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Selective detection of dopamine using a combined permselective film of electropolymerized (poly-tyramine and poly-pyrrole-1-propionic acid) on a boron-doped diamond electrode

Fengjun Shang,[a] Yali Liu,[b] Sabahudin Hrapovic,[b] Jeremy D. Glennon[a] and John H. T. Luong*[ab]

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An effective and robust electrochemical approach has been developed for selective detection of dopamine in the presence of 3,4-dihydroxyphenylalanine (L-DOPA), ascorbic acid, uric acid and other dopamine metabolites. A 'layer-by-layer' film of tyramine and pyrrole-1-propionic acid (PPA) was formed by subsequent electropolymerization on a boron-doped diamond (BDD) electrode with an overall thickness of ~33 nm as estimated by AFM. The formation of the electropolymerized homogeneous film was also confirmed by SEM and Raman spectroscopy. The modified BDD electrode exhibited rapid response to dopamine within 6 s and a detection limit of 50 nM with excellent reproducibility. The stable electropolymerized film was capable of excluding electroactive interference from 20 μM L-DOPA, 20 μM 3,4-dihydroxyphenylacetic acid (DOPAC), and ascorbic and uric acids at normal physiological conditions (100 μM each). The modified electrode could be used for several repeated analyses of dopamine at 5 μM, without noticeable surface fouling. A plausible mechanism for permselectivity was suggested and supported by pertinent experimental data.

Introduction

Dopamine (DA) plays an important role in the central nervous, renal, hormonal and cardiovascular systems.[1,2] It is a neurotransmitter in the brain, a neurotransmitter and a hormone within the gastrointestinal tract, and acts as a natriuretic hormone that affects electrolyte excretion by the kidney. Abnormal DA transmission is associated with neurological disorders such as schizophrenia, Parkinson's disease, and Huntington's disease.[3,4] Patients with Parkinson's disease have lost more than 80% of DA-producing cells in the substantia nigra. Only L-DOPA (3,4-dihydroxy-L-phenylalanine), the precursor of DA, can be used in therapy because DA cannot cross the blood–brain barrier.[3] Therefore, sensitive and selective determination of DA could be employed for molecular diagnostics of Parkinson's disease, the design of therapeutics and the evaluation of drug efficacy.[4] DA can be oxidized by glassy carbon and other metallic electrodes including Pt and Au. However, detection of DA is still challenging and problematical because of its coexistence with elevated ascorbic acid (AA), a major electroactive interfering species. Such electrode materials also produce comparable signal responses when they are invoked by L-DOPA and other DA metabolites such as DOPAC (3,4-dihydroxyphenylacetic acid), epinephrine, and norepinephrine.

Electropolymerization, an attractive method for the development of bioanalytical devices, allows reproducible, precise, uniform and thickness-controlled polymer coating without any limitation of the size, area and geometry of the surface.[5–7] Compared with conducting polymers,[5,8,9] non-conducting polymers often generate considerably thinner (~10–100 nm) membranes due to their self-limiting nature. They also possess impressive analytical performances such as fast response, excellent permselectivity, and high reproducibility.[6,7,10] Among various phenols and their derivatives, tyramine can be promising for producing a strongly adhering polymer film. With the amino group separated from the phenolic ring by two methylene groups, only the phenol moiety participates in polymerization.[11a] A few studies utilizing free amines on the polytyramine (PTy) backbone for covalent attachment of biomolecules have been reported.[11b,c] In contrast, polypyrrole (PPy) has been extensively studied because of its high conductivity, stability, and availability.[7,12] However, limited permeability of the PPy film hinders the diffusion of the target analyte.[13,14] Recently, pyrrole-1-propionic acid (PPA) has emerged as a novel material for immunosensor construction.[14–17] Due to its low conductivity and self-limiting growth, the hydrophilic PPA film enables rapid diffusion, fast response and high response signal.[14,16]

The boron-doped diamond (BDD) thin-film electrode, pioneered by Pleskov *et al.*,[18] has attracted considerable interest due to its superior features such as high current density, wide potential window, low background current, extreme electrochemical stability and high resistance to fouling.[19–21] Modified BDD electrodes for the selective determination of DA have been reported.[1,19,22,23] Of particular interest is the selectivity towards DA by anodical treatment,[23] and the electrodeposition of a poly(*N*,*N*-dimethylaniline) film.[1]

This paper describes an effective electrochemical approach for the selective detection of DA using a boron-doped diamond electrode. A one-step procedure with 95% yield is also developed for the synthesis of pyrrole-1-propionic acid (PPA). Tyramine and PPA are subsequently electropolymerized on the BDD

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surface to form a thin 'layer-by-layer' film with synergistic permselectivity against ascorbic and uric acids. The electropolymerized film, confirmed by AFM, SEM and Raman spectroscopy, is very stable and capable of eliminating/suppressing L-DOPA, DOPAC, epinephrine, and norepineprine. To date, no serious attempt has been made to explain the mechanism of permselectivity. Therefore, the rationale behind the permselective characteristic of the two-layered film is suggested and discussed in detail.

Materials and methods

Chemicals

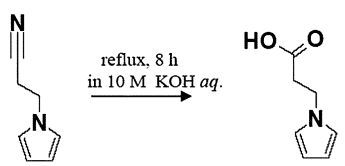
Pyrrole-1-propionitrile, tyramine hydrochloride, 4-hydroxyphenylacetic acid (HPA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxyphenylalanine (L-DOPA), ascorbic acid (AA), uric acid (UA), epinephrine (EP), norepinephrine (NEP) and other chemicals were purchased from Sigma-Aldrich (Dublin, Ireland). Deionized water (18.2 MΩ.cm) was obtained from a Milli-Q (Millipore, Bedford, MA) water purification system. All reagents were of analytical grade with highest purity.

Preparation of pyrrole-1-propionic acid (PPA)

A solution consisting of 2 g pyrrole-1-propionitrile in 100 mL of 10 M potassium chloride 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. EA was removed with a rotary evaporator in a temperature-regulated bath at 40 °C. The crystal precipitated at room temperature was redissolved in 2 mL of hot EA (65 °C) and left overnight, resulting in 2 g of PPA with a yield of 95%. This procedure was simpler than the synthesis of PPA from pyrrole with a yield of only 65% and facilitated the subsequent purification step.[14] The resulting PPA was confirmed by HPLC and identified by ^{1}H- and ^{13}C-NMR and mass spectrometry. ^{1}H-NMR ($CDCl_3$): δ 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 5 peak, 1H). ^{13}C-NMR ($CDCl_3$): δ 36.8, 45.0, 109.1, 121.1, 177.9 ppm. A schematic illustration for the synthesis of PPA is shown in Scheme 1.

Electrode preparation and electropolymerization

The BDD electrode was polished with wet silicon carbide paper, grit 2500 (Hand American Made Hardwood Products, South Plainfield, NJ), followed by 0.05 μm alumina slurry (Buehler, Markham, ON, Canada) on velvet to a mirror finish. After thorough rinsing with deionized water, the electrode was sonicated in 2-propanol and deionized water for 5 and 10 min, respectively and dried under a gentle nitrogen stream. Tyramine hydrochloride (0.1 M) dissolved in methanol containing 0.3 M NaOH was electropolymerized on the BDD electrode by cycling the potential between −0.1 and +1.7 V *vs.* Ag/AgCl at 500 mV/s for 20 cycles.[24] The polytyramine (PTy)-modified BDD electrode was then immersed in 50 mM phosphate buffer, pH 7.0 for 15 min. The PPA film was then electrochemically deposited on the PTy-modified BDD electrode from 50 mM PPA in 50 mM phosphate buffer, pH 7.0 by cycling the potential between −0.1 and 1 V *vs.* Ag/AgCl at 50 mV/s for 10 cycles. The prepared electrode was denoted as the PPA/PTy-modified BDD electrode. For comparison, the PTy-modified BDD, PPA-modified BDD and PTy/PPA-modified BDD electrodes were also prepared under similar conditions.



Scheme 1

Instrumentation

Electropolymerization, cyclic voltammetry (CV) and amperometric (I/t) measurements were performed using a CHI 1040A electrochemical workstation (CH Instruments, Austin, TX). All experiments were performed at room temperature using a three-electrode system consisting of a boron-doped diamond electrode (BDD, 3 mm diameter, 0.1% doped boron, Windsor Scientific, Slough, Berkshire, UK) as working electrode, an Ag/AgCl (3M NaCl) as reference electrode (BAS, West Layette, IN) and a platinum wire as counter electrode. SEM micrographs of the bare and modified BDD electrodes were obtained using a Hitachi scanning electron microscope, SEM (S-2600 N, Tokyo, Japan), operated in high vacuum and variable pressure (15 kPa) mode at 4–24 kV with a working distance of 4–20 mm. Atomic Force Microscopy (AFM) was used to estimate the thicknesses of the PTy and PPA films by subsequent electrodeposition of tyramine and PPA on a flat thin gold surface (2 mm^2). AFM micrographs were obtained using a Nanoscope IV (Digital Instruments, Veeco, Santa Barbara, CA) with a silicon tip operated in tapping mode. Raman spectra of the bare BDD, the PTy-modified BDD, and the PPA/PTy-modified electrodes were obtained using a custom-made adapter to align the electrode perpendicularly under the laser beam. Raman spectra were acquired by a micro-confocal Raman analyzer (LabRAM HR 800, Horiba/Jobin-Yvon, Longjumeau, France) equipped with a frequency-doubled Nd:YAG 532.1 nm laser operating at 30 mW.

Results and discussion

Electropolymerization of tyramine and pyrrole-1-propionic acid (PPA)

Cyclic voltammograms for the electropolymerization of 0.1 M tyramine in 0.3 M NaOH containing methanol at 500 mV/s revealed that an oxidation current was detectable at *ca.* 0.40 V with a well defined current peak at +1.43 V in the first cycle. Such behavior was attributed to the oxidation of the phenoxide ion to the free radical intermediate. In the reverse potential sweep, nearly zero current was observed, indicating the absence of polymer reduction, *i.e.* very strong passivation. Similar results were also reported for metal surfaces including platinum and gold.[25] In the second and third cycles, the anodic peak current

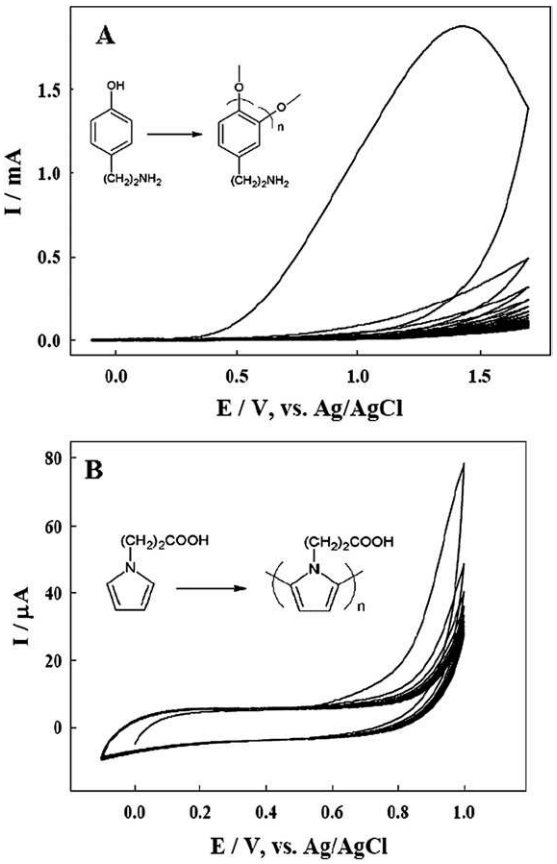
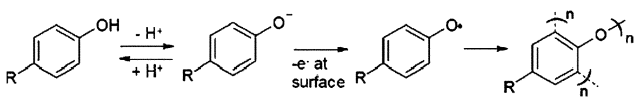


Fig. 1 (A) Electropolymerization of 0.1 M tyramine hydrochloride (dissolved in methanol with 0.3 M NaOH) on the BDD electrode. Cyclic voltammetry was performed from −0.1 to +1.7 V (*vs.* Ag/AgCl) at 500 mV/s for 20 cycles. (B) PPA electrodeposition was performed on the tyramine-modified electrode from 50 mM PPA in 50 mM phosphate buffer (pH 7.0) using cyclic voltammetry from −0.1 to +1.0 V at 500 mV/s for 10 cycles.

decreased more modestly, followed by a very small decrease in the peak current during the 17 subsequent cycles (Fig. 1A). Notice that the next 20 cycles did not result in any discernible change in the cyclic voltammogram (data not shown). The obtained results thus indicated that tyramine electrooxidation was irreversible and self-limiting, resulting in a very thin film. As a phenol derivative, the electrooxidation of tyramine is known to produce phenoxy radicals, which in turn react with a tyramine molecule to form a *para*-linked dimer. Further oxidation leads to oligomers and the eventual formation of a passivating insulating thin film. It is well known that polymers produced in more alkaline medium present self-limited growth.[26] The amino group is separated from the phenolic ring by two methylene groups; hence, only the phenol moiety is oxidized to perform the polymerization as shown in Scheme 2, where R = $(CH_2)_2NH_2$ for tyramine.



Scheme 2

Based on the CVs of Fig. 1A, it was conceivable that tyramine was first adsorbed on the BDD electrode and then oxidized to form a dimer with its neighboring molecules, followed by polymerization to form a thin electropolymerized film. The entire surface of the BDD electrode was mostly covered after the first few cycles of electrodeposition. To further improve the PTy film permselectivity, PPA was electropolymerized on the PTy-modified BDD electrode to form an additional layer to overcome ascorbic and uric acid interference. A significant current decrease was observed during the first 3 cycles followed by complete stabilization of the current value after 10 cycles (Fig. 1B). The amino groups of the PTy film are both neutral and protonated and the degree of protonation is dependent on the pH condition as addressed later.

Characterization of the PTy and PPA/PTy-modified electrodes

SEM micrographs of the pristine and PTy-modified BDD electrodes did not reveal any significant difference in their surface morphology (Fig. 2A). Such a result illustrated the formation of a low-conducting, compact, thin, smooth, and homogeneous layer on the BDD electrode. The electropolymerization of tyramine in methanol–sodium hydroxide solutions, the condition

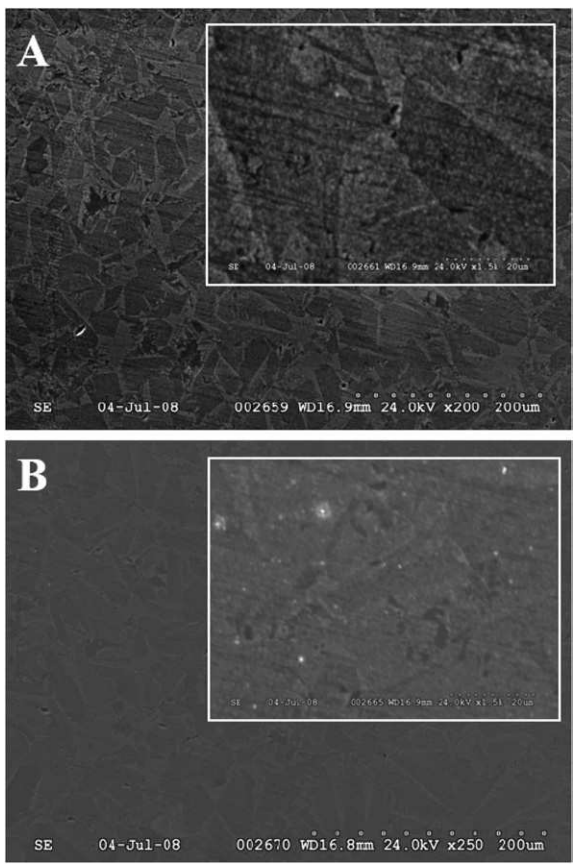


Fig. 2 (A) SEM micrographs of BDD electrode modified with the tyramine. Inset represents respective surfaces at ×1500 magnification. (B) SEM micrographs of the BDD electrode modified with tyramine and pyrrole-1-propionic (PPA) acid. Inset represents respective surface at ×1500 magnification. The same acceleration voltage, working distance, autofocus and auto-contrast were applied during the analysis.

used in this study, should result in a polymer film with very low conductivity.[26] In an acidic aqueous milieu, the amino group is protonated, leading to the formation of a polymer with a strong polycationic nature.[27a] SEM micrographs of the PPA/PTy-modified electrode revealed the identical topographical characteristic of the underlying BDD surface. However, the contrast was much lower and the micrographs appeared blurry compared to the PTy-modified surface. The inset at high magnification shows the presence of some white spots (sparks), which could be attributed to the charge effect of the combined PPA/PTy films (Fig. 2B). No appreciable defects (pinholes) were observed throughout the modified electrode surface. Raman spectroscopy was conducted to assess the formation of the electropolymerized film on the BDD electrode. Natural diamond displays a single sharp peak centered at 1332 cm^{-1} with a full width half-maximum (fwhm) of 2.3 cm^{-1}; *i.e.* the film is discontinuous to give rise to separate diamond crystals.[28] Any broad resonance at higher wave numbers, particularly at 1580 cm^{-1}, is considered as the presence of graphite-like non-diamond phases containing sp^2-bonded carbon atoms. Good-quality chemical vapor deposition diamond films should produce peaks with an fwhm of 4–10 cm^{-1}.[28] The Raman spectrum for the pristine BDD electrode featured one intense band at 1329 cm^{-1} (sp^3), the first-order phonon mode for diamond with an fwhm of 5.78 cm^{-1} (Fig. 3, curve a). The electrode displayed no broad band at 1550 cm^{-1}, indicating the absence of graphite or non-diamond carbon impurity in the film. The electrodeposition of tyramine on the BDD electrode only slightly affected the fwhm (6.75 cm^{-1}) and the peak position (1328 cm^{-1}). However, the peak intensity was significantly reduced from 10 312 to 8905 counts (Fig. 3, curve b). This phonon mode was much less intense due to the presence of the PTy film because the optical density is significantly higher, *i.e.* the penetration depth of the laser light was smaller. Similar behavior was also observed when PPA was electropolymerized on the PTy-modified electrode. However, peak intensity was reduced from 8905 counts to 6906 counts (Fig. 3, curve c) due to the additional formation of the thicker PPA layer as discussed below.

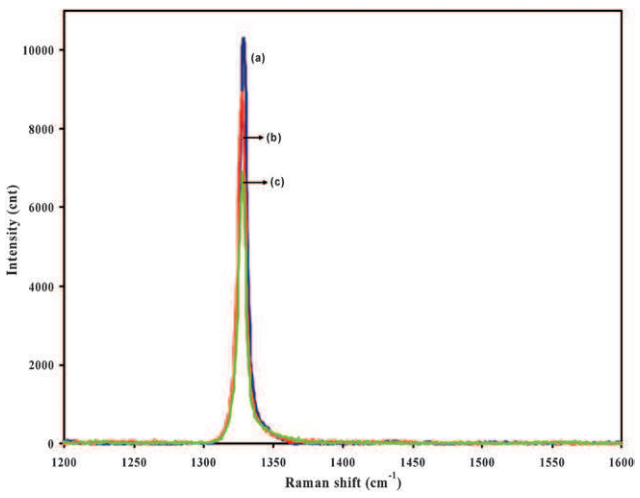


Fig. 3 Raman signatures of the bare BDD electrode (curve a), PTy-modified BDD (curve b) and PPA/PTy-modified BDD (curve c). The laser was focused on the center of the BDD electrode by an *XYZ* micromanipulator and Raman mapping was carried out to acquire 100 separate Raman spectra and the area of highest density was chosen for further analysis with the 5 highest values being averaged.

AFM could not be used to probe the morphology and the film thickness of the BDD electrode owing to its uneven surface and the oversized length (4 cm). Nevertheless, the thickness of the PTy film electropolymerized on a thin gold surface was estimated as 9.15 ± 1.09 nm (at 95% confidence interval, $n = 10$) (Fig. 4A). The obtained result was in agreement with the data obtained by

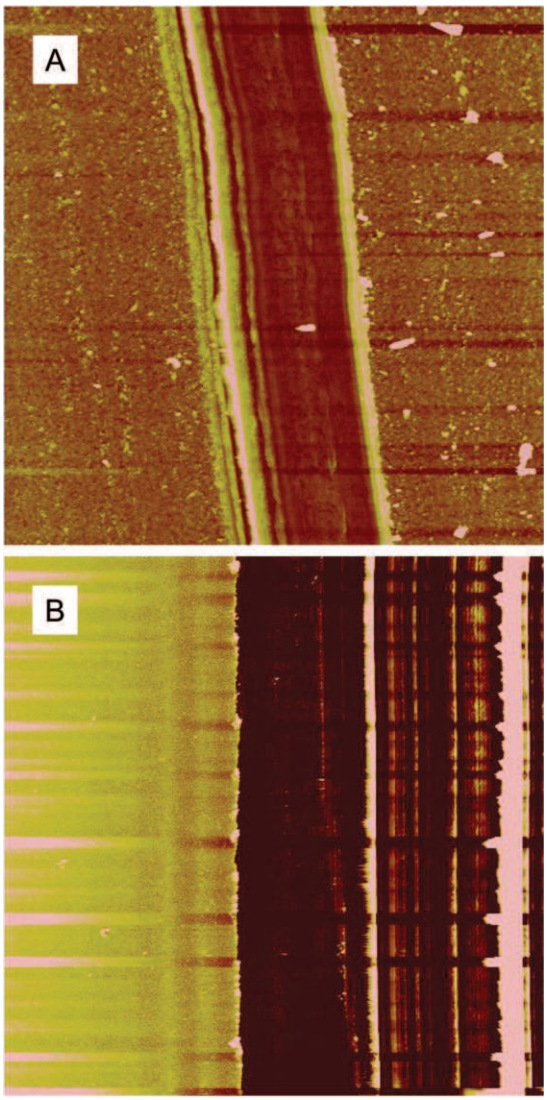


Fig. 4 (A) A typical AFM micrograph of the electropolymerized tyramine film on a thin gold surface (2 mm). 0.1 M tyramine hydrochloride (in methanol with 0.3 M NaOH) was electrodeposited by cyclic voltammetry from −0.1 to +1.7 V (*vs.* Ag/AgCl) at 500 mV/s for 20 cycles. The resulting surface was scratched using a sharp scalpel to enable the measurement of the resulting thickness. (B) A typical AFM micrograph of the electropolymerized PPA film on a thin gold surface (2 mm^2) pre-deposited with tyramine. 50 mM PPA in 50 mM phosphate buffer (pH 7.0) was electrodeposited using cyclic voltammetry from −0.1 to +1.0 V at 50 mV/s for 10 cycles *vs.* Ag/AgCl. Again, the resulting surface was scratched using a sharp scalpel to enable the measurement of the overall thickness.

profilometry (precision of ±5 nm) for an electropolymerized PTy film on bulk and patterned indium tin oxide (ITO) electrodes (15 ± 6 nm).[27a] The estimated film thickness was lower than the value reported by Dubois *et al.* (50 nm).[25] This deviation could be expected as the film thickness could be tuned mainly by pH and the number of sweeps performed and, to a lesser extent, the sweep rate and the magnitude of the forced voltage. Indeed, the PTy film thickness formed in $HClO_4$, pH 2 can be up to 1.2 μm. The formation of such a film is not self-limited but dependent on the number of deposition cycles.[27b] Thus, it is more difficult to control the film thickness and reproducibility of this preparation compared to the self-limited growth at alkaline pH or pH 7.[27c]

The resulting film from 0.1 M tyramine electropolymerization at 500 mV/s was more homogenous with improved permselectivity towards uric and ascorbic acids than the film formed at low scan rates (below 50 mV/s). No significant effect was noted at different tyramine concentrations, ranging from 0.01 to 0.1 M. Considering the thickness of the benzene ring (4.7 Å) and the PTy film (9.15 nm), a maximum of 20 PTy layers could be formed on the BDD electrode. The PTy film was stable even when subjected to boiling ethanol for several minutes and was only removed by extensive polishing of the electrode. Intermolecular π-to-π stacking and hydrophobic interaction of the phenol rings could be attributed to such remarkable stability. The total thickness of the PPA/PTy film was determined to be 32.94 ± 2.67 nm (at 95% confidence interval, $n = 10$), *i.e.* about 23.8 nm could be assigned for the PPA film thickness (Fig. 4B). Of interest was the comparison of the Raman and AFM data. The formation of the PTy film (9.15 nm) effected a decrease in the Raman peak intensity of 1050 counts. The formation of the second layer (PPA film, 23.8 nm), which was about double the PTy film thickness, decreased the Raman peak intensity by about double (2017 counts). Raman signatures of the PTy and/or PAA films were not detected due to the overwhelming signal of the intense band at 1329 cm^{-1} (sp^3), a typical characteristic of the pristine BDD electrode.

Analytical performance of the PTy and PPA/PTy-modified electrodes

As a key requirement in electrochemical bio/sensing, the electropolymerized film should be as thin as possible to minimize mass transfer diffusion but it must be stable for practical applications besides its permselectivity for target compounds. This was the main reason for the electrodeposition of tyramine at alkaline pH to attain a self-limited and thin film in this study. The bare BDD electrode exhibited a sensitive response to DA with a detection limit of 350 nM. As expected, it also produced comparable response signals to L-DOPA, DOPAC, epinephrine, and norepinephrine. Both ascorbic and uric acids at their physiological levels (0.1 mM) provoked phenomenal interference (Table 1). Such results confirmed the drawback or inapplicability of electrochemical detection for detecting DA in the presence of ascorbic and uric acids under normal conditions.

Fig. 5 shows a typical current–time plot and the calibration curve of the PPA/PTy-modified electrode at +0.8 V with the successive additions of 5 μM DA. A fast response time of 6 s was obtained, which could be attributed to the self-limiting thin film. As shown in Fig. 5A (inset), the detection limit was 50 nM (S/N = 3) with linearity up to 80 μM ($R^2 = 0.999$), compared to 350 nM and 45 μM obtained by the bare electrode. Such features could be attributed to a very low and stable background exhibited by the modified BDD electrode. No surface fouling was observed and the PPA/PTy-modified electrode did not require any surface cleaning steps during the course of repeated measurement. From time to time, Raman spectra of the repeatedly used electrode were examined which showed no appreciable difference from the freshly prepared counterpart with respect to intensity. Such a result implied that no film was formed on the electrode surface as a result of plausible DA polymerization. Notice also that the BDD electrode was reported for simultaneous measurement of DA and ascorbic acid,[23] and cyclic voltammetry must be performed in 0.1 M $HClO_4$ solution, a condition which is not compatible for *in vivo* monitoring or biological samples.

Alleviation of interferences

The combined PPA/PTy electropolymerized film completely blocked the diffusion of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ since no defined waves of this redox couple were observed even at 10 mM. Such behavior was indicative of a sieving feature of the film to exclude negatively charged compounds. A series of experiments was then conducted to evaluate the permselectivity of the PPA/PTy film. Ascorbic acid is the most severe interferent in the determination of DA in biological fluids with respect to its high concentration and low oxidation potential, compared to DA. Fig. 6 compares the signal response of the PTy-modified BDD and PPA/PTy-modified BDD electrodes at +0.8 V towards the consecutive addition of 20 μM of DA, 0.1 mM of AA, 0.1 mM of UA, and again 20 μM of DA (Fig. 6A) and 20 μM of other catecholamines (Fig. 6B). The relative permselectivity of different types of modified electrodes was presented in Table 1, calculated as the

Table 1 Permselectivity (%) of the PPA/PTy modified boron-doped diamond (BDD) electrode (calculated by the response signal ratio between each species relative to DA)

Analyte	Bare BDD (%)	PTy-modified BDD (%)	PPA/PTy-modified BDD (%)	pK_a
20 μM Dopamine, DA (MW = 153)	100	100	100	8.86–10.5
0.1 mM Ascorbic acid, AA (MW = 176)	1020	15	2.5	4.19
0.1 mM Uric acid, UA (MW = 168)	1050	17	5	5.8
20 μM L-DOPA (MW = 197)	80	10	4	8.72
20 μM DOPAC (MW = 168)	125	2.8	~0	4.08
20 μM EP (MW = 183)	160	58	26	8.86
20 μM NEP (MW = 169)	112	55	25	8.6
Tyramine (MW = 137)	n/a	n/a	n/a	9.74–10.52

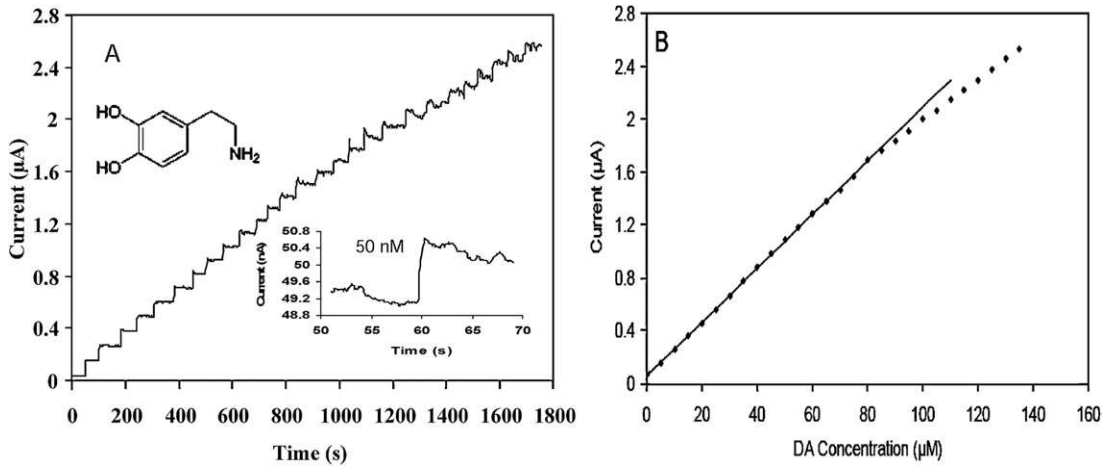


Fig. 5 (A) A typical current–time response of the PPA/PTy-modified BDD electrode upon successive additions of 5 μM DA in pH 7.0 phosphate buffer. (B) The calibration curve was replotted from the data obtained in Fig. 5A.

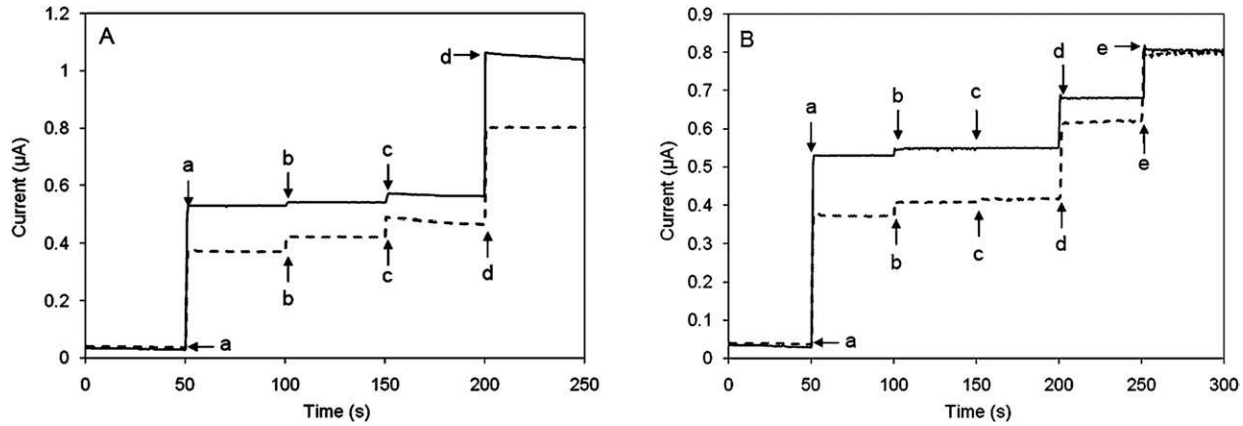


Fig. 6 (A) Amperometric response of the PPA/PTy-modified BDD (solid line) and PTy-modified BDD (dashed line) electrodes to the addition of (a) 20 μM DA, (b) 0.1 mM ascorbic acid, (c) 0.1 mM uric acid, and (d) 20 μM DA. (B) Amperometric response of the PPA/PTy-modified BDD (solid line) and PTy-modified BDD (dashed line) electrodes to the addition of 20 μM (a) DA, (b) L-DOPA, (c) DOPAC, (d) EP and (e) NEP.

response signal ratio for each species relative to DA. As mentioned earlier, the response of the bare BDD electrode to L-DOPA, DOPAC, EP and NEP was similar to that of DA at the same concentration (20 μM). The signals of AA and UA (0.1 mM) were 10-fold higher than that of DA (Table 1). The PTy-modified BDD electrode exhibited remarkable suppression (~40-fold) of both AA and UA. At the same concentration (20 μM), L-DOPA only invoked 10% of the signal response compared to that of DA whereas the response signal of EP or NEP was still over 50% of the DA signal. Further improvement in permselectivity was observed for the PPA/PTy-modified BDD electrode. DOPAC was eliminated whereas L-DOPA, UA, and AA only invoked 2.5–4% interference. The elimination of AA was an important finding since electrochemical methods such as cyclic voltammetry (CV) on solid electrodes or more sensitive differential pulse voltammetry (DPV) can be used to determine both DA and AA including *in vivo* monitoring. However, these two common methods do not permit continuous measurement but sampling with a frequency depending on the speed of the scan and the length of the regeneration period. The combined PPA/PTy film was very stable and strongly adhered to the BDD electrode surface. It was used for at least 30 repeated analyses of 5 μM DA without an electrochemical cleaning step during the course of measurement. For over 8 assays of 20 μM DA, some small changes in the signal response were noted. The modified BDD electrode was simply regenerated by electrochemical cleaning (−0.2 to +0.8 V at 100 mV/s for a few cycles) and ready for the next use for the detection of DA. It should be noted that Xiao *et al.*[29] modified a gold electrode with a polymer composite for selectively detecting DA in the presence of AA. After the self-assembly of an 11-mercaptoundecanoic acid (MUA) monolayer on the gold surface, poly(ethylene glycol) was applied and esterified with MUA. The modified electrode exhibits good reproducibility (±2%), a low detection limit for DA (10 nM), and a fast response time (<2 s). However, the system focused on DA and AA and it has not been tested for other electroactive neurotransmitters. Of also interest is the modification of glassy carbon (GC) with two polymer layers for the selective determination of DA.[30] The first layer was the electropolymerized macrocyclic nickel complex acting as an electrocatalyst for the dopamine oxidation and the second layer the polyurethane benzyl L-glutamate (PUBLG) for screening interfering species.

The hydrophobic PUBLG film was rendered to be more hydrophilic by immersing the electrode in a 1 : 3 (v/v) mixture of 4.0 M NaOH and methanol for 7 min at room temperature. After the treatment, carboxylate groups were developed into the PUBLG film. The resulting electrode exhibits improved selectivity with a detection limit of 80 nM for dopamine. Although several interferents were tested, the system was not challenged by other neurotransmitters.

Permselectivity of the BDD electrode modified with PPA, PPA/PTy and 4-hydroxyphenylacetic acid (HPA)

A series of experiments was conducted to decipher a plausible mechanism behind the PPA/PTy film permselectivity. Of importance to note that in order to attain the selectivity for DA detection, the PTy had to be formed first on the BDD electrode followed by the electropolymerization of PPA. Experimental data confirmed that the modified electrode formed by the reverse order (deposition of the PPA film first followed by tyramine electropolymerization) could not sufficiently suppress the interference caused by AA or UA, compared to the PPA/PTy electrode (Table 2). Unlike PTy, the PPA-modified BDD electrode displayed a well-defined feature of the ferri-/ferro-cyanide redox couple, similar to the result obtained with a PPA-modified glassy carbon surface.[14] Such behavior implied more porous morphology of the PPA membrane and its inferiority towards the suppression of AA and UA. The subsequent formation of the PTy layer on the PPA-modified electrode enhanced the permselectivity against both AA and UA, but still fell short of expectation, compared to the PPA/PTy-modified electrode. The experiment was also conducted to electropolymerize 4-hydroxyphenylacetic acid (HPA) on the BDD electrode to evaluate its permselectivity against uric and ascorbic acids. The CV was similar to the electrodeposition of tyramine, indicative of the creation of an electropolymerized film from HPA (Fig. 7). This film with COOH groups, however, behaved similar to the PPA film and could not suppress ascorbic and uric acids satisfactorily, compared to the PPA/PTy film as discussed earlier. Thus, the amino groups of the PTy film have played an important role in permselectivity against AA, UA, L-DOPA and DOPAC.

A plausible mechanism for permselectivity

The PTy film possessed a high density of amino groups, one NH_2 per monomer unit and should be partly protonated under pH 7 with respect to the pK_a of tyramine (9.74–10.52). When the film is prepared in $HClO_4$, pH 2, about 80% of the amino groups are protonated whereas 20% of the remainder are neutral.[27c] At pH 7, the neutral amino groups should be higher even though the overall surface was still positively charged. Based on their pK_a values (Table 1), the main forms of L-DOPA, DA, EP, and NEP

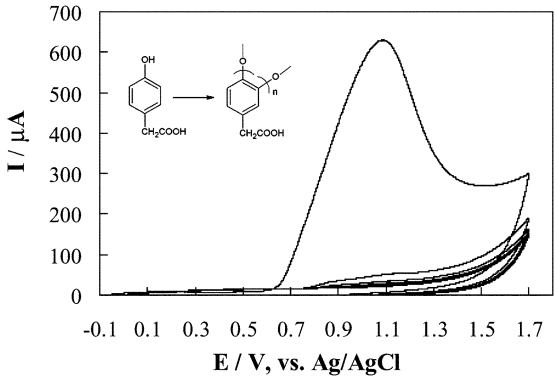


Fig. 7 Electropolymerization of 0.1 M 4-hydroxyphenylacetic acid (HPA) in 50 mM phosphate buffer, pH 7.0. Cyclic voltammetry was performed from −0.1 to +1.7 V (*vs.* Ag/AgCl) at 500 mV/s for 20 cycles.

at pH 7 would be cationic. In contrast, DOPAC, UA, and AA should have a negative charge. Table 1 illustrates significantly higher Faradaic responses of these neurotransmitters compared to the anionic species DOPAC, AA and UA. As the smallest compound in terms of molecular weight (MW = 153) and its structural similarity to tyramine, DA, existing mostly as a cation at physiological pK (pK_{a1} = 8.86 and pK_{a2} = 10.5)[31] provoked the highest response followed by EP and NEP. Apparently, the molecular fit or sieving effect played an important role to facilitate their penetration. Mass transfer limitation was expected to play a modest role since the film thickness was very small (~10 nm). However, the results obtained for L-DOPA and DOPAC were very intriguing. The presence of the COOH group in L-DOPA and DOPAC drastically diminished the Faradaic response of these two neurotransmitters. Similarly, the PTy film was able to exclude most uric and ascorbic acid even at 0.1 mM, *i.e.* 5-fold higher than the DA concentration tested. Uric and ascorbic acids with pK_a values of 5.8 and 4.19 respectively should exist predominately in urate and ascorbate forms. It was reasoned that the carboxyl group of DOPAC and L-DOPA was quickly oriented towards the PTy film to interact strongly with the protonated amino group whereas their oxidizable catechol tail was remote from the electrode surface. Hence, DOPAC and L-DOPA were effectively retained by the film and prevented their passage to the electrode surface. L-DOPA was not able to penetrate the PTy film due also to its bulkier structure compared to DA, *i.e.* hindrance effect. DOPAC with a pK_a[32] value of 4.08 would bind strongly to the outermost layer of the PTy film, resulting in an even smaller response signal. Similarly, urate and ascorbate were reasoned to bind to the outermost layer of the PTy electropolymerized cationic film *via* ionic interaction and extensive hydrogen bonding to form a double layer. Ascorbic acid is a very flat molecule with pK_{a1} = 4.19 and pK_{a2} = 11.57, and at pH 7 only one acidic hydroxy group should be dissociated

Table 2 Permselectivity (%) calculated by the ratio of signal response of each species relative to dopamine (DA)

Analyte	PPA/PTy-modified BDD (%)	PPA-modified BDD (%)	PTy/PPA-modified BDD (%)	HPA-modified BDD (%)
20 μM DA	100	100	100	100
0.1 mM AA	2.5	261	27	236
0.1 mM UA	5.3	81	46	76

to interact with the protonated amino group of the PTy film.[33] Its three remaining hydroxy groups then interacted with PTy film *via* hydrogen bonding to strengthen the complex. Consequently, a very small fraction of ascorbic acid could reach to the electrode surface to provoke the signal response. Experimental data confirmed that when 1 mM ascorbic acid was added to the detection cell, a signal response was comparable to that of 5 μM DA. The subsequent addition of ascorbic acid provoked no further signal response, owing to the saturation binding of ascorbic acid on the PTy film. The binding of ascorbic acid was reversible and easily dissociated by washing the PTy-modified electrode with the pH 7 phosphate buffer. Uric acid has 4 ionizable hydrogen ions (positions 1, 3, 7, and 9). Only the hydrogen ion on position 9 ($pK_a = 5.8$) is ionizable at physiological pH. Thus, it was able to bind strongly with the protonated amino groups of the PTy film *via* ionic interaction and hydrogen bonding. This mechanism was the rationale behind the observation that the redox couple, ferri/ferro(cyanide) provoked no signal response due to its high negative charge and bulky structure.

During the electrodeposition of PPA on the PTy-modified electrode, the first layer of PPA would adhere strongly to the PTy film *via* ionic interaction, π-to-π stacking and hydrogen bonding between the COOH of the former to the NH_2 of the latter. The second PPA layer with a negative charge favored the binding of DA, EP, and NEP while rejecting urate, ascorbate, DOPAC and L-DOPA from the active area of the electrode, a mechanism similar to the permselectivity of negatively charged Nafion.[34] It should be noted that unlike PTy or PPA, the preparation of the Nafion layer is attained by dropping and spreading this compound over the electrode surface. Therefore, it is somewhat difficult to precisely control the film thickness, uniformity, location, and reproducibility. Consequently, this approach cannot be used for the fabrication of microelectrodes and microelectrode arrays. In contrast, PTy or PPA is virtually electrodeposited on only the active area of the electrode with the above desired features.

Concluding remarks

The modification of boron-doped diamond electrodes with a combined electropolymerized film of tyramine and pyrrole-1-propionic acid has allowed the electrochemical detection of dopamine in the presence of dopamine metabolites and excess ascorbic acid, as would be the case in any human samples. This procedure is applicable for coating patterned substrates at the micro- and nano-meter scales towards the development of an electrochemical sensor/biosensor for *in vivo*/*in vitro* applications. This method of preparation would also be advantageous when the application requires long, thin, and insulated sensing tips. One such application is the recording of quantal neurotransmitters released from animal secretory cells in order to study cell behavior and physiology. In addition, probes of this type might be used in living tissues. The remarkable permselectivity of the combined PTy/PPA film obviates the requirement of a separation scheme such as HPLC or electrophoresis to circumvent electroactive interference. Diamond microelectrodes can be microfabricated in an array format to offer superior analytical performances, as compared to carbon fibers and metal wires, in terms of linearity, detection limit, response precision, and stability. The combined PPA/PTy film is also extremely versatile and offers new opportunities to link biofunctional molecules *via* the abundance of NH_2 and/or COOH functional groups on the polymer surface. This is an attractive procedure because the immobilization of the enzyme can be controlled closely, which is advantageous for microarray fabrication.

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