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PAPER

Sensitive determination of dopamine in the presence of uric acid and ascorbic acid using TiO₂ nanotubes modified with Pd, Pt and Au nanoparticles

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A simple modified TiO₂ nanotubes electrode was fabricated by electrodeposition of Pd, Pt and Au nanoparticles. The TiO₂ nanotubes electrode was prepared using the anodizing method, followed by modifying Pd nanoparticles onto the tubes surface, offering a uniform conductive surface for electrodeposition of Pt and Au. The performance of the modified electrode was characterized by cyclic voltammetry and differential pulse voltammetry methods. The Au/Pt/Pd/TiO₂ NTs modified electrode represented a high sensitivity towards individual detection of dopamine as well as simultaneous detection of dopamine and uric acid using 0.1 M phosphate buffer solution (pH 7.00) as the base solution. In both case, electro-oxidation peak currents of dopamine were linearly related to accumulated concentration over a wide concentration range of 5.0×10^{-8} to 3.0×10^{-5} M. However in the same range of dopamine concentration, the sensitivity had a significant loss at Pt/Pd/TiO₂ NTs electrode, suggesting the necessity for Au nanoparticles in modified electrode. The limit of the detection was determined as 3×10^{-8} M for dopamine at signal-to-noise ratio equal to 3. Furthermore, the Au/Pt/Pd/TiO₂ NTs modified electrode was able to distinguish the oxidation response of dopamine, uric acid and ascorbic acid in mixture solution of different acidity. It was shown that the modified electrode possessed a very good reproducibility and long-term stability. The method was also successfully applied for determination of DA in human urine samples with satisfactory results.

1. Introduction

Titanium dioxide (TiO₂) as a semiconductor metal oxide has attracted great attention in the past decade. Nanotubular structures of TiO₂ with high surface area have shown potential use in several applications such as biocompatible materials, photo-electrochemical solar cells, optical coating, photocatalysts and different type of sensors.^{1–3} These properties will be improved as the TiO₂ nanotubular structure is modified with modifiers such as metal nanoparticles and or carbon nanotubes. There are several reports describing the use of these architectures in electrochemical biosensors such as glucose biosensors.^{4,5}

Another important biomolecule which plays an important role in the function of central nervous, endocrine function, cognition and emotion systems is dopamine (DA).⁶ Dopamine is an

important neurotransmitter in the mammalian central nervous system for message transfer and exists at very low concentrations.^{7–9} Dopamine receptors belong to the superfamily of G protein-coupled receptors and play a crucial role in mediating the diverse effects of dopamine in the central nervous system.⁶ Any fluctuation in level of DA is very effective toward brain tissues, and the loss of DA-containing neurons may result in serious neurological disorders such as Parkinson's disease and addiction.^{10,11} The dopaminergic system is also the major target for antipsychotic medication in the treatment of schizophrenia.⁶

Electrochemical methods have always been a powerful tool for determination of DA at a low level in solution.¹² The major problem and difficulty in the determination of DA is due to the coexistence of ascorbic acid (AA) and uric acid (UA) with a similar oxidation potential and present in concentrations much higher than DA. At traditional electrodes, AA is oxidized at a potential close to that of DA, resulting in an overlapping voltammetric response.^{9,12,13} Considering all these conditions, a sensitive and accurate electrode for detection of DA in the presence of UA and AA is needed. Until now, it has been known that bare electrodes are far from sensitive enough for simultaneous detection of DA, UA and AA. Therefore there are several works which report using modified materials such as metal nanoparticles,^{14–16} carbon nanotubes,^{17,18} metal oxides,^{11,19–21} and

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polymers^{13,22} to improve the sensitivity, selectivity and stability of the bare electrode. Among them, metal nanoparticles such as Pt and Au modified electrodes have attracted much attention due to their high surface area, effective mass transport and catalytic activity. However, most of metal nanoparticles at the electrode surface are not conductive enough, so another strategy must be applied. In the following paper it is shown that Pd nanoparticles are able to uniformly deposit onto the surface of TiO₂ nanotubes, preparing a conductive and uniform surface for deposition of metal nanoparticles and which also improve the conductivity of the whole modified electrode. Therefore a mixed structure of catalytic and conductive metal nanoparticles will be more effective on the electrode surface. According to the literature, the most widely used electrodes are GCE (glassy carbon electrode)^{14,23} and metal electrodes such as Au and Pt¹⁹ which lack a large surface area. However, TiO₂ nanotubes electrodes possess an extra large surface area that prepares a suitable substrate for electrodeposition of metal nanoparticles. In order to enhance the conductivity of the TiO₂ nanotubes, Pd nanoparticles were deposited by a simple electrochemical pulse method. Then Pt and Au nanoparticles were electrodeposited from their single bath using the same pulse conditions. The final Au/Pt/Pd/TiO₂ NTs modified electrode was used for sensitive detection of DA alone, and in the presence of UA and AA using differential pulse voltammetry method. Compared to the other electrodes, this new electrode offers a very good selectivity of these three compounds without the use of any other modifiers.^{14,17,22,24} So it enables easier fabrication and is more stable after several times of use.

2. Experimental

2.1. Chemicals and reagents

Titanium foil (99.8% pure, 0.127mm thick) was purchased from Aldrich (Milwaukee, WI). Dopamine hydrochloride (purity > 99.0%), uric acid (purity > 99.0%), L-ascorbic acid (purity > 99.7%) were used as received without any further purification. Hexachloroplatinic (IV) acid, chloroauric (III) acid, and palladium chloride (II) of analytical reagent grade were purchased from commercial sources and used as supplied. Aqueous solutions were prepared with double redistilled water, and other chemicals were of analytic grade.

2.2. Equipment

A ZAHNER (IM6ex, Germany) working station was used for electrodeposition of metal nanoparticles. Electrochemical deposition was carried out in a three electrode configuration with a Pt sheet (Aldrich, 99.9% purity, 1 mm diameter) as counter electrode and SCE (saturated Calomel) as the reference electrode. Titanium sheet was used as the working electrode in whole experiment. Electrochemical characterization were carried out on a CHI 660D (CH Instruments Inc., Austin, TX) electrochemical working station with the same electrode configuration. The final topography of the electrode was studied using a scanning electron microscope (HITACHI S-4000, Japan). An energy dispersive X-ray (EDX) spectrometer fitted to the scanning electron microscope was used for elemental analysis.

2.3. Electrode preparation

Prior to anodization pure titanium foil was cut into 1 × 3 cm pieces and then cleaned in HF solution. The cleaned Ti sheets were immediately anodized in a solution containing 0.1 M NaF and 0.5M NaHSO₄ for almost 2 h. The anodizing cell was a two-electrode configuration with a Pt sheet as the cathode and the Ti sheet as the anode. After anodization, samples were rinsed several times with distilled water and dried in an air stream. The as-prepared TiO₂ nanotubes (TiO₂ NTs) were amorphous^{25,26} with pore diameter and average length of about 90 nm and 300 nm, respectively. Pd, Pt and Au were electrodeposited onto titania nanotubes using pulse electrodeposition method at -2 V for total duration of 1200 ms in a three-electrode configuration with TiO₂ NTs/Ti as the working electrode (1 cm² effective surface area), a Pt sheet as the counter electrode and a SCE (saturated Calomel electrode) as the reference electrode. Pd, Pt and Au nanoparticles were electrodeposited separately from single-bath solutions as reported in Table 1.

The electrocatalytic activity of the modified electrode in 1mM dopamine (DA) solution was determined by cyclic voltammetry (CV) with a potential sweep rate of 100mVs⁻¹. The supporting electrolyte in all electrochemical investigations was 0.1M pH 7 phosphate buffer solutions (PBS). The sensitivity of the electrode towards DA individually and in a solution containing uric acid (UA) was examined using differential pulse voltammetry (DPV) with pulse amplitude of 50 mV, pulse width of 0.2 s, scan rate of 20 mV s⁻¹ and accumulation time of 1 min without stirring. The oxidation current of each successive addition was measured in comparison to the DPV curve obtained in pure PBS. Solutions of DA, UA and ascorbic acid (AA) were prepared daily using double distilled water and then directly used for the detection. All the experiments were conducted at room temperature.

3. Results and discussion

3.1. Morphology of the Au/Pt/Pd/TiO₂ NTs modified electrode

Fig. 1A shows the microstructure of non-crystalline TiO₂ NTs modified with Pt nanoparticles. Obviously there is no evidence for uniform electrodeposition of Pt nanoparticles on the surface of as-prepared non-crystalline TiO₂ NTs. However, Fig. 1B shows the microstructure of non-crystalline TiO₂ nanotubes modified with Pd nanoparticle. These nanoparticles, with average diameter of 15 nm, have been uniformly deposited onto the TiO₂ NTs and form a chain-like structure. Comparing Fig. 1A and 1B, the metal loading is much more increased in case of Pd deposition (Fig. 1B). Because the TiO₂ nanotubes electrodes possess an extra large surface area with semi-conductive properties, it becomes a suitable substrate for the electrodeposition of metal nanoparticles.^{1,2} However, from Fig. 1A, one sees

Table 1 Composition of the electrolytes used for electrodeposition of nanoparticles

Steps	Electrolyte composition
Pd electrodeposition	2 mM PdCl ₂ + 0.1 M HCl
Pt electrodeposition	1 mM H ₂ PtCl ₆ + 0.5 M KCl
Au electrodeposition	1 mM HAuCl ₄

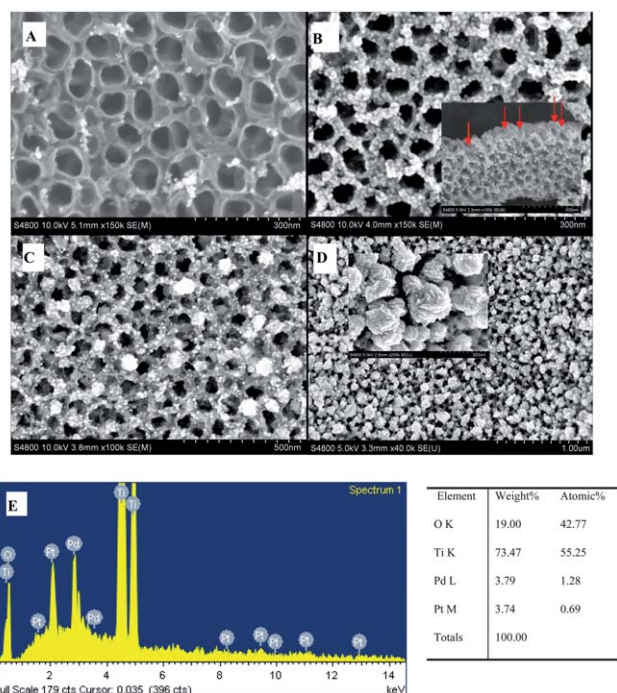


Fig. 1 SEM micrographs of (A) Pt/TiO₂ NTs, (B) Pd/TiO₂ NTs, (C) Pt/Pd/TiO₂ NTs, (D) Au/Pt/Pd/TiO₂ NTs electrodes under the same electrodeposition conditions with non-crystalline TiO₂ NTs, and (E) EDS spectrum of the Pt/Pd/TiO₂ NTs modified electrode. Inset of (B) shows the cross sectional image of Pd/TiO₂ NTs electrode.

that in non-crystalline TiO₂ there are not enough electroactive sites for nucleation of Pt to occur on the surface of the TiO₂ NTs, which results in the creation of small colonies of metal nanoparticles located far from each other. Meanwhile, the ability of Pd to deposit on the surface of amorphous silicon has been studied previously.²⁷ Consequently, it is expected that uniform deposition of Pd nanoparticles on the surface of amorphous TiO₂ NTs will take place without any need for a crystalline structure. In this case, the large surface area proposed by TiO₂ NTs substrate is uniformly covered with small and dispersed Pd nanoparticles, which then facilitate the electrodeposition of Pt nanoparticles. The inset of Fig. 1B confirms the presence of Pd nanoparticles inside the TiO₂ nanotubes. Uniform and deep deposition of the electroactive metal nanoparticles enhances conductivity due to the excellent electron transfer between electrode and adsorbents, which are also essential for high catalytic activity. Using non heat-treated TiO₂ NTs substrate; fabrication by this method is quick and efficient in the case of time and consuming of energy, respectively.

Fig. 1C shows the top view of Pd/TiO₂ NTs electrode after deposition of Pt nanoparticles. It seems that Pt nanoparticles have been deposited in the form of small colonies dispersed closely to each other over the surface. The corresponding energy dispersive X-ray spectroscopy (EDS spectrum) in Fig. 1E shows the presence of Pd and Pt in the electrode structure at 1.28 and 0.69 atomic%, respectively. Following further modification with Au, the surface of the nanotubes is uniformly covered with metal nanoparticles (Fig. 1D). The presence of cauliflower-like colonies of metals, uniformly dispersed all over the modified substrate is

shown in the inset (Fig. 1D). In this regard, it is expected that the Au/Pt/Pd/TiO₂ NTs modified electrode effectively acts as a biosensor for the detection of electrochemical reactions. This is due to the large surface area of the tubular structure, which is filled with nanoparticles both from the inside and top of the tubes. Such an architecture facilitates electron transfer due to the enhanced conductivity achieved by the uniform coverage of Pd which improves catalytic activity also due to the Pt and Au nanoparticles.

3.2. Electrochemical oxidation of dopamine at modified electrodes

As mentioned before, the as-prepared TiO₂ NTs are amorphous with good conductivity in an externally applied electric field.^{4,26} After heat treatment at 500 °C for 3 h in the air, it becomes crystalline.²⁸ However, there is no sign of electrocatalytic activity toward oxidation of DA on the surface of non-crystalline TiO₂ NTs electrode as shown in curve a of Fig. 2. In the previous section, it is discussed how Pd and Pt nanoparticles have different electrodeposition behaviour on the surface of non-crystalline TiO₂ NTs electrode under the same conditions. Accordingly, the resulting Pt/Pd/TiO₂ NTs electrode is supposed to represent different electrochemical behaviour toward DA oxidation when compared to Pt/TiO₂ NTs. Fig. 2 (curve b to d) shows the CV results of DA oxidation in 1.0×10^{-3} M DA 0.1M pH 7.0 PBS in three modified non-crystalline TiO₂ NTs substrate: (b) Pt/TiO₂ NTs, (c) Pt/Pd/TiO₂ NTs, and (d) Au/Pt/Pd/TiO₂ NTs electrodes. Obviously there is a low electrochemical response to oxidation of DA on the surface of Pt/TiO₂ NTs electrode (curve b). A pair of redox peaks is observed after modification of the electrode with Pd nanoparticles, representing improved conductivity and consequently, electrocatalytic activity of Pt/Pd towards DA oxidation (curve c). On the other hand, the high surface area of the TiO₂ NTs offers more reactive sites when compared with a solid support and high catalytic activity is therefore achieved by metal loading.^{4,29} In this reaction, the oxidation and reduction peaks (E_p) appear at 0.217 V and 0.075 V, respectively, with $\Delta E_p = 0.142$ V and oxidation current peak (I_p) of 0.591 mA. As discussed before, there is evidence of nanoparticles present inside the tubes. Such an architecture facilitates the electron transfer and enhances the conductivity. The performance of the electrode is much improved by modification with Au nanoparticles (curve

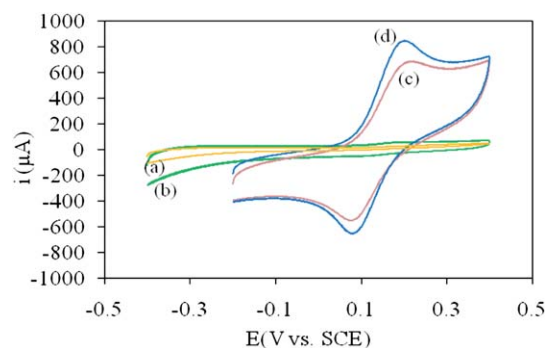


Fig. 2 Cyclic voltammograms obtained for 1×10^{-3} M DA in 0.1M pH 7.00 PBS at scan rate 100 mV s^{-1} . NTs electrodes: (a) TiO₂ NTs, (b) Pt/TiO₂ NTs, (c) Pt/Pd/TiO₂ NTs and (d) Au/Pt/Pd/TiO₂ NTs.

d) as the height of the oxidation/reduction peaks increases. It is obvious that the oxidation current peak (I_p) has sharply increased to 0.695 mA, which is significantly higher than that of Pt/Pd/TiO₂ NTs electrode with $I_p = 0.591$ mA. The oxidation peak also appeared at a less positive voltage 0.204 V and the reduction peak at 0.080 V, with $\Delta E_p = 0.124$ V. This small difference in redox peaks' potential indicates that the electrochemical oxidation of DA on the surface of TiO₂ NTs modified with metal nanoparticles has reversible behaviour. Moreover, in case of Au/Pt/Pd/TiO₂ NTs electrode sharp oxidation and reduction peaks have also the advantage of better sensitivity towards detection of DA, which we discuss in the following sections.

3.3. Effect of potential sweep rate on the electro-oxidation of dopamine

Fig. 3A shows the electrocatalytic activity of Au/Pt/Pd/TiO₂ NTs electrode towards oxidation of DA at different potential sweep rate. As the sweep rate increases the oxidation peak current also rises. The linearity behaviour of this increase is the result of a diffusion controlled process. Fig. 3B shows the oxidation peak current of DA vs. the square root of sweep rate on the surface of Au/Pt/Pd/TiO₂ NTs electrode. Obviously the oxidation peak current has increased linearly with a correlation coefficient of $R^2 = 0.99$, which ensures that the oxidation of DA on the Au/Pt/Pd/TiO₂ NTs electrode is a diffusion controlled process.

3.4. Effect of pH on the electro-oxidation of dopamine

Differential pulse voltammetry (DPV) was applied to study the effect of pH on the electro-oxidation of DA. Fig. 4A shows the DPV results obtained in 0.1M pH 7.0 PBS containing 0.5×10^{-4} M DA at different values of pH. It can be clearly seen that the electro-oxidation behaviour of DA is dependent on the pH of the solution. The peak potential for oxidation of DA shifts to more positive potentials as the pH of the solution changes from neutral to acidic values, with a linear relationship ($R^2 = 0.98$) between the oxidation peak potential and pH of the solution in range of 2–7 as shown in Fig. 4B.

The linear behaviour between the applied potential and pH with slope of 0.060 V/pH is close to the anticipated Nernstian value (0.059 V/pH) for a two electrons/two protons reaction,^{15,30}

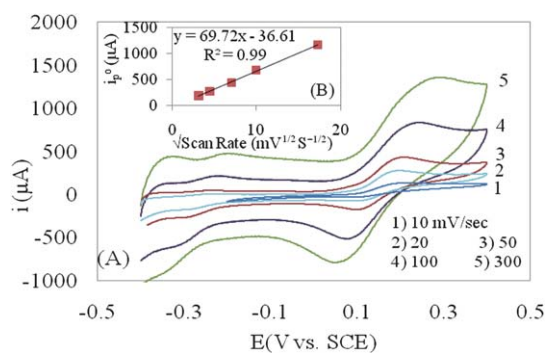


Fig. 3 (A) Cyclic voltammograms at Au/Pt/Pd/TiO₂ NTs obtained for 1×10^{-3} M DA in 0.1M pH 7.00 PBS at scan rate 10, 20, 50, 100 and 300 mV s⁻¹. (B) Oxidation peak current of DA vs. square root of scan rate.

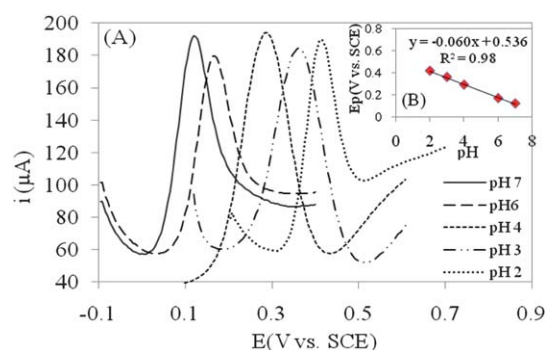
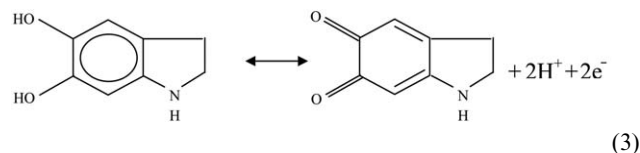
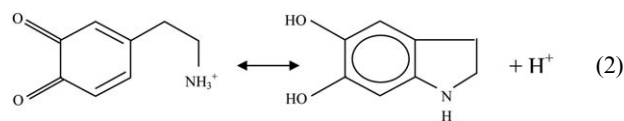
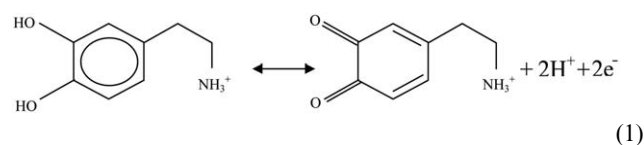


Fig. 4 (A) Differential pulse voltammograms obtained in the presence of 5.0×10^{-5} M DA at different acidity adjusted by H₂SO₄: (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 6 and (e) pH 7. The latter was the 0.1M pH 7.00 PBS base solution. The modified electrode was Au/Pt/Pd/TiO₂ NTs, under optimal condition scan rate 20 mV s⁻¹ and pulse amplitude 50 mV. (B) The calibration plot of peak potential for oxidation of DA vs. pH in the range of 2–7.

suggesting that DA oxidation is a two electrons/two protons process:^{7,15,11}



Accordingly, DA is oxidized to dopaminequinone in a two electrons oxidation reaction (eqn (1)). Dopaminequinone undergoes follow-up ring closure reaction (eqn (2)) leading to leucodopaminechrome, which in turn is oxidized to dopaminechrome (eqn (3)).^{7,15,11}

3.5. Sensitive detection of dopamine with binary/ternary metal modified TiO₂ NTs electrodes

In order to achieve a sensitive detection of DA at modified electrodes, DPV was applied to record anodic peak current of the reaction. This method offers much higher current sensitivity and better peak separation than CV.

Fig. 5A (A–I) shows the DPV results of DA oxidation at Pt/Pd/TiO₂ NTs electrode in 0.1M pH 7.0 PBS containing various concentrations of DA. Under optimised conditions the oxidation

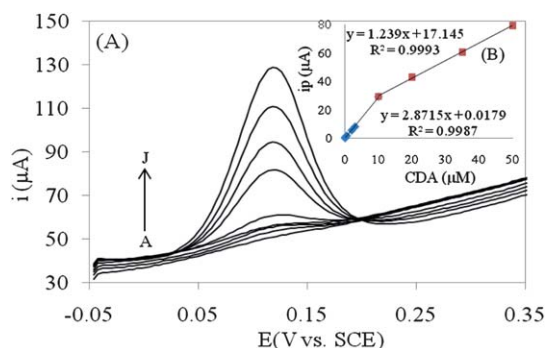


Fig. 5 (A) Differential pulse voltammograms of DA at Pt/Pd/TiO₂ NTs in 0.1M pH 7.00 PBS at scan rate 20 mV s⁻¹ and pulse amplitude 50 mV. The total concentration of DA in each step (A–J): 0, 5.0 × 10⁻⁸, 1.0 × 10⁻⁷, 5.0 × 10⁻⁷, 2.0 × 10⁻⁶, 4.0 × 10⁻⁶, 1.0 × 10⁻⁵, 2.0 × 10⁻⁵, 3.5 × 10⁻⁵ and 5.0 × 10⁻⁵ M. (B) The calibration plot of oxidation peak current vs. accumulated concentration of DA in each step.

peak potential of DA occurred at 0.120 V for all concentrations of DA in range of 5.0 × 10⁻⁸ to 5.0 × 10⁻⁵ M. However, as shown in Fig. 5B, the oxidation peak currents of DA experienced two linear ranges in this range of concentration. The oxidation peak currents of DA follow a sharp linear rise with slope of 2.87 μA μM⁻¹ (R² = 0.9987) in range of 5.0 × 10⁻⁸ – 1.0 × 10⁻⁵ M DA, suggesting a high sensitivity towards detection of DA in this range. When increasing the concentration of DA from 1.0 × 10⁻⁵ to 5.0 × 10⁻⁵ M, the oxidation peak currents experienced a slower rise with slope of 1.24 μA μM⁻¹ (R² = 0.9993) which is about 2.5 times less than previously.

Similar studies were carried out for the detection of DA at Au/Pt/Pd/TiO₂ NTs electrode in concentration ranges of 5.0 × 10⁻⁸ to 3.0 × 10⁻⁵ M (Fig. 6A). Under the same conditions, the oxidation peak potential of DA occurred at 0.122 V suggesting almost the same electrocatalytic behaviour for both modified electrodes. However, unlike the previous study the calibration curve (Fig. 6B) indicates a linear relationship between oxidation peak current of DA and accumulated concentration over the whole range, with a slope of 4.57 μA μM⁻¹ (R² = 0.9994) and

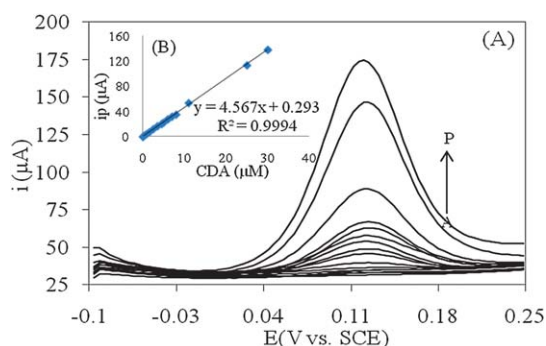


Fig. 6 (A) Differential pulse voltammograms of DA at Au/Pt/Pd/TiO₂ NTs in 0.1M pH 7.00 PBS at scan rate 20 mV s⁻¹ and pulse amplitude 50 mV. The total concentration of DA in each step (A–P): 0, 5.0 × 10⁻⁸, 1.0 × 10⁻⁷, 5.0 × 10⁻⁷, 1.0 × 10⁻⁶, 1.5 × 10⁻⁶, 2.5 × 10⁻⁶, 3.5 × 10⁻⁶, 4.5 × 10⁻⁶, 5.0 × 10⁻⁶, 6.0 × 10⁻⁶, 7.0 × 10⁻⁶, 8.0 × 10⁻⁶, 1.0 × 10⁻⁵, 2.5 × 10⁻⁵ and 3.0 × 10⁻⁵ M. (B) The calibration plot of oxidation peak current vs. accumulated concentration of DA in each step.

a detection limit of 3.0 × 10⁻⁸ M for S/N = 3. The obtained sensitivity is significantly higher than that of Pt/Pd/TiO₂ NTs and other modified electrodes which have been reported previously.^{10,16,30–32}

3.6. Differential pulse voltametry of a mixture of dopamine and uric acid with Au/Pt/Pd/TiO₂ NTs and Pt/Pd/TiO₂ NTs electrodes

The modified electrodes were also examined for detection of uric acid (UA) with a close oxidation potential to DA. Fig. 7 shows a series of DPV experiments in individual solutions of 1 × 10⁻³ M DA and 1 × 10⁻³ M UA in 0.1M pH 7.0 PBS. It seems that the oxidation peak current of DA is much higher than that of UA in case of both Au/Pt/Pd/TiO₂ NTs and Pt/Pd/TiO₂ NTs electrodes. Moreover, the oxidation currents of both DA and UA at the surface of Au- modified electrode are higher than Pt/Pd/TiO₂ NTs electrode. However the interesting point is -0.05 V shift in oxidation potential of UA at the surface of Au- modified electrode (curve c) compared to that of Pt/Pd/TiO₂ NTs electrode (curve b).

In order to study the sensitivity for simultaneous determination of DA and UA, DPV was applied to measure the anodic peak current on the modified electrodes. The electro-oxidation processes of DA and UA in the mixture was investigated when the concentration of one species changed, while the other was kept constant.

Fig. 8A shows the DPV obtained for different concentrations of DA in the presence of a large excess of UA *i.e.* 1.0 × 10⁻⁴ M in pH 7.0 PBS at Pt/Pd/TiO₂ NTs modified electrode. The oxidation peak potential for DA has been obtained at 0.122 V, indicating the same oxidation potential as the individual detection in Fig. 6A. It can also be seen that the location of the peak current for UA is almost constant during regular addition of DA. Like individual detection of DA, two ranges of linearity have been obtained from the calibration curve of oxidation peak current vs. accumulated concentration of DA in the range of 1.0 × 10⁻⁷ to 1.0 × 10⁻⁴ M. The first supports a short range of concentrations from 1.0 × 10⁻⁷ to 2.0 × 10⁻⁵ M DA with a slope of 2.43 μA μM⁻¹ (R² = 0.9985). Whereas, the second occurs in a relative wide range of concentrations from 2.0 × 10⁻⁵ M DA to 1.0 × 10⁻⁴ M DA with slope of 0.788 μA μM⁻¹ (R² = 0.9961),

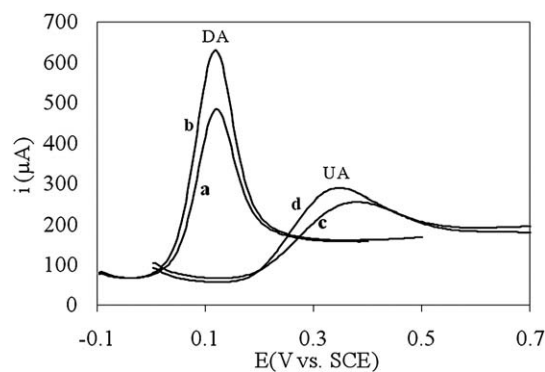


Fig. 7 Differential pulse voltammograms of 1 mM DA and 1 mM UA at (a) and (c) Pt/Pd/TiO₂ NTs and (b) and (d) Au/Pt/Pd/TiO₂ NTs in 0.1M, pH 7.00 PBS at scan rate 20 mV s⁻¹ and pulse amplitude 50 mV.

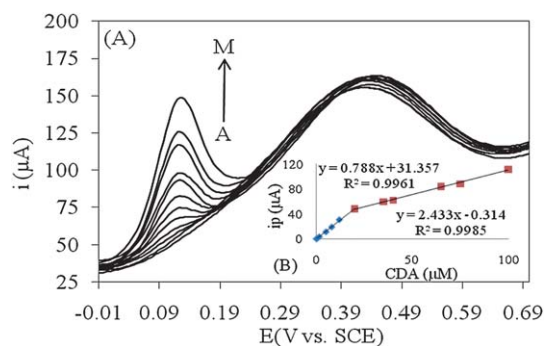


Fig. 8 (A) Differential pulse voltammograms of DA in the presence of 1.0×10^{-4} M UA at Pt/Pd/TiO₂ NTs in 0.1M pH 7.00 PBS at scan rate 20 mV s^{-1} and pulse amplitude 50 mV. The total concentration of DA in each step (A–M): $0, 1.0 \times 10^{-7}, 5.0 \times 10^{-7}, 1.5 \times 10^{-6}, 5.0 \times 10^{-6}, 8.0 \times 10^{-6}, 1.0 \times 10^{-5}, 2.0 \times 10^{-5}, 3.0 \times 10^{-5}, 4.0 \times 10^{-5}, 6.5 \times 10^{-5}, 7.5 \times 10^{-5}$ and 1.0×10^{-4} M. (B) The calibration plot of oxidation peak current vs. accumulated concentration of DA in each step.

indicating a 3-fold decrease in sensitivity of detection of DA at the Pt/Pd/TiO₂ NTs modified electrode. In a similar experiment, DPV determination of DA was carried out in the presence of 1.0×10^{-4} M UA in pH 7.0 PBS using Au/Pt/Pd/TiO₂ NTs. As shown in Fig. 9A the oxidation current of UA is almost constant as the concentration of DA increases. It was also found that the oxidation peak current of DA is proportional to its concentration over a wide range of concentrations from 1.0×10^{-7} to 4.0×10^{-5} M, with a sharp slope $4.05 \mu\text{A } \mu\text{M}^{-1}$ ($R^2 = 0.9988$). Considering these results, it can be concluded that Au nanoparticles have obviously improved the sensitive detection of DA both individually or simultaneously with UA. These results were also compared to those extracted from previous work as reported in Table 2, which confirms the sensing ability of the proposed electrode towards detection of DA.

3.7. Simultaneous detection of dopamine, uric acid and ascorbic acid at different pH using Au/Pt/Pd/TiO₂ NTs

It is known that simultaneous determination of DA, AA and UA is a problem due to their similar oxidation potentials, which

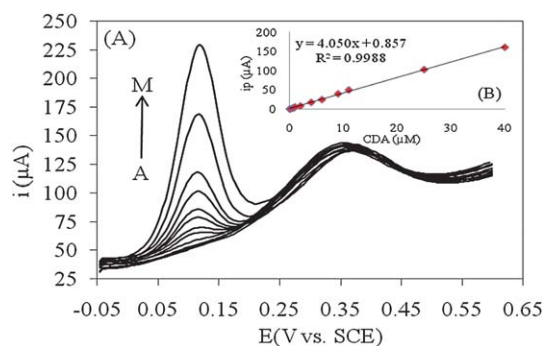


Fig. 9 (A) Differential pulse voltammograms of DA in the presence of 1.0×10^{-4} M UA at Au/Pt/Pd/TiO₂ NTs in 0.1M pH 7.00 PBS at scan rate 20 mV s^{-1} and pulse amplitude 50 mV. The total concentration of DA in each step (A–M): $0, 1.0 \times 10^{-7}, 5.0 \times 10^{-7}, 7.0 \times 10^{-7}, 9.0 \times 10^{-7}, 1.0 \times 10^{-6}, 2.0 \times 10^{-6}, 4.0 \times 10^{-6}, 6.0 \times 10^{-6}, 9.0 \times 10^{-6}, 1.0 \times 10^{-5}, 2.5 \times 10^{-5}$ and 4.0×10^{-5} M. (B) The calibration plot of oxidation peak current vs. accumulated concentration of DA in each step.

Table 2 Analytical parameters for detection of DA at several modified electrodes

Dopamine sensor	Linear range $\times 10^{-6}$ M	LOD \times 10^{-8} M	pH
TiO ₂ nanotubes modified with Au, Pt and Pd nanoparticles ^{Present work}	0.05–30	3	7.0
Carbon nanotubes paste electrodes modified with a melanic polymer ¹⁷	0.05–0.5	2	7.4
TiO ₂ nanoparticles modified carbon paste electrode ¹⁹	0.08–1.0 1.0–20.0	3.14	8.0
Titanate nanotubes modified glassy carbon electrode ¹⁰	4.45–155	10	7.4
Layer-by-layer modified multilayer films containing choline and Au nanoparticles ³⁰	0.2–80	12	7.0
Poly(3-methylthiophene)/Pd, Pt nanoparticle ¹⁶	0.05–1	0.8	7.4
Poly(Evans Blue) modified glassy carbon electrode ³¹	1–10	25	4.5
Polyaniline/polyelectrolyte/Au nanoparticles ³²	50–1000	5000	7.4

causes overlapped peaks on most common electrodes. Especially, in case of AA and DA, the oxidation peak potentials are very close together so that even modified electrodes are sometimes useless. Therefore, the oxidation of DA and UA was examined in the presence of AA at Au/Pt/Pd/TiO₂ NTs modified electrode using DPV techniques under the same conditions as in previous experiments. The experiment was carried out in 0.1M PBS solution containing 0.5×10^{-4} M DA, 1.0×10^{-4} M UA and 1.0×10^{-4} M AA. The test solution was adjusted to pH 7, 5 and 3 with H₂SO₄ in order to study the effect of pH on the peak separation of AA, DA and UA (Fig. 10A). It can be seen that at pH 7 the oxidation peak potential of DA and UA occurred at 0.122 V and 0.34 V, respectively, which is the same as previous results. While the oxidation peak of AA appears as a small curve before that of DA at 0.07 V. Although the oxidation peak of DA and AA are very close together, the Au/Pt/Pd/TiO₂ NTs modified electrode is capable of successfully separating their signals. Meanwhile, the oxidation of UA is shifted to a more positive potential. By increasing the acidity of the solution to pH 5, the oxidation peak potential of AA and DA are completely separated from each other at 0.18 V and 0.33 V, respectively. Comparing these results, the oxidation peak potential of AA experiences a shift of 0.11 V to more positive potentials, which is about two times bigger than that of DA. In other words, the peak potential of DA takes a bigger step in the movement of the peaks towards positive potentials, making the peak separation of DA and AA possible. An almost similar trend is observed as the pH of the solution decreases to 3, resulting in complete separation of the AA and DA peak potential at 0.24 V and 0.39 V, respectively. The peak separation between AA and DA, $\Delta E_p = E_p^{\text{DA}} - E_p^{\text{AA}}$, becomes greater in acidic solutions. At pH 5 and 3, the maximum separation of AA and DA was obtained $\Delta E_p = 0.15$, and thus AA and DA can be completely separated, so allowing simultaneous determination of AA and DA in the mixture. Cao *et al.* report that pH 4.7 provides best acidity of the solution for complete separation of AA and DA using a CPB/chitosan composite film electrode.²⁴

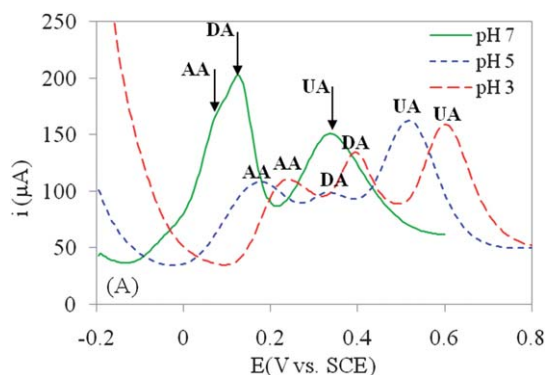


Fig. 10 Differential pulse voltammograms obtained in the presence of 0.5×10^{-4} M DA + 1.0×10^{-4} M UA + 1.0×10^{-4} M AA at three different acidities adjust by H_2SO_4 (a) pH 3, (b) pH 5 and (c) pH 7. The latter is 0.1M pH 7.00 PBS base solution. The modified electrode is Au/Pt/Pd/TiO₂ NTs under optimal conditions at scan rate 20 mV s⁻¹ and pulse amplitude 50 mV.

3.8. Reproducibility and stability of the Au/Pt/Pd/TiO₂ NTs electrode

In order to examine the reproducibility of the modified electrode, repeated DPVs experiment was run in 5.0×10^{-6} M DA in 0.1M pH 7.00 PBS. The relative standard deviation (RSD) was about 2.77% after 18 successive measurements indicating that the Au/Pt/Pd/TiO₂ NTs modified electrode has an excellent reproducibility. The long-term stability of the modified electrode was evaluated by measuring its CV response to 1.0×10^{-4} M DA in 0.1M pH 7.00 PBS and then repeating the same experiment after one month. When not in use, the electrode was stored at room temperature under normal condition. The oxidation current response maintained 93.02% of the original peak current value after one month.

3.9. Real sample analysis

The Au/Pt/Pd/TiO₂ electrode was tested in human urine samples for determination of DA. In this regard, 2mL of 0.1mM DA standard solution was injected into 18mL urine (The final concentration of DA is 0.01mM). Differential pulse voltammetry was applied for the detection of DA. Table 3 represent the results obtained for five measurements. These results were satisfactory enough to show the reliability of the proposed method for detection of DA in real samples.

Table 3 Determination of DA in human urine samples

Samples	DA spiking ($\times 10^{-6}$ M)	DA found ($\times 10^{-6}$ M)	Recovery (%)
1	10	11.09	110.9
2	10	11.26	112.6
3	10	10.92	109.2
4	10	10.58	105.8
5	10	10.80	108.0
Mean	10	10.93	109.3

4. Conclusions

A modified TiO₂ NTs electrode was fabricated by uniform electrodeposition of Pd, Pt and Au nanoparticles. It was shown that the simultaneous presence of Pt and Pd nanoparticles improved conductivity and also electrocatalytic activity towards detection of DA. Pd nanoparticles were essential to obtain uniform modification on the surface of non-crystalline TiO₂. Au nanoparticles were responsible for achieving good sensitivity, as high as $4.57 \mu\text{A} \mu\text{M}^{-1}$. Thus, the Au/Pt/Pd/TiO₂ NTs modified electrode showed high sensitivity towards detection of DA, individually and simultaneously with UA, under a wide range of DA concentrations. However, without Au nanoparticles the sensitivity exhibited a significant loss in the same ranges of DA concentration. Using a DPV method, the detection limit of DA was determined to be 3×10^{-8} M DA. The Au/Pt/Pd/TiO₂ NTs modified electrode improved not only sensitivity towards detection of DA, but was also able to distinguish the oxidation response of DA, UA and AA in a solution mixture of different acidities. The modified electrode offers very good reproducibility 2.77%, long-term stability 93.0%, and facile fabrication. The proposed method was applied to the determination of DA in real samples with satisfactory results.

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