Voltammetric Determination of L-Dopa on Poly(3,4-ethylenedioxythiophene)-Single-Walled Carbon Nanotube Composite Modified Microelectrodes

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Abstract
In the present communication, it is shown that platinum microelectrodes electrochemically coated with a composite of poly(3,4-)ethylenedioxythiophene and single-walled carbon nanotubes (PEDOT/SWNT) enable determinations of 3,4-dihydroxy-L-phenylalaines (L-dopa) in neutral phosphate buffer solutions containing an excess of ascorbic acid. The interpenetrated networked nanostructure of the composite was characterized by scanning electron microscope (SEM) and Raman spectroscopy. It is shown that the presence of the composite gives rise to an increase in the electroactive area of an order of magnitude in compared to the area for the bare microelectrodes. The composite film-coated microelectrode, which yielded reversible cyclic voltammograms for the ferro/ferricyanide redox couple for scan rates between 0.01 and 0.10 V s^{-1}, also gave rise to two well-resolved oxidation peaks for L-dopa and ascorbic acid (AA). The latter effect, which was not seen in the absence of the composite, enabled differential pulse voltammetric determinations of L-dopa in the concentration range between 0.1 to 20 μM with a detection limit of 100 nM.

Keywords: L-Dopa, Ascorbic acid, Microelectrode, Single-walled carbon nanotube, PEDOT, Voltammetry, Nanocomposites, Nanotubes

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1. Introduction

Electrochemical detection using microelectrodes enables fabrication of sensors with good precision and accuracy, high sensitivity and low cost [1 – 5]. The small size of the microelectrodes also makes in-vivo detection of easily oxidizable analyte molecules, e.g. neurotransmitters, in the extra cellular fluid of the brain in a very small sample volume possible [6 – 9]. For the latter type of applications, it is therefore interesting to investigate the properties of new sensing materials. Carbon fiber microelectrodes have traditionally been used for the in-vivo neurotransmission tests in the brain [10 – 11]. However, the applicability of micro sensors based on solid electrodes is often hampered by the poor selectivity, reproducibility due to fouling of the electrode surface and the mechanical stability of the electrode material. In particular, the complexity of real biological samples may result in overlapping voltammetric signals and the limited number of electrode materials available only makes a restricted number of analytes suitable for electrochemical detection.

Electrochemical detection with modified macro electrodes suitable for determinations of neurotransmitters in the presence of ascorbic acid has been reported extensively [12 – 14]. Among the neurotransmitters, L-dopa is an important precursor in the synthesis of dopamine and noradrenalin. As L-dopa is one of the catecholamine, it plays an important part in neurotransmission and too low concentrations of this compound in the brain tissue results in Parkinson's disease [15]. Many authors have reported on electrochemical detection of L-dopa using modified electrodes [16 – 18]. However, detection using modified microelectrodes [19] has so far not received that much interest for the detection of neurotransmitters although in-vivo measurements of these compounds rely on the availability of miniaturized electrodes.

In the present work, we present a modified microelectrode suitable for electrochemical detection of L-dopa in the presence of an excess of ascorbic acid. The electrochemical behavior of these microelectrodes, which are based on a composite layer composed of poly(3,4-ethylenedioxythiophene) and single walled carbon nanotubes, is investigated and the composites have been characterized using SEM and Raman spectroscopy. The results indicate that the present miniaturized electrodes constitute promising tool in-vivo electrochemical analysis of neurotransmitters.

2. Experimental

2.1. Chemicals

The chemicals used in the experiments, 3,4-ethylenedioxythiophene (EDOT), carbon nanotube-single-walled 50–70% basis, diam. $\times$ $L = 1.2–1.5$ nm $\times$ 2–5 μm, bundle dimensions (SWNT), L-dopa, ascorbic acid, tetrabutylammonium perchlorate (TBAPC), acetonitrile, potassium dihydrogen phosphate, were all purchased from Aldrich and were used as received. For the voltammetric studies, a 22 μm platinum working electrode was used together with a platinum wire combined auxiliary and reference electrode. Unless stated otherwise, the potential values given in the text are given with respect to the latter electrode. Phosphate buffer solutions (0.1 M) of pH 7.4 were employed as the electrolyte. During the electropolymerization of the composite in acetonitrile, a silver wire was instead used as a quasi reference electrode while the platinum wire was used as the counter electrode.

2.2. Synthesis of Polymer Composite

PEDOT was deposited on the platinum microelectrode from a solution of 5.0×10^{-3} M EDOT and 0.1 M TBAPC in acetonitrile by potential cycling between -0.6 and 1.6 V vs. an Ag wire. The PEDOT film was allowed to grow on the Pt microelectrode during a single cyclic voltammetric scan recorded at a scan rate of 0.05 V s^{-1}. In the PEDOT/SWNT case, 0.3% SWNT was also introduced into the 5.0×10^{-3} M EDOT and 0.1 M TBAPC solution, and the solution was sonicated for 5 to 10 minutes to obtain a good dispersion. The film was then grown on the microelectrode as indicated above.

2.3. Instrumentation

The voltammetric experiments were carried out using a μ-Autolab potentiostat/galvanostat (Eco Chemie, The Netherlands) at ambient temperatures. In the recordings of the differential pulse voltammograms (DPV), the following instrumental settings were used: scan rate: 0.03 V s^{-1}, sample-width: 17 ms, pulse-amplitude: 0.05 V, pulse-width (modulation time): 50 ms, pulse-period (interval):200 ms and quiet-time: 2 s.

The Raman spectra were obtained using a micro-Raman spectrometer (Renishaw-2000) equipped with a diode laser with a wavelength of 783 nm. The spectrophotometer was coupled to an optical microscope that focused the incident radiation down to an approximately 1 μm spot with incidence potency of 20 mW in the region between 100 and 3500 cm^{-1}.

The morphology of the polymers and composites were studied with a scanning electron microscope (LEO 1550) after depositing the films on fluorine doped indium tin oxide (FITO) conducting glass substrates (with <15 Ω cm conductivity) supplied by Nippon Glass.

3. Results and Discussion

Figure 1 shows SEM images of the PEDOT and PEDOT/SWNT composite films. It is seen that the electropolymerization resulted in the formation of an homogenous interpenetrated fibril network morphology. The diameter of the fibrils was approximately 10 nm and the films were highly porous. There was no noticeable difference between the structures of the PEDOT polymer and the PEDOT/SWNT composite. This is, however, not surprising since the SWNTs used in this study had a diameter of 1.2–1.5 nm and a length of 2–5 μm which make it is difficult to detect the carbon nanotubes with SEM. To ascertain that SWNTs had indeed been included into the polymer structure, Raman spectroscopy was hence also employed to characterize the composite

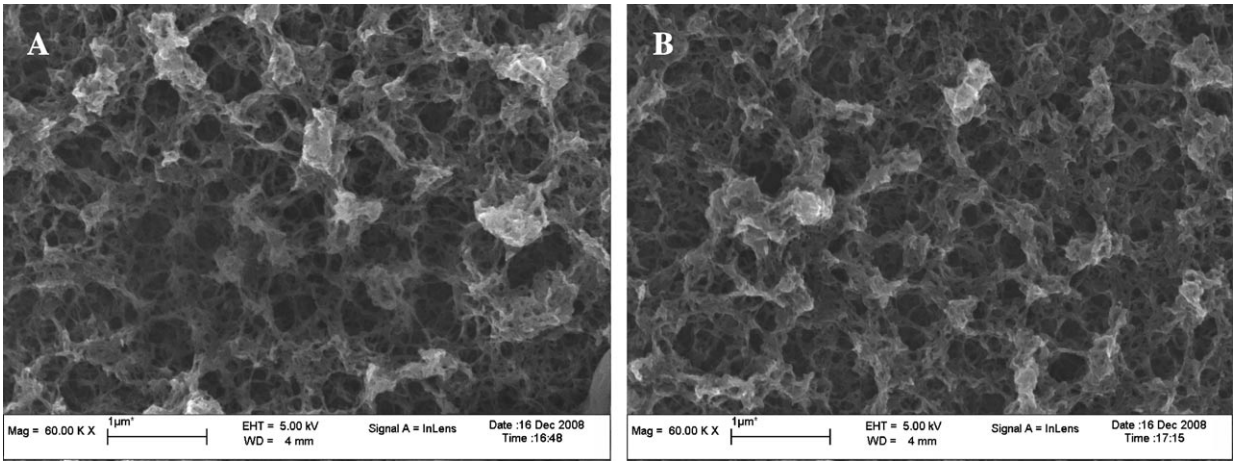

Fig. 1. SEM micrographs of (A) PEDOT and (B) PEDOT/SWNT composite.

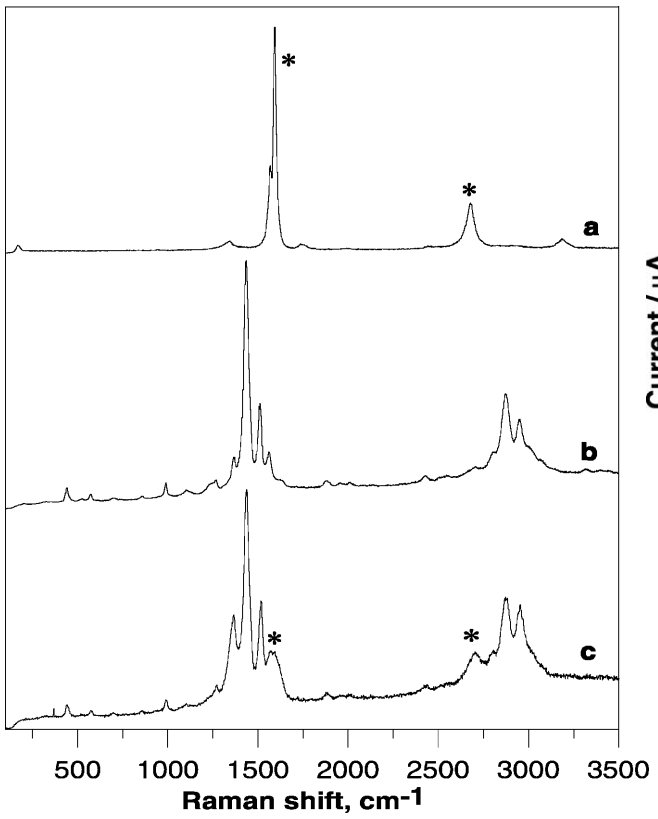

Fig. 2. Raman spectra of (a) SWNT, (b) PEDOT and (c) the PEDOT/SWNT composite, respectively.

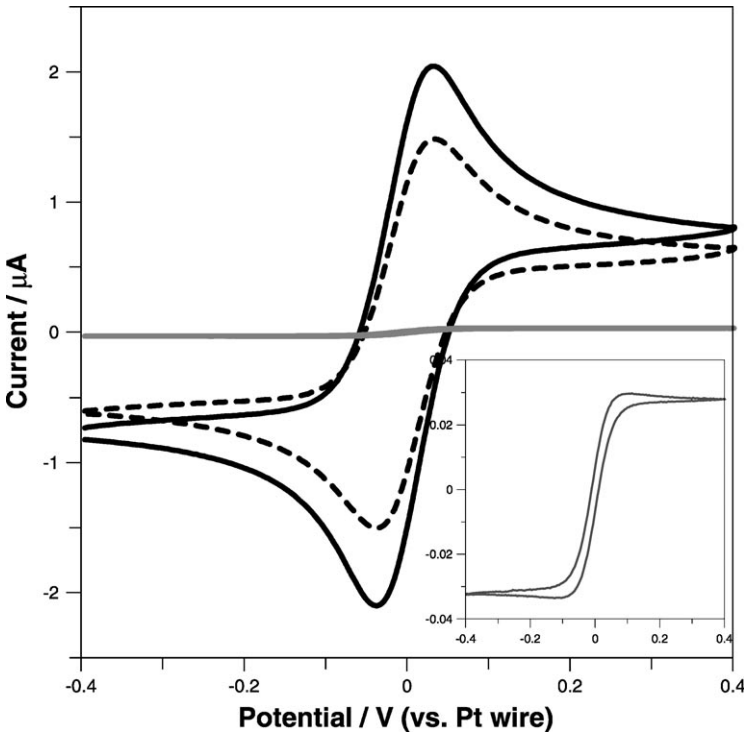

Fig. 3. Cyclic voltammograms recorded for a solution of 1.0×10^{-2} M $Fe(CN)_6^{3-/4-}$ in 0.1 M KNO_3 at a scan rate of 50 mV s^{-1} with the bare (Inset), PEDOT modified (broken line) and PEDOT/SWNT modified (solid line) Pt microelectrode.

as this technique is widely used for the characterization of carbon composite materials [20].

Figure 2 shows Raman spectra for SWNT, PEDOT and the PEDOT/SWNT composite films deposited on the microelectrode. The polymer film shows bands at 2951, 2872, 2430, 1561, 1512, 1437, 1367, 1271 and 989 cm^{-1}, which are the characteristics for the PEDOT polymer [21]. The spectrum for SWNT shows the characteristics D band at 1345 cm^{-1} (disordered sp^3 carbon structure), G band at 1595 cm^{-1} (sp^2 ordered crystalline graphite-like structures), and the second order G′ band at 2680 cm^{-1}. The spectrum for the PEDOT/SWNT composite clearly showed the presence of the SWNT characteristics peaks at 1575 and 2706 cm^{-1} with an approximate 20 cm^{-1} peak shift compared to the SWNT values, in addition to the polymer peaks. It should be stressed that the Raman spectra were collected by focusing the laser spot on different regions of the composite sample and that the results were always the same that as that showed in the figure.

As is seen in Figure 3, the electrochemical behavior of the modified microelectrodes was investigated by recording cyclic voltammograms for the different electrodes using a solution of ferri/ferro cyanide. While the bare Pt microelectrode showed the typical sigmoidal behavior (see the inset) expected for a microelectrode under steady state conditions, the modified electrodes gave rise to well-defined oxidation and reduction peaks. It is also seen that the obtained currents were much larger with the modified electrodes compared to with the bare Pt electrode. The latter can be explained by the enhancement of the electro active surface area of about one order of magnitude obtained with the polymer-modified electrodes, causing the modified electrodes to behave as macro- rather than microelectrodes (see Table 1). As is seen by comparing the voltammograms for the PEDOT and PEDOT/SWNT electrodes, there was also an additional smaller (i.e. 40%) enhancement of the peak current because of the inclusion of the carbon nanotubes. The electroactive areas of the modified electrodes seen in Table 1 were calculated using the well-known Randles – Sevcik equation [22],

$$I_p = 2.69 \times 10^5 \, n^{3/2} \, A \, C_o \, D^{1/2}{}_R \, \nu^{1/2} \qquad (1)$$

employing $n = 1$, $D = 6.70(\pm 0.02) \times 10^{-6}$ cm^2 s^{-1} [22] and $C = 1.0 \times 10^{-2}$ M. As is seen in Table 1, the corresponding large increase in the electroactive area was not seen when modifying a 3 mm glassy carbon electrode (GCE) due to the small relative increase in the electroactive surface area of such a large electrode.

Figure 4 shows cyclic voltammograms for modified microelectrodes obtained with 1.0×10^{-3} M L-dopa in a phosphate buffer solution of pH 7.4. It is seen that well-defined oxidation and reduction peaks were obtained for both the PEDOT and the PEDOT/SWNT electrodes while no clear peaks were obtained for the bare Pt microelectrode (see the inset in Fig. 4). With the PEDOT and PEDOT/SWNT

Table 1. Comparison of electrochemical active surface area: Modified macro vs. micro electrodes.

–	3 mm GCE (cm^2)	Pt 22 μm (cm^2)
Bare electrode	5.04×10^{-3}	3.80×10^{-6}
PEDOT modified	6.30×10^{-3}	3.15×10^{-5}
PEDOT-SWNT modified	7.56×10^{-3}	5.67×10^{-5}

modified microelectrodes, the L-dopa oxidation peak potential was found to be 0.113 and 0.122 V, respectively. The origin of the irreversible oxidation peak at about 0.6 V is presently not fully understood. In the latter case, a cathodic peak at about – 0.3 V was, however, seen on the reverse scan most likely due to the reduction of cyclodopa originating from a chemical reaction involving dopaquinone formed upon the oxidation of L-dopa [23].

The fact that the difference between the anodic and cathodic peak potentials in Figure 4 was only 48 and 35 mV, respectively, for the modified electrodes at a scan rate of 0.05 V s^{-1} indicates that L-dopa exhibited a close to reversible behavior on these electrodes although the relative small reduction peak indicates that the oxidation product undergoes chemical follow-up reactions. The presence of such reactions is in good agreement with previous findings [18]. These results clearly demonstrate that the modified microelectrodes are promising for determinations of L-dopa and that the main improvement of the response was due to the PEDOT modification although the response can be further improved upon the inclusion of SWNTs into the polymer matrix.

The influence of scan rate on the oxidation behavior of L-dopa was also studied using the PEDOT/SWNT-modified electrode. It was found that the oxidation peak current for L-dopa increased linearly with the square root of the scan rate for scan rates between 0.005 and 0.2 Vs^{-1}. The latter is in good agreement with the expected behavior for a diffusion-controlled process.

In oxidative determinations of neurotransmitters by electrochemical methods, ascorbic acid is the prime interfering species mainly as ascorbic acid is oxidized at the more or less the same potential as the neurotransmitters and the relative high concentrations of ascorbic acid in biological samples [24]. It is therefore interesting to investigate the electrochemical behavior of ascorbic acid on the PEDOT and PEDOT/SWNT modified microelectrodes.

Figure 5 depicts cyclic voltammograms obtained with a solution containing 1.0×10^{-3} M L-dopa and 1.0×10^{-3} M ascorbic acid with a bare Pt, PEDOT and PEDOT/SWNT modified microelectrodes, respectively. With the bare electrode (see the inset), the oxidations of L-dopa and ascorbic acid overlap significantly to give rise to a single broad wave. This means that it would be difficult to determine L-dopa in the presence of an excess of ascorbic acid with the bare Pt microelectrode. Two distinct oxidation peaks due to the oxidation of ascorbic acid and L-dopa were, on the other hand, observed at around – 0.03 and 0.2 V using the PEDOT modified microelectrode. An analogous voltammogram with two clear peaks (0.02 and 0.23 V) was also

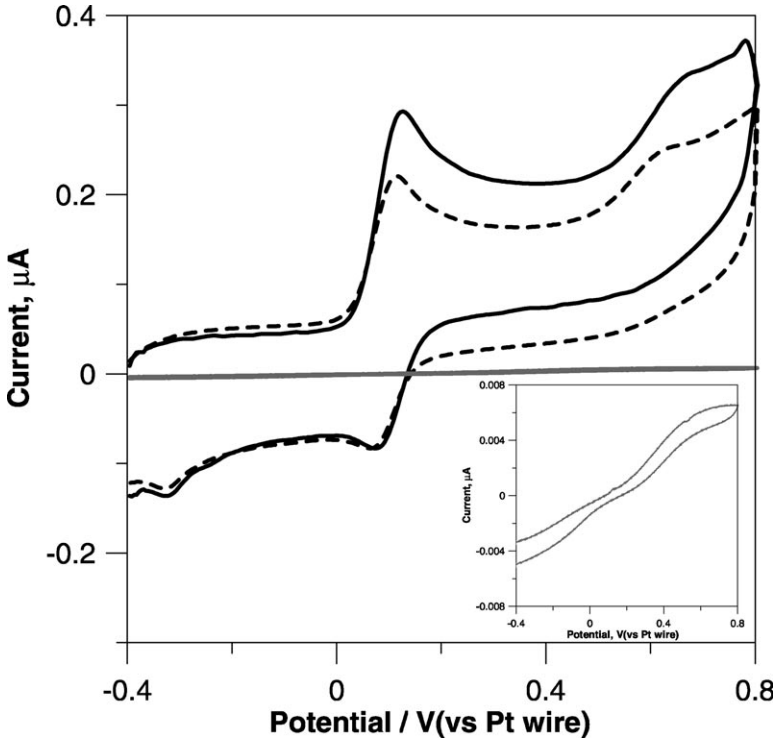

Fig. 4. Cyclic voltammograms for 1.0×10^{-3} L-dopa recorded at a scan rate of 50 mV s^{-1} with a bare (see inset), PEDOT modified (broken line) and PEDOT/SWNT modified (solid line) platinum microelectrode in a phosphate buffer of pH 7.4.

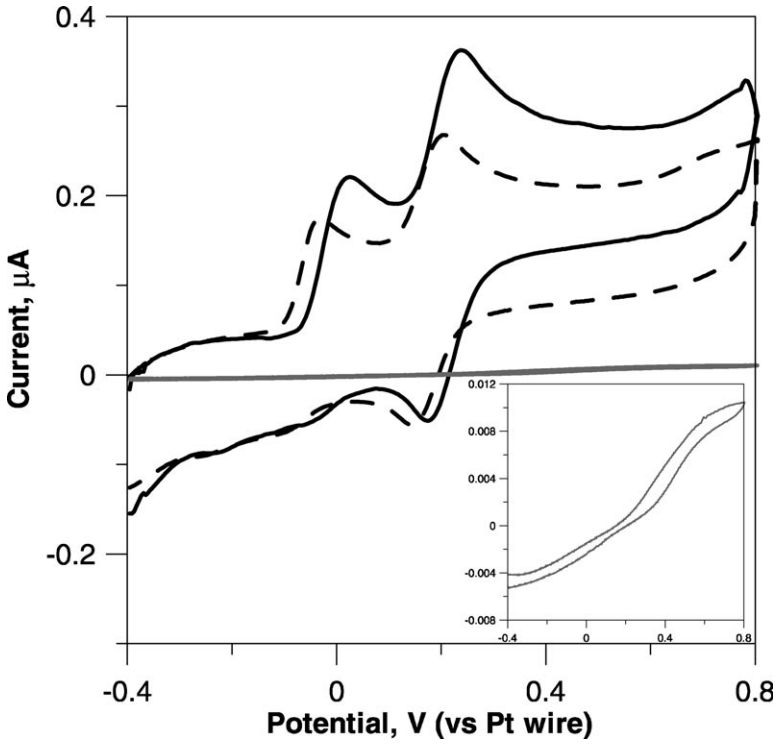

Fig. 5. Cyclic voltammograms recorded in a solution containing 1.0×10^{-3} M L-dopa and 1.0×10^{-3} M ascorbic acid at a scan rate of 50 mV s^{-1} employing a bare (see inset), PEDOT modified (broken line) and PEDOT/SWNT modified (solid line) platinum microelectrode in a phosphate buffer of pH 7.4.

obtained with the PEDOT/SWNT modified electrode. These results indicate that it is possible to determine L-dopa also in the presence of an excess of ascorbic acid.

To determine the detection limit for L-dopa, at physiological conditions, with the modified PEDOT/SWNT electrode, differential pulse voltammetric experiments were carried out. Fig. 6 shows the voltammograms for different concentrations (i.e. 1.0×10^{-7} to 2.0×10^{-5} M) of L-dopa recorded in the presence of an excess of ascorbic acid. It was found that the ascorbic acid was oxidized at a potential approximately 0.2 V less positive that the L-dopa oxidation potential and that the L-dopa oxidation peak currents in the absence and presence of AA were found to be almost the same. This suggests that the L-dopa oxidation at the PEDOT/SWNT composite electrode was unperturbed by the ascorbic acid concentration used the present experimental conditions. By plotting the L-dopa oxidation current as a function of the L-dopa concentration, a linear calibration curve with a correlation coefficient of 0.9982 was also obtained.

The present sensor linear behavior over the 0.1 to 20 μM L-dopa range examined ($R = 0.9982$), with a detection limit of 10 nM. A highly reproducible response was observed for 50 repetitive measurements with a *RSD* of 1.6%, while the effect of ascorbate upon the voltammetric signal for L-dopa was negligible. It was also found that the fouling of the electrode because of adsorption of the oxidized product of ascorbic acid on the electrode surface was eliminated with the modified electrodes. The latter ensured that the responses for AA and L-dopa were reproducible with the modified electrode. It was thus found that the same electrode could be used for 40 successive experiments

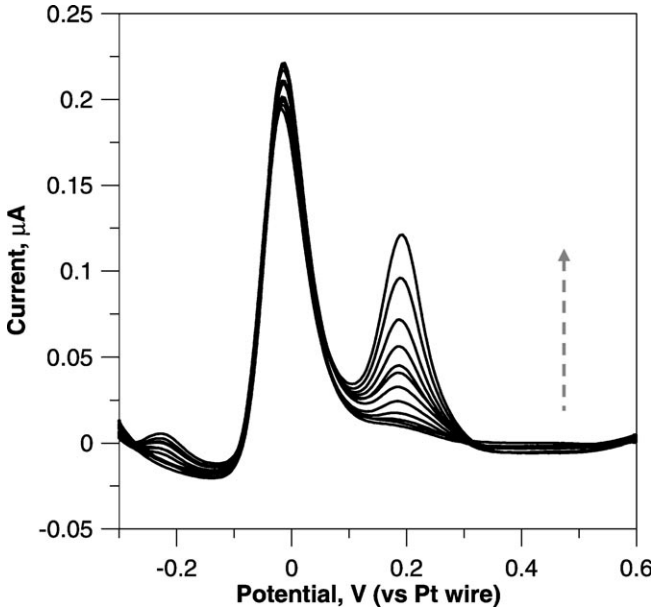

Fig. 6. Differential pulse voltammograms for ascorbic acid and L-dopa recorded using the PEDOT/SWNT modified microelectrode in a phosphate pH 7.4 buffer. The L-dopa concentration was increased from 1.0×10^{-7} to 2.0×10^{-5} M while maintaining a constant ascorbic acid concentration of 1.0×10^{-3} M.

yielding a mean current of 1.22 μA and a relative standard deviation of 1.7% for a solution of 20 μM L-dopa in the presence of 1 mM ascorbic acid. Since the modified electrodes can measure low (i.e. 100 nM) concentrations of L-dopa in the presence of 1.0×10^{-3} M ascorbic acid (which corresponds to a much larger concentration difference than under physiological conditions), it is reasonable to assume that PEDOT/SWNT modified microelectrodes may be used for in-vivo determination of neurotransmitters and ascorbic acid in biological systems.

4. Conclusions

It has been shown that PEDOT/SWNT modified platinum microelectrodes can be used for electrochemical detection of L-dopa in pH 7.0 phosphate buffer solutions even in the presence of an excess of ascorbic acid. The latter is due to the fact that with these electrodes, two well-defined peaks are seen for the oxidation of ascorbic acid and L-dopa, respectively. Since two separate oxidation peaks were also seen for PEDOT modified electrodes it is evident that the interference of ascorbic acid, which is a main interfering species in biological fluids, can be avoided using PEDOT modified electrodes also in the absence of single-walled carbon nanotubes. Inclusion of the latter, however, gives rise to an additional increase in the oxidation currents. The electroactive surface area of the modified electrodes was found to be approximately one order of magnitude larger than that of the bare platinum microelectrode. The increased surface area ensures that the average current density on the modified electrodes will be significantly smaller than on bare platinum microelectrodes. The latter can explain the decreased overpotentials and better defined voltammograms obtained with the modified electrodes. It can be concluded that the modified electrodes enable sensitive and reproducible determinations of L-dopa even in the presence of a large excess of ascorbic acid with little fouling of the electrodes. The present modified microelectrodes should therefore be well suited for use in in-vivo determinations of selected neurotransmitters in biological systems.

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6. References

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