Electropolymerization for Dopamine Detection

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Abstract:
As investigations for development of better selectivity and sensitivity of dopamine detection continued, functionalized polymers like poly(m-aminobenzene sulfonic acid), poly(metanilic acid), poly(alizarin yellow R), poly(glutamic acid), poly(solochrome cyanine) and many conductive polymers with nanoparticle modifiers were used to fabricate polymer modified electrodes by electropolymerization method was successfully achieved. Catalytic effects produced by electropolymerized electrode modified films clearly attributed to an increment of detection level of dopamine even at micron level which could insignificantly affected by the most common co-existing interference species of ascorbic acid and uric acid with unique structural and excellent electrochemical properties make it promising candidate for the construction of more sensitive and highly selective sensors. Besides to the enhanced electrochemical catalytic ability produced by electropolymerized electrode modified films created better physical stability or free from subject of electrode fouling which alleviates one of the main limitations of bare electrode. The results indicated that the polymer modifiers on the surface of carbon based electrodes provided a mimic environment for the determination of dopamine and the electron transfer rates were gently enhanced compared with those involving electroactive protons alone at the bare based electrode.

Keywords: Dopamine, Electropolymerization, Electrochemical activity and Modified electrodes.

1. Introduction
Dopamine is one of the most significant biogenic amines belonging to the family of catecholamine, found in mammalian central nervous systems which play an important role in medication and physiological events involved in the functioning of renal, cardiovascular, hormonal and nervous systems. However, high dopamine levels are cardio toxic, leading to rapid heart rate, high blood pressure, and possible death of the heart muscles. A measure of this regulation is the concentration of dopamine released to the neurons [1]. Generally extreme abnormalities of dopamine levels are symptoms of several neurological diseases such as Parkinson’s, Alzheimer’s disease, Schizophrenia and Huntington, leading to motor dysfunction. It has been also suggested that dopamine plays a role in drug addiction and some manifestations of HIV [2,3].

Dopamine is an electrochemically active compound that can be directly oxidized at an appropriate potential and a suitable electrode material. Especially, carbon-based electrodes are shown to exhibit negligible electrode fouling upon dopamine oxidation. Dopamine is present at low concentrations in the human body (nM range) compared to other electroactive counterparts such as ascorbic acid and uric acid are normally present together with dopamine in human body samples which may interfere with dopamine signal due to their often higher concentrations and lower oxidation potentials [2,4]. Therefore, efforts have been orientated towards finding a sensitive, selective and reproducible method for the quantification of dopamine becomes requisite in the fields of clinical and analytical chemistry [5].

In the detection of dopamine, electrochemical techniques have the advantages over other methods (e.g., highly selective, less time consuming, less costly) where the concentrations of dopamine and its metabolites are monitored rather than measuring the electrical signals. As dopamine possesses very strong electrochemical activity, electrochemical detection of dopamine has received much interest [6]. For selective detection of dopamine, many different strategies including direct adsorption, cross-linking, covalent modification, and polymer film or carbon paste are now devised for the isolation and the quantification of dopamine have been used.
2. The Need of Electropolymerization for Dopamine Detection

Many analytical methods, including electrochemistry, chemiluminescence, fluorescence, spectrophotometry, gas, liquid and capillary chromatography, and thin layer chromatography with fiber optic detection, have been reported for dopamine detection. In brief, fluorometry is sample-consuming and lacks selectivity, whereas chromatography combined mostly with mass spectrometry requires sample pretreatment, lengthy analysis times, and high costs preventing them from being applied in routine analysis. Electrochemical methods, with the advantages of rapidity and cost effectiveness, have been widely employed for the detection of highly electroactive dopamine. The method, however, suffers from a major problem with the severe electroactive interference caused by endogenous ascorbic acid, uric acid and other dopamine metabolites, such as 3,4-dihydroxyphenylalanine, epinephrine and norepinephrine [7].

Electropolymerization technique is a preferable electrochemical method which enables detection of a range of chemicals from agricultural, pharmaceutical, fermentation industries and biological metabolites produced due to complex reaction sequences that has been the subject of considerable research efforts over the past three decades. Electropolymerization avenue, an attractive method for development of bioanalytical devices, allows reproducible, precise, leads to uniform, and thickness-controlled polymer coatings without limitations of the size, area, and geometry of the surfaces which increase effectiveness in rejecting the interfering anions like ascorbate compared to non-uniform Nafion coating methods [8].

As both dopamine and ascorbic acid exhibit nearly identical redox potential ranges and comparable sensitivities; thus, it is not possible to detect dopamine selectively by the known plain electrodes. Electrode fouling problems associated with dopamine polymerization can be circumvented by using modified electrodes that inhibit polymerization either physically, as, for example, by using self-assembled monolayers, or by a combination of this and modification of potential distribution as occurs in most electropolymerized films, including polypyrrole, poly aniline, polythiophene, overoxidized polypyrrole, overoxidized poly(1,2-phenylenediamine), poly(3-methylthiophene), clay modified electrodes and poly(2-picolinic acid) to increase the selectivity of the oxidation of dopamine [9].

Selectivity for electrochemical detection can also be realized by forming an electropolymerized film with a uniform and controllable thickness to block the passage of interferences from ascorbic acid and uric acid to the active area of the sensing electrode. Conducting polymers like polypyrrole and its derivatives have been widely utilized; however, their limited permeability hinders the diffusion of the target analyte [10]. In contrast, nonconductive polymers have emerged as attractive candidates for fabricating considerably thinner membranes owing to their self-limiting formation. Electropolymerized polyyramidine is a strongly adhering film with excellent permselectivity against anionic species. In addition, the hydrophilic poly(pyrrole-1-propionic acid) film is able to realize rapid diffusion, fast response with high resulting response signal. Therefore, a combination of such polymeric films could provide selectivity and sensitivity for the measurement of dopamine without compromising its detection sensitivity [11].

To this end, the general experimentation, different results and discussions are presented on the need for electropolymerization for dopamine detection in comparison with some other methods and results.

3. Experimental Part

Looking at the envisaged applications there is a common need to work with film electrodes fabricated via electropolymerization methods have attracted considerable interest due to their versatility in electrochemical bio/sensors for dopamine, ascorbic acid and uric acid detection in mixed and clinical samples. The electrochemical sensors have been widely used in detection of biogenic molecules because of their high sensitivity and selectivity, portable field-based size, rapid response time and low-cost.

Aniline, pyrrole, metanilic acid, glutamic acid, solochrome and many other derivatives were widely electropolymerized on a glassy carbon electrode, carbon paste electrode or some other metal nanoparticles or metal arrays by cyclic voltammetry, differential pulse voltammetry, square wave voltammetry and electrochemical impedance spectroscopy method in phosphate or acetate buffer solutions.

The morphology, composition, and optical properties of the resulting products were characterized by scanning electron microscopy, transmission electron microscopy, X-ray diffraction, thermogravimetric analysis, X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, and ultraviolet-visible absorption spectra.
4. Result and Discussion

The electrochemical behavior of dopamine in glassy carbon electrode (GCE) was found less reversible compared with different polymer modified and nanoparticle doped electrodes. For example if results of gold nanoparticle(AuNP)-modified GCE are compared with bare GCE, two oxidation peaks were observed for AuNP-modified GCE in the presence of dopamine at 0.43 and 0.56 V, which were clearly attributed to the formation of a polaron and the oxidation of dopamine to dopamine-o-quinone (scheme 1), respectively. This enhancement of the electrochemical response of the dopamine was due to the catalytic role of the AuNPs: the electronic transference between the redox pair and the AuNPs provokes the lowering of the oxidation peak of the neurotransmitter.

Scheme 1. Electrochemical oxidation of dopamine

\[
\text{dopamine} \xrightarrow{-2e^-} \text{dopamine-o-quinone} + 2H^+ 
\]

As the results of cyclic voltammograms of poly(N-methylpyrrole) (PNMPy) and PNMPy/AuNP-modified GCEs in the absence and presence of dopamine concentrations ranging from 1 to 10mM are displayed in figure 1, there is clear difference in the oxidation potential and current density of dopamine with in the presence of nanoparticle modifiers. Whereas figure 2 displays the results corresponding to the poly[N-(2-cyanoethyl)pyrrole] (PNCPy) and PNCPy/ AuNP-modified GCEs.

Figure 1. Cyclicvoltammograms for the oxidation of a. bare PNMPy and b. PNMPy/AuNP-modified GCEs in the absence and presence of different dopamine concentrations (from 1 to 10mM). Scan rate: 100 mV/s. Initial and final potential: -0.40 V; reversal potential: +0.80 V. Each graphic labels a-e refers to dopamine concentrations of 0, 1, 3, 6, and 10 mM, respectively.

Figure 3a and b represents the variation of the oxidation potential and the current density of the neurotransmitter against the dopamine concentration for the polymer modified GCE electrode systems. These results indicate that in both cases metal nanoparticle modified GCE electrodes improve the electrochemical oxidation response of the neurotransmitter. The oxidation potential of dopamine is clearly lower for the PNMPy/AuNP system than for the PNMPy without AuNPs (figure 3a). This effect is not so evident when PNCPy/AuNP and PNCPy are compared, even though the oxidation peak is much more pronounced in the former than in the latter (figure 2). Cathodic and anodic current densities are significantly higher for PNCPy/AuNP than for the bare PNCPy (figure 3b).
Figure 2. Cyclic voltammograms for the oxidation of a. PNCPy and b. PNCPy/AuNP-modified GCEs in the absence and presence of different dopamine concentrations (from 1 to 10 mM) at a scan rate of 100 mV/s. Initial and final potential: -0.40 V; reversal potential: +0.80 V. Each graphic, labels a-e refer to dopamine concentrations of 0, 1, 3, 6, and 10 mM, respectively.

However, although detectable, this effect is much less pronounced for PNMPy-containing systems. This difference should be attributed to the fact that the interaction of AuNPs with PNCPy is better than that with PNMPy, which is probably due to the combination of two effects: the higher roughness of PNCPy matrix and the electron-withdrawing behavior of the cyano group.

Figure 3. Variation of a. the oxidation potential and b. the current density for the oxidation peak of dopamine against the neurotransmitter concentration measured using PNMPy (▲), PNMPy/AuNP (●), PNCPy (∆), and PNCPy/AuNP-modified GCEs (□).

Figures 1b and 2b show the presence of a second oxidation peak (0.67 and 0.65 V, respectively) and the corresponding reduction peak (0.30 and 0.52 V, respectively) when the concentration of dopamine is 10 mM. The same behavior is also displayed by the PNMPy system for concentrations of 6 and 10 mM (figure 1a), whereas such a second peak is not observed in the voltammograms recorded using the PNCPy-modified GCE. The second peak has been attributed to the oxidation of dopamine-o-quinone molecules to dopamine chrome (scheme 2).
Comparing the oxidation and reduction processes, it is evidence that the oxidation of dopamine is not a completely reversible process because of its polymerization. Despite this, some reduction peaks, which should be attributed to the reduction of the conducting polymer chains, are observed. This behavior is evidenced by the anodic current density of the first oxidation peak, which is higher than the cathodic current density of the corresponding reduction peak. For the PNCPy-modified GCE, the current density ranges from 1.10 to 3.80mA/cm$^2$ when the dopamine concentration ranges from 1 to 10 mM, whereas the cathodic current density of the corresponding reduction peak is of only $\sim$0.1 mA/cm$^2$. In the case of PNCPy/AuNP-modified GCE, there is no reduction peak in the cyclic voltammograms with the exception of that recorded for a dopamine concentration of 10 mM, which shows a current density of $-0.13$ mA/cm$^2$.

Additionally, the oxidation potential increases with the concentration of dopamine for the four modified electrode systems (figure 3a). This behavior is very evident for concentrations up to 6 mM but practically inexistent when dopamine increases from 6 to 10mM, which should be attributed to the saturation of the electrode. Thus, after a threshold (6mM), the accumulation of oxidized dopamine molecules in the surface makes difficult, or even precludes, the oxidation of other neurotransmitter molecules [12]. As comparison to the effect of both conductive polymers besides to the catalytic effects produced by AuNPs in the dopamine detection process, due to the fact that charge migration through PNMPy and PNCPy is facilitated by the charge hopping in the conductor which clearly attributed to an increment of detection level of dopamine even at micron level even though the sensitivities of the polymers varied due to differences in electron availabilities. After several efforts it is possible to simultaneously and selectively detect dopamine in the presence of ascorbic acid and uric acid not only on metal based modified electrodes but also using polymer modified carbon paste electrodes and carbon nanotubes by different voltammetry technique has been successfully achieved.

As pulse voltammograms of dopamine oxidation at poly glutamic acid-carbon nanotube (PGA-SWCNT) film modified electrode exhibited a sharp peak that increased with increasing dopamine concentration (figure 4). The peak current was linear from dopamine concentrations of 3.3 to 26.6 $\mu$M which is comparable to previous reports of dopamine detection by modified gold-glassy carbon electrodes.

**Figure 4.** Different pulse voltammetry responses of dopamine on the PGA-SWCNT film electrode at various dopamine concentrations.
Investigation of electrocatalytic oxidation of dopamine mixed with ascorbic acid and uric acid gave different pulse voltammograms under optimum conditions (as shown in figure 5). The dopamine response was not influenced by increasing ascorbic acid concentration; likewise, in the presence of both ascorbic acid and uric acid, dopamine oxidation on the PGA-SWCNT film electrode changed the peak current only slightly. Mixed dopamine, ascorbic acid and uric acid showed two peaks: one for dopamine oxidation at 0.140V and another for uric acid at around 0.280V (figure 5b).

Figure 5. Different pulse voltammetry responses at the PGASWCNT film electrode on detecting dopamine a. in the presence of ascorbic acid (16, 32, 64, or 80 μM) or b. both ascorbic acid and uric acid (each analyte concentration was 32, 48, or 64 μM). Dopamine concentrations were 74 μM and 23 μM in (a) and (b), respectively.

The negatively charged PGA completely blocked ascorbic acid oxidation on the PGA-SWCNT film electrode. This effect could occur because both ascorbic acid (pKa = 4.1) and uric acid (pKa = 5.75) are negatively charged, but dopamine (pKa = 8.89) is positively charged at physiological pH (7.2) which is attracted by the negative charges on the free carboxylate groups of PGA modified SWCNT film surface. However both ascorbic acid and uric acid, which have negative charges, are repelled by the PGA-SWCNT electrode surface [13, 14].

Therefore, dopamine is attracted by the negative charges on the free carboxylate groups of PGA-SWCNT film surface which made it a promising analytical platform for detecting dopamine selectively from common counter parts such as ascorbic acid and uric acid and free from subject to surface fouling and remained stable. Had it been tested with MWCNTs, it would be much easier in the synthesis method and surface modification can easily be supported by the functionalization process. Its applicability and production costs can be minimized.

As well known, the major problem encountered with the detection of dopamine is the interference from ascorbic acid, which largely coexists and has an overlapping oxidation potential on the solid electrodes. As investigations for development of better selectivity and sensitivity of dopamine detection continued, functionalized polymers like poly(m-aminobenzene sulfonic acid) (m-ABSA) was used to fabricate polymer modified electrodes by electropolymerization method which is contained electron-rich N atom and high electron density of sulfonic group having the negatively charged polymer film make ascorbic acid no interference for detection of dopamine at physiological pH with better stability and reproducibility.

Cyclic voltammogram response of dopamine and ascorbic acid at bare GCE at pH 7 phosphate buffer solution has shown an anodic peak at the potentials of 0.192 and 0.184 V, respectively (figure 6). The peak potentials are very close and nearly overlap. But at the poly (m-ABSA) film modified electrode, it could be observed that the modification clearly shifts the oxidation potentials of dopamine and ascorbic acid toward significantly negatively potentials.
Figure 6. Differential pulse voltammograms of dopamine and ascorbic acid A. at a bare GCE and B. poly(m-ABSA) film modified GCE in 0.1 M phosphate buffer solution (pH 7) A. a: blank; b: 1 mM dopamine; c: 1 mM ascorbic acid B. a: blank; b: 1 mM dopamine; c: 1 mM dopamine + 1 mM ascorbic acid.

At pH 7 dopamine exists as a cationic amino group (pKa= 8.9) while m-ABSA is nonprotonated. Hence, oxidation of dopamine might be ascribed to the electrostatic attraction interaction between dopamine cations and the high electron density of sulfonic group of m-ABSA, such an interaction would lead to the increase in concentration of dopamine around the surface of the modified electrode.

\[
\text{O} + 2\text{H}^+ + 2e^- + \text{NH}_3\text{HO} + \text{HO}\text{O} + \text{NH}_3
\]

\[
\text{O} + \text{NH}_3\text{N} + 2\text{H}^+ + 2e^- \quad (1)
\]

\[
\text{N} + \text{HO} + \text{HO} + \text{N} + 2\text{H}^+ \quad (2)
\]

Scheme 3. Schematic determination of dopamine

In the extracellular fluid of the central nervous system, dopamine exists in only a nanomolar to micro molar range compared with the concentration of counter parts like ascorbic acid and uric acid which is very high. As a result ascorbic acid is the main interference that hinders the accurate detection of dopamine because the oxidized dopamine product, dopamine-o-quinone, can be catalytically reduced to dopamine by ascorbic acid that again becomes available for oxidation (as can be seen in figure 6 B, the dopamine oxidized current increased when ascorbic acid exists); however, when the concentration ratio of ascorbic acid to dopamine is greater than one, this interference is constant. Carefully examination of the oxidation currents of dopamine at the poly(m-ABSA) modified GCE in the presence of increasing concentrations of ascorbic acid (figure 7a) did not obviously change in the oxidation currents of dopamine when the concentration of ascorbic acid changed. Moreover, there was hardly any response for ascorbic acid oxidation at the poly(m-ABSA) modified electrode.
Figure 7. A. Differential pulse voltammograms at pH 7 phosphate buffer solution at poly (m-ABSA) modified GCE containing 50 μM dopamine in the presence of different concentrations of ascorbic acid: a. 0 μM b. 500 μM c. 600 μM d. 700 μM e. 800 μM f. 900 μM and g. 1000 μM. B. Differential pulse voltammograms at poly (m-ABSA) modified GCE containing 1 mM ascorbic acid in the presence of different concentrations of dopamine: a. 0 μM b. 5 μM c. 10 μM d. 15 μM e. 20 μM f. 25 μM and g. 30 μM.

As can be seen in figure 7b, the oxidation currents of dopamine increased proportionally with dopamine’s concentration while the peak current of ascorbic acid remained constant, indicating that the poly (m-ABSA) electrode was sensitive only to dopamine. This means that in the real biological matrixes, where the ascorbic acid level is usually more than three orders of magnitude larger than dopamine, the poly(m-ABSA) film modified electrode could be used for the determination of dopamine in the real sample. One of the problems of determination of dopamine by the bare electrode is the fouling of electrode surface, but in this work the peak current of dopamine remained constant after the scan cycles of up to 7 times [15,16].

Simultaneous determination of dopamine, ascorbic acid and uric acid is of critical importance not only in the field of neurochemistry and biomedical chemistry but also in diagnostic and pathological research. As a result, different researchers are still working to overcome the poor selectivity and sensitivity of the common electrodes which is a timely question to look for alternative modifiers of electrode surface through electropolymerization, employing active dopant nanoparticles, self-assembled monolayers or surface modifying thin films of conjugated polymers.

The easily oxidizable neurotransmitters showed higher sensitivity at palladium doped poly(solochrome cyanine) modified carbon paste electrode (polySCCy/PdNPs/CPE) (curve d) when compared with polySCCy film/CPE (curve c) and bare CPE (curve b) respectively as it is easily in figure 8.
The cyclic voltammograms of dopamine at polySCCy/PdNPs/CPE showed its anodic peak potential (Epa) and cathodic peak potential (Epc) at 320mV and 200mV with peak difference of 120 mV, respectively. Both anodic and cathodic peak currents of dopamine obtained shows 15-fold increments at polySCCy/CPE and 25 fold increments at polySCCy/PdNPs/CPE in comparison with bare CPE. The doped Pd nanoparticles in the polySCCy/CPE film had drastically enhanced number of the electroactive sites on the limited electrode surface and hence the peak currents were increased at polySCCy/PdNPs/CPE in comparison with polySCCy/CPE. This result indicated that the modifiers on the surface of CPE provided a mimic environment for the determination of dopamine and the electron transfer rates were gently enhanced compared with those involving electroactive protons alone at the bare carbon paste electrode.

The polySCCy/PdNPs/CPE was employed towards the electrocatalytic oxidation of dopamine by varying its concentrations from $5 \times 10^{-6}$ M to $1 \times 10^{-3}$ M by cyclic voltammetry and differential pulse voltammetry methods and as it was shown in figure 9 A and B there is direct correlation between the peak current and concentration of dopamine.

Figure 9A. Cyclic voltammograms of dopamine for the different concentrations (M) a. $5 \times 10^{-6}$, b. $6 \times 10^{-6}$, c. $7.0 \times 10^{-6}$, d. $8 \times 10^{-6}$, e. $9 \times 10^{-6}$, f. $1 \times 10^{-5}$, g. $2 \times 10^{-5}$, h. $3 \times 10^{-5}$, i. $4 \times 10^{-5}$, j. $5 \times 10^{-5}$, k. $6 \times 10^{-5}$, l. $7 \times 10^{-5}$, m. $8 \times 10^{-5}$, n. $9 \times 10^{-5}$, o. $1 \times 10^{-4}$.
p. 2x10^{-4}, q. 3x10^{-4}, r. 4x10^{-4}, s. 5x10^{-4}, t. 9 x 10^{-3}, u. 3 x 10^{-3}. B. differential pulse voltammmograms of dopamine for the different concentrations (M) a. 5x10^{-6}, b. 6 x 10^{-6}, c. 7.0 x 10^{-6}, d. 8 x 10^{-6}, e. 9 x 10^{-6}, f. 1 x 10^{-5}, g. 2 x 10^{-5}, h. 3 x 10^{-5}, i. 4 x 10^{-5}, j. 5 x 10^{-5}, k. 6 x 10^{-5}, l. 7 x 10^{-5}, m. 8 x 10^{-5}, n. 9 x 10^{-5}, o. 1 x 10^{-4}, p. 2 x 10^{-4}, q. 4 x 10^{-4}, r. 6 x 10^{-4}, s. 8 x 10^{-4}, t. 1 x 10^{-3}, u. 3 x 10^{-3}.

The developed method, nanoparticle-polymer modified electrode was tested in the cyclic and differential pulse voltammetric determination of mixtures of biological compounds such as dopamine (4 x 10^{-5} M), ascorbic acid (10 x 10^{-5} M) and uric acid (4 x 10^{-5} M) simultaneously and selectively. Figure 10A and B shows CV and DPV for the mixture of dopamine, ascorbic acid and uric acid at the bare CPE (curve b) and polySCCy/PdNPs/CPE. The oxidation peaks of dopamine, ascorbic acid and uric acid were unable to be separated at bare CPE. The polymer film modified chemical sensor reduces the over potential, required for the oxidation of dopamine, ascorbic acid and uric acid. CV and DPV at polySCCy/PdNPs/CPE showed good separation with well-defined peaks for dopamine, ascorbic acid and uric acid with distinct and different oxidation potentials. The separation of oxidation peak potentials between dopamine, ascorbic acid and uric acid played an important role in the analysis of dopamine. This result was sufficient to recognize and detect dopamine, in the presence of ascorbic acid and uric acid, at polySCCy/PdNPs/CPE.

Figure 10. Voltammograms for simultaneous determination of 4.0 x 10^{-5} M dopamine, 10 x 10^{-4} M ascorbic acid and 4 x 10^{-5} M uric acid, at polySCCy/PdNPs/CPE. A. CV B. DPV C. differential pulse voltammograms of a. 2 x 10^{-5} M b. 4 x 10^{-5} M c. 6 x 10^{-5} M d. 8 x 10^{-5} M e. 1 x 10^{-4} M f. 1.2 x 10^{-4} M g. 1.6 x 10^{-4} M h. 2.2 x 10^{-4} M of dopamine in ABS of pH 5 in the presence of 2 x 10^{-5} M ascorbic acid and 4 x 10^{-5} M uric acid at polySCCy/PdNPs/CPE. D. differential pulse voltammograms of a. 2 x 10^{-5} M b. 4 x 10^{-5} M c. 6 x 10^{-5} M d. 8 x 10^{-5} M e. 1.0 x 10^{-3} M f. 1.2 x 10^{-3} M g. 1.6 x 10^{-3} M h. 2.0 x 10^{-3} M i. 2.6 x 10^{-3} M j. 3.2 x 10^{-3} M of uric acid in ABS of pH 6 in the presence of 2 x 10^{-5} M dopamine and 4 x 10^{-5} M ascorbic acid at polySCCy/PdNPs/CPE. E differential pulse voltammograms of a. 5 x 10^{-4} M b. 1.0 x 10^{-3} M c. 1.5 x 10^{-3} M d. 2 x 10^{-3} M of ascorbic acid in ABS of pH 5 in the presence of 4 x 10^{-5} M dopamine and 4 x 10^{-5} M uric acid at polySCCy/PdNPs/CPE.
Ascorbic acid and uric acid coexist with dopamine in the extracellular fluid of the central nervous system and their concentrations are much higher than dopamine. The resolution of dopamine in the presence of ascorbic acid and uric acid at bare CPE was not an easy process since the oxidation potentials of all three biological compounds were close, but with the application of polySCCy/PdNPs/CPE the resolution was easier, since all three compounds had well separated oxidation potentials. The resolution of dopamine, ascorbic acid and uric acid in the mixture solution was investigated by DPV technique because it provides a better peak resolution and sensitivity in comparison to CV. The investigation was carried out by changing the concentration of each individual and by keeping the concentration of remaining two species constant.

Under the suitable conditions, the DPV current was proportional to dopamine concentrations, over the concentration ranges of $2 \times 10^{-5}$ M to $2.2 \times 10^{-4}$ M, when keeping the concentration of ascorbic acid $2 \times 10^{-5}$ M and uric acid $4 \times 10^{-5}$ M constant (figure 10C). It can be seen that, there was no change in peak currents for ascorbic acid and uric acid. Similarly in the same way results of figure 10D and E show various concentrations of ascorbic acid ($5 \times 10^{-4}$ M to $2 \times 10^{-3}$ M) and uric acid ($2 \times 10^{-5}$ M to $3.2 \times 10^{-4}$ M). These results show that at polySCCy/PdNPs/CPE the overlapping and inference from ascorbic acid and uric acid was greatly reduced in the determination of dopamine.

Figure 11.A. EIS spectrum 0.1M ABS of pH (5.)/1mM K$_3$[Fe(CN)$_6$]/K$_4$[Fe(CN)$_6$]/ 2 x 10$^{-4}$M dopamine a. bare CPE b. at polySCCy/PdNPs/CPE. B. Equivalent circuit at polySCCy/PdNPs/CPE.

Electrochemical impedance spectroscopic technique, an emerging method, was used in identifying the surface nature of the electrode. Nyquist diagram of $2 \times 10^{-4}$ M dopamine in 1 mM [Fe(CN)$_6$]$^{3-}$/4$^{-}$ and 0.1 M acetate buffer solution (pH 5) at bare and modified electrodes are illustrated in figure 11. At polySCCy/PdNPs/CPE the interfacial electron transfer rate (Ret) was increased greatly (curve b) in comparison with bare CPE (curve a). This indicated that the polySCCy/PdNPs/CPE modified electrode showed less resistance and faster electron transfer rate. The Randles equivalence circuit for polySCCy/PdNPs/CPE is shown in figure 11B, where Rs represents solution resistance, ‘Ret’ represents electron/charge transfer resistance and ‘Cd1’ represents double layer capacitance and ‘Q’ is constant phase element [17, 18].

More sensitive and selective method based on a poly(alizarin yellow R)-modified carbon paste electrode (PAYR/CPE) was successfully established to detect dopamine and uric acid. The developed modified electrode, PAYR/CPE shows excellent electrocatalytic activities towards the oxidation of dopamine as it can be visualized in the cyclic voltammograms of figure 12 at a bare CPE (curve a) and a PAYR/CPE (curve b). At the bare CPE, a weak oxidation peak potential of 0.4 V was observed and almost no reduction peak was exhibited. In contrast, a couple of well-defined redox peaks were obtained at PAYR/CPE, accompanied with a six-fold enhanced anodic peak current (Ipa). The anodic peak potential (Epa) has shifted negatively to 0.170V, and the cathodic peak potential(Epc) appeared at 0.132V, which resulted in a well-defined redox peak of dopamine with a separation of peak potentials of 0.038 V. The greatly enhanced peak current and smaller peak separation strongly indicated the excellent catalysis ability of PAYR film and the faster electron transfer of dopamine. The voltammetry results demonstrated that the PAYR film is conductive and the PARY/CPE could greatly increase the electron transfer rate of the analyte rather than blocking it. This suggested that the PAYR/CPE shows good electrochemical oxidation towards dopamine.
As differential pulse voltammetry has higher current sensitivity and better resolution than cyclic voltammetry, DPV was used to detect different concentrations of dopamine and uric acid and their responses at the PAYR/CPE were recorded in figure 13 A and B.

The individual determination of dopamine or uric acid in their mixtures was performed by DPV on PAYR/CPE with the concentration of one species changed and that of the other species kept constant. Figure 13A shows the DPV curves for different concentrations of dopamine at pH 7 of phosphate buffer solution coexisting with 0.1 mM uric acid. The results showed that the peak current of dopamine was proportional to its concentration, while the oxidation peak current for uric acid remained nearly unchanged. Similarly, as shown in figure 13 B, keeping the concentration of dopamine unchanged, the anodic peak current of uric acid increased linearly with the concentration of uric acid and without obvious influence on the peak response of dopamine. The results indicate that the responses to dopamine and uric acid at the PAYR/CPE are comparatively independent which in turn led to conclude the detection
of dopamine could insignificantly affected by the most common interfering species of ascorbic acid, uric acid, glucose, citric acid and tartaric acid at PAYR/CPE.

Figure 14. Electrochemical impedance spectroscopy on a. bare CPE and b. PAYR/CPE. EIS was obtained in a 5 mM\( \text{K}_3\left[\text{Fe(CN)}_6\right]\)/\( \text{K}_4\left[\text{Fe(CN)}_6\right] \) (1:1) mixture containing 0.1M KCl, and the applied perturbation amplitude was 0.005 V.

Electrochemical impedance spectroscopy (EIS) was carried out to further study the efficiency of the modified electrode and clarifies the electrochemical performance differences between bare CPE and PAYR/CPE. EIS, a powerful tool for probing the features of surface-modified electrodes, provided useful information on the impedance changes of the modified electrode surface. This resistance controls the electron transfer kinetics of the redox-probe at the electrode interface as displayed in the Nyquist plots: curve (a) shows a large diameter of semicircle for the bare CPE. However, curve (b) shows an arc the diameter of the semicircle diminished when PAYR/CPE were employed. The Nyquist diameter of the PAYR/CPE is much smaller than that of the bare CPE, which suggests that the PAYR film coated on the CPE can further accelerate the electron transfer of the redox probe [19]. Electropolymerization of alizarin yellow-R played a great role on modifying uniform surface with improved sensitivity and selectivity of low level dopamine detection.

Conductive polymers such as polypyrrole and polyaniline have been widely used to alleviate the problems with dopamine detection. Their widespread popularity arises not only because of their easier fabrication processes and good electrical conductivity but also because it is possible to increase the functional density and facilitate electron exchange through chemical substitution on the polymer chain. The multi-functionality of metal/conductive polymer composites is particularly useful, as they are endowed with enhanced electrochemical catalytic ability and better physical stability.

Combination of the benefits of metal electrodes such as gold cavity array (GCA) electrode and functionalized polymers like poly(metanilic acid) helped to develop more sensitive and highly selective electrochemical dopamine sensors. Electropolymerized thin film of poly(metanilic acid) deposited on the concave bowl-shaped surface of the GCA electrode which drastically enhanced the surface area available for the sensitive detection of the target analyte and the electrocatalytic ability of the gold nanoparticles (AuNPs) and their strong interaction with the polyaniline backbone significantly improved the stability of the poly(metanilic acid).

The comparative cyclic voltammograms of 0.5 mM dopamine at the bare Au and GCA electrodes modified with poly(metanilic acid) are shown in figure 15.
A couple of definite reversible redox peaks, located at 0.132 and 0.272 V, were clearly observed at the GCA electrode. It is obvious that the same redox peaks could also be obtained at the bare Au electrode with little resolution. However, the peak current for dopamine oxidation at the GCA electrode was 74.66 mA, which was 9 times larger than that recorded at the bare Au electrode (8.43 mA) under identical working conditions. The better electrochemical response exhibited at the poly(metanilic acid) modified GCA electrode which is resulted to the combination of the contribution of effects of larger surface area of the GCA, excellent anti-fouling property of the poly(metanilic acid) thin film against the oxidation products, easy diffusion of dopamine through the thin film to the surface of GCA, and the possibility of the electrostatic interaction between the negatively charged sulfonic group of the polymeric modifier and the positively charged dopamine (dopamine is positively charged at pH 7.4 or in a neutral environment). Thus, the composite film with unique structural and electrochemical properties could be a promising candidate for the construction of more sensitive and highly selective sensors.

Although the surface area of the GCA electrode was enlarged as a result of its modification, with a concomitant increase in faradaic current, the capacitive current also showed an increase, and thus when using cyclic voltammetry, the overall signal-to-noise ratio would not be improved significantly. Consequently, square wave voltammetry (SWV) technique was preferred and employed for the quantitative determination of dopamine, because it can minimize the influence of the capacitive current and render a real improvement in sensitivity. As displayed in figure 16, parts of the SWV responses of dopamine with different concentrations at the poly(metanilic acid) modified GCA electrode, the oxidation peak currents increased linearly with the concentrations of dopamine ranging from 0.2 to 100 mM. However, when the concentrations of dopamine were greater than 100 mM, the responses of the SWV currents gradually deviated from linearity as it can be seen from the calibration curve. According to the IUPAC definition, the limit of detection was calculated to be 0.08 mM. The lower detection limit and wider linear range are due to the excellent electrochemical activity of the GCA based composite film towards dopamine.
Figure 16. Typical SWVs of the poly(metanilic acid) modified GCA electrode in 0.1 M PBS (pH 7) solution containing increasing concentrations of dopamine (0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 mM). The inset is the linear relationship between dopamine peak current and concentration.

It is well known that ascorbic acid and uric acid usually have a higher concentration than dopamine in biological samples and are oxidized at approximately similar redox potential, resulting in an overlapped voltammetric response. The oxidation of coexisting dopamine and uric acid at the poly(metanilic acid) modified GCA electrode was carried out by SWV curves and the results show dopamine in the presence of 0.2 mM uric acid in the buffer solution was simultaneously detected at various concentration ranges (figure 17a). As observed, the oxidation peak currents of dopamine at +0.17 V increased with the increasing dopamine concentration from 2 to 25 mM in the presence of uric acid, while the oxidation peak currents of uric acid at +0.46 V remained unchanged.
Figure 17. SWVs of the modified GCA electrode in 0.1 M PBS (pH 7) solution containing a. 0.2 mM uric acid and increasing concentrations of dopamine (2.0, 5.0, 10.0, 15.0, 20.0, 25.0 mM), b. 20.0 mM dopamine and increasing concentrations of uric acid (2.0, 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 100.0 mM).

The signal of dopamine can be clearly detected, even when the concentration of uric acid is 500 times higher than that of dopamine. Figure 17 b shows the SWV obtained at the modified electrode for the different concentrations of uric acid in the presence of 20 mM dopamine. The uric acid peak current increased with increasing concentration of uric acid from 2 to 100 mM and the dopamine peaks were essentially unchanged. These results verified that dopamine and uric acid could be individually and simultaneously detected at the modified electrode with high sensitivity and selectivity [20-22].

5. Outlook

Had it been tested with MWCNTs instead of SWCNTs, its synthesis process would be much easier and surface modification of an electrode can easily be facilitated by different functional groups and defects in MWCNT. Its applicability and production costs can be minimized. Catalytic effects produced by metal nanoparticles in the polymer composite film modified electrode played great role for the construction of more sensitive and highly selective sensors for dopamine detection. But the effect of the types of polymer modifiers has no clear pattern on facilitating the electrochemical activities with different contributing factors such as conductivities of the polymers and nature of functional density. Generally, the multi-functionality of polymer/nanoparticle/carbon based composite electrode is particularly useful, as they are endowed with enhanced electrochemical catalytic ability and better physical stability or free from subject of fouling. This led to conclude the detection of dopamine could insignificantly affected by the most common interfering species of ascorbic acid, uric acid, glucose, citric acid and tartaric acid with in the developed of polymer/nanoparticle/carbon based composite electrodes.

6. References


