Yttria-Modified Titania-CNTs Composite as an Electrochemical Biosensor for Lysine

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Summary: A composite of carbon nanotubes with yttrium modified titania particles (Y/TiO₂-CNT) is synthesized and characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and Fourier transform infrared spectroscopy (FTIR). The electrochemical behavior of amino group (Lysine) is investigated by cyclic voltammetry (CV). The response mechanism of the synthesized biosensor is proposed to be the combination of electrostatic and electrical interactions of CNTs with amino group. Excellent sensitivity, repeatability, stability, selectivity and recovery for the potentiometric determination of Lysine in the range of 5mM to 25mM concentrations are obtained.

Keywords: Carbon nanotubes, Amino group, cyclic voltammetry, Sensors, Yttrium.

Introduction

The determination of amino acids is important in several applications, and often it is the determination of a single amino acid which is desired. Lysine is the most easily damaged of the essential amino acids and therefore, Lysine is a vital marker for the nutritional value of food. Lysine is often added as a dietary supplement to food and drugs in order to correct possible deficiencies of this amino acid in humans [1, 2]. Amino acid biosensing systems with a wide range of detection mechanisms have already been developed [3-5]. The accurate and rapid determination of Lysine is of great interest owing to its importance in human alimentation.

Carbon nanotubes (CNTs) possess interesting electrochemical properties. Several works have established that the electroactivity of CNTs is due to the presence of reactive groups on the surface [6-8]. In general, lower over voltages and higher peak currents are observed in the voltammetric response of several molecules at electrodes modified with CNTs. Due to these unique properties, CNTs have received enormous attention for the preparation of electrochemical sensors, as it was largely reviewed [9-11]. For the electrochemical biosensor applications, the electrode modifying material is expected to possess several characteristics such as good electron transduction capability, physical or chemical environment for the stable immobilization of enzyme, bioactivity, easy accessibility towards the analyte and large surface area. Literature reveals that all these important characteristics cannot be inbuilt in a single material. Hence, there is always a demand for the development of composite materials, comprising two or more components, to achieve adequate sensitivity and stability for the biosensors [12-14].

One of the problems for the preparation of sensors based on the use of CNTs is their insolubility in usual solvents. Therefore, CNTs based composites have continuously been investigated for sensors and biosensor application [15-17]. Recently, CNTs and TiO₂ composite materials have attracted considerable interest in several fields, [17-19] especially biosensors [20-22]. Combining the superior characteristics of TiO₂ and CNTs, the TiO₂-CNT composite materials are expected to construct a good electrochemical sensor for sensitive determination of Lysine. When the target analyte binds to the receptor molecule there is a persuade change in the electrical conduction of the CNTs, thus providing the mechanism for detecting and transducing the electrical signal. Our specific objectives are therefore to functionalize the CNTs with several types of molecular receptor to achieve sensors that display a high selectivity towards the analyte of interest.

In this study we have developed a new TiO₂-CNT composite material by hydrothermal method. High response current owing to Y-modification in TiO₂-CNT composite for selective and rapid Lysine determination makes this material a valuable electrochemical biosensor, with simple instrumentation, low production cost and fast response. The performance of the sensor, such as linear response
range, detection limit, stability and reproducibility, are evaluated and discussed in detail.

Results and Discussion

Fig. 1 shows the XRD pattern of TiO$_2$ particles, TiO$_2$-CNT composite and Y/TiO$_2$-CNT composite. The average crystallite size of the samples TiO$_2$, TiO$_2$-CNT and Y/TiO$_2$-CNT as calculated by the Scherer’s equation [23] are 17nm, 12nm and 14nm respectively. The reduction in crystallite size can be attributed to relatively low crystallization, due to the high density of oxygen functional groups, including carboxylic and hydroxyl groups, on CNT surface to hinder diffusion, crystallization and growth. The peaks at 25.3°, 37.8°, 48.0° and 62.5° (2θ) for the diffraction angles of (101), (004), (200) and (204) planes of anatase TiO$_2$ broaden with Y-modification in TiO$_2$-CNT, as crystallite size decreases. A new reflection at 29.2° (2θ) emerge and indicate characteristic (222) diffraction of Y$_2$O$_3$ particles along with some additional Y$_2$O$_3$ diffractions (400), (411), (134) and (440) at 33.8°, 35.9°, 43.8° and 48.7° (2θ), respectively.

Further, some of the diffraction peaks attributed to TiO$_2$, Y$_2$O$_3$ and those of CNTs support are also overlapped. Although the most intense peaks of CNT corresponding to (002) reflection overlaps the anatase (101) reflection, Y-modified TiO$_2$ still present peaks of anatase reflection in their diffraction patterns without any extra phase, which means Yttrium is well dispersed on TiO$_2$. The other intense peak due to (100) reflection between 40° and 45° (2θ), where no peak can be attributed to TiO$_2$ is assigned to CNTs.

Fig. 2 shows the morphology of TiO$_2$ particles, TiO$_2$-CNT composite and Y/TiO$_2$-CNT composite. As shown, TiO$_2$-CNT and Y/TiO$_2$-CNT composite with clearly observable CNTs are significantly different from regular cubic TiO$_2$. The particle size has reduced for composites in comparison to pure TiO$_2$, which is anticipated to be crucial for contact between TiO$_2$ and CNTs and facilitate the electron transfer by forming conductive network. Y-modification has an eminent effect on higher dispersion of CNTs and in turn improved conductivity for Y/TiO$_2$-CNT composite. In the report of Zhang, [24] a good dispersion of particles can provide more reactive sites than aggregated particles and the conductivity of CNTs network can enhance the electron transfer rate.

Fig. 3 shows the FTIR spectra of TiO$_2$ particles, TiO$_2$-CNT composite and Y/TiO$_2$-CNT composite. The band due to stretching and bending modes of Ti-O and O-Ti-O appeared below 850 cm$^{-1}$ which indicates the formation of titania [25]. However, the band becomes broader for composites as compared to pure TiO$_2$. The broadening is probably due to the inclusion of CNTs. For TiO$_2$-
CNT composite, as shown in Fig. 3b, a broad band peak centered at 3406 cm\(^{-1}\) is attributed to the OH stretching of physisorbed water on the composites surface and OH groups resulting from the HNO\(_3\) oxidation. A relatively sharp band at 1630 cm\(^{-1}\) corresponds to the OH bending modes of water molecules [26]. From these, the FTIR spectra strongly indicate the presence of abundant hydroxyl groups on composites surface, which plays an important role in the Lysine detection. The peak at 1382 cm\(^{-1}\) could be assigned to the stretching vibrations of –CH\(_3\) groups [27].

Electrochemical Detection of Lysine

The electrochemical detection of Lysine at the TiO\(_2\), TiO\(_2\)-CNT and Y/TiO\(_2\)-CNT electrode was investigated by CV. Fig. 4 shows the CV plots of 10mM Lysine concentration at various electrodes in 0.1M Phosphate buffer solution (PBS) (pH 7.0). No oxidation/reduction peak is observed for TiO\(_2\) electrode, indicating its inadequacy for Lysine detection. For Y/TiO\(_2\)-CNT electrode two oxidation peaks with an isolated reduction peak at 0.035V, 0.356V and -0.19V are observed respectively. While for TiO\(_2\)-CNT electrode small oxidation peak current at relatively low potential of 0.298V is observed. The appearance of oxidation peak at 0.298V for TiO\(_2\)-CNT electrode and 0.356V for Y/TiO\(_2\)-CNT electrode indicate conversion of carboxyl groups at the end of the shortened CNTs into active Schiff base, while oxidation/reduction peak for Y/TiO\(_2\)-CNT electrode at 0.035V/-0.19V may be due to surface active OH groups promoted by Y-modification.

From the EDX spectra of TiO\(_2\)-CNT composite and Y/TiO\(_2\)-CNT composite, the elements C, O, Ti and Y are confirmed and their weight percentages are listed in Table-1, which are according to our synthesis procedure. Y could only be found in Y/TiO\(_2\)-CNT composite representing its successful association in the composite.

Table-1: EDX elemental microanalysis of TiO\(_2\)-CNT and Y/TiO\(_2\)-CNT composites.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Ti</th>
<th>C</th>
<th>O</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO(_2)-CNT</td>
<td>53.3</td>
<td>18.3</td>
<td>26.4</td>
<td>-</td>
</tr>
<tr>
<td>Y/TiO(_2)-CNT</td>
<td>42.2</td>
<td>17.3</td>
<td>33.5</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Fig. 4: Cyclic voltammograms of 10mM Lysine detection on the (a) TiO\(_2\) Electrode (b) TiO\(_2\)-CNT Electrode (c) Y/TiO\(_2\)-CNT electrode.
The high oxidation peak current of Lysine due to Y-modification is proposed to be due to (i) increased electronic conductivity of CNTs with high electron concentration caused by Y-modification; (ii) the particle size of TiO\textsubscript{2} in Y/TiO\textsubscript{2}-CNT is much smaller than that in pure TiO\textsubscript{2} as evident from SEM and stabilizing effect of Y and CNTs on the anatase phase and crystallite dimensions of TiO\textsubscript{2}.

**Fig. 5:** Cyclic voltammograms of Lysine detection on the Y/TiO\textsubscript{2}-CNT electrode [the Lysine conc. 0, 5, 10, 15, 25mM]

**Electrochemical Detection of Lysine on Y/TiO\textsubscript{2}-CNTs Electrode**

pH 7.0 was selected as the optimal pH value for Lysine detection since maximum detection response was observed at this pH, after comparing the effect of pH for various experiments.

As shown in Fig. 5, cyclic voltammograms of Y/TiO\textsubscript{2}-CNT electrode was investigated at different Lysine concentrations (0mM, 5mM, 10mM, 15mM, and 25mM). The results clearly indicate that peak currents are function of Lysine concentration. For the sake of comparison the linear response curves and the percent increase in peak currents on Y/TiO\textsubscript{2}-CNT electrode are also shown in Fig. 6a and b. From the results it is clear that Y-modification and CNTs incorporation has increased sensitivity and detection capability even at very small Lysine concentration.

We propose that molecular interactions with the plane of CNTs via π-stacking through the Lysine with the defects of CNTs. The geometry created during this interaction is highly reactive to nucleophilic substitution by primary and secondary group of Lysine and change in the stochiometry results in the detection of Lysine on Y-modified CNTs.

**Analytical Characteristics of the Electrode**

The peak current and peak potential for different concentrations of Lysine on Y/TiO\textsubscript{2}-CNTs electrode are summarized in Table-2. It may be noted that the Y/TiO\textsubscript{2}-CNTs electrode has highest sensitivity as the concentration of Lysine is high. The stability of the electrode was evaluated by measuring the current response at 10mM Lysine concentration for ten days. It was found that only 0.5% of the response signal of the electrode was lost in ten days. The reproducibility of the Y/TiO\textsubscript{2}-CNTs electrode was investigated by analyzing ten samples at a fixed concentration of 10mM of Lysine. A relative standard deviation of 1.13% was obtained. These
results prove that Y/TiO$_2$-CNT electrode possesses excellent reproducibility for Lysine detection and are comparable to other reported materials for Lysine detection [3-5, 29-31].

Table-2: Peak current and peak potential for different concentrations of Lysine on Y/TiO$_2$-CNT electrode.

<table>
<thead>
<tr>
<th>Lysine Conc.</th>
<th>Ip (i) (µA)</th>
<th>Ip (ii) (µA)</th>
<th>Ip (iii) (µA)</th>
<th>Ep (i) (V)</th>
<th>Ep (ii) (V)</th>
<th>Ep (iii) (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5mM</td>
<td>2.95</td>
<td>5.43</td>
<td>-4.43</td>
<td>0.038</td>
<td>0.362</td>
<td>-0.21</td>
</tr>
<tr>
<td>10mM</td>
<td>3.17</td>
<td>5.65</td>
<td>-4.60</td>
<td>0.035</td>
<td>0.356</td>
<td>-0.19</td>
</tr>
<tr>
<td>15mM</td>
<td>3.39</td>
<td>5.73</td>
<td>-4.65</td>
<td>0.034</td>
<td>0.349</td>
<td>-0.18</td>
</tr>
<tr>
<td>25mM</td>
<td>3.55</td>
<td>5.74</td>
<td>-4.74</td>
<td>0.026</td>
<td>0.341</td>
<td>-0.17</td>
</tr>
</tbody>
</table>

Furthermore, the selectivity of Y/TiO$_2$-CNTs electrode was determined by the current response of Lysine in the presence of other amino acids. For all other amino acids no demonstrable response was observed. The results suggest that the Y/TiO$_2$-CNTs electrode has good selectivity for Lysine detection.

From the voltammograms electrochemical informations can be obtained. Factually whether Lysine is present or not, the peak currents without Lysine addition are linear. This indicates that on addition of Lysine in different concentration in to the nano composite the process is controlled by the adsorption. Moreover in the case of absence of Lysine, the reduction potential peaks are proportional to the natural logarithim for scan rates greater than 1 V s$^{-1}$. According to the formula proposed by Laviron [32].

$$E_p = E^0 + \frac{RT}{nF} \ln \frac{RTK_s}{anF} - \frac{RT}{anF} \ln v$$

The estimated number of electrons transferred during the reaction and the standard rate constant of the surface reaction are 20 and 45 s$^{-1}$ respectively. This is an indication the electrochemical reaction occurred during the process.

On the basis of our discussions we suggest a mechanism (Fig. 7) which possibly could generate Lysine detection during the electrochemical reaction. The presence of Ti$^{3+}$ sites might be related to the longer recovery time of TiO$_2$-CNT composite, given the large tendency of Ti$^{2+}$ sites to trap holes. This is in accordance with previous XPS studies of TiO$_2$ based composites where the abundance of Ti$^{3+}$ and Ti$^{2+}$ was observed in composites annealed below 600 $^\circ$C [33]. Regarding the sensing mechanism, the type of weak or strong detection response is connected with the amount of charge transferred, the stability of the species before and after adsorption, and the presence of adsorption/desorption barriers. In the mechanism proposed, the sizes of the arrows in Fig. 7 illustrate the magnitude of charge transfer. Our hypothesis is that the abundance Ti$^{3+}$ is indicative of the strong interaction between CNTs and titania, causing a larger charge transfer process by the coordinating Lysine with Ti$^{3+}$ during which it is oxidized to Ti$^{4+}$ and signal is detected on CV curve. While a much better sensing due to Y-modification is proposed due to synergistic effect of Ti and Y undergoing simultaneous charge transfer process.

Fig. 7: Schematic of the sensing mechanism proposed for TiO$_2$-CNT electrode and Y/TiO$_2$-CNT electrode while the arrow size represents the amount of charge transferred.

**Experimental**

Homemade CNTs (US patent 2009208403) were used. Lysine (≥98%), Yttrium acetate (99.9%), Titanium tetrachloride (99.9%) were purchased from Sigma-Aldrich and stored at 4 $^\circ$C. All other chemicals were of analytical grade and used without further purification. The supporting electrolyte was 0.1M PBS (pH 7.0) in distilled water.

Morphology, structure, elemental composition and functional group assessment of the samples were carried out by scanning electron microscopy (SEM, JEOL 5910/Japan), X-ray diffraction with Cu Kα (1.50598Å) radiation (XRD Barkke, D8/Germany), energy dispersive X-ray spectroscopy (INCA 200/Oxford) and Fourier transform-infrared spectroscopy (Thermo Nicolet 6700/USA) respectively. Cyclic voltammograms were recorded on Sp-150 biologic software package GPES 4.9. Three electrode cell with modified GC working electrode (3.0mm diameter), a reference Ag/AgCl electrode and Platinum wire as counter electrode were used.

**Preparation of Y/TiO$_2$-CNTs**

In a typical hydrothermal synthesis of Y/TiO$_2$-CNT composite, 0.34g TiCl$_4$ was dissolved
in H$_2$O, in which 1.3mL concentrated H$_2$SO$_4$ was added in. After functionalizing by dispersing in HNO$_3$ (3M), at room temperature in an ultrasound bath for 2 h [34], CNTs were dispersed in the above solution by ultrasonication for 0.5 h and stirred for another 0.5 h. 0.08g Y(CH$_3$COO)$_3$.4H$_2$O was then added to the mixture, and transferred into autoclave, sealed, and maintained at 100°C for 5 h. The resulting black solid products were separated by centrifugation, washed with distilled water (Fig. 8), and dried in vacuum at 60°C for 6 h.

Fig. 8: Dispersion of Y/TiO$_2$-functionalized CNTs.

For comparative study, pure TiO$_2$ and TiO$_2$-CNTs samples were also prepared by the above procedure.

Modification of Electrode and Analytical Procedure

The bare GC electrode with a tip surface of 3mm in diameter was polished and rinsed thoroughly with distilled water. Using 10µl Nafion as a binder TiO$_2$ based sample (2.0mg) was placed to the tip of the GC electrode and dried in air. Different concentrations of Lysine (5mM, 10mM, 15mM, 25mM) were added to 5.0mL of 0.1M PBS (pH 7.0) in Argon atmosphere with stirring. Cyclic voltammograms in the range of -0.6 - +0.7V with oxidation, reduction peak currents were recorded to detect Lysine.

Conclusion

In this study, we presented the fabrication and characterization of TiO$_2$ particles, TiO$_2$-CNT composite and Y/TiO$_2$-CNT composite and their electrochemical response towards Lysine detection. The SEM micrographs of TiO$_2$-CNT and Y/TiO$_2$-CNT composite showed that TiO$_2$ particles were distributed uniformly with small regions of aggregates in the CNTs network while Y-particles were fixed on the surface. From the EDX data, the main elements such as C, O, Ti and Y existed. FTIR measurements confirmed that nitric acid led to functional groups such as carbonyl, carboxyl and hydroxyl on CNTs. CNTs were covered with an anatase TiO$_2$ thin layer and Y-coating of 12nm and 14nm in size respectively. The composites were rich in surface hydroxyl groups. An electrochemical sensor for Lysine detection had been developed based on these composites. The results demonstrated that the Lysine detection could be attributed to synergic effects of TiO$_2$, the electron assistance of the CNTs network, the enhancement of Y and a function of the applied potential.

References