**Compressed multiwall carbon nanotube composite electrodes
provide enhanced electroanalytical performance for determination
of serotonin**

Aidan Fagan-Murphy and Bhavik Anil Patel

School of Pharmacy and Biomolecular Sciences, University of Brighton, UK

Corresponding Author

*Email: b.a.patel@brighton.ac.uk, Fax: +44 (0)1273 643 333

Abstract

Serotonin (5-HT) is an important neurochemical that is present in high concentrations within the intestinal tract. Carbon fibre and boron-doped diamond based electrodes have been widely used to date for monitoring 5-HT, however these electrodes are prone to fouling and difficult to fabricate in certain sizes and geometries. Carbon nanotubes have shown potential a suitable material for electroanalytical monitoring of 5-HT but can be difficult to manipulate into a suitable form. The fabrication of composite electrodes is an approach that can shape conductive materials into practical electrode geometries suitable for biological environments. This work investigated how compression of multiwall carbon nanotubes (MWCNTs) epoxy composite electrodes can influence their electroanalytical performance. Highly compressed composite electrodes displayed significant improvements in their electrochemical properties along with decreased internal and charge transfer resistance, reproducible behaviour and improved batch to batch variability when compared to non-compressed composite electrodes. Compression of MWCNT epoxy composite electrodes resulted in an increased current response for potassium ferricyanide, ruthenium hexaammine and dopamine, by preferentially removing the epoxy during compression and increasing the electrochemical active surface of the final electrode. For the detection of serotonin, compressed electrode has a lower limit of detection and improved sensitivity

31 compared to non-compressed electrodes. Fouling studies were carried out in 10 μ M
32 serotonin where the MWCNT compressed electrode was shown to be less prone to
33 fouling than non-compressed electrodes. This work indicate the compression of
34 MWCNT carbon-epoxy can result in a highly conductive material that can be
35 moulded to various geometries, thus providing scope for electroanalytical
36 measurements and the production of a wide range of analytical devices for a variety
37 of systems.

39 1.1 Introduction

40 Carbon electrodes are found in a wide array of different applications ranging from
41 biochemical to environmental analysis where they are involved in the sensing of a
42 variety of important molecules [1-3]. These carbon electrodes are widely used due to
43 their compatibility to a variety of matrices and are perceived to be more
44 biocompatible than metal based electrodes. Previously glassy carbon, carbon fibre
45 and graphite based electrodes have been most commonly utilised [4, 5], however
46 these carbon materials are known to be prone to fouling [6, 7]. Newer materials such
47 as boron-doped diamond, carbon nanotubes and graphene have emerged as
48 materials that may have the potential to be utilised for electrochemical
49 measurements in complex matrices [6, 8-13].

50 Serotonin (5-HT) is an important neurotransmitter that is known to play a key role in
51 the central and enteric nervous system [14, 15]. However, monitoring of serotonin in
52 the intestinal tract using electroanalytical approaches has recently been conducted
53 and at present are hindered by two key issues. Firstly concentrations are high,
54 micromolar levels, in the intestinal tract [16, 17]. Secondly, serotonin oxidative by-
55 products are known to have a great affinity to adsorb on the electrode surface, this
56 limits the lifespan of the electrode by preventing electron transfer at the electrode
57 surface as these by-products occupy the electrochemically active sites[6, 18]. These
58 two issues are linked, as fouling by 5-HT has also shown to be flux dependent
59 becoming a significant problem when monitoring 5-HT in the intestinal tract [19, 20].
60 To date the majority of electrodes used for detection of 5-HT in the intestinal tract are
61 carbon fibre microelectrodes [21, 22], but these are prone to electrode fouling which
62 means that they can only be used for a limited time period before they are rendered

unresponsive. Recently, boron-doped diamond electrodes have been utilised for measurements of 5-HT from intestinal tissue and these have shown promise in reducing the degree of electrode fouling that occurs [23, 24]. However these electrodes are difficult to fabricate and are limited to certain sizes and geometries making them less applicable for monitoring in all biological environments. In recent years, some studies in other biological areas have utilised carbon nanotubes to monitor both dopamine and serotonin as an alternative to other commonly utilised carbon based materials [11, 25, 26]. A significant hurdle in using carbon nanotubes is the difficulty in fabricating them into a practical electrode geometry that can be used for biological sensing. Work in this area has approached this problem by either growing carbon nanotubes on surfaces or by incorporating them within polymers to coat on the surface of electrodes [11, 25-27].

Composite electrodes have for many years provided a means of making conventional electrode geometries from conductive powders [28-30]. Conductive materials, mainly focused graphite to date, are mixed with non-conductive polymers or resins and cast in moulds to create solid state electrodes. This method of electrode fabrication has been widely used but often results in variable electrode behaviour between different batches. These electrodes also have reduced conductive performance due to the non-conductive polymers used to provide structural support to the conductive material. Enhanced conductivity is often difficult to obtain due to limitation on the percentage of conductive material that can be used to create a composite electrode without compromising the structural stability. Limited studies have investigated if nanotube composite electrodes [31-33] offer enhanced electrochemical performance for stable monitoring of key neurochemicals.

In this manuscript we investigate if compression of multiwall carbon nanotube epoxy resin electrodes can enhance electroanalytical performance and display improved electrochemical behaviour. We utilised multi-wall carbon nanotube (MWCNT) carbon nanotubes and carried out a light and heavy compression of the composite material. These electrodes were then all investigated for their electrochemical performance and surface reproducibility. Studies were also carried out to investigate the sensitivity and limit of detection for the various electrodes against 5-HT. Fouling studies with serotonin were conducted to understand if the compressed electrode showed enhanced performance to normally fabricated composite electrodes.

96

97 **1.2 Experimental**

98 **1.2.1 Chemicals and solutions**

99 Potassium chloride, ruthenium hexaammine trichloride, potassium ferricyanide,
100 dopamine hydrochloride, serotonin (Sigma-Aldrich, USA), epoxy resin and hardener
101 (Robnor Resins Ltd, UK) were used as received. Solutions for potassium ferricyanide
102 and ruthenium hexamine were prepared in 1 M KCl while dopamine and serotonin
103 were prepared in 0.1 M phosphate buffered saline (PBS, pH 7.4). MWCNT-epoxy
104 composites sensors were prepared from 10-20 nm outside diameter, 3-6 nm inside
105 diameter length 10-30 μm MWCNT (obtained from ALIT, Ukraine). Nanotubes were
106 produced using chemical vapour deposition. Removal of unwanted materials was
107 accomplished by refluxing in 4 M NH_4OH for 36 hours.

108

109 **1.2.2 Fabrication of various MWCNT-epoxy composite sensors**

110 Three individual fabrication methods were employed for the production of these
111 MWCNT-epoxy composites. Each approach consisted with a 25% MWCNT : 75%
112 Epoxy resin (w/w) mixture. This mixture was carried out by hand and often results in
113 the formation of a paste (Supplementary Figure 1). This mixture was then placed into
114 a 5 cm section of PTFE tubing (internal diameter of 3.2 mm, external diameter 6.4,
115 working pressure 375 psi). Three different fabrication methods were employed; For
116 the 'Std' method half the PTFE tubing, 2.5 cm was packed with the MWCNT:epoxy
117 resin mixture and left to set, the 'Gel' method used previously prepared 3.2 mm
118 diameter, 2 mm thick epoxy disks to compress the material slightly (only 1-2 mm
119 reduction in the initial packaging was observed) by hand. The 'ViC' method
120 employed previously prepared 3.2 mm diameter, 2 mm thick epoxy disks and 3.2
121 mm diameter ~3 cm long rods to provide compression through the use of a vice to
122 the composite reducing the packed content to half the original volume. The tubing
123 had a working pressure value of 375 psi and the pressure was kept between 300-
124 375 psi to avoid deforming the tubing. Figure 1 displays the composite material
125 method before and after their fabrication steps. The composite mixtures were left to
126 set for 48 hours at ambient room temperature.

The MWCNT-epoxy composite-packed tubing was cut into 3.2 mm diameter, 2 mm thick disks using a diamond wafer blade (Buehler saw). Electrical contact was achieved using a wire connected to one side of the composite disc using silver-loaded epoxy resin (Circuit Works, RS Components, Corby, UK). The finished electrode was produced by encasing the electrode in epoxy in a cylindrical mould reminiscent of a commercial electrode casing. This was left to set for 48 hours at ambient room temperature in order for the epoxy to set. It was then cut once again at its base, removing 1 mm from the disks' thickness, with the diamond wafering blade to expose a MWCNT-epoxy composite disc electrode and to ensure that the edge of the composite disc were sealed within the epoxy. The dimensions of the final electrode disk are 1 mm in thickness and 3.2 mm in diameter. The electrode was polished sequentially in 1, 0.3 and 0.05 micron alumina slurry to reduce surface roughness to acceptable levels.

1.2.3. Electrochemical assessment of MWCNT electrodes

All voltammetric measurements and data extraction were carried out using CHI 1206 potentiostat (CH Instruments, Austin, TX) controlled using CH Instruments software. All electrochemical measurements were carried out with a three electrode system, which consisted of a Ag|AgCl (3 M KCl) reference electrode, a platinum wire auxiliary electrode and the various different MWCNT-composite working electrodes as well as glassy carbon (GC) and boron doped diamond (BDD) commercial electrodes for comparison.

Electrochemical impedance spectroscopy (EIS) was performed at 270 mV with an amplitude of 10 mV at a frequency from 1×10^6 Hz to 1 Hz in an aqueous solution of 0.5 mM potassium ferricyanide and 0.5 mM potassium ferrocyanide in 1 M KCl. The working electrodes was either a Std, Gel, ViC fabricated composite electrode or a commercial boron-doped diamond (BDD) or a glassy carbon (GC) electrode with a Pt counter electrode and 3 M KCl Ag/AgCl reference electrode.

The electrochemical characteristics of the different MWCNT-epoxy composite electrodes were assessed with 1 mM potassium ferricyanide and 1 mM ruthenium hexaammine trichloride in 1 M KCl and 1 mM dopamine in 0.1 M PBS. 1 mM

potassium ferricyanide was assessed between -0.2 and 0.6 V at a 0.1 V s^{-1} scan rate. Ruthenium hexaaamine was assessed between 0.2 and -0.5 V at a 0.1 V s^{-1} scan rate. For dopamine, voltammograms were carried out between -0.5 and 1 V at a 0.1 V s^{-1} scan rate. Measurements of the background capacitance of these electrodes were estimated from the background currents at 0 V and were performed in 1 M KCl between -0.8 and 0.8 V at a scan rate of 0.1 V s^{-1} . Prior to electrochemical studies the electrodes were polished with 0.05 micron alumina slurry then washed with D.I. water.

1.2.4 Investigation of electrode surface variability

The variability of the heterogeneous surface of the different MWCNT-epoxy composite electrodes was assessed using 1 mM potassium ferricyanide in 1 M KCl through cyclic voltammetry. Each electrode was repeatedly polished with 0.05 micron alumina between scans to remove the upper layer of the composite material and exposing the underlying layers. This was repeated five times with three electrodes produced from each of the three different fabrication techniques.

1.2.5 Electrode sensitivity and stability of 5-HT detection

The sensitivity of each of the MWCNT composite electrodes was assessed by addition of varying concentration of 5-HT from 0.25 to 10 μM into 0.1 M PBS buffer under convection. Amperometric measurements were carried out at +0.7 V vs. Ag|AgCl. The change in the current amplitude from baseline was recorded for each step change and a calibration response was obtained.

To observe fouling of the electrodes the change in the electrochemical behaviour response of 1 mM potassium ferricyanide was monitored following exposure to 5-HT. Cyclic voltammograms of potassium ferricyanide were performed before and after the electrode had been subjected to a potential of +0.7 V (versus Ag|AgCl, 3 M KCl) in a solution of 10 μM 5-HT in 0.1 M PBS for 30 minutes. Following fouling of the electrode, the ease of electrode rejuvenation after fouling was assessed by 2 wipes

of the fouled electrode surface with an ethanol soaked lens cloth and conducting cyclic voltammograms in potassium ferricyanide.

1.2.6 Data Analysis

Electrode capacitance was measured as the difference in the current observed at 0 V over the scan rate. For electrochemical data, the anodic peak potential, anodic peak current or difference in the amperometric current were monitored and compared. Comparison of the data was carried out with either one-way or two-way ANOVA test with ad hoc Tukey tests.

1.3 Results and Discussion

1.3.1 Characterisation of MWCNT composite

Figure 1 shows representative examples of the fabricated electrode casts under the various types of compression. All composite material shown in Figure 1A consist of 25% MWCNT and 75% epoxy resin (w/w) mixture, where initial half of the polymer tubing was packed initially with the material. Following the various approaches of compression there are clearly differences in the Gel and ViC electrodes. Based on the volume loss on compression no significant reduction in volume is observed in Gel electrodes, however an approximately 50% reduction in the packed volume was observed for the ViC electrode, which on visual expectation was felts to be predominantly the epoxy resin component. During visual inspection of the electrode surface the roughness of the exposed electrode area was greatly reduced in Gel and even further reduced in ViC electrodes compared to Std electrodes.

Figure 2A displays a representative CV trace in 1 M KCl on each electrode type to monitor the capacitive current of the electrode. The capacitance was measured as the difference in the current observed at 0 V over the scan rate. There is a significant increase in the capacitive current of the ViC electrode when compared to the Std electrode ($p < 0.05$, $n = 6$, Figure 2C). There is a greater amount of variability on the Gel electrodes, which may be due to the heterogeneity with the composite material at this degree of compression. The increased capacitive signal at the ViC electrode

is suggestive of a greater electroactive surface area as the observed current is twice the size of that observed at the Std electrodes. The ViC electrode also shows a greatly increased capacitive current in comparison to GC and BDD electrodes ($p < 0.001$, $n = 6-4$).

Figure 2B displays the real and imaginary impedance for the different electrodes. The Std and Gel electrodes display similar regions under kinetic control. ViC electrodes display very low dependence on kinetic effects and are almost entirely mass transfer dependent for the frequencies examined. The charge transfer resistance (R_{ct}) for the ViC electrode was significantly lower than that for the Std and Gel electrodes ($p < 0.05$, $n = 3$, Figure 2E). The Std and Gel electrodes shows a high degree of variability in the response, which is most likely due to the heterogeneous nature of the electrode surface, which can potentially hinder electron transfer. The ViC electrode had a significantly lower R_{ct} than the GC electrode ($p < 0.05$, $n = 3$) and also BDD electrode ($p < 0.01$, $n = 3$). It is expected that the BDD has a higher R_{ct} as the material has sluggish kinetics, but this result indicates the ViC electrode is more favourable for electron transfer than GC electrodes, which supports the enhanced performance of MWCNTs.

The internal resistance (R_{Ω}) for the ViC electrode was significantly lower than that of Std ($p < 0.001$, $n = 3$, Figure 2D) and the Gel electrode ($p < 0.05$, $n = 3$). Std and Gel electrodes show similar R_{Ω} values, but have high variability suggestive of their heterogeneous composite nature. The ViC electrode however showed values similar to commercial BDD and GC electrode indicative of minimal internal resistance and thus supports a uniform conductive pathway from electrical contact to the solution. Recently the number of connections between individual CNTs and the porosity of the overall mass has been shown to affect the overall resistance of the sample [13, 34].

1.3.2 Standard redox probe behaviour at the different electrodes

Three redox couples were used to examine the electrochemical behaviour of these composite electrodes, ruthenium hexaamine, potassium ferricyanide and dopamine; these cover surface insensitive, surface sensitive and adsorption –based processes

respectively. Figure 3 shows voltammetric responses on the three composite electrodes in the various redox couples.

For potassium ferricyanide the currents observed at the ViC electrode was significantly greater than those observed at the Std or Gel electrode ($p < 0.001$, $n = 3$). The anodic peak current was $16.4 \pm 0.5 \mu\text{A}$ on the ViC electrode and was approximately half this for the Std and Gel electrode. There was no significant difference in the current observed between the Std and Gel electrode. Kinetic studies examining the behaviour of potassium ferricyanide display anodic peak current values proportional to the square root of the scan rate indicating a non-surface confined process at all electrode types. For the ViC electrode, double the anodic peak current was observed in comparison to the Std and Gel electrodes. This would suggest that the compression approach is potentially doubling the percentage of conductive material present, as all electrodes were fabricated with 25 % MWCNT, by preferentially removing the epoxy during compression. This may be due to the mesh of nanotubes creating an interspersed mesh to selectively allow for the loss of the epoxy binder. Assuming that the compression methods have doubled the amount of conductive material present, we investigated if a 50 % MWCNT would achieve the same response. However as shown in Supplementary Figure 1 it would not be feasible to fabricate a 50 % MWCNT composite electrode as the epoxy agglomerates nanotubes into larger particles but overall does not provide sufficient capacity for the binding of the powder to make a composite electrode. Therefore not only does this approach improve the conductive properties of the composite electrode but also provides a higher load of conductive material which is normally not feasible during normal mixing of conductive and non-conductive. The anodic to cathodic peak separation (ΔE_p) was measured as to study the electrochemical performance of the electrode. The ΔE_p was 75 ± 4 for the ViC electrode, 113 ± 9 mV for the Gel electrode and 116 ± 3 mV for the Std electrode. There was a significant reduction in the ΔE_p on the ViC electrode, when compared to the Gel and Std electrode when studying potassium ferricyanide ($p < 0.001$, $n = 3$).

A similar trend was observed with ruthenium hexaammine, where the ViC electrode displayed a greater cathodic peak current than the Gel and Std electrode ($p < 0.001$, $n = 3$). The performance of the ViC electrodes was also better compared to the Std and Gel electrodes when assessing the ΔE_p . There was a significant reduction in the

ΔE_p in the ViC electrode when compared to the Std and Gel electrodes ($p < 0.01$, $n = 3$).

For dopamine there was a significant difference between the three composite electrodes. When monitoring the anodic peak current, the Vic electrode outperformed the Gel ($p < 0.05$, $n = 3$) and Std electrode ($p < 0.001$, $n = 3$). Overall this behaviour suggests that while the differences are primarily due to the increased conductive surface area of the electrodes there are additional considerations with regards to the surface structure of the composite electrodes as the adsorption based oxidation is hindered on the Std electrode but not on the Gel electrode. This varies from the more similar behaviour of ruthenium hexaammine and potassium ferricyanide at the Std and Gel electrodes. The ΔE_p was monitored for dopamine on the electrodes, however dopamine is a kinetically slow process with a cathodic peak that cannot be completely resolved at the Std electrodes but a fully resolved cathodic peak was observed on the Gel and ViC electrodes. The ΔE_p was significantly lower in the ViC electrode, when compared to the Gel electrode ($p < 0.05$, $n = 3$).

Overall the ViC electrodes show significant improvement over the Gel or Std electrodes arising from both their increased electroactive surface area, improved thermodynamic properties, reduced ohmic drop and more rapid kinetic responses for the three trial species investigated.

1.3.3. Electrode surface variability

Although composite electrodes are fabricated with a homogenous mixture of the conducting and insulating components they often have significant variability. This variability can be between different electrodes and as routine polishing uncovers new layers of the underlying composite material on an individual electrode. Both occurrences can greatly impact the reproducibility of any single composite electrode and between different electrodes. The electrochemical characteristics of potassium ferricyanide at the three different electrodes were assessed in-between polishing. Figure 4 displays the CVs for an individual electrode from each fabrication method and the relative error of the mean (R.E.M.) of the anodic peak current measured between a batch of electrodes between polishing. There was a significantly lower

R.E.M. for the ViC electrode when compared to the Std ($p < 0.001$, $n = 5$) and Gel electrodes ($p < 0.01$, $n = 5$). The R.E.M. was also lower for the Gel electrode when compared to the Std electrode ($p < 0.01$, $n = 5$). This would suggest that ViC electrode offer enhanced batch-to-batch reproducibility with minimal variation between the layers of the composite material.

1.3.4. Sensitivity and detection limits for serotonin

The electrodes were assessed for their suitability for the detection of 5-HT. The current response were detected at varying concentration of 5-HT as shown in Figure 5A. The mean data for each concentration at the various electrodes is displayed as a calibration plot in Figure 5B. The sensitivity of the ViC electrodes was 92.52 ± 2.07 nA μM^{-1} , which decreased to 64.96 ± 2.88 nA μM^{-1} for the Gel electrodes and further decreased to 52.98 ± 2.15 nA μM^{-1} for the Std electrodes. At lower concentrations (below 2 μM) there was no significant decrease in the current response between the three electrodes. The limit of detection was 800 nM on the Std electrode, 450 nM on the Gel electrode and 300 nM on the ViC electrode. The ViC electrode showed enhanced performance compared to Gel and Std electrode. For a marcoelectrode, this limit of detection for 5-HT is greater than anticipated, however is this mainly due to the low capacitive current observed on CNT electrode compared to graphite based electrodes. Similar responses have been shown where on “pristine” carbon nanotube networks the limit of detection for serotonin was significantly lower than that for boron-doped diamond and glassy carbon electrode [26].

1.3.5 Bio-fouling and electrode recovery

5-HT oxidative by-products are known to reduce the lifespan of the electrode which also limits the timescale of electroanalytical measurements. The different composite electrodes were investigated to examine the extent of electrochemical fouling that occurs at this manner of electrodes. Figure 6 shows electrochemical responses of potassium ferricyanide on all three electrodes before exposure to 5-HT, following exposure to 10 μM 5-HT in 0.1 M PBS for 30 minutes and after cleaning the electrode by wiping with an ethanol soaked lens cloth. The percentage current loss

from the anodic peak current of the initial response in potassium ferricyanide was monitored on all three electrodes and shown in Figure 6D. Following fouling of the electrode in 5-HT, the current response at the Std and Gel electrodes diminished to a greater extent than that at the ViC electrode ($p < 0.05$, $n = 3$). Following regeneration of the electrode with ethanol, the ViC electrode recovered to a greater extent than the Std and Gel electrode ($p < 0.001$, $n = 3$) and the Gel electrode recovered to a greater extent than the Std electrode ($p < 0.01$, $n = 3$).

For the Std electrode, ~ 70 % of anodic peak current was lost following fouling in 5-HT and recovery in ethanol did not significantly regenerate the electrode surface. In the Gel electrode ~ 70 % of anodic peak current was also lost and as observed on the Std electrodes, there was no significant improvement in performance following regeneration in ethanol. For the ViC electrode there was a ~50 % loss in the current response after fouling occurred in the 5-HT solution, however ethanol treatment significantly recovered the current loss from initial to ~20 % ($p < 0.05$, $n = 3$). These results indicate that MWCNT have enhanced resistance to electrochemical fouling as the ViC electrode by compression has the greatest density of MWCNT. However, this also suggests that composite surface microstructure may act in a means to enhance fouling of the electrode as the degree of fouling on the Std electrode is greater than that on the Gel electrode

Various studies have shown that carbon nanotubes have the potential to reduce the rate of fouling from oxidative by-products of dopamine and serotonin[25, 26], however our data clearly indicates that the carbon nanotubes play a vital role in achieving this behaviour as increased amounts of nanotubes in our ViC composite electrode display the greatest resistance to fouling. Due to the flexibility in electrode manufacture using composite materials, ViC electrodes may provide practical electrode geometries suitable for bioanalytical monitoring in a variety of situations while maximising the electroanalytical performance of carbon nanotubes.

1.4 Conclusions

This study has shown that pressure compression of MWCNT epoxy composite electrodes enhances the electrochemical performance of the final electrodes. This is

observed along with decreased internal resistance of the electrodes and increased capacitance at the electrodes. The compressed ViC electrodes have reduced surface variability compared to Std and Gel fabricated composite electrodes. For the detection of serotonin, the ViC electrodes have a significantly greater limit of detection than both Gel and Std MWCNT composite electrodes. The ViC electrode is also less prone to fouling from 5-HT oxidative by-products when compared to the Std and Gel electrodes. Only the ViC electrodes display the ability to be regenerated easily using ethanol cleaning.

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FIGURE LEGENDS

FIGURE 1. Photographs showing 25% MWCNT : 75% Epoxy resin (w/w) mixture utilised for the fabrication of electrodes. (A) shows packing of ~2 cm depth of the composite mixture. (B) shows the decrease in the volume of the packing material following compression. Scale bar shows 1 cm.

FIGURE 2. Electrochemical characterisation of MWCNT composite electrodes. (A) shows current response of the three composite electrodes, GC electrode and BBD electrodes in 0.1 M PBS buffer solution, where the capacitive current was monitored at 0 V. Cyclic voltammograms carried out at 0.1 V s^{-1} (B) shows real and imaginary impedance spectroscopy responses for the different electrodes. (C) shows the average capacitance from all the electrodes. (D) shows the internal resistance (R_{Ω}) and (E) shows the charge transfer resistance (R_{ct}) for all the electrodes. Data shown as mean $\pm$ S.D where $*p<0.05$, $**p<0.01$ and $***p<0.001$ vs. ViC electrodes.

FIGURE 3. Electrochemical performance of MWCNT composite electrodes on various redox couples. Responses are shown for 1 mM ruthenium hexaammine (A) and 1 mM potassium ferricyanide (B) in 1 M KCl and 1 mM Dopamine (C) in 0.1 M PBS. All measurements carried out 0.1 V s^{-1} scan rate.

FIGURE 4. Investigate of the variability of the electrode surface following polishing. (A) shows response of the Std electrode in 1 mM potassium ferricyanide in 1 M KCl. The electrodes were polished and monitored for five consecutive runs. Similar responses are shown for (B) Gel and (C) ViC electrodes. The relative error of the mean anodic current is shown for the three electrodes. Where $**p<0.01$ and $***p<0.001$ vs. Std electrodes and $^{++}p<0.001$ vs. Gel electrodes.

FIGURE 5. Assessment of electrode sensitivity and detection limit for the monitoring of 5-HT. (A) shows amperometric response carried out at +0.7 V vs Ag|AgCl on the three electrodes. The arrows indicate the addition of various concentrations of 5-HT. All are micromolar concentrations. The mean obtained current was plotted as a calibration curve in (B). Where $**p<0.01$ and $***p<0.001$ vs. Std electrodes; $^{++}p<0.01$ and $^{+++}p<0.001$ vs. Gel electrodes.

FIGURE 6. Study of electrode fouling in 5-HT. Responses of 1 mM potassium ferricyanide in 1 M KCl on (A) Std, (B) Gel and (C) ViC electrode are shown. The initial response, the response after fouling, where the electrode was placed in $10 \mu\text{M}$ 5-HT in 0.1 M PBS for 30 minutes at a potential of +0.7 V vs Ag|AgCl and following regeneration of the electrode surface using ethanol. The percent current loss from the initial response was shown in (D). $\#p<0.05$ vs Std electrode after fouling; $^{++}p<0.01$ and $^{+++}p<0.001$ vs. Std electrodes after recovery with ethanol; $^{+++}p<0.001$ vs. Gel electrodes after recovery with ethanol and $*p<0.05$ electrode fouling vs. electrode recovery in ethanol.

529 **FIGURE 1**

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FIGURE 4

FIGURE 5

FIGURE 6