

The Effect of Electrochemical Pretreatment on the Performance of Single Walled Carbon Nanotubes

Subbiah Alwarappan¹, Shradha Prabhulkar¹, Andriy Durygin², and Chen-Zhong Li^{1,*}

¹Department of Biomedical Engineering, Nanobioengineering/Bioelectronics Lab,
Florida International University, Miami, Florida, 33174, USA

²Centre for the Study of Matter at Extreme Conditions, Florida International University,
University Park, Miami, Florida, 33174, USA

In this paper, we report the effect of subsequent electrochemical anodization and cathodization subjected to single walled carbon nanotubes. The performance of the single walled carbon nanotubes before and after activation was evaluated electrochemically using different charged redox probes and a biologically important neurotransmitter-dopamine. Further, for the first time, the surface of single walled carbon nanotubes before and after electrochemical activation was also evaluated using Raman Spectroscopy. Electrochemical results indicated that the pretreatment method employed in this work greatly enhanced the performance of single walled carbon nanotubes towards the detection of the model analytes employed. The observed enhancement in the signal is attributed to the rupture of the basal plane found on the end caps of carbon nanotubes giving rise to a larger density of edge-plane like defects on the single walled carbon nanotubes surface. A detection limit of 10 nM (based on three times the standard deviation of the slope) was also estimated when dopamine was monitored using electrochemically activated single walled carbon nanotube electrodes. In addition, the electrochemically activated carbon nanotubes exhibited an improved selective and sensitive detection of our model analyte dopamine with a minimal electrode fouling of about $3\% \pm 1.2\%$ ($N = 4$).

Keywords: Electrochemistry, Biosensor, Single Wall Carbon Nanotubes, Electrochemical Pretreatment, Neurotransmitter, Dopamine.

1. INTRODUCTION

It is a well known fact that the electrochemical activation of carbon based electrodes will result in improved performance during electroanalysis. Usually, in all the electrochemical pretreatment methods, researchers initially apply large positive potentials to the working electrode followed by the application of desired negative potentials. The exact amplitude and the duration of the applied potential depend on the type of carbon material employed and it varies considerably. Engstrom et al.¹ demonstrated the performance of the electrochemically pretreated electrodes were better than their counterparts freshly polished on emery paper and alumina. There are varied reasons for the enhanced activity of the electrochemically pretreated electrode.^{2–7} Though, it is widely accepted that electrochemical pretreatment introduces or alters the nature of surface active groups that might serve as mediators of electrons between the electrode surface and electroactive species.^{1,8–9} Amongst

various surface active groups introduced or altered during this pretreatment method, quinone and quinone alike functionalities appear to be the likely candidate that mediates or enhances the electron transfer process.^{8–13} On the other hand, several research groups demonstrated the effect of electrochemical pretreatment with highly oriented pyrolytic graphite (HOPG) which possesses a well-defined layer. It has been demonstrated by Rice et al.¹⁴ and Bowling et al.¹⁵ that upon the application of anodic pulse for a definite time interval to HOPG, the less reactive basal plane of HOPG surface cleaves, resulting in the exposure of larger quantity of reactive edge planes (graphitic surface), that possibly enhances the electron transfer rate. Electrochemical pretreatment is often performed to generate reproducible surface and to achieve improved electron transfer behaviors. Similar to carbon based electrodes, electrodes made out of carbon nanotube (CNT) have been recently employed in many electroanalytical applications.¹⁶ Their unique electronic property is widely useful in electrochemistry for promoting the electron transfer process occurring in biological compounds such as

*Author to whom correspondence should be addressed.

proteins, peptides, neurotransmitters and NADH.^{17–20} The electron transfer electrochemical property of the CNT's varies markedly with the method of its preparation.²¹ For example, if we compare the electrochemical reactivity of CNT's obtained by CVD and ARC process, the former exhibits a greater reactivity.²¹ This observed phenomenon is attributed to the presence of larger density of edge plane defects witnessed in the CNT's obtained by CVD technique.¹⁶ The origin of the electrochemical reactivity of the CNT's has been recently investigated in detail by Banks et al.^{22,23} In addition, they also demonstrated the similarities existing between edge plane graphites and CVD-CNT's in their electrochemical behavior. Recently, the effect of electrochemical pretreatment of ARC-CNT's and CVD-CNT's was studied by Wang et al.¹⁶ Their results indicated that ARC-MWCNT's is electrochemically reactive than the CVD-MWCNT's. According to Wang et al.,¹⁶ the observed property of CVD-MWCNT's is due to breaking up of the end caps during pre-anodisation resulting in the formation of reactive new edge plane like sites, similar to those that occur at the open-ends of untreated CVD-MWCNT's. However, the fundamentals of how electro-activation impacts the structure and functions of carbon nanotube still remained unclear.

In this work, we investigated in detail the effects of electrochemical pretreatment on the functionalities and structural changes of SWCNT. The redox activities and sensing function of SWCNT's before and after electrochemical pretreatment were compared using different charged redox probes and a neurotransmitter-dopamine. In addition, the nano structure of SWCNT's was also physically characterized using Raman spectroscopy. Results indicated that the electrochemical pretreatment was effective and resulted in the breaking up the end caps of CNT's creating a larger density of open ends that contributed for the observed enhanced electrochemical reactivities.

2. EXPERIMENTAL DETAILS

2.1. Chemicals

Potassium chloride, dopamine, Potassium ferricyanide were all purchased from Sigma-Aldrich, USA and are employed without any further purification. Required redox solutions were freshly prepared using de-ionised water every day before the start of experiments. Single walled carbon nanotubes (diameter 2 nm, length 0.5 μm –2 μm , 90% Pure) were purchased from Sigma and then subjected to acid treatment as explained by Rusling et al.²⁴

2.2. Electrochemical Set-Up

Cyclic voltammetric and differential pulse voltammetric measurements were carried out using CHI-630A electrochemical analyzer (CH Instruments, Inc., Austin, TX). A conventional three electrode cell consisting of a

Ag|AgCl reference electrode (3 M KCl) (Bioanalytical Systems, IN, USA) and a platinum wire as an auxiliary electrode was employed.

2.3. CNT's Immobilization Method

SWCNT's purchased were subjected to acid treatment by ultrasonating them in a 1:3 H_2SO_4 - HNO_3 mixture for 2 hours at 70 °C. According to Rusling et al.^{24,25} this treatment will shorten and functionalize the SWCNT's. Following the acid treatment SWCNT's were filtered, washed with de-ionized water and dried for about 10 hours at room temperature. The dried SWCNT's were then dissolved in DMF and used for immobilization. In this work, 3 mg of SWCNT's was dissolved in 1 mL of DMF. The solution was then sonicated for about 3 hours to facilitate the complete dissolution of SWCNT's. About 5 μL of this solution was then placed on the polished glassy carbon electrode and dried under IR-lamp for about ten hours.

2.4. Electrochemical Pretreatment

SWCNT's immobilized on to the electrode surface were initially subjected to an optimized anodization by applying a potential of +1.6 V for about 600 seconds. This was then followed by optimized cathodization in which a potential of –1.2 V was applied for 300 seconds. The pretreatment process was performed in a pH 7.2 PBS buffer.

2.5. Raman Spectroscopy

Raman spectroscopic measurements were conducted at room temperature by using Raman spectrometer in the back scattering configuration. The 514.5 nm Ar^+ laser was operating at 50 mW. Raman spectra were collected with 10 min exposure time by using high throughput holographic imaging spectrograph with volume transmission grating, holographic notch filter and thermoelectrically cooled CCD detector with the resolution of 4 cm^{-1} .

3. RESULTS AND DISCUSSION

3.1. Voltammetry of Positively Charged Redox Probe at SWCNT's Before and After Electrochemical Pretreatment

Initially cyclic voltammetry of 1.0 mM $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in 1.0 M KCl were performed before and after electrochemical activation of SWCNT's. Figure 1(a) represents the cyclic voltammograms obtained using SWCNT's before electrochemical pretreatment. As a consequence of electrochemical pretreatment, we expected that there will be some negative charge on the activated SWCNT's and this will enhance the reduction of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ which will give rise to a greater peak current. However, the cyclic voltammogram of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ following electrochemical

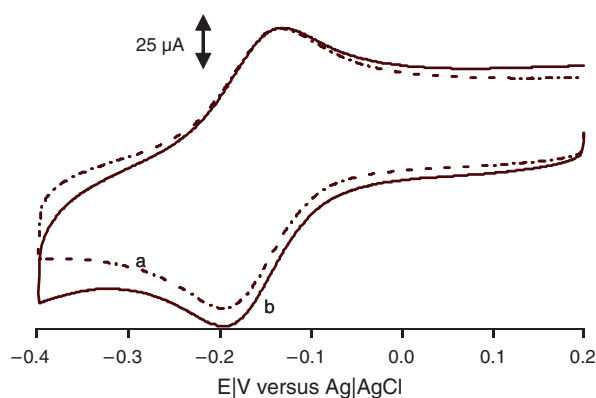


Fig. 1. Cyclic voltammetry of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ at SWCNT's (a) before pretreatment, (b) after electrochemical pretreatment.

pretreatment indicated that there is only a minimal change in the peak current as shown in Figure 1(b). From this, it is evident that the negative charge present on the SWCNT's following electrochemical activation has negligible effect in mediating the reduction of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ or there is only an insufficient amount of negative charge present on the activated SWCNT's.

3.2. Voltammetry of Negatively Charged Redox Probe at SWCNT's Before and After Electrochemical Pretreatment

In order to probe the effect of electrochemical pretreatment, we then ventured to use a different redox probe possessing different charge. Chen et al.² and McCreery et al.⁸ demonstrated that cyclic voltammetry of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is very sensitive to the electrochemical properties and surface chemistry, particularly the hydrogen or oxygen functional groups, on carbon based electrodes. According to Yuan et al.,²⁶ carbon nanotube can also be considered as a "Rolled-up" structure of graphite with basal and edge planes. Therefore, $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is useful redox probe for evaluating the electron kinetics at the SWCNT's before and after pretreatment. In our work, cyclic voltammetry and differential pulse voltammetry of SWCNT's in 1.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 1.0 M KCl were performed before and after electrochemical pretreatment of SWCNT's. Figure 2(a) represents the cyclic voltammogram obtained using SWCNT's prior to electrochemical pretreatment. On the other hand, Figure 2(b) represents the cyclic voltammogram obtained using electrochemically pretreated SWCNT's. From the figure, it is evident that the CV obtained at the pretreated SWCNT's exhibited a higher

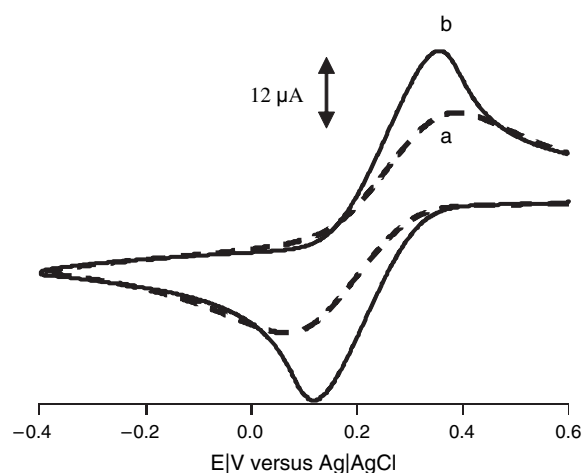


Fig. 2. Cyclic voltammetry of $[\text{Fe}(\text{CN})_6]^{3+}$ at SWCNT's (a) before pretreatment, (b) after electrochemical pretreatment.

current than the untreated SWCNT's. In addition, the onset of $[\text{Fe}(\text{CN})_6]^{3-}$ reduction starts to occur a little earlier in the pretreated SWCNT's than the untreated SWCNT's. The observed increase in current is possibly due to the greater density of "edge-plane" like defects present in the SWCNT's that resulted in an enhanced electroactivity due to electrochemical pretreatment. Pretreatment, results in altering the nature of functional groups on the electrode surface which can act as a possible mediator for electron transfer occurring between the electrode surface and the electroactive species. Previous investigations have suggested that quinone like functional groups are the possible candidates that serve as mediators enhancing the electrochemical activity of the pretreated electrodes. The electrochemical parameters corresponding to this redox reaction was tabulated in Table I. A less positive ΔE_p value, following the pretreatment is an indication of the faster electron transfer occurring at the modified surface as explained by Chen et al.^{2,8}

3.3. Voltammetry of Dopamine at SWCNT's Before and After Electrochemical Pretreatment

Dopamine (DA) is a catecholamine neurotransmitter that has been extensively studied all these years as a result of its important role within neurobiological systems. Aside from being a precursor to epinephrine and nor-epinephrine, dopamine also has an essential role in the neurological reward centers and pathways; specifically the substantia nigra, caudate-putamen, ventral tegmental area and

Table I. Calculated electrochemical parameters of SWCNT's before and after electrochemical pretreatment.

Redox system	$[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$		$[\text{Fe}(\text{CN})_6]^{3-/4-}$		Dopamine	
	ΔE_p (mV)	$I_p \times 10^{-5}$ (A)	ΔE_p (mV)	$I_p \times 10^{-4}$ (A)	ΔE_p (mV)	$I_p \times 10^{-4}$ (A)
Before pretreatment	50 ± 8	4.2 ± 0.8	248 ± 23	7.65 ± 0.002	118 ± 8	0.83 ± 0.86
After electrochemical pretreatment	49 ± 6	4.8 ± 1.5	223 ± 15	9.45 ± 0.001	114 ± 4	1.18 ± 0.15

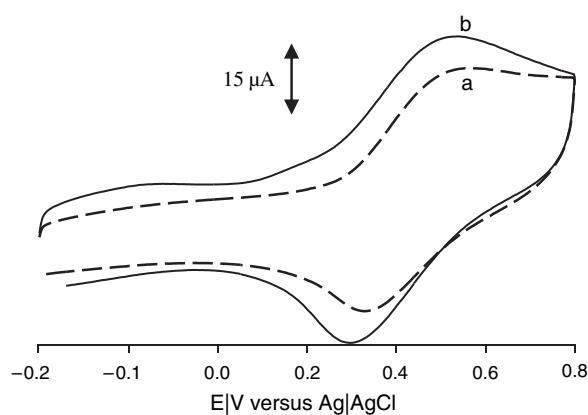


Fig. 3. Cyclic voltammetry of dopamine at SWCNT's (a) before pretreatment, (b) after electrochemical pretreatment.

nucleus accumbens.^{27–30} Therefore, detection and continuous monitoring of the level of dopamine *in vivo* is of considerable interest. As dopamine is easily oxidised at an electrode surface, electrochemical techniques such as voltammetry and chronoamperometry, in conjunction with anatomical, physiological and pharmacological evidence, provide a sensitive and rapid means of detection.^{31,32} In this work, we compared the performance of SWCNT's towards the detection of dopamine before and after electrochemical pretreatment. Figure 3(a) and (b) are the CV response of the SWCNT's towards the detection of 1.0 mM dopamine in pH 7.2 PBS buffer before and after electrochemical pretreatment respectively. Figure 4(a) and (b) represents the DPV responses of the SWCNT's towards the detection of 1.0 mM dopamine pH 7.2 PBS buffer before and after electrochemical pretreatment respectively. In both these techniques, we noticed a higher current corresponding to the oxidation of dopamine with pretreated SWCNT's. The electrochemical parameters corresponding to this redox reaction were tabulated in the Table I.

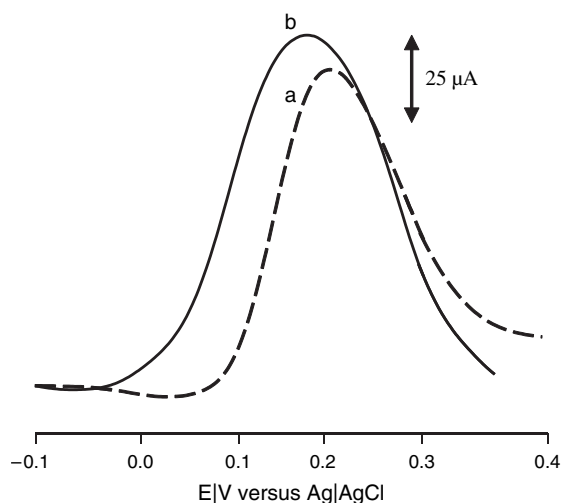


Fig. 4. Differential pulse voltammetry of dopamine at SWCNT's (a) before pretreatment, (b) after electrochemical pretreatment.

The observed increase in the oxidation current without altering its ΔE_p to a greater extent is possibly attributed to the increased electroactivity of the SWCNT's following electrochemical pretreatment as reported by Wang et al.¹⁶ The enhanced electrochemical activity of the SWCNT's, following pretreatment, is attributed to the edge plane defects at the open ends of the CNT rather than on their side walls that resembles basal plane pyrolytic graphite.^{16,26} The electrochemical pretreatment performed resulted in breaking up the end caps and expose new edge plane sites that favor catecholamine electrochemistry that was obvious with the enhanced voltammetric behavior of the pretreated SWCNT's. Thus, the edge plane like defects originated as a result of electroactivation serve as a favorable electron transfer site for catecholamine electrochemistry.

3.4. Comparison of Raman Spectra of the SWCNT's Before and After Pretreatment

Raman Spectra of SWCNT's before and after electrochemical pretreatment is shown in Figure 5(a) and (b) respectively. In both the spectra, we noticed the SWCNT's characteristic peaks centered at 220 cm^{-1} , 1335 cm^{-1} and 1590 cm^{-1} . The peak centered at 220 cm^{-1} is the radial breathing mode (RBM) of SWCNT and is shown on the inset of the Figure 5. The peak centered at 1335 cm^{-1} is the disordered D-band of SWCNT's and the peak centered at 1590 cm^{-1} is the tangential G-mode of SWCNTs. The intensity ratio of D-band to G-band was calculated for SWCNT's before and after activation. Prior to electrochemical pretreatment the SWCNT's exhibited an intensity ratio (I_D/I_G) of 0.35, whereas after pretreatment SWCNT's exhibited an intensity ratio of 0.23. The decrease in the ratio following pretreatment is possibly attributed to the surface modification taken place after the electrochemical pretreatment. According to Choi et al.,³³ the D-band and the G-band correspond to sp^2 and sp^3 carbon stretching

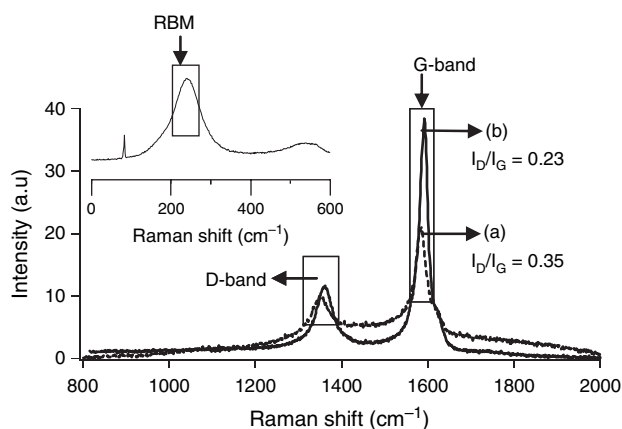


Fig. 5. Raman spectrum of SWCNT's (a) before pretreatment, (b) after electrochemical pretreatment.

modes and their intensity ratio is a measure of the amount of disorder in the CNTs. A decrease in the I_D/I_G ratio after pretreatment in our work is possibly attributed to a greater sp^3 character in the SWCNT's as compared to that of sp^2 character. Thus the electrochemical pretreatment of SWCNT's resulted in a greater percentage of sp^3 like structure onto the SWCNT's which will be helpful for enhanced electrochemical reactivity and greater resistance towards the attack of biological molecules (less electrode fouling character).

3.5. Evaluation of Electrode Fouling Before and After Electrochemical Activation

In order to evaluate the fouling of electrodes during the detection of dopamine, differential pulse voltammograms were performed using 0.1 mM DA using unactivated SWCNT and electrochemically activated SWCNT's separately and is shown in Figure 6. With each electrode, the difference in current between the first and the twenty fifth scan was evaluated and from this the percentage of electrode fouling was evaluated. Results indicated that the electrochemically activated SWCNT's exhibited $3\% \pm 1.2\%$ ($N = 4$) decrease in the peak current whereas the unactivated SWCNT's showed a $42\% \pm 7\%$ ($N = 4$) decrease. The better performance of the electrochemically activated SWCNT's is due to the greater sp^3 like character associated with it than the sp^2 kind character associated with unactivated SWCNT's. Electrochemically activated SWCNT's is expected to possess a greater sp^3 like character and as a result the de-localization of pi-electrons is not as effective as the one witnessed in unactivated SWCNT's. As a result, electrochemically activated SWCNT's shows a better stability after multiple scans than its unactivated counterpart.

3.6. Effect of Scan Rate and Concentration on the Redox Behavior of DA

In order to investigate the mass transport phenomenon of dopamine, cyclic voltammetry was performed using

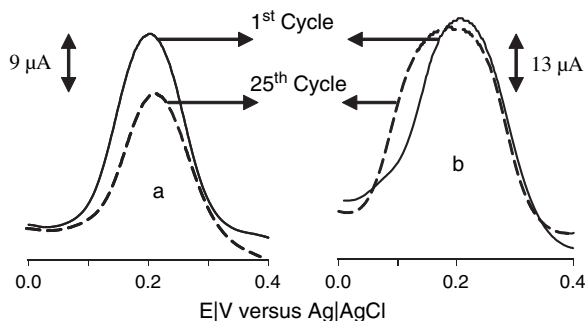


Fig. 6. Comparison of differential pulse voltammograms of the 1st and 25th cycle of dopamine at SWCNT's (a) before pretreatment, (b) after electrochemical pretreatment.

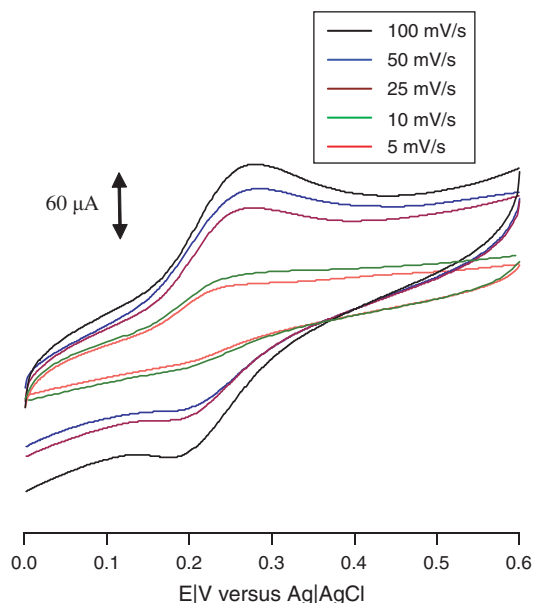


Fig. 7. Cyclic voltammetry of dopamine at SWCNT's at various scan rates.

0.1 mM DA at electrochemically activated SWCNT's. In this experiment we witnessed an enhanced anodic peak current with increase in scan rate and is shown in Figure 7. A plot of anodic peak current versus the square root of scan rate is linear in the range $0.001\text{--}0.1 \text{ mVs}^{-1}$ with a correlation co-efficient of 0.9971 indicating diffusion controlled process and is shown in Figure 8. If the scan rate is increased further the anodic peak current tends to remain constant and this is possibly due to the sluggish mass transfer kinetics arising from improper or insufficient diffusion of DA occurs at a faster scan rates.

Following this, the activated SWCNT's were used for the analytical detection of dopamine at different concentration (Fig. 9). A linear plot was observed when the chronoamperometric peak current was plotted against different concentration of DA employed (An aliquot of $25 \mu\text{L}$ of $1 \mu\text{M}$ DA was added in to the cell containing 5 mL of pH 7.2 PBS during each step). Figure 10 depicts the linear calibration plot obtained in our experiment with a

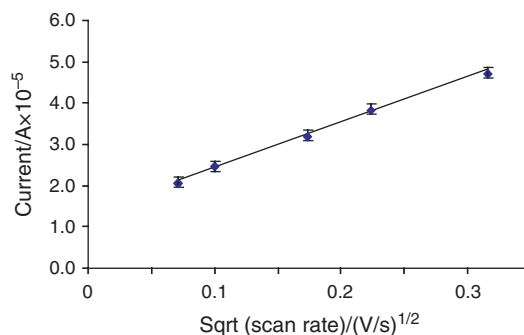


Fig. 8. Calibration plot showing linear relation between sqrt scan rate and peak current.

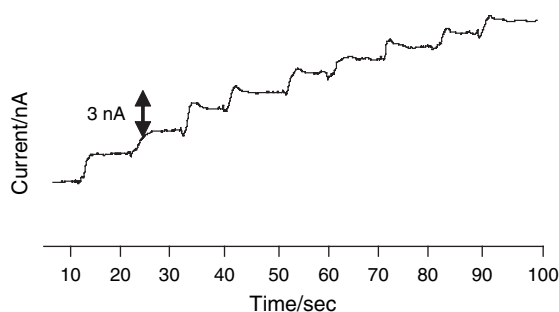


Fig. 9. Chronoamperometric response at activated SWCNT's at an applied potential of 250 mV with the injection of 1 μ M DA in to 5 mL of PBS (pH 7.2).

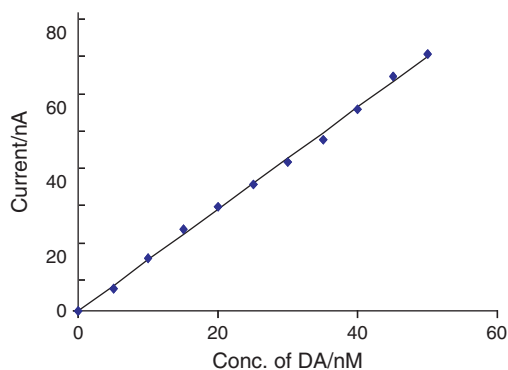


Fig. 10. Calibration plot obtained at activated SWCNT's during the successive addition of dopamine (Range 5 nM–50 nM).

correlation co-efficient of 0.9877 and it was found to be statistically significant at 95% confidence level. Based on three times the standard deviation of the blank signal we estimated a detection limit of 10 nM which is almost fifty times more sensitive than the detection limit obtained using the unactivated carbon nanotubes.

4. CONCLUSION

In this work, we have described a method for the electrochemical activation of SWCNT's. In addition, we have also performed a comparative study towards the detection of different charged redox couples and the neurotransmitter dopamine using the unactivated and activated SWCNT's. In the foretold analysis, activated SWCNT's exhibited better performance towards the detection of the analytes, which can be attributed to the rupture of basal planes first evidenced by Raman characterization. Based on three times the standard deviation of slope the activated SWCNT's exhibited a detection limit of about 10 nM for dopamine. In conjunction with the sensitivity of activated SWCNT's, future studies will focus on the detection of dopamine *in vivo*.

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References and Notes

1. R. C. Engstrom and V. A. Strasser, *Anal. Chem.* 56, 136 (1984).
2. P. Chen and R. L. McCreery, *Anal. Chem.* 68, 3958 (1996).
3. I. F. Hu and T. Kuwana, *Anal. Chem.* 58, 3235 (1986).
4. D. T. Fagan, I. F. Hu, and T. Kuwana, *Anal. Chem.* 57, 2759 (1985).
5. R. C. Engstrom and V. A. Strasser, *Anal. Chem.* 56, 136 (1984).
6. J. F. Evans and T. Kuwana, *Anal. Chem.* 49, 1632 (1977).
7. M. R. Deakin, K. J. Stutts, and R. M. Wightman, *J. Electroanal. Chem.* 182, 113 (1985).
8. P. Chen, M. A. Fryling, and R. L. McCreery, *Anal. Chem.* 67, 3115 (1995).
9. W. J. Blaedel and R. A. Jenkins, *Anal. Chem.* 46, 1952 (1974).
10. I. F. Jones and R. C. Kaye, *J. Electroanal. Chem.* 20, 213 (1969).
11. H. V. Drushel and H. V. Hallum, *J. Phys. Chem.* 62, 1502 (1958).
12. K. F. Blurton, *Electrochim. Acta* 18, 869 (1973).
13. D. Laser and M. J. Ariel, *J. Electroanal. Chem.* 52, 291 (1974).
14. R. J. Rice and R. L. McCreery, *Anal. Chem.* 61, 1637 (1989).
15. R. J. Bowling, R. T. Packard, and R. L. McCreery, *J. Am. Chem. Soc.* 111, 1217 (1989).
16. M. Musameh, N. S. Lawrence, and J. Wang, *Electrochem. Commun.* 7, 14 (2005).
17. J. J. Davis, R. J. Coles, and H. A. O. Hill, *J. Electroanal. Chem.* 440, 279 (1997).
18. M. Musameh, J. Wang, A. Merkoci, and Y. Lin, *Electrochem. Commun.* 4, 743 (2002).
19. J. Wang, M. Li, Z. Shi, N. Li, and Z. Gu, *Electroanalysis* 14, 225 (2002).
20. Z. Wang, J. Liu, Q. Liang, Y. Wang, and G. Lou, *Analyst* 127, 653 (2002).
21. N. S. Lawrence, R. P. Deo, and J. Wang, *Electroanalysis* 17, 65 (2005).
22. R. R. Moore, C. E. Banks, and R. G. Compton, *Anal. Chem.* 76, 2677 (2004).
23. C. E. Banks, R. R. Moore, T. J. Davies, and R. G. Compton, *Chem. Commun.* 16, 1804 (2004).
24. X. Yu, D. Chattopadhyay, I. Galeska, F. Padimitrakopoulos, and J. F. Rusling, *Electrochem. Commun.* 5, 408 (2003).
25. X. Yu, S. N. Kim, F. Papadimitrakopoulos, and J. F. Rusling, *Mol. Biosyst.* 1, 70 (2005).
26. Y. H. Yun, A. Bange, W. R. Heineman, H. B. Halsall, V. N. Shanov, Z. Dongd, S. Pixley, M. Behbehani, A. Jazieh, Y. Tue, D. K. Y. Wong, A. Bhattacharya, and M. J. Schulz, *Sens. Actuators, B* 123, 177 (2007).
27. M. T. Bardo, *Crit. Rev. Neurobiol.* 12, 37 (1998).
28. K. C. Berridge, *Neurosci. Biobehav. Rev.* 20, 1 (1995).
29. S. Kurumiya and S. Nakajima, *Brain Res.* 448, 1 (1988).
30. J. D. Salamone, M. S. Cousins, L. D. McCullough, and R. J. Carriero, *Pharmacol. Biochem. Behav.* 49, 25 (1994).
31. R. A. Clark, S. E. Zerby, and A. G. Ewing, *Electrochemistry in Neuronal Microenvironments: Electroanalytical Chemistry*, edited by A. J. Bard and I. Rubinstein (1996), p. 227.
32. S. Alwarappan, S. K. Butcher, and D. K. Y. Wong, *Sens. Actuators, B: Chemical* 128, 229 (2007).
33. W. S. Choi, S. H. Choi, B. Hong, D.-G. Lim, K.-J. Yang, and J.-H. Lee, *Mater. Sci and Eng. C* 26, 1211 (2006).

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