Selective detection of dopamine using glassy carbon electrode modified by a combined electropolymerized permselective film of polytyramine and polypyrrole-1-propionic acid

Zhou, Lin; Shang, Fengjun; Pravda, Mila; Glennon, Jeremy D.; Luong, John H. T.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1002/elan.200804504
Electroanalysis, 21, 7, pp. 797-803, 2009-04

Full Paper

Selective Detection of Dopamine Using Glassy Carbon Electrode Modified by a Combined Electropolymerized Permselective Film of Polytyramine and Polypyrrole-1-propionic Acid

Lin Zhou, *Fengjun Shang*, *Mila Pravda*, *Jeremy D. Glennon*, *John H. T. Luong**

Analytical and Biological Research Facility (ABCRF), Department of Chemistry, University College Cork, Cork, Ireland
*e-mail: john.luong@cnrc-nrc.gc.ca; j.luong@ucc.ie

Received: November 25, 2008
Accepted: January 7, 2009

Abstract
A sensitive and selective electrochemical method for the determination of dopamine using a combined electropolymerized permselective film of polytyramine and polypyrrole-1-propionic acid on a glassy carbon (GC) electrode was developed. The formation of a "layer-by-layer" film has allowed for selective detection of dopamine in the presence of 3,4-dihydroxyphenylalanine (L-DOPA), DOPAC, ascorbic acid, uric acid, epinephrine and norepinephrine. The modified electrodes exhibited a detection limit of 100 nM with linearity ranging from 5×10^{-6} to 5×10^{-5} M. No cleaning step was required during the course of repeated measurement.

Keywords: Dopamine, Electropolymerization, Polytyramine, Polypyrrole-1-propionic acid, Glassy carbon electrode

DOI: 10.1002/elan.200804504

1. Introduction

Since its discovery in the 1950s, tremendous attempts have been made to detect dopamine (DA), an important catecholamine neurotransmitter, widely distributed in brain tissues which activates dopamine receptors in mammalian central nervous, renal, cardiovascular and hormonal systems [1–3]. Dopamine can be supplied as a medication that acts on the sympathetic nervous system, increasing heart rate and blood pressure. Insufficient DA level caused by the degeneration of dopaminergic neurons in the nigrostriatal pathway is related with neurological diseases such as Parkinsonism, schizophrenia and Huntington, leading to motor dysfunction [4–7]. Therefore, sensitive and selective determination of DA in vitro and in vivo could be useful for the diagnostics of such diseases and the evaluation of drug efficacy [7].

Many analytical methods, including electrochemistry, chemiluminescence [8], fluorescence [9], spectrophotometry [10–11], gas, liquid and capillary chromatography [12–13], and thin layer chromatography with fiber optic detection [14], have been reported for dopamine determination. However, the fluorometric method is sample-consuming and lacks selectivity, while chromatography requires sample pretreatment, long analysis times and high costs, preventing them from being applied in routine analysis [5]. 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 (AA) [15–17], uric acid (UA) and other DA metabolites, such as 3,4-dihydroxyphenylalanine (DOPAC) [18], epinephrine (EP) and norepinephrine (NEP) [19]. Various approaches have been applied to overcome these difficulties, i.e. pretreated electrodes [20–21], self-assembled monolayers [22] and metal nanoparticle arrays [23]. However, a preconcentration step is still a prerequisite since these procedures are based on adsorption phenomenon or electrostatic interaction.

Selectivity for electrochemical detection can be realized by forming an electropolymerized film with a uniform and controllable thickness to block the passage of such interferences 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. In contrast, nonconductive polymers have immerged as attractive candidates for fabricating considerably thinner membranes owing to their self-limiting formation. Electropolymerized polytyramine is a strongly adhering film with excellent permselectivity against anionic species [24–26]. In addition, the hydrophilic poly(pyrrole-1-propinonic acid) (PPA) film is able to realize rapid diffusion, fast response with high resulting response signal. Therefore, a combination of these two films could provide selectivity and sensitivity for the measurement of dopamine without compromising its detection sensitivity.

This paper describes a simple electrochemical strategy for selective detection of DA using a modified glassy carbon electrode. A composite polymer film of tyramine and PPA was subsequently electrodeposited on a glassy carbon electrode. Such a resulting film exhibited synergistic perm-

selectivity against ascorbic and uric acids at normal physiological levels. The signal responses invoked by L-DOPA, DOPAC, epinephrine, and norepinephrine were also eliminated or significantly suppressed.

2. Experimental

2.1. Reagents and Materials

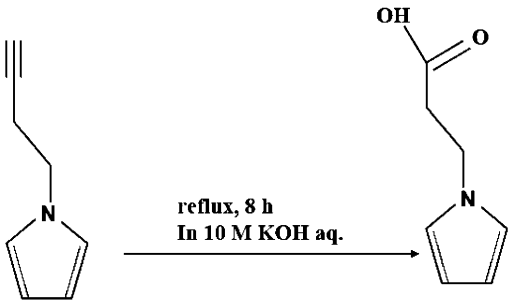
Dopamine, DA (3-hydroxytyramine), L-DOPA (3,4-dihydroxy-L-phenylalanine), DOPAC (3,4-dihydroxyphenylacetic acid), epinephrine, norepinephrine, pyrrole-1-propionenitrile, tyramine hydrochloride, ascorbic acid (AA) and uric acid (UA) were purchased from Sigma-Aldrich (Dublin, Ireland). Pyrrole-1-propionic acid was synthesized according to the method of Dong et al. [27] with some modifications to improve the product yield and facilitate the product purification. A 50 mM phosphate buffer solution (PB) pH 7.0 was employed as the supporting electrolyte. Deionized water (18.2 MΩ cm) obtained from a Milli-Q (Millipore, Bedford, MA) water purification system was used throughout. All reagents were of analytical grade with highest purity.

2.2. Apparatus

Amperometric measurement (*I/t*), electropolymerization, and cyclic voltammetry (CV) were performed using a CHI 1040A electrochemical workstation (CH Instruments, Austin, TX) at room temperature. The three-electrode system consists of a glassy carbon working electrode (diameter = 3 mm, BAS, West Layette, IN), an Ag/AgCl (3 M NaCl) reference electrode (BAS, West Layette, IN) and a Pt wire counter electrode. The convective transport during the amperometric determination was performed with magnetic stirring at 800 rpm.

2.3. Preparation of Pyrrole-1-Propionic Acid (PPA)

A solution consisting of 2g pyrrole-1-propionenitrile in 100 mL of 10 M potassium hydroxide was refluxed for 8 h. After cooling, concentrated HCl was added to the resulting solution on ice to pH 2.5. PPA was extracted from aqueous solution five times using 20 mL ethyl acetate (EA). After washing twice with 20 mL saturated NaCl, the collected organic layer was dried over 4 g of anhydrous Na_2SO_4. Ethyl acetate was removed with a rotary evaporator in a temperature regulated bath at 40 °C. The crystals precipitated at room temperature were redissolved in 2 mL of hot EA (65 °C) and left overnight, resulting in 2 g of PPA with a yield of 95%. This single-step procedure was much simpler than the synthesis of PPA from pyrrole with a yield of only 65% and facilitated the subsequent purification step as shown in Scheme 1. The resulting PPA was confirmed by HPLC and identified by ^{1}H- and ^{13}C- NMR and mass spectrometry. ^{1}H-NMR ($CDCl_3$):2.89 (t, 2H, J = 6.7 Hz), 4.27 (t, 2H, J = 6.7 Hz), 6,25 (t, 2H, J = 2.0 Hz), 6.76 (t, 2H, J = 2.0 Hz), 10.99 ppm (s, bd peak, 1H). ^{13}C-NMR($CDCl_3$): 36.8, 45.0, 109.1, 121.1, 177.9 ppm.



Scheme 1. Synthesis of PPA from pyrrole-1-propionenitrile with a product yield of ca. 95%.

2.4. Electrode Preparation

The glassy carbon electrode was cleaned with wet silicon carbide paper, grid 1500 (Hand American Made Hardwood Products, South Plainfield, NJ), followed by polishing with 0.05 μm alumina slurry (Buehler, Markham, ON, Canada) on velvet to a mirror finish. After 5 min of sonication in deionized water, the electrode was immersed in concentrated H_2SO_4 for 3 min followed by thorough rinsing with deionized water and ethanol. The resulting electrode was then transferred to 50 mM phosphate, pH 7.0 for cleaning by cyclic voltammetry between −0.5 and +1.5 V at 0.1 V s^{-1} until a stable CV profile was obtained.

2.5. Electropolymerization

The polytyramine film (PTy/GC) was electrogenerated from 0.1 M tyramine dissolved in 0.3 M NaOH-containing methanol by cycling the potential from −0.1 to +1.7 V for 40 cycles at 0.5 V s^{-1}. The PPA film was then electrodeposited on the PTy modified GC electrode from 50 mM PPA in 50 mM phosphate buffer, pH 7.0 by cycling the potential between −0.2 and +1.0 V at 0.05 V s^{-1} for 20 cycles. The resulting electrode was referred to as the (PPA/PTy/GC) electrode.

3. Results and Discussion

3.1. Electropolymerization of Tyramine and Pyrrole-1-Propionic Acid (PPA)

Electrochemical polymerization of tyramine has been employed for sensor and biosensor construction [26, 28, 29] and the mechanism has been discussed in detail [30]. The film thickness could be adjusted by pH, electropolymerization cycles, scan rate and the extention of the forced voltage.

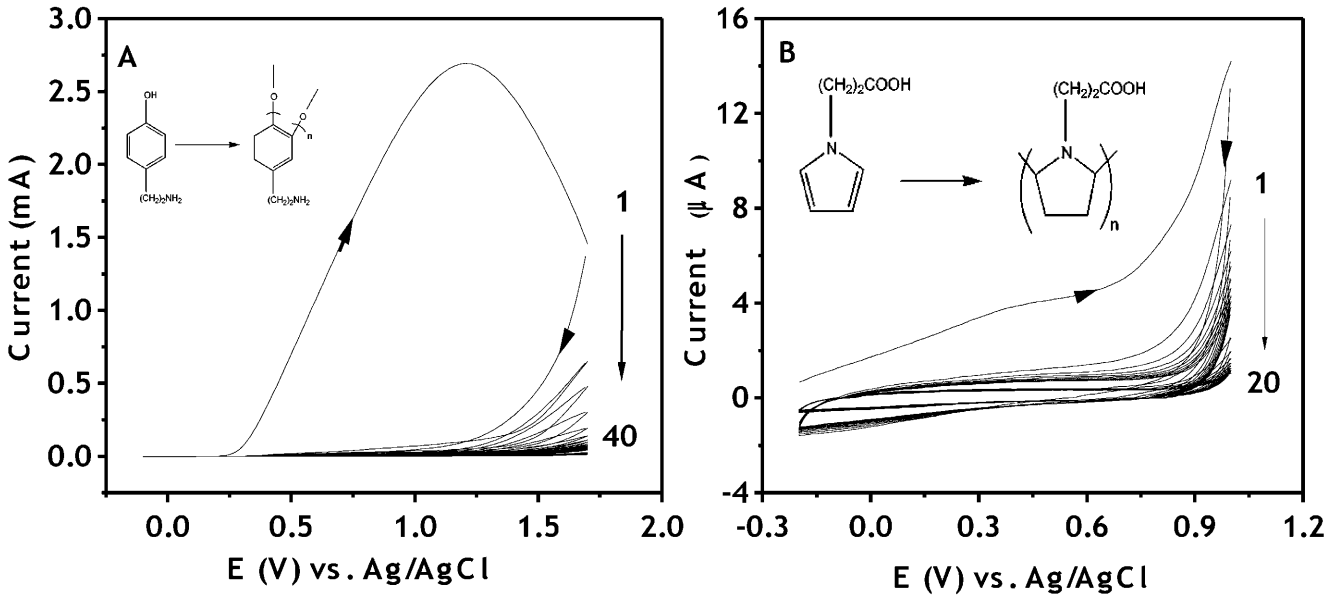
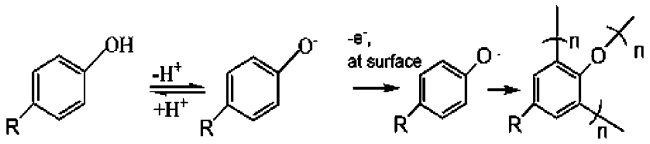


Fig. 1. A) Electropolymerization of 0.1 M tyramine hydrochloride (dissolved in 0.3 M NaOH-methanol) on the glassy carbon electrode. Cyclic voltammetry was performed from −0.1 to +1.7 V (vs. Ag/AgCl) at 0.5 V s^{-1} for 40 cycles. B) Electropolymerization of 50 mM PPA (dissolved in 50 mM phosphate buffer, pH 7.0) on the polytyramine modified glassy carbon electrode (PTy/GC). Cyclic voltammetry was performed from −0.2 to +1.0 V (vs. Ag/AgCl) at 0.05 V s^{-1} for 20 cycles.

The resulting films prepared from methanol-sodium hydroxide [26] and methanol-phosphate buffer [28] display very high resistivity with the thickness of several tens of nanometers [30]. In contrast, the film thickness prepared in $HClO_4$ (pH 2) varies from 50 nm to 1.2 μm, depending on the deposition cycles [29]. A positive shift in E_{pa} with decreasing pH suggests a slower electron transport across the resulting electropolymerized PTy [31]. The response current to $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ of the PTy film is also much higher compared to basic pH, implying higher porosity of the resutling film [31]. Therefore, the PTy film prepared in this study at alkali pH should be porous and thin (ca. 30–40 nm), in agreement with the result reported by Situmorang et al. [26].

As shown in Figure 1A, the oxidation peak of tyramine at +1.2 V was observed in the first sweep segment, indicating the oxidation of the phenoxide ion to the free radical intermediate. No reduction was observed throughout the reverse potential sweep with nearly zero current, reflecting irreversible passivation. In subsequent cycles, the oxidation currents of tyramine continually diminished, ascertaining the formation of a nonconducting polymer film and hindered the further film growth. The entire electrode surface was almost covered after the first few cycles of electrodeposition as only very low oxidation currents were observed. By direct-eye visualization, a transparent and strongly adhering polytyramine film was formed on the electrode surface. Notice that the amount of PTy deposited on the electrode surface decreases with increasing scan rate, i.e., more permeable towards DA whereas the resulting film grown at 0.01 V s^{-1} or lower is not homogenous and possesses considerable pin-holes. Therefore, 0.5 V s^{-1} was applied for the electrochemical deposition of tyramine in this study. No significant effect was noted at different tyramine concentrations ranging from 0.01 M to 0.1 M.



Scheme 2. Schematic oxidation and polymerization of tyramine, where R = $(CH_2)_2$ NH_2 for tyramine.

In brief, during the course of tyramine electrooxidation, phenoxy radicals were formed and reacted with a neighboring tyramine molecule to form a para-linked dimer. Since the amino group is separated by two methylene groups, the polymerization should be realized by the phenol moiety. Futhermore, the overoxidation of the electropolymerized film resulted in oligomers, leading to the formation of a passivating insulating film (Scheme 2).

The PTy film possesses a remarkable stability and antifouling ability attributed to the intermolecular π-to-π stacking and hydrophobic interaction of the phenol rings. To further improve the film permselectivity, PPA was electropolymerized on the PTy modified GC electrode (PPA/PTy/GC). It was reasoned that the first layer of PPA would adhere strongly to the PTy via ionic interaction, π-to-π stacking and hydrogen bonding between carboxyl groups and the amino groups remaining on the outer surface of the latter. After a gradual decrease in the first 10 cycles, the anodic current became constant, evincing the conductive characteristic of the PPA film (Fig. 1B).

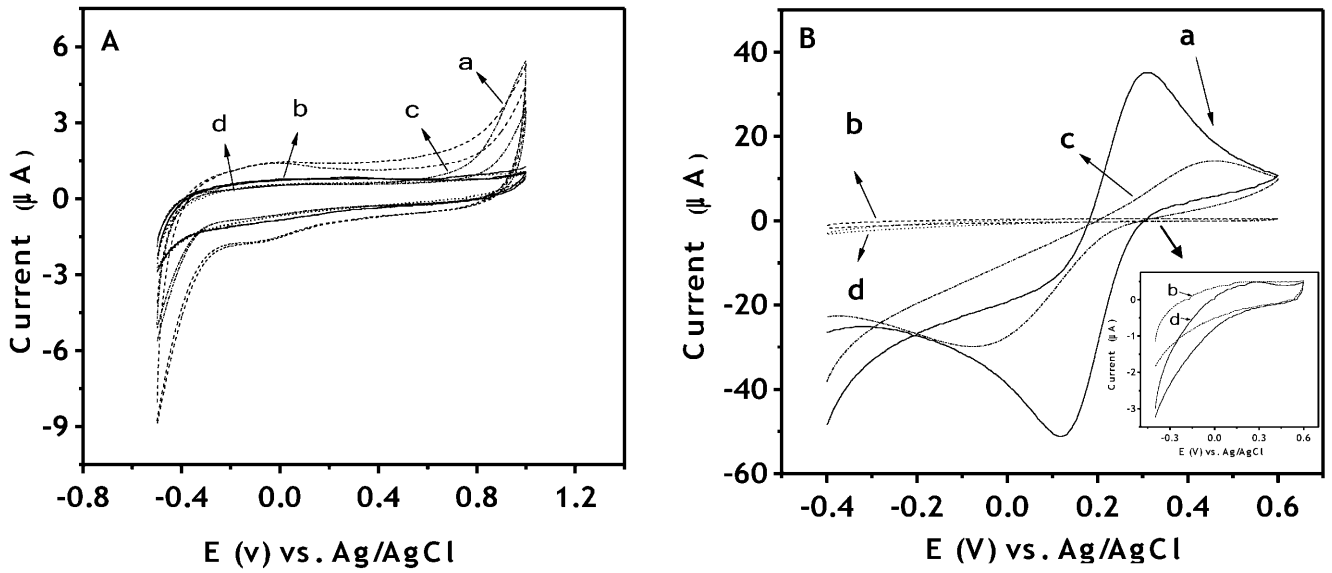


Fig. 2. A) Cyclic voltammograms for bare GC (a), PTy/GC (b), PPA/GC (c), and PPA/PTy/GC (d) electrodes in 50 mM phosphate buffer, pH 7.0 at 0.1 V s^{-1}. B) Cyclic voltammograms for bare GC (a), PTy/GC (b), PPA/GC (c), and PPA/PTy/GC (d) electrodes in 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ and 0.1 M KCl at 0.05 V s^{-1}.

3.2. Characterization of the PTy and PPA/PTy Modified Electrodes

Figure 2A compares the cyclic voltammograms of different GC electrodes in 50 mM PB, pH 7.0. As expected, the current of the bare GC electrode (Fig. 2A, curve a) was higher than the PTy/GC electrode (Fig. 2A, curve b), due to the passivation of the electrode surface by the non-conductive polytyramine. In contrast, the formation of the low-conductive PPA film alone on GC only slightly reduced the resulting current (Fig. 2A, curve c). The current of the PPA/PTy/GC (Fig. 2A, curve d) electrode remained the same as the PTy/GC electrode (Fig. 2A, curve b). It was further observed that for the bare GC electrode, the oxidation and reduction peaks of the ferri/ferrocyanide redox couple were observed at +0.306 V and +0.128 V, respectively (Fig. 2B, curve a). For the PTy/GC (Fig. 2B, curve b) electrode, the redox peak current sharply decreased, indicating severe diffusion limitation of the redox couple. The PPA modified GC electrode still displayed the feature of the ferri/ferrocyanide redox couple (Fig. 2B, curve c), i.e., more porous morphology of the PPA film. The redox peak current of the PPA/PTy/GC electrode (Fig. 2B, curve d) electrode was comparable to that of the PTy/GC (Fig. 2B, curve b) electrode, confirming the conductivity of the PPA film.

3.3. Analytical Performance of the PTy and PPA/PTy Modified Electrodes

Figure 3A shows the calibration curve for the PPA/PTy modified electrode poised at +0.8 V in response to 5 μM DA at pH 7.0 (50 mM PB). The electrode exhibited linearity up to 50 μM (I (nA) = 0.0366 C_{DA} (μM) + 0.0378) (Fig. 3B) with a detection limit of 100 nM (detectable signal at S/N = 3) (Fig. 3A, Inset), compared with 20 μM and 700 nM for the bare GCE. Such features were achieved because the modified electrode displayed low and stable background current. A response time of 6 s affirmed that these films were self-limiting and very thin. No surface fouling was noticed after repeated analysis, therefore, no cleaning-reconditioning steps were required during the extended course of measurement.

3.4. Interference Study

As anticipated, the bare GC electrode exhibited comparable response signals for DA, L-DOPA, DOPAC, EP and NEP and both AA and UA at their physiological levels provoked significant interference. Figure 4 shows the signal response of the PTy modified GC electrode (solid line) and PPA/PTy modified GC electrode (dotted line) at +0.8 V with the consecutive addition of 20 μM DA, 20 μM L-DOPA, 20 μM DOPAC, 20 μM EP, 20 μM NEP, 100 μM AA and 100 μM UA. The permselectivity of different types of the modified GC electrodes was presented in Table 1. The response signal ratio of each species was calculated relative to DA. The response of the metabolites of DA, such as L-DOPA, DOPAC, EP, NEP were about the same as DA whereas the signals of AA and UA were 3-fold higher than that of DA. The PTy modified GC electrode exhibited great rejection of DOPAC, without compromising its detection sensitivity for DA detection. Compared to DA, the signal response to 20 μM L-DOPA was ca. 33% and other metabolites of DA were also suppressed. In comparison to the bare GC electrode, the signal response to AA and UA

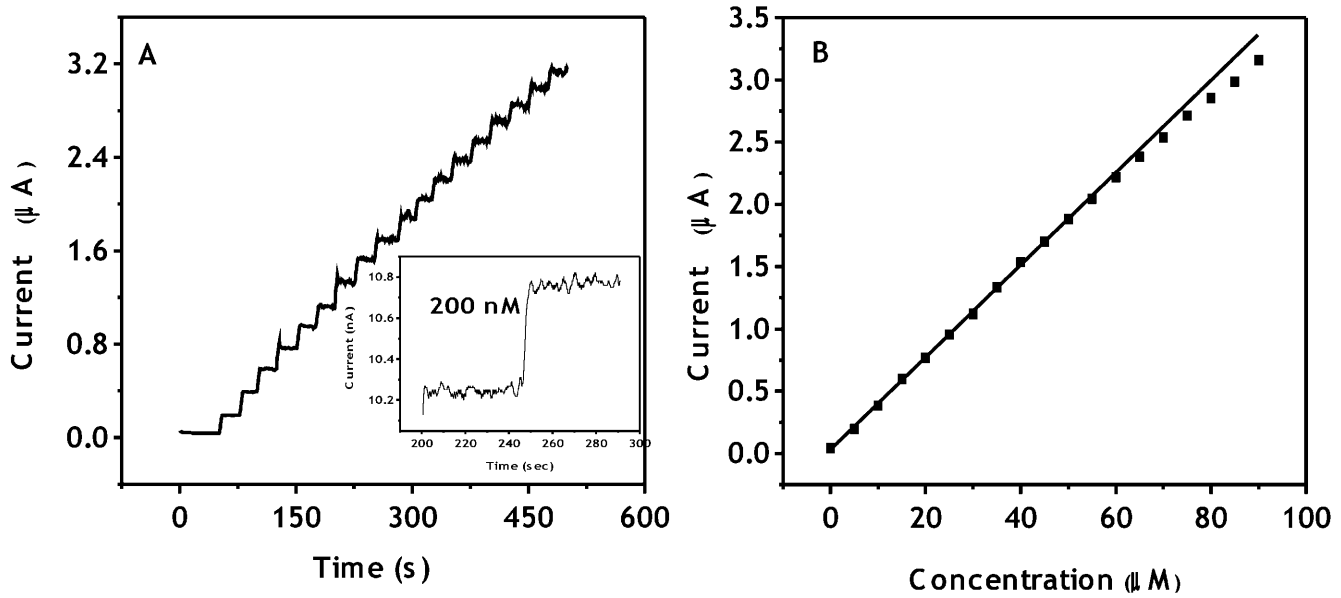


Fig. 3. A) A typical current-time response of the PPA/PTy modified GC electrode upon the successive addition of 5 μM DA in 50 mM phosphate buffer, pH 7. The inset shows the detection limit for DA, detectable signal response at the lowest DA concentration at $S/N = 3$. B) The calibration curve was replotted from the data obtained in Figure 3A.

were greatly circumvented. The response signal to UA signal was ca. 30% of DA. The PTy film possessed a high density of amino groups which should be partly protonated under pH 7 since the pKa of tyramine is 9.74 – 10.52. Based on the pK_a values, the main species of DA, L-DOPA, EP, and NEP are positively charged whereas DOPAC, AA and UA are negatively charged at pH 7. Thus, the signals of DOPAC, AA and UA were suppressed by the PTy film.

Although a mechanism for selective determination of dopamine in the presence of such electroactive species warrants further studies, UA and AA were reasoned to bind to the outermost layer of the PTy cationic film via ionic interaction and extensive hydrogen bonding to form a double layer. The COOH group in L-DOPA and DOPAC drastically reduced the Faradic response. This was due to the carboxyl group of L-DOPA and DOPAC which was quickly oriented towards the PTy film to interact with the protonated amino group whereas their oxidizable catechol tail was remote from the electrode surface. Thus, L-DOPA and DOPAC were effectively retained by the PTy film.

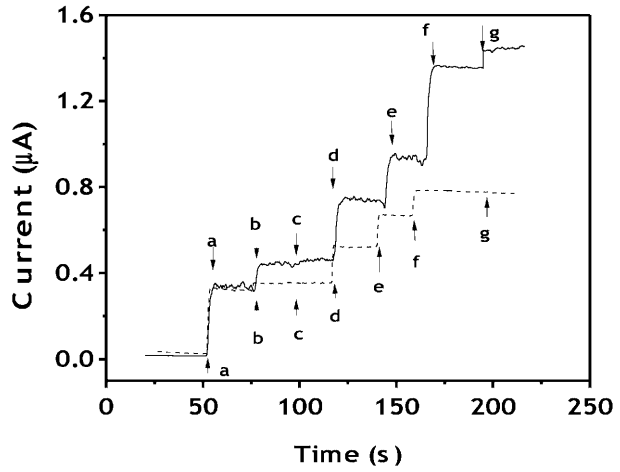


Fig. 4. Amperometric response of the PTy modified GC electrode (solid line) and the PPA/PTy modified GC electrode (dashed line) in 50 mM phosphate buffer, pH 7.0 to the addition of a) 20 μM DA, b) 20 μM L-DOPA, c) 20 μM DOPAC, d) 20 μM EP, e) 20 μM NEP, f) 100 μM AA, and g) 100 μM UA.

Further improvement in permselectivity was observed for the PPA/PTy modified GC electrode. DOPAC and UA were completely eliminated. L-DOPA only invoked 10% interference. The signal of EP, NEP and AA were about 50% of DA. The PPA/PTy modified GC electrode was efficient for blocking the interference from UA and DOPAC. The combined PPA/PTy film was very stable and strongly adhered to the GC electrode surface. Thus, the resulting modified electrode was used for at least 30 repeated analyses of 5 μM DA without electrochemical cleaning during the course of measurement. Apparently, the molecular sieving effect played an important role to facilitate their penetration. The smallest compound DA ($MW = 153$), structurally similar to tyramine, provoked the highest response followed by EP and NEP. Nevertheless, L-DOPA was not able to effectively penetrate the PTy film due to its bulkier structure. L-DOPA and DOPAC were also retained as the carboxyl groups were rapidly anchored towards the film to interact strongly with the protonated amine groups, leaving the oxidizable catechol tails remote from the electrode surface. DOPAC (pKa = 4.08) would bind more strongly resulting in an even smaller response signal (ca. 0). The formation of the PPA film on the PTy modified GC electrode induced further improvement on the permselectivity. The first layer of PPA strongly adhered to the PTy film via ionic interaction, π-to-π stacking and hydrogen bonding

Table 1. Permselectivity (%) of the PPA/PTy modified glassy carbon electrode (GC) (calculated by the response signal ratio between each species relative to DA).

	Bare GCE (%)	PTy modified GCE (%)	PPA modified GCE (%)	PPA/PTy modified GCE (%)	pK_a
20 μM Dopamine, DA ($MW = 153$)	100	100	100	100	8.86 – 10.5
20 μM L-DOPA ($MW = 197$)	74	33	107	11	8.72
20 μM DOPAC ($MW = 168$)	111	0	83	0	4.08
20 μM EP ($MW = 183$)	137	88	169	57	8.86
20 μM NEP ($MW = 169$)	100	65	65	50	8.6
100 μM Ascorbic acid, AA ($MW = 176$)	289	130	218	40	4.19
100 μM Uric acid, UA ($MW = 168$)	333	28	12	0	5.8
Tyramine ($MW = 137$)	n/a	n/a	n/a	n/a	9.74 – 10.52

between the COOH and NH_2 groups while the outermost layer with a negative charge favored cationic DA, EP and NEP and rejected anionic DOPAC, AA and UA (Fig. 5). Consequently, the preferential passage of DA was observed on the combined PPA/PTy film.

In order to attain the selectivity for DA detection, the PTy had to be formed first on the GC electrode followed by the electrodeposition of PPA. Experimental data confirmed that the PPA modified electrode exhibited similar response to all neurotransmitters (Table 1) and only UA was suppressed. Similar to the mechanism of the Nafion layer, the negatively charged PPA film favored the positively charged DA, EP and NEP while rejecting the negatively charged L-DOPA, DOPAC, UA and AA. Indeed, the subsequent depositon of PTy on the PPA modified electrode could not sufficiently suppress the interference caused by AA compared to the PPA/PTy electrode (data not shown).

Selective measurement of dopamine in the presence of ascorbic acid can be realized using a Nafion coated glassy carbon electrode modified with catechin hydrate as a natural antioxidant [32]. Similarly, clay modified electrodes must be coated with Nafion for selective determination of dopamine [33]. As the Nafion layer is formed by dropping on the electrode surface, it is difficult to precisely control the film thickness, uniformity and location. Consequently, reproducibility from one electrode to another during preparation is always problematical. In most cases, cyclic voltammetry (CV) or differential pulse anodic stripping voltammetry (DPASV) must be used for measurement of dopamine [17]. Therefore, the measurement is not continuous and less attractive for in situ monitoring. In addition, the Nafion layer only rejects the negatively charged L-DOPA, DOPAC, UA and AA and does not provide any selectivity for DA over EP and NEP because of the positive charge of these three analytes. Wang et al. [34] reported that the Nafion and overoxidized polypyrrole-modified Pt electrodes exhibit fast response to both DA and NE (0.1 mM) as well as a small response signal to AA (1 mM). UA and other neurotransmitters were not investigated in such a study. Although overoxidized poly(pyrrole-*co*-[3-(pyrrol-1-yl)-propanesulfonate]-coated platinum electrodes provide better discrimination than Nafion or overoxidized polypyrrole coatings, such electrodes exhibit response to both DA and NE [34]. Fast-scan cyclic voltammetry (FSCV) can be used for monitoring dopamine fluctuation within the mammalian brain. In addition to the background current drift, its large amplitude relative to the currents for the solution species of interest is one of the serious drawbacks of this technique. A procedure termed analog background subtraction has been recently reported to overcome this drawback. The background is recorded, and its inverse is played back to the current transducer during data acquisition to cancel the background in subsequent scans. Although background drift still persists,

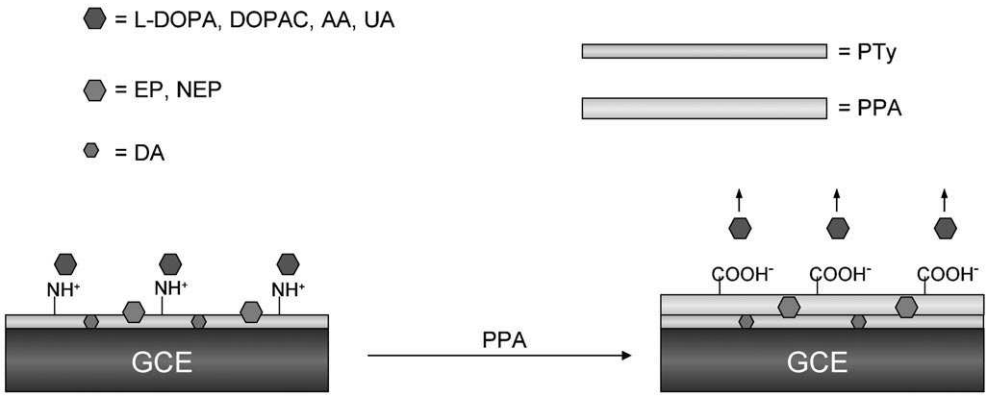


Fig. 5. Schematic representation of the layer-by-layer deposition of PTy and PPA on a glassy carbon (GC) electrode.

its magnitude is small compared to the original background. Different kinds of electrodes can be used to sensitively detect DA in the presence of AA and UA. Using FSCV, gold microelectrodes were found to be more sensitive to DA than carbon fiber microelectrodes [35].

In this work, the PPA film thickness can be controlled by the electropolymerization procedure. The PTy and PPA films are only electrodeposited on the active area of the electrode surface. Notice also that Xiao et al. [2] described a highly sensitive and selective method to detect dopamine in the presence of ascorbic acid by a new polymeric composite film. This system has not been tested for other interfering species such as L-DOPA, DOPAC, epinephrine and norepinephrine.

4. Conclusions

In brief, the glassy carbon electrode modified by a combined electropolymerized film of polytyramine and polypyrrole-1-propionic acid has been developed for selective detection of dopamine in the presence of endogenous ascorbic acid and uric acid. This attractive procedure, with great clinical significance, is advantageous for microelectrode modification and microarray fabrication as this permselective film is generated only onto the active sites of the detecting electrode regardless of their sizes and shapes. The resulted polytyramine film eliminates or suppresses the interference of L-DOPA, DOPAC, epinephrine and norepinephrine. This simple and inexpensive procedure could offer a good alternative to existing analytical methods for detection of dopamine in a very short analysis time. The combined PPA/PTy film is also extremely versatile, which can be used for the immobilization of enzyme and immunoassays.

5. Acknowledgements

This work was financially supported by the Irish Research Council for Science, Engineering and Technology (IRCSET), Ireland, under the Embark Initiative Postgraduate Research Scholarship Scheme (LZ & FS) and the Science Foundation of Ireland (SFI), the Walton Visitor Award (JHTL).

6. References

[1] A. Mascia, J. Afra, J. Schoenen, *Cephalalgia* **1998**, *18*, 174.
[2] Y. Xiao, C. Guo, C. M. Li, Y. Li, J. Zhang, R. Xue, S. Zhang, *Anal. Biochem.* **2007**, *371*, 229.
[3] P. R. Roy, T. Okajima, T. Ohsaka, *Bioelectrochem.* **2003**, *59*, 11.
[4] T. C. Pritchard, K. D. Alloway, *Medical Neuroscience*, 1st ed., Fence Creek Publishing, LLC, Madison, Connecticut **1999**, pp. 38 – 39.
[5] J. Kim, M. Jeon, K.-J. Paeng, I. R. Paeng, *Anal. Chim. Acta* **2008**, *619*, 87.
[6] J. I. Routh, R. E. Bannow, R. W. Fincham, J. L. Stoll, *Clin. Chem.* **1971**, *17*, 867.
[7] S. R. Ali, Y. Ma, R. R. Parajuli, Y. Balogun, W. Y.-C. Lai, H. He, *Anal. Chem.* **2007**, *79*, 2583.
[8] L. Zhang, N. Teshima, T. Hasebe, M. Kurihara, T. Kawashima, *Talanta* **1999**, *50*, 677.
[9] A. H. Anton, D. F. Sayre, *J. Pharmacol. Exp. Ther.* **1962**, *138*, 360.
[10] I. da Cruz Vieira, O. Fatibello-Filho, *Talanta* **1998**, *46*, 559.
[11] P. Nagaraja, R. A. Vasantha, K. R. Sunitha, *Talanta* **2001**, *55*, 1039.
[12] F. Musshoff, P. Schmidt, R. Dettmeyer, F. Priemer, K. Jachau, B. Madea, *Forensic Sci. Int.* **2000**, *113*, 359.
[13] T. J. Panholzer, J. Beyer, K. Lichtwald, *Clin. Chem.* **1999**, *45*, 262.
[14] B. A. Patel, M. Arundell, K. H. Parker, M. S. Yeoman, D. O'Hare, *J. Chromatogr. B* **2005**, *818*, 269.
[15] S.-M. Chen, W.-Y. Chzo, *J. Electroanal. Chem.* **2006**, *587*, 226.
[16] R. Aguilar, M. M. Davila, M. P. Elizalde, J. Mattusch, R. Wennrich, *Electrochim. Acta* **2004**, *49*, 851.
[17] S. Alpat, S. K. Alpat, A. Telefoncu, *Anal. Bioanal. Chem.* **2005**, *383*, 695.
[18] P. R. Roy, M. S. Saha, T. Okajima, S.-G. Park, A. Fujishima, T. Ohsaka, *Electroanalysis* **2004**, *16*, 1777.
[19] S.-M. Chen, K.-T. Peng, *J. Electroanal. Chem.* **2003**, *547*, 179.
[20] K. Pihel, Q. D. Walker, R. M. Wightman, *Anal. Chem.* **1996**, *68*, 2084.
[21] J.-W. Mo, B. Ogorevc, *Anal. Chem.* **2001**, *73*, 1196.
[22] F. Malem, D. Mandler, *Anal. Chem.* **1993**, *65*, 37.
[23] C. R. Raj, T. Okajima, T. Ohsaka, *J. Electroanal. Chem.* **2003**, *543*, 127.
[24] S. A. Miscoria, G. D. Barrera, G. A. Rivas, *Sens. Actuators B* **2006**, *115*, 205.
[25] E. V. Suprun, H. C. Budnikov, G. A. Evtugyn, Kh. Z. Brainina, *Bioelectrochemistry* **2004**, *63*, 281.
[26] M. Situmorang, J. J. Gooding, D. B. Hibbert, D. Barnett, *Biosens. Bioelectron.* **1998**, *13*, 953.
[27] H. Dong, C. M. Li, W. Chen, Q. Zhou, Z. X. Zeng, J. H. T. Luong, *Anal. Chem.* **2006**, *78*, 7424.
[28] M. Situmorang, J. J. Gooding, D. B. Hibbert, *Anal. Chim. Acta* **1999**, *394*, 211.
[29] L. D. Tran, B. Piro, M. C. Pham, T. Ledoan, C. Angiari, L. H. Dao, F. Teston, *Synth. Met.* **2003**, *139*, 251.
[30] A. M. Tenreiro, C. Nabais, J. P. Correia, F. M. S. S. Fernandes, J. R. Romero, L. M. Abrantes, *J. Solid State Electrochem.* **2007**, *11*, 1059.
[31] C. M. de Castro, S. N. Vieira, R. A. Gonçalves, A. G. Brito-Madurro, J. M. Madurro, *J. Mater. Sci.* **2008**, *43*, 475.
[32] A. Salimi, K. Abdi, G.-R. Khayatian, *Microchim. Acta* **2004**, *144*, 161.
[33] J.-M. Zen, P.-J. Chen, *Anal. Chem.* **1997**, *69*, 5087.
[34] J. Wang, P. V. A. Pamidi, G. Cepria, S. Basak, K. Rajeshwar, *Analyst* **1997**, *122*, 981.
[35] M. K. Zachek, A. Hermans, R. M. Wightman, G. S. McCarty, *J. Electroanal. Chem.* **2008**, *614*, 113.

