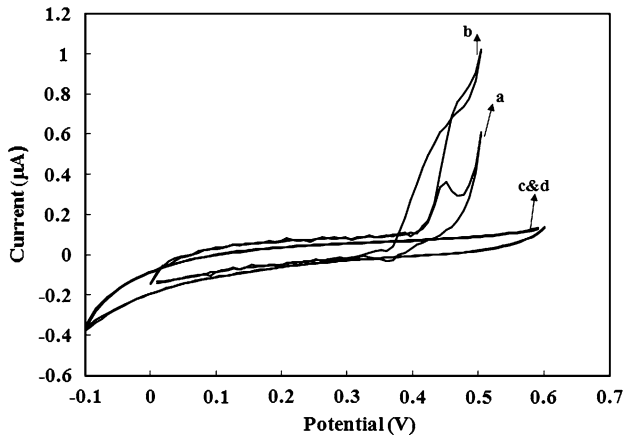

Fig. 5 Cyclic voltammograms in a pH 13 solution at scan rate of 10 mV s^{-1} for GCE modified by NiONPs in the absence (*a*) and the presence (*b*) of 1 mM Gly solution and for the bare GCE in the absence (*c*) and in the presence (*d*) of 1 mM Gly solution

possessed a slope of 24.3 nA μM^{-1} (sensitivity) and a correlation coefficient of 0.9989. The detection limit was 0.9 μM, at a signal to noise ratio of 3. The NiONPs modified GCE imparts higher stability for amperometric measurements of Gly. Figure 7b shows the amperometric response of a 22 μM solution of Gly, recorded over a continuous period of 19 min. The chronoamperograms, similar to those for Gly which are shown in Figs. 6 and 7 and taken under the same experimental conditions, are given in Figs. 8, 9, 10, 11 for L-Serine and L-Alanine. The respective linear concentration range, detection limit, and sensitivity for the determination of L-Serine and L-Alanine were 1–400 μM, 0.85 μM, 12.4 nA μM^{-1} and 30–200 μM, 29.67 μM, 0.4 nA μM^{-1}, respectively. Therefore, the modified NiONPs/GCE possesses excellent, stable, and strong mediation properties along with the facility for the low potential amperometric detection of simple amino acids.

3.4 Reproducibility and interferences

Figure 12 shows the steady-state responses of the modified electrode for the oxidation of Gly, L-Serine, and L-Alanine. The steady-state currents for the oxidation of these analytes were examined at a constant potential of electrode (0.42 V) in solutions of pH 13. For the first and the second injections, the current responses for the analytes were very similar, indicating the high reproducibility of the responses.

In order to investigate the selectivity of the modified NiONPs/GCE, several amino acids were tested in the amperometric determination of 5.0 μM Gly, 5.0 μM

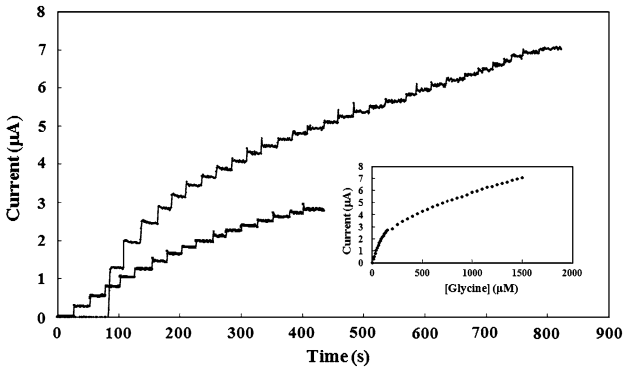

Fig. 6 Amperometric response for a rotating modified NiONPs/GCE at a rotation speed of 1,500 rpm and 0.42 V in a pH 13 solution for successive addition of 50 μM Gly. *Inset* shows the plot of chronoamperometric current response versus Gly concentration

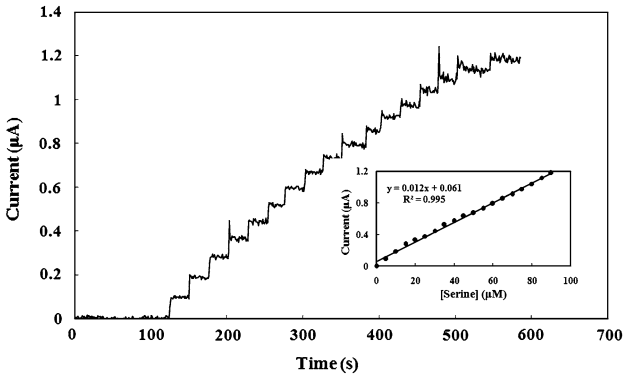

Fig. 9 Amperometric response for a rotating modified NiONPs/GCE at a rotation speed of 1,500 rpm and 0.42 V in a pH 13 solution for successive addition of 5 μM L-Serine. *Inset* shows a plot of chronoamperometric current response versus L-Serine concentration

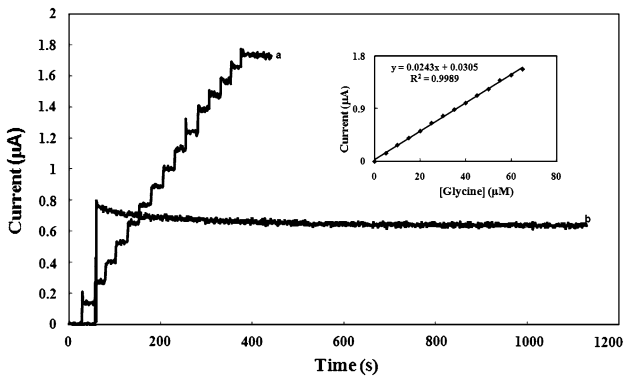

Fig. 7 Amperometric response for a rotating modified NiONPs/GCE at a rotation speed of 1500 rpm and 0.42 V in a pH 13 solution for successive addition of 5 μM Gly (*a*). *Inset* shows the plot of chronoamperometric current response versus Gly concentration. (*b*) is the recorded chronoamperogram for 22 μM Gly during a long time period of 1,140 s; other conditions were similar to (*a*)

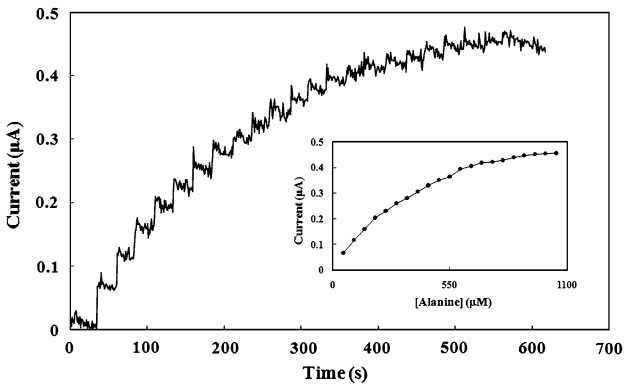

Fig. 10 Amperometric response of rotating modified NiONPs/GCE at a rotation speed of 1,500 rpm and 0.42 V in a pH 13 solution for successive addition of 50 μM L-Alanine. *Inset* shows a plot of chronoamperometric current response versus L-Alanine concentration

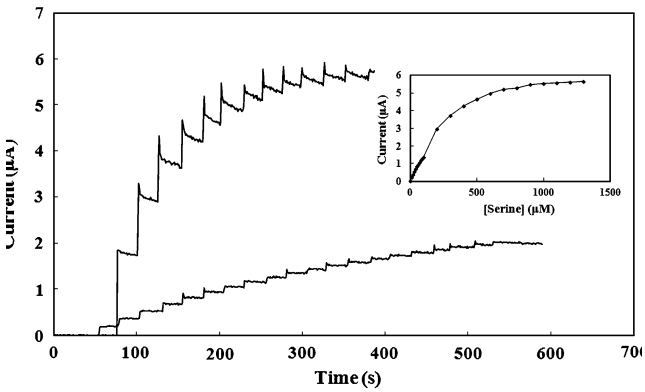

Fig. 8 Amperometric response for a rotating modified NiONPs/GCE at a rotation speed of 1,500 rpm and 0.42 V in a pH 13 solution for successive addition of 100 μM L-Serine. *Inset* shows a plot of chronoamperometric current response versus L-Serine concentration

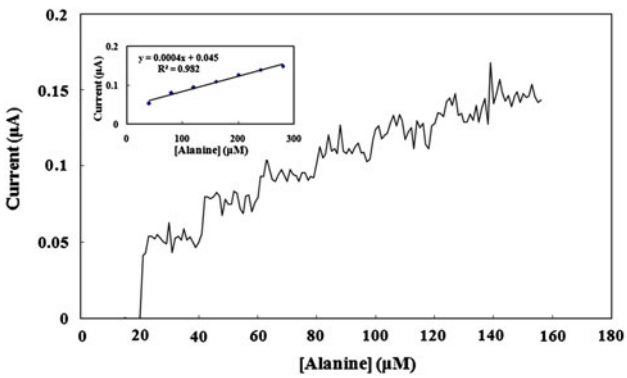

Fig. 11 Amperometric response of rotating modified NiONPs/GCE at a rotation speed of 1,500 rpm and 0.42 V in a pH 13 solution for successive addition of 40 μM L-Alanine. *Inset* shows a plot of chronoamperometric current response versus L-Alanine concentration

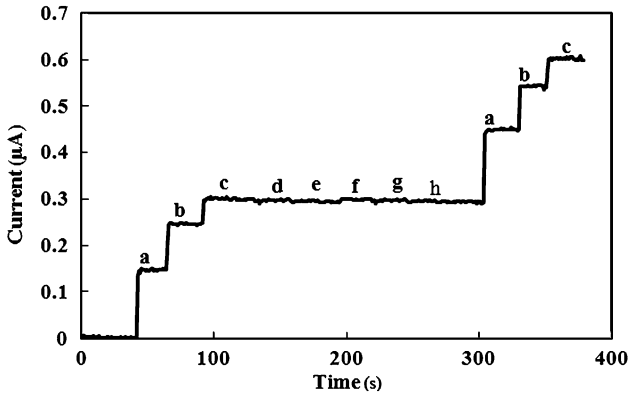

Fig. 12 Steady-state current responses corresponding to oxidation of Gly, L-Serine, and L-Alanine at NiONPs modified GCE at pH 13 upon the injection of 5.0 μM of Gly (*a*) and L-Serine (*b*), 40 μM of L-Alanine (*c*), 100 μM Threonine (*d*), 200 μM Asparagine (*e*), 200 μM Histidine (*f*), 200 μM Glutamine (*g*), and 200 μM of Proline (*h*); applied potential was 0.42 V and other conditions were similar to Fig. 10

L-Serine, and 40.0 μM L-Alanine. As could be seen from Fig. 12, the presence of 100 μM Threonine, 200 μM Asparagine, 200 μM Histidine, 200 μM Glutamine, and 200 μM of Proline resulted in low relative errors of 1.2, 1.2, 1.9, 1.8, and 1.3 %, respectively. These results confirm the acceptable selectivity of the proposed modified electrode.

3.5 Individual determination of Gly, L-Serine, and L-Alanine

Determination of mutual interferences is very important for the individual measurement of Gly, L-Serine, and L-Alanine. The NiONPs/GCE showed that L-Serine and L-Alanine had no interference effect on the oxidation signals of Gly. Also, Gly and L-Alanine revealed no interference on the oxidation signals of L-Serine (Figs. 12, 13). These findings confirm that there is no mutual interference between Gly, L-Serine, and L-Alanine during their measurement by the NiONPs/GCE.

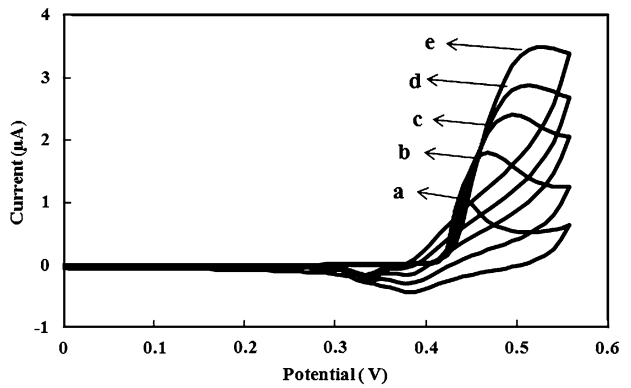

Fig. 13 Cyclic voltammograms for modified NiONPs/GCE at pH 13.0 and scan rate of 30 mV s^{-1} for stepwise addition of 0.0 (*a*), a mixture 5.0 mM of L-Serine and L-Alanine (*b*), 0.50 mM Gly (*c*), 1.0 mM Gly (*d*), and 1.5 mM Gly (*e*)

4 Conclusion

GCE modified by NiO nanoparticles was successfully applied to the sensitive and stable constant-potential amperometric detection of amino acids Gly, L-Alanine, and L-Serine. The detection process was found to vary in sensitivity depending on the nature of amino acid analytes. The modified electrode exhibited excellent sensitivity and stability for determination of amino acids based on increasing anodic peak current upon increasing concentrations of amino acids.

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