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Evaluation of physically small *p*-phenylacetate-modified carbon electrodes against fouling during dopamine detection *in vivo*

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ABSTRACT

In this paper, the effectiveness of anionic *p*-phenylacetate film was evaluated in protecting physically small conical-tip carbon electrodes (~2 μm radius and ~4 μm axial length) from fouling during dopamine detection *in vivo*. After characterising *p*-phenylacetate film-modified carbon electrodes in several redox systems *in vitro*, they were found to exhibit an 11% loss in dopamine oxidation signal over a 40-day storage period in ambient laboratory conditions, compared to over a 90% loss at bare carbon electrodes. In addition, by incubating in a synthetic laboratory solution containing the fouling reagents, 1.0% (v/v) caproic acid (a lipid), 0.1% (w/v) bovine serum albumin and 0.01% (w/v) cytochrome C (both are protein) and 0.002% (w/v) human fibrinopeptide B (a peptide), film-modified carbon electrodes showed a 29% reduction in the limit of detection and a 25% decrease in sensitivity for dopamine over 7 days, compared to undeterminable results arising from a severely degraded surface at bare carbon electrodes. During dopamine detection *in vivo*, 70–95% of the dopamine oxidation current remained after the first 40 min of the experiment, and at least 50% over the next 20 min. In contrast, constant degradation in the dopamine oxidation signal was observed at bare carbon electrodes throughout the experiment. An average electrode surface fouling rate of 0.54% min^{-1} was estimated at the *p*-phenylacetate film-modified carbon electrodes during the first 40 min of the experiments.

1. Introduction

Dopamine is a neurotransmitter widely present in the central nervous system, where it modulates several aspects of the brain circuitry. It also plays a crucial role in the functioning of the central, nervous, cardiovascular, renal and hormonal systems. As dopamine can be easily oxidised, electrochemistry, in conjunction with anatomical, physiological and pharmacological evidence, has been developed as a sensitive, real-time detection method for dopamine. For example, research groups of Wightman [1–4], Ewing [5–8], Blaha [9–12] and others [13–15] have significantly contributed to the electrochemical detection of dopamine *in vivo*. A common challenge in electrochemical detection of dopamine *in vivo* is electrode fouling caused by adsorption of hydrophilic high molecular-weight proteins, lipids and peptides present in biological matrices on the electrode, prohibiting dopamine from making direct contact with the electrode surface for electron transfer reaction. This results in a diminishing transient electrode response, distorted voltammetric signals and suppressed electrode sensitivity [16]. Considerable research efforts including fast scan voltammetry [17], a protective organic film on the electrode surface [18], completely altered surface functionalities [19], nanocrystalline diamond coated electrodes [20] have been developed to address electrode fouling problems.

Downard et al. [18] previously demonstrated the anionic *p*-phenylacetate film as an effective coating on 3-mm diameter glassy carbon electrodes for the detection of dopamine. As dopamine exists as a cation (pK_a 8.9) under physiological pH 7.4, dopamine is electrostatically attracted to the film-modified electrodes, giving rise to a dopamine oxidation peak that was sixfold enhanced compared to that obtained at bare electrodes. Similarly, a 1.5-fold enhancement of the dopamine oxidation signal was observed at film-modified carbon microdisc electrodes relative to that at bare microelectrodes. On the other hand, as ascorbic acid is anionic (pK_a 4.1) at pH 7.4, the response of this neurotransmitter was suppressed at the film-modified glassy carbon electrodes. As the oxidised product of dopamine, dopamine quinone, is reduced back to dopamine by ascorbic acid, this gives rise to an amplified dopamine oxidation signal in the presence of ascorbic acid. Therefore, Downard et al. conducted chronoamperometry at a short timescale (20 ms) so that only 18% of the dopamine signal was catalytically enhanced by ascorbic acid. Similar to *p*-phenylacetate, Nafion is also an anionic

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film known to exhibit electrode protection from fouling during electrochemical detection of biological species [21,22]. However, there is a lack of study on the effectiveness of *p*-phenylacetate film in protecting electrodes from fouling, particularly during dopamine detection *in vivo*.

Our laboratory has been developing and characterising carbon electrodes that are physically small for detection of dopamine *in vivo* [23–25]. These electrodes are fabricated by thermally pyrolysing acetylene gas in and on the shank of a quartz capillary already pulled down to a fine tip. Our recent simulation work [26] indicates that these electrodes consist of a cone-shaped carbon film with a typical tip diameter of 2–5 μm and an axial length of 4 μm around the pulled capillary. Owing to the quartz substrate used, these conical-tip carbon electrodes are mechanically strong and their sharp tips aid in easy membrane penetration during implantation in an *in vivo* experiment. Notably, a carbon fibre electrode often used in detecting dopamine *in vivo* is constructed by sealing a μm-sized carbon fibre in a pulled capillary such that an ~100-μm length protrudes the capillary. The insulating plane at this finite fibre-capillary junction limits mass transport of analyte to the base edge of the electrode, whereas the open-ended base edge on a conical-tip electrode is more accessible. Therefore, compared to carbon fibre electrodes of a similar dimension, these conical-tip electrodes displayed an improved signal-to-noise ratio in detecting dopamine *in vivo* [25].

In this study, we aim at evaluating the effectiveness of *p*-phenylacetate film-modified physically small conical-tip carbon electrodes in resisting fouling during dopamine detection *in vivo*. We begin by studying the characteristics of these electrodes in several redox systems including $Ru(NH_3)_6^{3+}$, $Fe(CN)_6^{3-}$ and dopamine, followed by their analytical dopamine detection *in vitro* in the presence of deliberately added fouling reagents such as proteins, peptides, and lipids. Finally, the degree of electrode fouling at *p*-phenylacetate film-modified carbon electrodes during dopamine detection *in vivo* will also be estimated.

2. Experimental

2.1. Reagents

Ultrapure (Milli-Q) water (18.2 MΩ cm at 25 °C) was used to prepare all solutions. Analytical grade dopamine, citric acid, potassium chloride, potassium ferricyanide, sodium phosphate dibasic heptahydrate, hexanoic acid, human fibrinopeptide B, cytochrome *C*, bovine serum albumin and graphite powder were purchased from Sigma Aldrich. Tetrabutylammonium tetrafluoroborate and 4-aminophenyl acetic acid were obtained from Aldrich, while sodium fluoroborate was acquired from Fluka. Hydrochloric acid and diethyl ether were obtained from Univar. Ultra high purity acetylene and nitrogen gases were purchased from BOC Gases, Australia. Hexamineruthenium(III) chloride was obtained from Stremm Chemicals (Newport, USA). All chemicals and reagents were used without further purification. All redox solutions and supporting electrolytes were prepared daily and purged with nitrogen for 5 min preceding any analysis. The compound, 4-phenylacetic acid diazonium fluoroborate, was electrochemically reduced to form a *p*-phenylacetate film on conical-tip carbon electrodes. 4-Phenylacetic acid diazonium fluoroborate was synthesised using a previously reported procedure [27]. The identity of the compound was verified by ^{1}H nuclear magnetic resonance spectroscopy before use.

2.2. Apparatus

Quartz capillaries (1-mm outside diameter, 0.5-mm inside diameter and 75-mm length, Sutter Instruments, Novato, CA) were pulled into a fine tapered end using a Model P-2000 Sutter Puller (Sutter Instrument Co.). Electrochemical measurements involving cyclic voltammetry and chronoamperometry were performed using a low-current potentiostat (eDAQ Pty Ltd., Sydney, Australia) operated using EChem version 2.1.2 software on a PC *via* an E-corder interface (eDAQ Pty Ltd.) A single compartment, three-electrode glass cell that accommodates a Ag|AgCl reference electrode, a platinum wire counter electrode, and either a bare or a *p*-phenylacetate film-modified conical-tip carbon working electrode was used.

2.3. Fabrication of conical-tip carbon electrodes

The procedure for fabricating physically small conical-tip carbon electrodes was as reported by McNally and Wong [24]. Briefly, quartz capillaries were pulled down to a fine tip using a laser micropipette puller. The pulled quartz capillary was housed in a larger nuclear magnetic resonance quartz tube such that acetylene gas was delivered into the former at 50 kPa and a nitrogen stream was counter-flowing through the latter at 60 mL min^{-1}. The acetylene gas was thermally pyrolysed to form carbon in and on the shank of pulled capillaries. Following pyrolysis, the capillary was left to cool for 20 s before its tip was rinsed with distilled water. To accomplish electrical connection, graphite powder was packed and a tin-coated copper fuse wire (10 A) was introduced through the larger end before the capillary was sealed with epoxy.

2.4. Electrochemical deposition of p-phenylacetate

The procedure reported by Downard et al. [18] was adopted to electrochemically deposit a *p*-phenylacetate film on conical-tip carbon electrodes. In this procedure, a 5.0 mM solution of 4-phenylacetic acid diazonium fluoroborate was prepared in 0.1 M Bu_4NBF_4 in acetonitrile. Then, 20–50 repeated cyclic voltammetric scans of tetrabutylammonium tetrafluoroborate at carbon electrodes in acetonitrile were carried out from 200 mV to −1200 mV at 100 mV s^{-1} to electrochemically reduce phenylacetate salt on the electrode surface. The electrodes were sonicated in acetone for 5 min before being dried and stored for analysis.

2.5. In vivo dopamine detection

All experiments were conducted by adhering to ethics approved by the Animal Ethics Committee at Macquarie University. A male Sprague Dawley rat (303–330 g) was mounted on a stereotaxic frame and housed inside a Faraday cage to isolate noise. Next, a concentric bipolar stimulating electrode was implanted into the ventral tegmental area. A 31-g stainless-steel guide infusion cannula was implanted in the left substantia nigra pars compacta, and an Ag|AgCl reference and stainless-steel auxiliary electrode combination was placed in surface contact with contralateral cortical tissue approximately 2.0 mm posterior to bregma. Either a bare or a *p*-phenylacetate film-modified conical-tip carbon working electrode was then implanted in the left striatum. Approximately 5 min following implantation of the recording electrode, a series of 0.5 ms duration cathodic monophasic current pulses (800 μA) was delivered to the stimulating electrode *via* an optical isolator and programmable pulse generator. Data acquisition was conducted using a potentiostat. A fixed potential of +0.9 V was applied and the dopamine released was chronoamperometrically monitored for 60 min. Peaks were compared at the start and end of the monitoring period to evaluate the degree of fouling. The current was sampled at 10,000 bits s^{-1}. Where needed, Gaussian peak fitting was performed to distinguish overlapping signals. All signals were corrected for the 50 Hz mains cycle contribution.

2.6. Data analysis

Waveslopes of all cyclic voltammograms were estimated from a plot of potential *versus* $\log_{10}[(I_{lim} - I)/I]$ (where I denotes current at a specific potential and I_{lim} the limiting current on the voltammogram), with the half-wave potential ($E_{½}$) being the intercept on the potential axis. The statistical significance of all correlation coefficients at the 95% confidence level was evaluated based on Student's t-test. Uncertainties associated with the slope and ordinate intercept of all linear plots were expressed as confidence intervals at the 95% level.

3. Results and discussion

3.1. p-Phenylacetate film-modified conical tip carbon electrodes

In this work, conical-tip carbon electrodes used for detection of dopamine were fabricated by pyrolysing acetylene in and on the shank of a quartz capillary that was pulled down to a fine tip. As previously reported by us [23], only electrodes that displayed a sigmoidal-shaped cyclic voltammogram in 1.0 mM $Ru(NH_3)_6^{3+}$ (1.0 M KCl supporting electrolyte) with a small charging current between the forward scan and the backward scan were used in further work. Based on analysis of chronoamperometric results [26], the average radius of these electrodes was estimated to be 2.1 μm with a standard deviation of 0.5 μm and an average axial length of 4 μm with a standard deviation of 0.1 μm (N = 14).

3.2. Electrodeposition of p-phenylacetate film

Next, *p*-phenylacetate film was deposited on conical-tip carbon electrodes by performing multiple cyclic voltammetry of 0.01 M 4-phenylacetic acid diazonium fluoroborate in 0.1 M Bu_4NBF_4 in acetonitrile. The cyclic voltammogram obtained in 0.1 M Bu_4NBF_4 in acetonitrile at an electrode, displayed in Fig. 1(a), shows a featureless voltammogram, indicating a lack of oxidation or reduction of any species in the supporting electrolyte that would otherwise affect the subsequent electro-modification of the electrode. Cyclic voltammetry was repeated in 0.01 M 4-phenylacetic acid diazonium fluoroborate in 0.1 M Bu_4NBF_4 (in acetonitrile) and the voltammogram obtained is shown in Fig. 1(b). In this voltammogram, a reduction current peak was observed between −0.6 V and −1.2 V. With subsequent cyclic voltammetric scans, this reduction current was observed to decrease. This was attributed to the formation of a film on the electrode surface, inhibiting further reduction of 4-phenylacetic acid diazonium fluoroborate at the electrode surface [28]. Typically, a total of 20–50 scans was conducted to obtain a featureless voltammogram, indicating film coverage on the electrode.

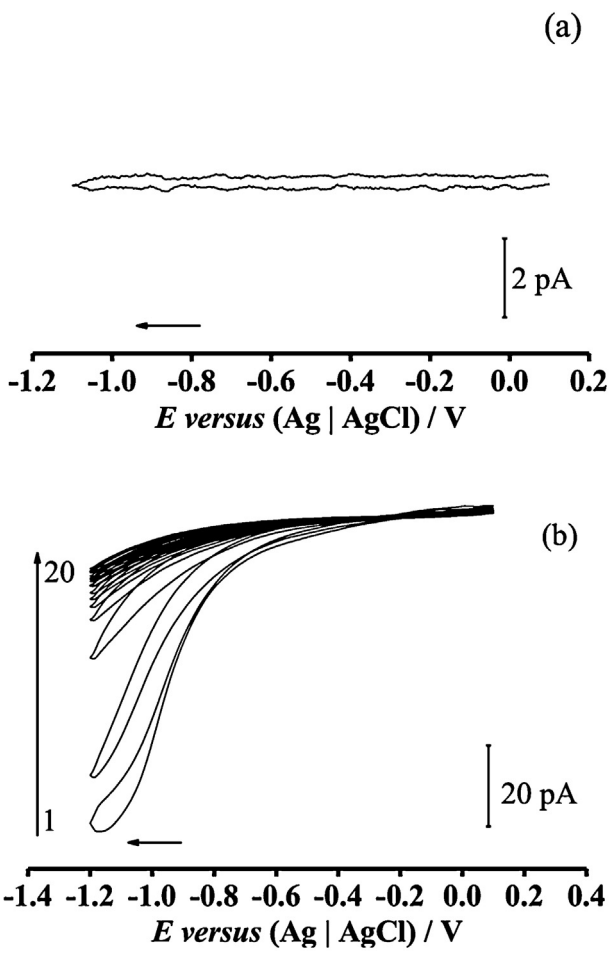

Fig. 1. Cyclic voltammetry of (a) 0.1 M Bu_4NBF_4 in acetonitrile and (b) 0.01 M 4-phenylacetic acid diazonium fluoroborate in 0.1 M Bu_4NBF_4 in acetonitrile. Scan rate: 100 mV s^{-1}. Horizontal arrows indicate initial potential scan direction.

3.3. Electrochemical characterisation of p-phenylacetate film-modified carbon electrodes

In our work, we have initially characterised *p*-phenylacetate film-modified conical-tip carbon electrodes by comparing the cyclic voltammeric responses of these electrodes to those of bare conical-tip carbon electrodes in three redox systems: (i) 1.0 mM $Ru(NH_3)_6^{3+}$ in 1.0 M KCl (pH 7.0), (ii) 1.0 mM $Fe(CN)_6^{3-}$ in 1.0 M KCl (pH 7.0) and (iii) 1.0 mM dopamine in pH 7.4 citrate/phosphate buffer. All results obtained are displayed in Fig. 2.

As shown in Fig. 2(a), a sigmoidal-shaped cyclic voltammetric signal for $Ru(NH_3)_6^{3+}$ obtained with a 54% (with a standard deviation of 7%; N = 6) increase in the limiting current at the film-modified carbon electrode, compared to the corresponding bare carbon electrode. As expected, the incorporated anionic carboxylic-rich *p*-phenylacetate film attracts the cationic redox marker towards the electrode for reduction, giving rise to a larger limiting current. We then determined the waveslope and $E_{½}$ of the cyclic voltammograms to assess the electrochemical reversibility of the reaction at the electrodes. The results are tabulated in Table 1. Based on a 4% increase in the waveslope and a 4% decrease in $E_{½}$, the *p*-phenylacetate film appeared to exhibit very little effect on the electrochemical reversibility of $Ru(NH_3)_6^{3+}$ reduction at *p*-phenylacetate film-modified carbon electrodes relative to bare carbon electrodes.

Next, *p*-phenylacetate film-modified electrodes were characterised using $Fe(CN)_6^{3-}$ and the results displayed in Fig. 2(b) indicate a 4% decrease (with a standard deviation of 1%; N = 6) in the limiting current of $Fe(CN)_6^{3-}$ at film-modified electrodes relative to bare carbon electrodes. This is attributed to electrostatic repulsion of $Fe(CN)_6^{3-}$ by the negatively charged film. However, the small decrease could be due to an uneven *p*-phenylacetate film on the electrodes, possibly with pinholes within the coating that allowed the $Fe(CN)_6^{3-}$ reduction to take place. As shown in Table 1, a 24% increase in waveslope following film application indicates improved reversibility of the reduction reaction, and the $E_{½}$ remained relatively unchanged from 107 mV to 102 mV.

Finally, cyclic voltammograms of dopamine are depicted in Fig. 2(c). The limiting oxidation current at the *p*-phenylacetate film-modified electrode was observed to increase by ~71% (with 3% standard deviation; N = 7), relative to the corresponding bare carbon electrode. Here, cationic dopamine is electrostatically attracted by anionic carbonyl and ether functionalities on the film, yielding

Table 1
Waveslope and $E_{½}$ estimated from cyclic voltammograms of the redox systems at bare and *p*-phenylacetate film-coated conical-tip carbon electrodes.

Redox system		Waveslope (mV)	$E_{½}$ *versus* Ag|AgCl (mV)
$Ru(NH_3)_6^{3+}$	Bare carbon electrodes	68 (11)[a]	−166 (13)[a]
	Film-coated carbon electrodes	71 (4)	−173 (8)
$Fe(CN)_6^{3-}$	Bare carbon electrodes	224 (49)	107 (49)
	Film-coated carbon electrodes	279 (76)	102 (78)
Dopamine	Bare carbon electrodes	124 (7)	258 (25)
	Film-coated carbon electrodes	104 (14)	252 (12)

[a] All parenthesised values denote standard deviations estimated from 6 repeated experiments.

an increased oxidation current at film-modified electrodes. As indicated in Table 1, the waveslope decreased by 16% and the $E_{½}$ decreased by 2% after the *p*-phenylacetate film was immobilised on the electrodes. These suggest *p*-phenylacetate film exhibited minimal effect on the reversibility of dopamine oxidation at these electrodes.

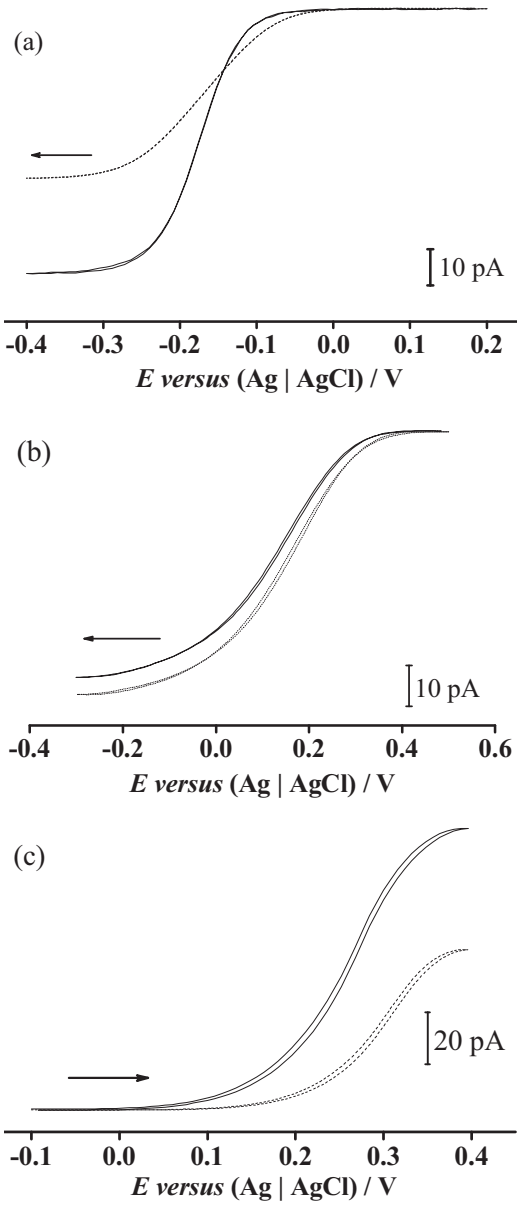

Fig. 2. Cyclic voltammetry of (a) 1.0 mM $Ru(NH_3)_6^{3+}$ in 1.0 M KCl supporting electrolyte, (b) 1.0 mM $Fe(CN)_6^{3-}$ in 1.0 M KCl supporting electrolyte, and (c) 1.0 mM dopamine in pH 7.4 citrate/phosphate buffer at *p*-phenylacetate film-modified (solid line) and bare (dashed line) conical-tip carbon electrodes. Scan rate: 100 mV s^{-1} in all voltammograms.

3.4. Stability of p-phenylacetate film-modified conical-tip carbon electrodes

To determine stability, *p*-phenylacetate film-modified carbon electrodes were stored under laboratory conditions (1 atm pressure, 25 °C temperature) for a period of 40 days, after which their cyclic voltammetric responses to dopamine were determined. These electrodes displayed approximately 11% ($N = 4$) decrease in their response to dopamine, compared to over 90% loss in signal at bare carbon electrodes. There was no significant deterioration of the voltammetric character (waveslope = 95 mV, $E_{½}$ = 239 mV), compared to bare carbon electrodes (see Table 1). These results support stability of the film-modified electrode surface for at least a 40-day period.

3.5. Analytical performance of p-phenylacetate film-modified electrodes

Dopamine calibration based on I_{lim} of dopamine cyclic voltammograms at both *p*-phenylacetate film-modified electrodes and bare carbon electrodes was then established. In these experiments, a 1.7-fold increase in the dopamine oxidation current was measured at *p*-phenylacetate film-modified electrodes compared to that at bare electrodes. This result is similar to a 1.5-fold increase achieved at carbon microdisc electrodes by Downard et al. [18]. Fig. 3 shows the calibration plots with the corresponding linear expression and correlation coefficient obtained at these electrodes, which were of different dimensions from those used to obtain the data in Fig. 2(c). At film-modified electrodes, the limit of detection (based on a signal-to-noise ratio of 3) and sensitivity (from the slope of the calibration plot) were estimated to be 541 pM and 16 pA nM^{-1}, respectively. The limit of detection values in our study compare favourably with values reported elsewhere for other film-modified electrodes, such as 80 nM at non-activated Nafion [29], 5 nM using Nafion/carbon nanotube composite [30,31], 2.5 nM at Nafion/multi-walled carbon nanotubes [22] and 10 nM at Pt electrodeposited Nafion-coated glassy carbon electrodes [32]. Thus, *p*-phenylacetate film-modified conical-tip electrodes are expected to be able to detect low dopamine concentration approaching 0.2–2.0 μM generally encountered *in vivo* [33,34].

3.6. Electrode performance in solutions containing fouling reagents

Electrode fouling often causes distortion of the voltammetric signal and suppression of electrode sensitivity. It is thus useful to evaluate and compare the limit of detection and sensitivity before and after any fouling takes place. Accordingly, we have calibrated electrodes using cyclic voltammetry of dopamine in citrate/phosphate buffer before and after they were incubated in a solution consisting of 1.0% (v/v) caproic acid (a lipid), 0.1% (w/v)

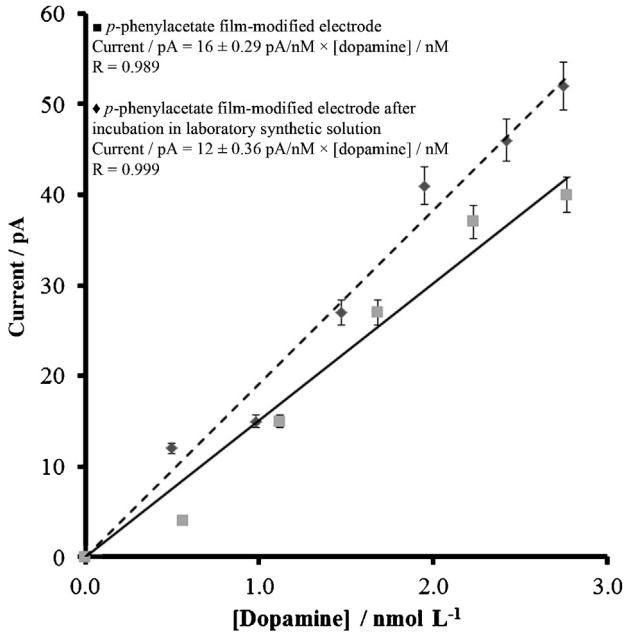

Fig. 3. Calibration plots based on I_{lim} of dopamine cyclic voltammograms in pH 7.4 citrate/phosphate buffer solution at *p*-phenylacetate film-modified conical-tip carbon electrodes before and after a 7-day incubation in 0.1% (w/v) bovine serum albumin + 1.0% (v/v) caproic acid + 0.002% (w/v) human fibrinopeptide B + 0.01% (w/v) cytochrome C in pH 7.4 citrate/phosphate buffer. Scan rate: 100 mV s^{-1}.

bovine serum albumin and 0.01% (w/v) cytochrome C (both are protein) and 0.002% (w/v) human fibrinopeptide B (a peptide) for 7 days. Such a solution comprising these large molecular weight hydrophilic species was used to mimic a simple matrix of extracellular fluid in a brain. The calibration plots, and their linear expressions, obtained at film-modified electrodes are shown in Fig. 3. These electrodes yielded a limit of detection of 385 pM and sensitivity of 12 pA nM^{-1} dopamine after a 7-day incubation, compared to 541 pM and 16 pA nM^{-1} before they were incubated. Unfortunately, upon incubation in the laboratory synthetic solution, the dopamine calibration plot obtained at bare carbon electrodes displayed widely scattered data points with a statistically non-significant correlation coefficient. Hence the limit of detection and sensitivity could not confidently be determined. We attribute this to extreme surface degradation, leading to almost non-observable sensitivity to dopamine. These results show that the film-modified electrodes exhibited a degree of fouling resistance in the laboratory synthetic solution compared to bare electrodes.

3.7. Dopamine detection at p-phenylacetate film-modified electrodes in vivo

In order to provide a more realistic assessment, the effectiveness of *p*-phenylacetate film-modified carbon electrodes in resisting fouling was also evaluated in *in vivo* dopamine detection. Electrode fouling is among factors responsible for distortion of the dopamine oxidation signal [23,35]. In these experiments, electrodes were implanted in the left striatum of a rat brain, where abundant dopamine vesicles are located, and the oxidation signal between the time of implanting the electrode in the striatum and a defined period later was monitored to assess the degree of fouling. In these experiments, the dopamine oxidation signals arising in response to electrical stimulation were recorded at the start of stimulations and every 15 min subsequent to implantation for up to 60 min. The first stimulation was commenced within 1 min of implanting the electrode in the striatum. For clarity, only Gaussian-fitted

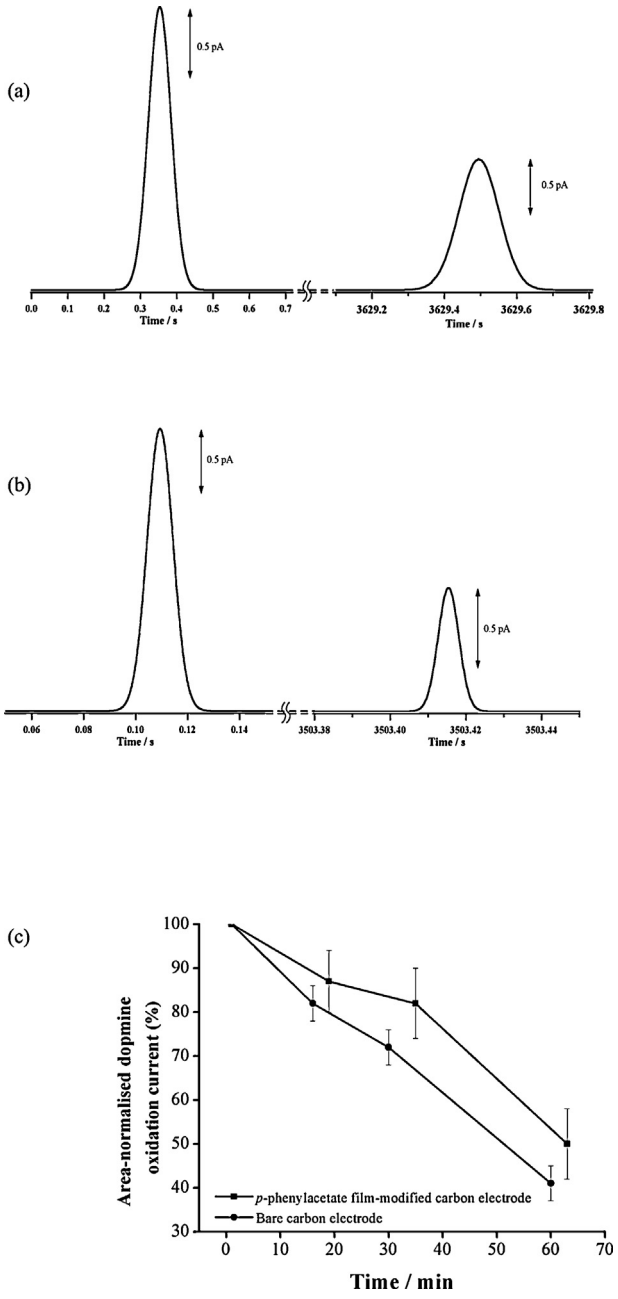

Fig. 4. Gaussian-fitted dopamine oxidation signals obtained upon repeated electrical stimulations in the rat striatum at the start and after 60 min of monitoring at (a) *p*-phenylacetate film-modified and (b) bare conical-tip carbon electrodes; (c) a plot of electrode area-normalised current measured at 60-min interval at *p*-phenylacetate film-modified and bare conical-tip carbon electrodes implanted in the rat striatum.

responses obtained at 1 min and 60 min after electrical stimulations are shown in Fig. 4(a), ignoring those obtained at repeated 15-min simulations. Prior to stimulation, negligible current was flowing in the absence of any stimulus from 0.00 s to approximately 0.26 s. In the brain, upon appropriate stimulation such as an action potential, dopamine was released at the synaptic cleft between two neurons. As it reached the implanted working electrode in the striatum, dopamine was immediately oxidised, giving rise to an increase in oxidation current, followed by a current decay upon depletion effects through processes such as diffusion away from the synapse, interaction with cell receptors or uptake by membrane-bound proteins [36]. With increasing adsorption of large molecular weight hydrophilic species on the electrode surface, less dopamine

oxidation occurs at the surface, leading to a transient decline in the oxidation peak feature height. Therefore, the extent of fouling at an electrode surface can be evidenced by a gradual decrease in the oxidation peak height. The corresponding results obtained at bare carbon electrodes are displayed in Fig. 4(b), where a transient decrease in peak current is apparent.

Fig. 4(c) shows a plot of the mean percentage of electrode area-normalised oxidation current remained against time over which detection of dopamine was monitored at several *p*-phenylacetate film-modified electrodes implanted in the rat brain. A limited number of rats used in the experiments has most likely caused the fluctuations of results observed in the figure. Nonetheless, we assume negligible fouling took place between the time the electrode was implanted and measurement of dopamine after the first series of stimulations. Accordingly, the current measured was assigned 100%. Then, approximately 70–95% of current was still measurable after the first 40 min, while at least 50% of the current was evident after the next 20 min, indicating more severe fouling took place towards the end of the experiment. Typically, the electrodes displayed ~19% to 56% of dopamine current after 40 min. Notably, the varied percentage of dopamine current could have been caused by varied reproducibility among such films on different electrodes [37]. Finally, the rate of fouling, based on the slope of the linear plot of the averaged current decay results depicted in Fig. 4(c) was estimated to be 0.54% min^{-1} during the first 40-min period. Results obtained at bare carbon electrodes, also shown in Fig. 4(c), indicate a constant degradation in oxidation signal throughout the experiment, suggesting that severe fouling was occurring at the bare carbon surface without any impedance. The absence of a protective cover on electrodes was expected to ease adsorption of hydrophilic molecules that in turn retarded dopamine oxidation. In Fig. 4(c), the average rate of bare carbon electrode surface fouling over the first 40-min period was estimated to be 0.94% min^{-1}. These results indicate that incorporation of *p*-phenylacetate film on an otherwise bare carbon surface does provide an initial degree of protection from fouling, and enables the analysis of dopamine *in vivo*. The rate of fouling, as determined from rate of deterioration of the dopamine signal is also slower at the *p*-phenylacetate film-modified surface.

4. Conclusions

In this work, *p*-phenylacetate film-modified and bare conical-tip carbon electrodes were used to detect dopamine *in vivo*. The former was found to offer some protection against fouling (0.54% min^{-1}) relative to the latter (0.94% min^{-1}) during the first 40-min after implantation. Initially, these modified electrodes displayed electrochemical behaviour consistent with that expected of an anionic film. In addition, they showed stability upon storage for at least 40 days, while bare electrodes demonstrated 90% loss in dopamine signal in the same period. Furthermore, when incubated in a laboratory synthetic solution containing protein, peptides and lipids, film-modified electrodes demonstrated only slight changes in limit of detection of and sensitivity to dopamine. This compares favourably to bare carbon electrodes, which demonstrated an unmeasurable limit of detection for and sensitivity towards dopamine. These findings suggest that the absence of the protective *p*-phenylacetate film on bare carbon electrode results in extreme surface degradation.

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