Functionals Aspects and Simultaneous Detection of Dopamine, Ascorbic Acid and Uric Acid Using Chitosan-Catechol Graphene and Carbon Nanotube Modified Electrodes

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Abstract  Dopamine functions as a neurotransmitter in the brain. The dysfunction of the dopaminergic system is the leading cause of numerous diseases such as Parkinson’s disease. Hence, it is important to find selective and sensitive detection methods for the early diagnosis of diseases related to the abnormal levels of dopamine. In this study, we show a new electrochemical sensing platform based on carbon nanotube (SWCNT) and a sheet of graphene (GRA). The novelty of our sensor is the coating of the substrates with chitosan-catechol (CC) by electrodeposition, enhancing the dopamine response by 70%. The dose-response for each set of electrodes (bare CNT, bare GRA, and coated CNT as well as coated GRA) was measured. Finally, the electrodes were tested in cerebrospinal fluid (artificial and human), for the detection of millimolar to nanomolar levels of dopamine. The electrodes exhibited high sensitivity (2.03mA mol·L⁻¹, 1.45 mA mol·L⁻¹, 0.0298 mA mol·L⁻¹, and 0.0559 mA mol·L⁻¹ for the modified CNT, bare CNT, modified GRA and bare GRA, respectively, for the oxidation of DA). The oxidation peak current was proportional to the concentration of DA in the range from 50×10⁻⁶ to 50x10⁻⁹ M (n= 6, r² =0.98). The dopamine recovery in human CSF were, 49- 78% and 65-65% with coated graphene and CNT electrodes, respectively. Our results indicate that the CC modified CNT electrodes achieved the best recovery, sensitivity, limit of detection, and selectivity compared to the uncoated CNT as well as the coated and uncoated graphene electrodes.

Keywords: dopamine, uric acid, ascorbic acid, cyclic voltammetry, limit of detection, sensitivity, cerebrospinal fluid

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1. Introduction

Dopamine (DA) is a neurotransmitter in the human brain. It can be found in the ventral tegmental area of the midbrain, the substantia nigra pars compacta, and the arcuate nucleus of the hypothalamus [1]. It is a critical puzzle piece especially when it comes to understanding of the neural behavior and in developing therapeutic intervention technologies for neurological disorders. The dynamics of dopamine concentration in the extracellular space can be studied for addiction, learning and even memory processes. Extracellular DA concentration is thus deemed as a biomarker that is clinically relevant for specific disease states as well as a gateway to monitor treatment efficacy [1]. Inconvenience could significantly be reduced using rapid and efficient dopamine testing methods using small sample volumes. This could also help regain safe and effective levels and make rapid adjustment of dosage when necessary. Direct electrochemical detection of the redox-active dopamine is an attractive testing mechanism [2], especially for patients with Parkinson’s disease. It is therefore relevant to address issues of robustness, reliability, and materials which can be leveraged for chronic implants and that present biocompatible characteristics. An issue for dopamine sensing is the presence of two of the interferent species, ascorbic (AA) and uric acid (UA), that foul the sensor due to similar responses as dopamine. Ascorbic acid (AA) is an important compound especially when it comes to health care i.e skin, connective tissues
and the immune system [3]. Uric acid (UA) is a compound that can be excreted in urine and is considered as a final oxidation product of urine metabolism. Detection and quantification of AA, DA and UA are important in the diagnosis, monitoring, prevention and treatments of some certain diseases including arthritis, Parkinson’s disease, schizophrenia and hyperuricemia [3]. These acids are present in most structures of the brain at concentrations at least one thousand times higher than dopamine. In order to measure a signal that is buried within such interference from other sources, the problem of tracking dopamine in the extracellular space becomes almost intractable. It is well known that at traditional bare electrodes, AA, DA and UA exhibit oxidation peaks at potentials very close to each other resulting in an overlapping voltammetric response [4]. However, graphene, carbon nanotube materials, chitosan and catechol have received attention for their great electrocatalytic properties and as biosensors.

During the past decade, various preparation methods and characteristics of chitosan–catechol have been reported, and researchers have proposed various biomedical applications such as drug/gene delivery depots and tissue engineering scaffolds [5]. Chitosan–catechol has exhibited not only excellent solubility and biocompatibility in neutral pH solutions but also strong adhesiveness on tissue surfaces. Since chitosan has been shown to be a suitable polysaccharide for biomedical applications, as it exhibits properties of high biocompatibility, biodegradability, tissue adhesion, and anti-microbial activities [5]. Chemical tethering of catechol derivatives to chitosan backbones can dramatically affect the properties of chitosan.

Due to their inherent electrocatalytic nature, the accelerated the rate of electron transfer between specific molecules and electrodes; nanoparticles and graphene-based electrodes have received attention in recent years [6]. Graphene is one of the most promising materials for electrochemical applications because of its high electrical conductivity, large surface area, biocompatibility [7] and relatively low cost of preparation. Conductive electrodes can be modified with graphene suspension, powder, graphene composites, and even graphene sheets can also be used as electrodes for in vivo applications [8]. The use of reduced graphene oxide (rGO) for DA detection has recently been proposed. The reduced GO enhanced the electrochemical response of any sensor as a result of the presence of oxygen-containing groups on its surface. The DA sensors with the best detection limit (2.3 x 10^{-8} mol/L) and linear range (3 x 10^{-8} – 4 x 10^{-4} mol/L) were obtained by electrodes modified with graphene prepared by GO reduction [8]. On the other hand, carbon nanotubes (CNTs) have generated a great deal of interest for various applications based on their field emission and electronic transport properties, their high mechanical strength, and their chemical properties. From this arises an increasing potential for the use of CNTs as field emission devices, nanoscale transistors, tips for scanning microscopy or components for composite materials [9]. For the monitoring and quality control of noxious substances in both medicinal and environmental chemistry, Chemical sensors have become an increasingly attractive tool. The use of CNTs for sensors applications has also led to greater research activities due to their advantages as “one shot” sensors that can be disposed after usage [9].

Nonetheless, no report is available concerning the use of graphene and carbon nanotube materials or their composites as electrode modifying material using chitosan and catechol for simultaneous determination of biological compounds such as AA, DA and UA. So far, separation of the differential pulse voltammetry (DPV) peak potentials of the voltammetric responses between AA-DA, DA-UA and AA-UA was calculated to be 165, 90 and 255 mV, respectively from Han et al [10]. Other articles [2, 11-13] have also demonstrated separation of anodic peaks for the AA–DA and DA–UA pairs where the best separation results were obtained by use of Pd nanoparticle-modified electrodes—PdNP/CNF (0.244 V, 0.148 V) and PdNC/PMPy/Pt (0.205 V, 0.201 V), leaving room for improvements. Therefore, simultaneous determination of these three species is of great significance not only in biomedical chemistry and neurochemistry but also for diagnostic and pathological investigations.

The improvement of electrode materials, in conjunction with electroanalysis techniques, resulted in the improved detection of DA at nanomolar levels [2]. This improved sensing performance has been achieved using techniques such as cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, and fast-scan cyclic voltammetry [6]. Using electrodes to measure DA in the presence of interfering molecules such as UA and AA under physiological conditions is still a work in progress. Despite these techniques, lifetime is one of the main shortcoming of electrochemical sensors that will need to be tackled in order produce long-term implants that can reliably detect neurotransmitters [6].

![Figure 1.](image)

**Figure 1.** DA as an oxidizing mediator in the catechol-modified chitosan system. (a) Schematic of the system with the diffusing DA. (b) Continuous oxidation of DA in the presence of catechol (Q) reduction. (c) DA acts as an oxidizing mediator of QH$_{2}$, and Ru$^{2+}$ as a reducing mediator regenerating the Q. Electrochemical potential bar represents standard reduction potential of Ru$^{2+}$, Q and DA. [2]
The application of the catechol-modified chitosan redox cycling system was recently presented to amplify and detect the electrochemically active clozapine (CLZ) in undiluted human serum [2] ranging between 0.350 µg/mL and 1µg/mL [14]. In this study, our new concept was presented for the detection of dopamine (DA) in situ due to its electrochemically-active nature. We show the generated concept as a selective detection method for dopamine in the presence of interferences such as uric acid and ascorbic acid. This provides for DA oxidation amplification. Chitosan and electrochemically-active catechol was embedded as a layer in which the redox cycling can occur (Figure 1). The generated oxidizing current of DA was amplified through a continuous cycle of DA reduction by the catechol followed by re-oxidation at the electrode. The redox cyclic system allowed for an increase in the total charge transferred by DA oxidation.

2. Experimental Methods

2.1. Reagents and Instruments

Carbon nanotube threads were purchased from Cheaptubes (CTI-400, Cheaptubes, Grafton, VT, USA) [30]. The CNT threads are 100 µm ± 10 % in diameter. Graphene electrodes were purchased from Cambridge Graphene Centre, UK [31]. Ascorbic acid (AA), 3-hydroxytyramine hydrochloride (dopamine, DA), uric acid (99%, UA), hexaammineruthenium(III) chloride (HARu), ferrocene and phosphate buffer saline (0.1 M PBS, pH 7) were purchased from Sigma-Aldrich (St. Louis, MO). Artificial cerebrospinal fluid (aCSF) was prepared using and containing (in mm): 126 NaCl, 0.3 NaH2PO4, 2.5 KCl, 0.3 KH2PO4, 1.6 CaCl2, 1.0 MgCl2, 0.4 MgSO4, 26.2 NaHCO3, and 11 d-glucose saturated with 95% O2 and 5% CO2. The osmolarity of the aCSF was 300 ± 5 mOsm.

CSF from human donors was purchased from Lee Biosolutions (Maryland Heights, MO). All chemicals were of analytical reagent grade and used as received. Cyclic voltammetry (CV) was also performed with voltages from -0.4 V to 0.7 V at a scan rate of 100 mV/s for a total of 5 cycles using a CHI 660D potentiostat (CHI, Warminster, PA).

2.2. Preparations of Chitosan-catechol Modified Electrodes

Electrodeposition was utilized to modify both the graphene (0.625 mm²) and CNT electrodes (0.5 mm²) using chitosan and catechol. Chitosan deposition was achieved in an electrochemical macro-scale cell (1 ml) (Working Electrode (WE) – macro-electrode; Counter Electrode (CE)+ Reference Electrode (RE) – Pt foil) filled with 1.5ml of RT 1% chitosan solution, a constant current was applied (calculate according to the current density 28.709 µA/m² (for graphene) and 18.943 µA/m² (for CNT) for 45s (chronopotentiometry; cathodic current). Then, the electrode was cleaned by dipping in PBS (10 mM Phosphate buffer saline. pH at ~7.0) and was left in PBS to prevent dehydration if needed. The chitosan film thickness and roughness can be characterized by dehydrating the electrodeposited film.

Catechol deposition was performed in an electrochemical macro-scale cell with a chitosan-coated macro-electrode as a working electrode (CNT or graphene electrode); a platinum foil as a counter electrode and a silver-silver chloride as a reference electrode. Then, the Open Circuit Potential (OCP vs. Ag/AgCl) was left to stabilize the redox species and the potential was started when OCP is ~0.063V. The cell was filled with 1ml of 5mM catechol in 0.1 M PB. Chronoamperometry was performed with double potential pulse of 0.5V to 0.6V to 0.5V. The pulse width was set to 180s with a pulse interval of 0.1, steps of 1, a quiet time of 2s, and a sensitivity of 10⁻⁵ A/V.

To determine if deposition has occurred, the color of the surface of the working electrode will change to a more brown color during catechol grafting. The electrode was immersed in DI to remove ungrafted catechol for 5 minutes then moved to fresh PBS in a beaker. The catechol modification procedure was repeated for other electrodes with fresh solution for each electrode.

A redox validation is also performed to assure that deposition was successful. This was done in an electrochemical macro-scale cell using a catechol-chitosan macro-electrode, a platinum foil as a counter electrode and a silver-silver chloride as a reference electrode. The cell was filled with 1ml of 25µM HARu + 25µM Fc(MeOH)2. Cyclic voltammetry was initiated with an initial potential of 0, a high potential of 0.7, a low potential of -0.4 and a final potential of 0. The scan rate was set to 0.1V/s with a sample interval of 0.001V, quiet time of 5s and sensitivity of 10⁻⁵. The OCP was allowed to equilibrate (0.044V – 0.067V) and was performed using the parameters stated. This procedure is repeated for other electrodes in a fresh solution for each electrode.

The experiment was conducted on four groups of electrodes: modified CNT (n=6), modified graphene (n=6), bare CNT (n=6), and bare graphene (n=6). The solutions that these electrodes were tested in are PBS (phosphate buffered saline), artificial cerebrospinal fluid (aCSF), and human-CSF. The tested concentrations of dopamine were: 10⁻⁶ M, 10⁻⁷ M, 10⁻⁸ M, and 10⁻⁹ M. For AA and US, the concentrations were either 200 nM or 200 mM. Electrodes were tested in varying concentrations of dopamine and UA and AA when immersed in aCSF and in (human) CSF.

Cyclic voltammetry (CV), unless where otherwise noted, was performed with a three-electrode setup (counter electrode is a platinum foil of 5 mm² area, and reference electrode is a silver-silver chloride pellet), with voltages from -0.4 V to 0.7 V at a scan rate of 100 mV/s for a total of 5 cycles.

3. Results

3.1 Characterization of Chitosan-catechol Graphene Composite

The characterization of chitosan-catechol graphene electrodes begun after the redox validation. The redox validation was performed to assure deposition. Electrodes
were immersed in a mixture of hexaammineruthenium(III) chloride (HARu) and ferrocene. To ensure that the graphene electrodes’ deposition was successful, a PBS test was performed for each electrode. To do so the electrode was lowered, with a micro-manipulator, 5 mm past the surface of the beaker containing PBS before and after the deposition. This assured that a change or increase in charge storage capacity as well as in capacitance (Figure 2) was noticed after the deposition. The modified graphene electrode and the unmodified graphene electrode showed an increase in the CV curve after the deposition (Figure 2).

3.2. Characterization of Chitosan-catechol Carbon Nanotube Composite

Similarly, the chitosan-catechol carbon nanotube electrode what characterizes using PBS after the redox validation occurred. To determine if validation was visible using CV, the PBS test was also used. The modified electrode showed a greater increasing the surface area or charge \((99.14 \times 10^{-6} \text{ C})\) versus the unmodified electrode \((71.02 \times 10^{-6} \text{ C})\) (Figure 3) averaging an increase of \(28 \pm 5\%\) for each electrode. That in conjunction with the redox validation showed that chitosan and catechol were deposited on the surface of electrodes.

3.3. Voltammetric Responses for Different Electrodes

After the redox validation was assured using the PBS test, voltammetric responses were determined for all the electrodes for ascorbic acid (AA) dopamine (DA) and uric acid (UA) separately. Figure 4, Figure 5, Figure 6, Figure 7 shows modified CNT (n=6) and graphene electrodes (n=6) and bare CNT (n=6) and graphene electrodes (n=6), in artificial cerebrospinal fluid (aCSF) and in 4 concentrations \((10^{-6} \text{ M}, 10^{-7} \text{ M}, 10^{-8} \text{ M}, \text{ and } 10^{-9} \text{ M})\) of DA + aCSF, UA + aCSF, AA + aCSF. As noticed in for the CNT modified electrodes in Figure 4, AA and UA show irreversible oxidation peaks at -0.159 and 0.393, respectively. DA, on the other hand, depicts a reversible redox couple with anodic and cathodic peaks at 0.125 and 0.02. For the CNT unmodified electrodes, the corresponding oxidation peak potentials of AA, DA, UA are shifted to n/a, 0.163, 0.415. The current observed on the modified CNTs approximately doubled in value. This maybe due to the increased surface area and/or the catalytic activity of the chitosan-catechol CNT electrodes. In comparison to the unmodified electrodes, the DA and UA oxidation peaks what also negatively shifted indicating a catalytic activity of the electrode material. For the modified graphene electrodes the CV curve shows consistent distinct peaks for a DA only and occasional peaks for UA. The oxidation peaks well well-defined at 0.228 mV. However, the CV curves shows indistinctive peaks from both UA and AA where peaks will interfere and end up in the region where DA is oxidized or not even shown at all. The unmodified graphene electrodes showed an even worse ability to detect UA and AA at the different concentrations. Not only where the oxidation peaks of DA the only noticeable peaks, but not every electrode was able to effectively detect it.
Figure 7. Cyclic voltammogram of a bare graphene electrode in a solution containing 200 μM AA, 50 μM DA, 200 μM UA in artificial cerebrospinal fluid (aCSF)

Cyclic voltammograms with different scan rates for each electrode type at the nanomolar concentration in the same solution was determined. The plots of the oxidation peak current as a function of the scan rate are shown in Figure 8. In all four cases of good linear relationship ($R^2 = 0.98 \pm 0.01$) of current response against the scan rate was observed indicating an absorption control process for all four types as well as that the redox reaction are due species on the surface and not due to diffusion from the bulk.

Figure 8. Graph shows the linearity of peak current vs. scan rate as proof of absorption of 50 nM DA present in a CSF fluid on different electrodes as indicated

3.4. Statistical Analysis

A statistical analysis was performed to determine the behavior of each electrode type before performing a simultaneous detections. Cyclic voltammetry curves between electrode types were examined for significance using a 4 x 4 repeated measures ANOVA (four electrode types by four dopamine, uric acid and ascorbic acid. Concentrations). This will allow us to determine how each electrode should perform in the presence all interferences as well as solidify our findings. When assessing the main effects of each dependent variable, Mauchly’s test was used to determine if the data violated the assumption of sphericity [28]. Sphericity is indicated when the variances of the differences between all combinations of related groups measured are equal. When a violation of sphericity occurs, the variances of the differences between all combinations of related groups are not equal. In this case, if Mauchly’s test indicated that the assumption of sphericity was not met, the Greenhouse-Geisser [29] correction was applied. All main effects utilized a Tukey confidence interval. Two-tailed p-values less than 0.05 were considered significant.

3.4.1. Response of DA in Different Types of Electrodes

The assumption of sphericity was met using Mauchly’s test. There is a significance difference between the peak current of DA concentrations (p<0.001) whereas no significant difference was determine between each electrode type per concentration (p = 0.081). This means that the way that DA is interacting with each electrode is the same, therefore batch and electrode fabrication is consistent across all electrode types. There is also no difference at each peak current per electrode type but a significance is seen in the between-subject effects (p < 0.001); this shows that each electrode type is different and that each electrode can differentiate between each peak current of the DA concentrations. The results also revealed that the difference in electrode type can be seen between the CC-CNT and CC-GRA (p < 0.001) as well as the CC-CNT and B-GRA (p < 0.001). Since the difference was observed between the CC-CNT and CC-GRA as well as the CC-CNT and B-GRA, we can infer that the electrode types are able to show a decrease in DA peaks as the concentration goes down. The results (Figure 9) also show that the highest peak current per concentration was detected with the CC-CNT electrodes, proving that the coating as well as the material of the electrode can provide a better distinction in the peak current per DA concentration by increasing the peak current.

Figure 9. Mean value of peak current (volts) per DA concentration for each chitosan-catechol CNT (CC-CNT), bare CNT (B-CNT), chitosan-catechol graphene (CC-GRA), bare graphene (B-GRA).

3.4.2. Response of UA in Different Types of Electrodes

Significance of the Greenhouse-Geisser showed that a different peak current is generated per concentration (p < 0.001) as well as that each electrolyte is able do distinguish between these values differently (p < 0.001). The between-subjects effects also shows that there is a difference in each electrode type validating that they are different (p < 0.001). Moreover in the detection of UA peaks, CC-CNT showed a difference in measuring peak current in comparison to B-CNT (p = 0.008). This means that the coating could have an effect on the measurements. The same was not shown with GRA electrodes. In this case, the CC-GRA and B-GRA did not have a significant difference (p = 0.903) in their measurement of UA peak current. When comparing the types of electrodes, CC-CNT and B-CNT electrodes measured differently than GRA electrodes. Additionally, the comparison of the performance of each electrode type to the measured peak current revealed that the coating effect between the CNT electrode allows for the CC-CNT to measure a higher UA current peak per concentration versus the rest of the
electrode types. This is also noticed in the graph (Figure 10), where CC-CNT shows the highest difference in peak current and shows its ability to measure and differentiate between peaks while the remaining had a lower difference or could not especially at lower concentrations. This shows that the coating is imperative.

Figure 10. Mean value of peak current (volts) per UA concentration for each chitosan-catechol CNT (CC-CNT), bare CNT (B-CNT), chitosan-catechol graphene (CC-GRA), bare graphene (B-GRA)

3.4.3. Response of AA in Different Types of Electrodes

The assumption of sphericity was met using Mauchly’s test. The AA peak currents per concentrations are significant (p = 0.026) however each electrode type is not able to measure the peak current per concentration differently (p = 0.059). This means that there’s a difference in the measurement that each electrode type detects per concentration, concluding that the coating as well as the material does not affect the peak current as well as does not amplify the AA signal in the solution. The pairwise comparison does show a difference in CC-CNT and CC-GRA electrodes (p = 0.025) as well as CC-CNT and B-GRA (p = 0.036) but each electrode type is not different in measuring the peak current per concentration of AA. They cannot differentiate between the different peaks regardless of the coating and material. This is confirmed in the collected data as only data was able to be collected for the micro molar concentration. All of the CC-CNT electrodes, 3 of the B-CNT, 1 of the CC-GRA and 1 of B-GRA electrodes were able to detect 200x10^-6 M peaks while only one of the CC-CNT electrode was able to detect a 200x10^-7 M peak. This is also evident in the graph (Figure 11) where CC-CNT showed a difference in the peaks of the first two concentrations where the other electrodes did not even show a difference.

Figure 11. Mean value of peak current (volts) per AA concentration for each chitosan-catechol CNT (CC-CNT), bare CNT (B-CNT), chitosan-catechol graphene (CC-GRA), bare graphene (B-GRA)

3.5. Simultaneous Determination of AA, DA, UA

The dynamics of availability and clearing of DA in the brain is an important factor for determining neurological disease. However, the existence of interferences such as AA and UA at high concentrations prevents proper measurements of the time course of DA availability in the extracellular space. Figure 12 and Figure 13 show CVs of bare CNT, chitosan-catechol CNT, bare graphene, chitosan-catechol graphene electrodes in a CSF solution containing the mixture of 50 μM DA, 200 μM AA and 50 nM DA, 200 nM AA and DA, respectively. As it can be seen in the Figure 12 and Figure 13, only the distinct peak of DA can be seen for the bare CNT at 0.191 V, 0.104 V for the millimolar and nanomolar concentration respectively, chitosan-catechol CNT at 0.201 V, 0.082 V, bare graphene showed no peaks at both concentrations, and chitosan-catechol graphene showed a peak at 0.259 V for the millimolar concentration of DA. As shown in Figure 12 and Figure 13, the voltammetric response at bare CNT and bare graphene electrodes were observed as weak and broad peaks, which suggests the both of the electrodes were very poor in selectivity and sensitivity. As for the modified graphene electrode, the current increased as founded in the CV technique in Figure 12, but simultaneous detection of AA, UA and DA was still hard to achieve having some of the peaks not being shown at all. While in Figure 12, for the modified CNT electrode, three distinct and well-defined voltammetric peaks at -0.159 V, 0.451 V, 0.201 V for AA, UA and DA respectively, were observed at the millimolar concentration.

Figure 12. Cyclic voltammogram of bare CNT, chitosan-catechol CNT, bare graphene, chitosan-catechol graphene electrodes in aCSF solution containing the mixture of 50 μM DA, 200 μM UA and AA

Figure 13. Cyclic voltammogram of bare CNT, chitosan-catechol CNT, bare graphene, chitosan-catechol graphene electrodes in a CSF solution containing the mixture of 5 50 nM DA, 200 nM UA and AA

Separation of the voltammetric responses between AA-DA, DA-UA and AA-UA was calculated to be 0.360 V, 0.250 V, 0.760 V, as seen in Table 1. The separations are large enough to allow simultaneous
determination of these three species in the same solution especially at high concentrations; however, at low concentrations, peaks are disappearing leaving only the DA peak to be achieved using the chitosan-catechol CNT electrodes. The lack of peak separation is due to the decrease in amplitude of the AA or UA peaks while DA peaks are apparent at lower concentrations. Determination of AA, DA, UA in their mixtures is employed using the CV technique. In the experiments performed concentration of one component was continuously decreased from a millimolar concentration to a nanomolar of concentration by diluting 1.5 mL of the previous solution with 13.5 mL of aCSF. The results are shown in Figure 14. All the results are summarized in Table 1. The catalytic properties of CNT are affecting the separation of the redox peaks of the analytes thus enabling their determination in a mixture as well as improving the sensitivity and selectivity.

Table 1. Averages of separations of peaks for chitosan-catechol CNT, bare CNT, chitosan-catechol graphene and bare graphene at a) 10⁻⁶ M b) 10⁻⁷ M c) 10⁻⁸ M d) 10⁻⁹ M

| Electrodes → | Chitosan-catechol CNT (mV) | Bare CNT (mV) | Chitosan-catechol graphene (mV) | Bare Graphene (mV) |
|--------------|---------------------------|---------------|-------------------------------|-------------------|
| AA-DA        | 356±13.6                  | 327±17.9      | No AA peak                    | No AA peak        |
| DA-UA        | 262±11.1                  | 273±25.8      | 165±21.3                      | 153±22.1          |
| UA-AA        | 618±10.3                  | 600±13.2      | No UA peak, no AA peak        | No UA peak, no AA peak |

| Electrodes → | Chitosan-catechol CNT (mV) | Bare CNT (mV) | Chitosan-catechol graphene (mV) | Bare Graphene (mV) |
|--------------|---------------------------|---------------|-------------------------------|-------------------|
| AA-DA        | No AA peak                | No AA peak    | No AA peak                    | No AA peak        |
| DA-UA        | 255±23.2                  | 205±32.3      | 163±35.4                      | 190±23/4          |
| UA-AA        | No AA peak                | No AA peak    | No AA peak                    | No AA peak        |

| Electrodes → | Chitosan-catechol CNT (mV) | Bare CNT (mV) | Chitosan-catechol graphene (mV) | Bare Graphene (mV) |
|--------------|---------------------------|---------------|-------------------------------|-------------------|
| AA-DA        | No AA peak                | No AA peak    | No AA peak                    | No AA peak        |
| DA-UA        | 219±9.2                   | 198±20.2      | 140±23.1                      | 162±17.8          |
| UA-AA        | No AA peak                | No AA peak    | No AA peak                    | No AA peak        |

| Electrodes → | Chitosan-catechol CNT (mV) | Bare CNT (mV) | Chitosan-catechol graphene (mV) | Bare Graphene (mV) |
|--------------|---------------------------|---------------|-------------------------------|-------------------|
| AA-DA        | No AA peak                | No AA peak    | No AA peak                    | No AA peak        |
| DA-UA        | 227±5.8                   | 158±10.4      | No UA peak                    | 165±25.7          |
| UA-AA        | No AA peak                | No AA peak    | No UA peak, no AA peak        | No AA peak        |
3.5.1. DA Detection in hCSF Solution

Furthermore, each electrode was tested in human cerebrospinal fluid. Because there was little to no sign of DA, AA and UA, DA was spiked up by adding 5 µL and 50 µL of dopamine hydrochloride to the hCSF solution. With the addition of the DA in the solution, results showed the modified CNT electrode was able to detect DA by having a recovery percentage of 78% after a 50 µL addition and 49% with a 5 µL addition and the chitosan-catechol graphene electrode generated 65% and 66% recovery for the 5 µL and 50 µL addition respectively. The recovery percentage of all electrodes increase with the added chitosan and catechol coating on the bare CNT and graphene. The nanomolar limit of detection from the chitosan-catechol CNT electrodes showed that the modified CNT electrodes have the highest high affinity for DA as well as being able to separate the oxidation peaks of the other species. All results are shown in Table 2. The limit of detection (LOD = 3 S/N) was determined using the formula ((k * SD)/m), where k is chosen to be 2 or 3; k-value 2 corresponds to a confident level of 92.1% and k-value 3 corresponds to a confident level of 98.3%. SD is the standard deviation of the background signal and m is the calibration sensitivity that is slope of the linear plot between concentrations versus current and well as the sensitivity of each electrodes type.

Table 2. Recovery of analyte concentration with chitosan-catechol CNT, bare CNT, chitosan-catechol graphene, and bare graphene electrodes

| Electrodes     | 5 µM addition of DA - % Recovery | 50 µM addition of DA - % Recovery |
|----------------|-----------------------------------|-----------------------------------|
| Chitosan-catechol CNT | 47%                             | 78%                               |
| Bare CNT        | 52%                             | 50%                               |
| Chitosan-catechol graphene | 65%                            | 66%                               |
| Bare graphene electrodes | 0%                              | 4%                                |

The information about the analytical data (sensitivity, detection limit, interferences) of a variety of biosensors was determined. The best characteristics for dopamine biosensors were obtained by Wang et al. [24] and Zhu et al. [25]. They achieved a low-level (nM) detection limit for dopamine. However, it is very difficult to compare the results, because of lack of information about the surface properties of the graphene-modified electrodes and the lack of information pertaining to the size of the electrode used in Zhu et al. The remaining electrodes showed a greater LOD with a greater electrode size. Though they might be considered suitable for DA detection in the presence of interferences, there is still a lack of research in their miniaturized form. In comparison to the list of biosensors, our CNT/CC biosensor was able to achieve a low LOD of 5 × 10^8 mol L⁻¹ whilst using a small diameter and surface area something not yet observed with other sensors.

4. Discussion

The results exhibited that CC-CNT and B-CNT provided a higher DA peak across all concentrations separately and simultaneously. This not only validates them as a better distinctive material for the detection but also the coating seems to provide a higher affinity for DA, which is seen in the increase of peak current per DA concentration in comparison to the GRA electrodes. In terms of UA, the coating does not seem to affect the UA peaks current but rather seems to measure the peak current due to the change in concentration similarly. On the other hand, CNT electrodes show that the modification does affect the measurement. Since CC-CNT does measure a higher peak current, we can conclude that it is able to identify the concentration UA molecule on its surface than the remaining electrodes. As for AA detection, electrodes material and modification did not have an effect on the peak current, neither by amplifying it or distinguishing between each peak current per concentration. This shows that each electrode has a lower affinity to AA in the solution. Since CC-CNT electrodes were the only one able to detect more peak current, chances are better when using this electrode to detect some concentrations. Overall, the CC-CNT electrode not only had a higher affinity for DA due to the higher peak current in comparison to all electrodes but also exhibited a low LOD. This bettered its ability to distinguish between the UA, DA, and AA simultaneously. Additionally, the peak potential across all electrodes differed significantly and exuded batch and electrode fabrication stability. In conclusion, the CC-CNT performed as a better electrode in the detection of DA in the presence of UA and AA.

5. Conclusion

In this work we blended two chemicals, chitosan and catechol, for a new composite to be synthesized as coating on carbon nanotube and graphene electrodes. Chitosan played a key role as dispersant and stabilizer in the composite such as seen in the modified graphene and CNT electrodes. By adjusting the electrodeposition, well dispersed and stabilized electrodes were fabricated. The electrochemical investigation of the application of chitosan and catechol on the graphene and CNT bare electrodes showed novelty of CV as a technique for successful deposition of chitosan and catechol and simultaneous detection of AA, DA and UA. It generated excellent electrochemical catalytic activities towards AA, DA, and UA compared to the bare electrodes and other electrodes studied. In addition to that, well defined voltammetric responses where observed in the simultaneous detection of AA, UA, and DA. The chitosan-catechol CNT composite in particular shows good electrochemical catalytic activity to the reactions studied by considerably decreasing the over-potential of the oxidation reactions studied.

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