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Sensors for 5-hydroxytryptamine and 5-hydroxyindole acetic acid based on nanomaterial modified electrodes

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ABSTRACT

Simultaneous voltammetric determination of serotonin and 5-hydroxyindole acetic acid has been described at single walled carbon nanotube modified glassy carbon electrode and gold nanoparticles modified indium tin oxide electrode. The method described is fast, simple, accurate with detection limits as low as 32 nM for 5-hydroxytryptamine (5-HT) at single-walled carbon nanotube modified glassy carbon electrode and 27 nM for 5-hydroxyindole acetic acid (5-HIAA) at gold nanoparticles modified indium tin oxide electrode. Linear variation of peak current with change in concentration was observed in the concentration range 0.1–100 μM for 5-HT and 5-HIAA. Application of the method for non-invasive determination of the compounds in urine samples has also been described. The inter- and intra-day stability of the electrodes were also determined and electrodes were found to be sufficiently stable for 1 week.

1. Introduction

Serotonin (5-hydroxytryptamine, or 5-HT) is a well-known neurotransmitter which primarily functions as chemical messenger between nerve cells. It is believed to play an important role in wide variety of activities such as inhibition of aggression, appetite, cardiovascular function, muscle contraction, endocrine regulation and depression [1–3]. 5-Hydroxyindoleacetic acid (5-HIAA) is a product of 5-HT breakdown and is excreted in the urine. 5-HT and 5-HIAA together have a regulatory action on the homeostatic state of human body [2]. Sudden infant death syndrome (SIDS) is claimed to be associated with abnormal serotonergic neurons [4,5]. Extremely high levels of 5-HT have toxic and potentially fatal effects known as serotonin syndrome [6]. Phenomenon of natural ageing has been found to exhibit profound effect on 5HIAA/5-HT ratio in human body [7]. Certain tumorous cells have been found to produce excess 5-HT (I) and 5-HIAA (II) and their altered urinary level may act as a marker of the starting stage of these tumors [8]. Hence, simultaneous determination of 5-HT (I) and 5-HIAA (II) is considered of vital importance as their altered level indicates disturbance in human physiology. In addition, some chemicals included in prohibited list of WADA have also been reported to disturb 5-HT and HIAA level [9–11]. Hence, athletes showing altered level of these two compounds should be monitored for doping, which further strengthens the need of simultaneous determination of these compounds.

Several chromatography-based techniques have been applied from time to time for simultaneous determination of 5-HT (I) and 5-HIAA (II). Yoshitake et al. described liquid chromatography coupled with fluorescence detection [12] while HPLC coupled with electrochemical detection was demonstrated by several research groups [13–16]. Takeda et al. [17] determined the biologically important compounds by coulometric HPLC method. However, chromatography-based techniques require costly instrument and solvents along with complex time consuming pretreatment process. The importance of 5-HT/5-HIAA level in day-to-day related matters requires a simple cost effective method for rapid monitoring of the two compounds. Electroanalytical methods meet the above requirements but normally have low sensitivity at conventional electrodes. However, low sensitivity and poor selectivity of electrochemical methods can be overcome by electrode modification. In past few years, nanogold and fullerene modified electrodes have been used by our research group for simple, fast and sensitive voltammetric determination of various compounds of physiological importance [18–20] and an enhanced sensitivity has been observed with shift of peak potential to less positive potentials.

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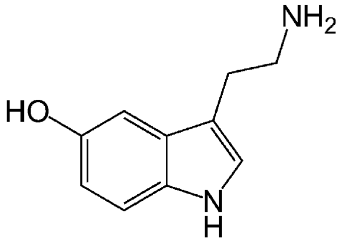
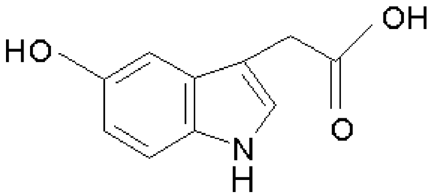


(I)



(II)

In this paper the response of single walled nanotube modified glassy carbon electrode (NTGC) and gold nanoparticles modified indium tin oxide (NGITO) electrode is compared for simultaneous determination of 5-HT and 5-HIAA. The effect of common interferents such as dopamine, uric acid and ascorbic acid on determination has also been evaluated.

2. Experimental

2.1. Reagents

5-HT and 5-HIAA were purchased from Sisco Research Laboratory, Mumbai (India). Single walled carbon nanotubes of purity >95% were obtained from Bucky, USA. Indium tin oxide (ITO) spurted glass sheets of size 10 mm × 20 mm × 2 mm and resistivity $50\,\Omega\,\mathrm{cm}^{-2}$ were obtained from CBC Optics Co. Ltd., Japan. Cetyltrimethylammonium bromide (CTAB), $HAuCl_4$ and $NaBH_4$ were purchased from Aldrich (USA). Other solvents and chemicals used were of analytical grade. All the chemicals were used as received. Double distilled water was used for making stock solutions.

2.2. Apparatus and procedure

BAS (Bioanalytical Systems, West Lafayette, USA) CV-50W voltammetric analyzer was used for electrochemical measurements. Glassy carbon electrode (area $\sim 2.0\,\mathrm{mm}^2$) was obtained from BAS, USA. Conventional single compartment cell having three electrodes was used. Platinum wire electrode acts as counter, Ag/AgCl (3 M NaCl) as reference electrode (Model BAS MF-2052 RB-5B) and NGITO (geometric area $\sim 0.0314\,\mathrm{cm}^2$) or NTGC as working electrode. JEOL JSM-7400 F field emission scanning electron microscopy instrument was used to characterize the growth of gold nanoparticles on the ITO surface. The Osteryoung squarewave voltammetric (OSWV) experiments were carried out at room temperature (27 ± 2 °C). Phosphate buffers in the pH range 3.35–9.96 (ionic strength, $\mu = 0.1$ M) were prepared according to the method reported by Christian and Purdy [21].

2.3. Fabrication of the modified electrodes

2.3.1. Single walled nanotube modified glassy carbon electrode

Glassy carbon electrode surface was cleaned to a mirror like finish by gently rubbing it with zinc oxide (Aldrich) on polishing pads (BAS). The electrode surface was then polished with alumina on polishing pads. The electrode was then dipped in 1 M H_3PO_4 to remove any sticked matter, followed by washing several times with double distilled water. Single walled nanotubes were dispersed in dimethylformamide (5.0 mg/10.0 mL) and different volumes of SWNT/DMF suspension were casted on electrode surface. The peak current of analyte was found to increase up to 80 μL of the suspension and then became constant. Hence, 80 μL of the suspension was coated onto the surface of the clean glassy carbon electrode using a microsyringe and dried in a stream of hot air (45 °C). The modified electrode has a uniformly covered surface with single walled carbon nanotubes as confirmed by FE-SEM (Fig. 1A) and the electrode was ready for experimental use.

2.3.2. Gold nanoparticles modified ITO electrode

Modification of ITO surface with gold nanoparticles was carried out in stepwise manner. In the first step, a sheet of ITO is washed with water, acetone, ethanol and then again with distilled water. The dried ITO substrate was then immersed in a seed solution (mixture of 0.5 mL of 10 mM $HAuCl_4$, 2 mL of 10 mM trisodiumcitrate and 0.5 mL of 0.1 mM $NaBH_4$ solution and 18 mL of distilled water). After the seeding procedure, the ITO sheet was thoroughly rinsed with distilled water and dried with nitrogen. The ITO substrate was then put into a growth solution (mixture of cetyltrimethylammonium bromide (CTAB), $HAuCl_4$, ascorbic acid and NaOH solution) for further crystal growth of nanoparticles [22]. After 24 h, ITO substrate was taken out, flushed with distilled water and then dried with nitrogen. The ITO sheet was then connected to a copper strip and molded between two strips of scotch tape. A hole of 1 mm dia was made on one side of tape for contact with the solution. The electrode was then ready for use. Field emission scanning electron micrographs showed that spherical nanogold particles (diameter 50–60 nm) were evenly dispersed on the surface of ITO (Fig. 1B). The prepared electrode was stored in air under dry conditions.

3. Results and discussion

3.1. Voltammetric behaviour of 5-hydroxytryptamine (serotonin) at modified electrodes

Serotonin exhibits a well-defined but slightly broad electrooxidation peak at ~400 mV at bare glassy carbon electrode while at NTGC; 5-HT shows a sharp oxidation peak at ~348 mV in square wave voltammetry. Similarly a large negative shift was observed for oxidation potential of 5-HT at NGITO (410 mV) in comparison to its oxidation potential at bare ITO electrode (650 mV). Also a significant increase has been observed for the oxidation peak currents of 5-HT at modified electrodes in comparison to bare electrode. Such electrocatalytic behavior improves sensitivity of the electrochemical method and lowers the detection limit of analyte. Fig. 2(A) compares voltammogram recorded at bare glassy carbon electrode and NTGC, while Fig. 2(B) compares voltammetric response of bare ITO and NGITO, for 25 μM 5-HT solution at pH 7.2.

The Osteryoung squarewave voltammograms of 5-HT were also recorded in phosphate buffer solutions of different pH using modified electrodes. It was observed that oxidation potential of 5-HT at both the modified electrodes shifts to less positive potential with increase in pH. The dependence of E_p on pH was linear. Eqs. (1) and (2) show dependence of peak potential of 5-HT on pH at NTGC and NGITO electrodes, respectively.

$$E_\mathrm{p}(\mathrm{mV}\ \textit{versus}\ \mathrm{Ag/AgCl}) = -59.21\,\mathrm{pH} + 782.8 \qquad (R^2 = 0.9830) \tag{1}$$