

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

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10
11 **Abstract**

12 Serotonin (5-HT) is an important neurochemical that is present in high
13 concentrations within the intestinal tract. Carbon fibre and boron-doped diamond
14 based electrodes have been widely used to date for monitoring 5-HT, however these
15 electrodes are prone to fouling and difficult to fabricate in certain sizes and
16 geometries. Carbon nanotubes have shown potential a suitable material for
17 electroanalytical monitoring of 5-HT but can be difficult to manipulate into a suitable
18 form. The fabrication of composite electrodes is an approach that can shape
19 conductive materials into practical electrode geometries suitable for biological
20 environments. This work investigated how compression of multiwall carbon
21 nanotubes (MWCNTs) epoxy composite electrodes can influence their
22 electroanalytical performance. Highly compressed composite electrodes displayed
23 significant improvements in their electrochemical properties along with decreased
24 internal **and charge transfer** resistance, reproducible behaviour and improved batch
25 to batch variability when compared to non-compressed composite electrodes.
26 Compression of MWCNT epoxy composite electrodes resulted in an increased
27 current response for potassium ferricyanide, ruthenium hexaammine and dopamine,
28 by preferentially removing the epoxy during compression and increasing the
29 electrochemical active surface of the final electrode. For the detection of serotonin,
30 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.

38

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

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

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

87 In this manuscript we investigate if compression of multiwall carbon nanotube epoxy
88 resin electrodes can enhance electroanalytical performance and display improved
89 electrochemical behaviour. We utilised multi-wall carbon nanotube (MWCNT) carbon
90 nanotubes and carried out a light and heavy compression of the composite material.
91 These electrodes were then all investigated for their electrochemical performance
92 and surface reproducibility. Studies were also carried out to investigate the sensitivity
93 and limit of detection for the various electrodes against 5-HT. Fouling studies with
94 serotonin were conducted to understand if the compressed electrode showed
95 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_3 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 \sim 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.

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

140

141 **1.2.3. Electrochemical assessment of MWCNT electrodes**

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

149 Electrochemical impedance spectroscopy (EIS) was performed at 270 mV with an
150 amplitude of 10 mV at a frequency from 1×10^6 Hz to 1 Hz in an aqueous solution of
151 0.5 mM potassium ferricyanide and 0.5 mM potassium ferrocyanide in 1 M KCl. The
152 working electrodes was either a Std, Gel, ViC fabricated composite electrode or a
153 commercial boron-doped diamond (BDD) or a glassy carbon (GC) electrode with a
154 Pt counter electrode and 3 M KCl Ag/AgCl reference electrode.

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

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

166

167 **1.2.4 Investigation of electrode surface variability**

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

174

175 **1.2.5 Electrode sensitivity and stability of 5-HT detection**

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

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

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

189

190 **1.2.6 Data Analysis**

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

196

197 **1.3 Results and Discussion**

198 **1.3.1 Characterisation of MWCNT composite**

199 Figure 1 shows representative examples of the fabricated electrode casts under the
200 various types of compression. All composite material shown in Figure 1A consist of
201 25% MWCNT and 75% epoxy resin (w/w) mixture, where initial half of the polymer
202 tubing was packed initially with the material. Following the various approaches of
203 compression there are clearly differences in the Gel and ViC electrodes. Based on
204 the volume loss on compression no significant reduction in volume is observed in
205 Gel electrodes, however an approximately 50% reduction in the packed volume was
206 observed for the ViC electrode, which on visual expectation was felts to be
207 predominantly the epoxy resin component. During visual inspection of the electrode
208 surface the roughness of the exposed electrode area was greatly reduced in Gel and
209 even further reduced in ViC electrodes compared to Std electrodes.

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

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

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

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

242

243 **1.3.2 Standard redox probe behaviour at the different electrodes**

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

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

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

276 A similar trend was observed with ruthenium hexaammine, where the ViC electrode
277 displayed a greater cathodic peak current than the Gel and Std electrode ($p < 0.001$,
278 $n = 3$). The performance of the ViC electrodes was also better compared to the Std
279 and Gel electrodes when assessing the ΔE_p . There was a significant reduction in the

280 ΔE_p in the ViC electrode when compared to the Std and Gel electrodes ($p < 0.01$,
281 $n = 3$).

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

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

299

300 **1.3.3. Electrode surface variability**

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

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

316

317 **1.3.4. Sensitivity and detection limits for serotonin**

318 The electrodes were assessed for their suitability for the detection of 5-HT. The
319 current response were detected at varying concentration of 5-HT as shown in Figure
320 5A. The mean data for each concentration at the various electrodes is displayed as a
321 calibration plot in Figure 5B. The sensitivity of the ViC electrodes was 92.52 ± 2.07
322 $\text{nA } \mu\text{M}^{-1}$, which decreased to $64.96 \pm 2.88 \text{ nA } \mu\text{M}^{-1}$ for the Gel electrodes and further
323 decreased to $52.98 \pm 2.15 \text{ nA } \mu\text{M}^{-1}$ for the Std electrodes. At lower concentrations
324 (below $2 \mu\text{M}$) there was no significant decrease in the current response between the
325 three electrodes. The limit of detection was 800 nM on the Std electrode, 450 nM on
326 the Gel electrode and 300 nM on the ViC electrode. The ViC electrode showed
327 enhanced performance compared to Gel and Std electrode. For a macroelectrode,
328 this limit of detection for 5-HT is greater than anticipated, however is this mainly due
329 to the low capacitive current observed on CNT electrode compared to graphite
330 based electrodes. Similar responses have been shown where on “pristine” carbon
331 nanotube networks the limit of detection for serotonin was significantly lower than
332 that for boron-doped diamond and glassy carbon electrode [26].

333

334 **1.3.5 Bio-fouling and electrode recovery**

335 5-HT oxidative by-products are known to reduce the lifespan of the electrode which
336 also limits the timescale of electroanalytical measurements. The different composite
337 electrodes were investigated to examine the extent of electrochemical fouling that
338 occurs at this manner of electrodes. Figure 6 shows electrochemical responses of
339 potassium ferricyanide on all three electrodes before exposure to 5-HT, following
340 exposure to $10 \mu\text{M}$ 5-HT in 0.1 M PBS for 30 minutes and after cleaning the
341 electrode by wiping with an ethanol soaked lens cloth. The percentage current loss

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

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

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

369

370 **1.4 Conclusions**

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

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

381

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384

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489 **FIGURE LEGENDS**

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

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

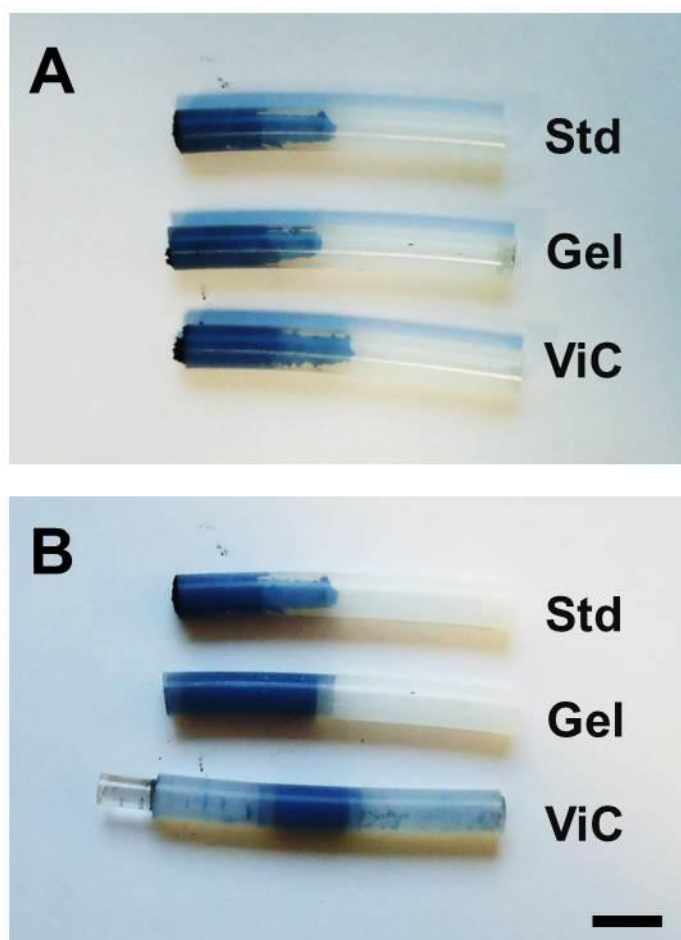
503 **FIGURE 3.** Electrochemical performance of MWCNT composite electrodes on
504 various redox couples. Responses are shown for 1 mM ruthenium hexaammine (A)
505 and 1 mM potassium ferricyanide (B) in 1 M KCl and 1 mM Dopamine (C) in 0.1 M
506 PBS. All measurements carried out 0.1 V s⁻¹ scan rate.

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

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

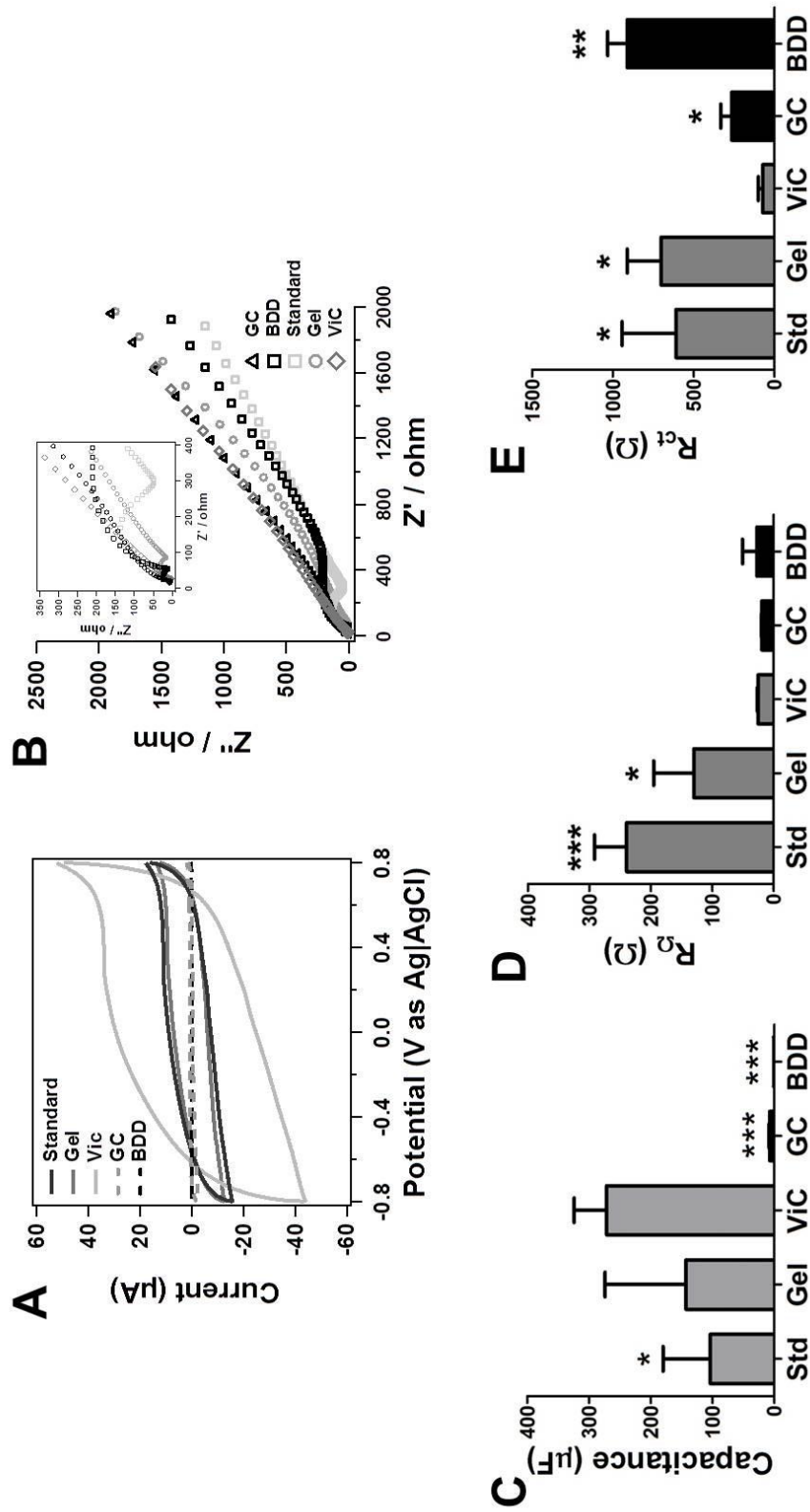
519 **FIGURE 6.** Study of electrode fouling in 5-HT. Responses of 1 mM potassium
520 ferricyanide in 1 M KCl on (A) Std, (B) Gel and (C) ViC electrode are shown. The
521 initial response, the response after fouling, where the electrode was placed in 10 μ M
522 5-HT in 0.1 M PBS for 30 minutes at a potential of +0.7 V vs Ag|AgCl and following
523 regeneration of the electrode surface using ethanol. The percent current loss from
524 the initial response was shown in (D). # $p < 0.05$ vs Std electrode after fouling;
525 †† $p < 0.01$ and ††† $p < 0.001$ vs. Std electrodes after recovery with ethanol; ††† $p < 0.001$
526 vs. Gel electrodes after recovery with ethanol and * $p < 0.05$ electrode fouling vs.
527 electrode recovery in ethanol.

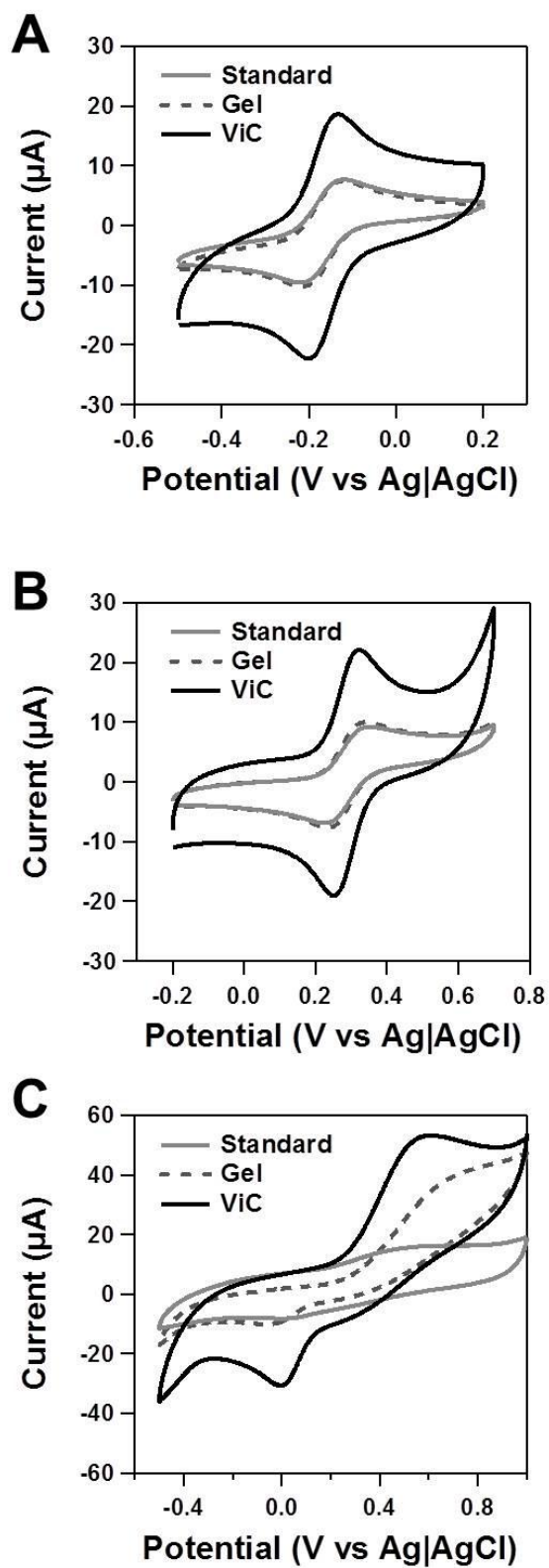
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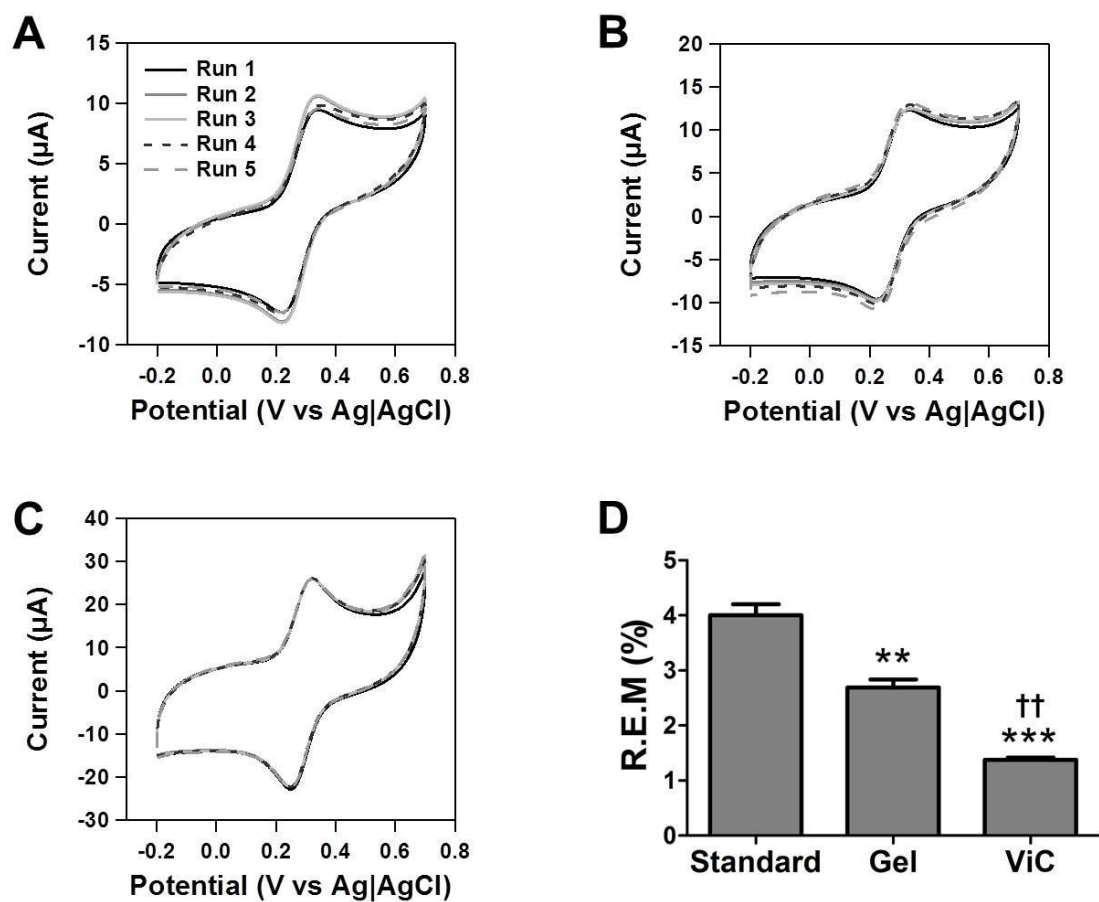
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538 **FIGURE 4**

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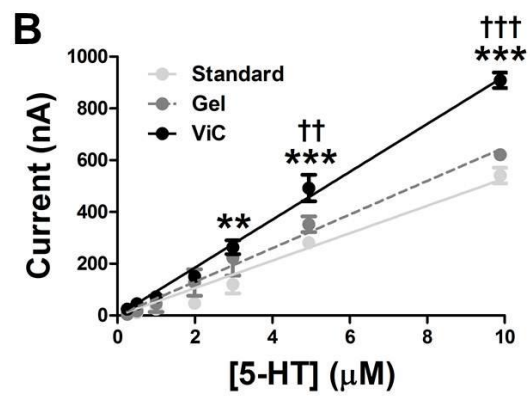
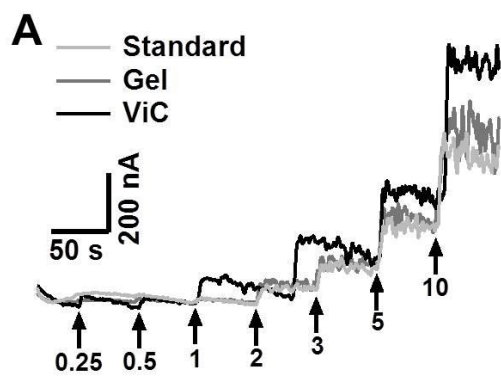


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542 **FIGURE 5**

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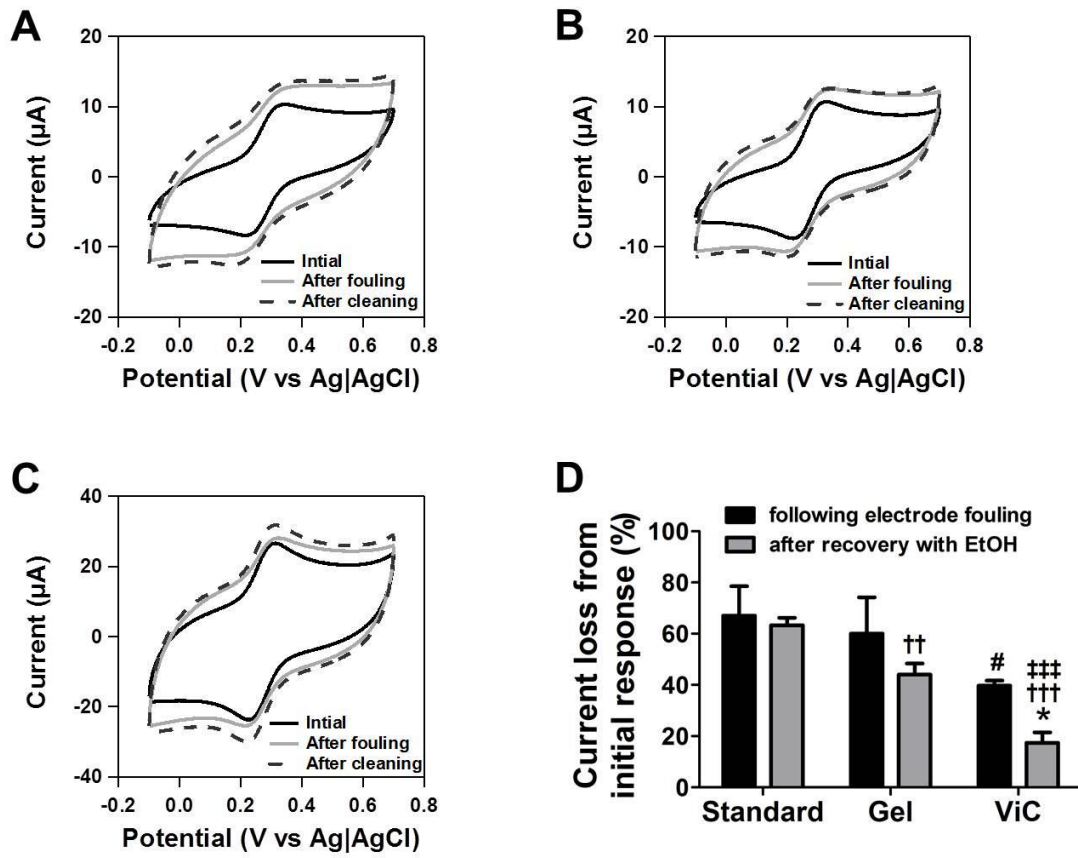
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547 **FIGURE 6**

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