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Simultaneous electrochemical detection of the catecholamines and ascorbic acid at PEDOT/S-β-CD modified gold electrodes

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ABSTRACT

Poly (3,4-ethylene dioxythiophene)/sulphated β-cyclodextrin (PEDOT/S-β-CD) films, deposited onto gold working electrodes, were investigated for ascorbic acid (AA) and catecholamine detection using cyclic voltammetry, hydrodynamic voltammetry and amperometry. The thin PEDOT/S-β-CD films were fabricated *via* three electropolymerisation cyclic voltammetry cycles, on gold working macroelectrodes. A limit of detection (LoD) of 1.3×10^{-7} M was determined at PEDOT/S-β-CD modified gold electrodes, *via* cyclic voltammetry, for dopamine (DA) in the presence of 1×10^{-3} M of AA in human urine samples.

1. Introduction

Poly (3,4-ethylene dioxythiophene) (PEDOT) has good electrochemical stability, a small electronic bandgap, and thus, high inherent conductivity, and is biocompatible [1,2]. For these reasons PEDOT is an ideal candidate for biomedical/sensor applications. A detailed review on PEDOT and its derivatives has recently been published [3]. A problem with ethylene dioxythiophene (EDOT) is its solubility in aqueous solution and some of the literature to date has reported on the electropolymerisation of EDOT in the presence of surfactants [4]. Numerous authors have electropolymerised EDOT from organic media but dopant solubility can be limited in organic solvents. Recent literature findings report on the use of cyclodextrins (CDs) to electropolymerise thiophenes from aqueous solution [5–8]. The host–guest properties of CDs are well known [9,10] and can be used to increase EDOT solubility in H_2O [11].

When detecting catecholamines, at physiological pH, the prominent cationic monoamines–dopamine (DA), serotonin, epinephrine and norepinephrine, oxidise at very similar potentials making electrochemical signal separation difficult. Ascorbic acid (AA) is also a major interferent in the electrochemical detection of catecholamines, as it can be present in as much as 1000-fold excess [12,13] in the body. Polymeric materials that operate on the principle of charge separation [14–16] and size exclusion [17,18] can block anionic AA, thereby eliminating AA interference. PEDOT and blends of PEDOT have been used in the simultaneous detection of DA and AA due to the intrinsic hydrophobic and hydrophilic properties of the film [6,19,20]. In general, authors have used a separate anion when electrosynthesising polymer composites, with β-CD (usually a neutral form) in the same solution, so that co-immobilisation of the macrocycle occurs [21,22]. Here we report on the cyclic voltammetric electropolymerisation of EDOT from aqueous solution, using an anionic sulphated-β-CD (S-β-CD, 7–11 anionic sulphated groups) as the sole dopant. While PEDOT can facilitate the electrochemical separation of the AA and DA oxidation potentials, the possibility of some host–guest complexation between catecholamines and the immobilised S-β-CD was not discounted, and could also contribute to the selectivity of the polymer composite.

Many quantitative techniques have been successfully used in DA detection. Usually careful sample preparation is required and has been achieved, for example, *via* analyte derivation [23–25] or separation. Capillary electrophoresis and high performance liquid chromatography are commonly used to separate analytes, and techniques such as UV–*vis* [26], fluorescence [27–32], photodiode arrays [33], voltammetry [34–40], and chemiluminescence [41,42], are employed for detection and quantification. Alternatively selectivity and sensitivity can be enhanced with detection techniques based on enzyme amplification strategies [43,44]. While some of these techniques report excellent selectivity and limits of detection down to sub-nanomolar levels [45,46], they do not provide real time data, the equipment can be expensive, and sample work up can be laborious. Electrochemical sensors provide a cheap alternative, are easily fabricated and have the ability

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to detect analytes in real time, which is of particular importance in the detection of dynamic concentration levels in biological systems. The fabricated PEDOT/S-β-CD reported here was used to determine AA and DA concentrations in spiked human urine samples.

2. Experimental

Sulphated-β-CD, EDOT, NaCl, and all analytes were used as received from Sigma–Aldrich. Milli-Q™ water was used to prepare all solutions. A Solartron 1285 potentiostat was used for the electrochemical experiments and data analysis was performed using Corrview 2 software. RDE voltammetry experiments were performed using a PAR Model 630 unit in conjunction with the Solartron 1285. For cyclic voltammetry experiments the gold working electrodes (diam = 2 mm) were obtained from *IJCambria*, while the gold RDE (diam = 4 mm) was obtained from Pine Instruments. The electrodes were polished using 0.1 μm and 0.05 μm grade aluminium oxide paste on a Buehler Microcloth, and subsequently sonicated in ethanol and Milli-Q™ water, respectively. A platinum counter electrode and a SCE reference electrode were employed. All solutions were purged with nitrogen for 15 min prior to use. The electrolyte used was 0.1 M NaCl, pH ~ 5.8, and experiments were carried out at room temperature (295 K).

3. Results and discussion

3.1. PEDOT/S-β-CD/Au film fabrication and behaviour in 0.1 M NaCl

PEDOT/S-β-CD film fabrication was achieved by cycling a gold macroelectrode in aqueous solutions of EDOT and S-β-CD. EDOT is irreversibly oxidised at potentials ⩾+0.8 V (EQCM) which leads to deposition of an insoluble PEDOT layer on the electrode surface (Fig. S-1, SI). The PEDOT/S-β-CD film properties were very sensitive to the upper (anodic) potential of the voltammetric sweep used, as well as the EDOT/S-β-CD ratio, when fabricating the polymer film. In this body of work, polymer films formed *via* three electropolymerisation cycles between −0.50 and +1.08 V, from a 10:1 EDOT/S-β-CD solution ratio, were found to yield optimum results for the detection of analytes, in terms of sensitivity and reproducibility.

The voltammetric response of the PEDOT/S-β-CD/Au electrode when cycled from −0.8 to 0.75 V is shown in Fig. 1a. We observe a PEDOT oxidation peak, A_1, at *ca* −0.16 V, and a corresponding well defined reduction peak, C_1, at −0.39 V. A second more cathodic reduction peak, C_2, appears at −0.8 V and this may be coupled to the shoulder at −0.45 V in the oxidation sweep. At the redox potentials of conducting polymers, charge transfer is co-dependent on the flux of ions through the film to maintain charge electroneutrality. The oxidation process generates positive regions in the polymer matrix, which are charge compensated by the influx of anions from solution [47]. However, if the anion is large and, thus, immobilised within the film, cation exchange is required to maintain electroneutrality, as described by Eq. (1) [48]:

$$PEDOT^0S\text{-}\beta\text{-}CD^-Na^+ \rightleftarrows PEDOT^+S\text{-}\beta\text{-}CD^- + Na^+ \quad (1)$$

$PEDOT^0$ and $PEDOT^+$ represent the polymer in the reduced and oxidised states, respectively. EQCM was employed to prove that PEDOT/S-β-CD behaves as a cation exchange composite and a plot of polymer mass change as a function of potential is shown in Fig. 1b. This data was obtained for the oxidation sweep of the CV shown in Fig. 1a. For comparison we also cycled the polymer film in 0.1 M $NaNO_3$. The mass of NO_3^- is greater than that of Cl^- (62 g mol^{-1} and 35.5 g mol^{-1}, respectively), thus, if anions were the exchange species then differences in the EQCM mass changes would indicate this. With the onset of oxidation at −0.8 V we observe a decrease in mass up to 0 V. This observation is consistent with the egress of Na^+ ions from the polymer composite. The polymer is fully oxidised at 0 V and the egress of Na^+ ions ceases as charge electroneutrality is achieved. The similarity in the mass change traces for both electrolytes indicates that Na^+ ions, and not anions, are indeed involved in the redox process. At potentials more anodic than 0 V we observe a large and similar mass increase in the polymer films cycled in the different electrolytes. This mass increase in the polymer film can be attributed to ion uptake, solvent uptake, or indeed coupled ion/solvent uptake. At potentials where the polymer is fully oxidised, i.e. $E > 0$ V, the charge electroneutrality requirement for conduction is satisfied, and the exchange of cations with the electrolyte solution is not expected. If the species entering the film were Na^+Cl^- or $Na^+NO_3^-$ ion pairs, a difference in the EQCM mass changes between the two electrolyte solutions should be apparent. One possibility for the observed mass increase is that Na^+ or solvated Na^+ ions pair with the negatively charged CD sulphate groups immobilised in the polymer film. This process may be independent of polymer charge transport and a similar mass change on cycling in the different electrolytes would then be ex-

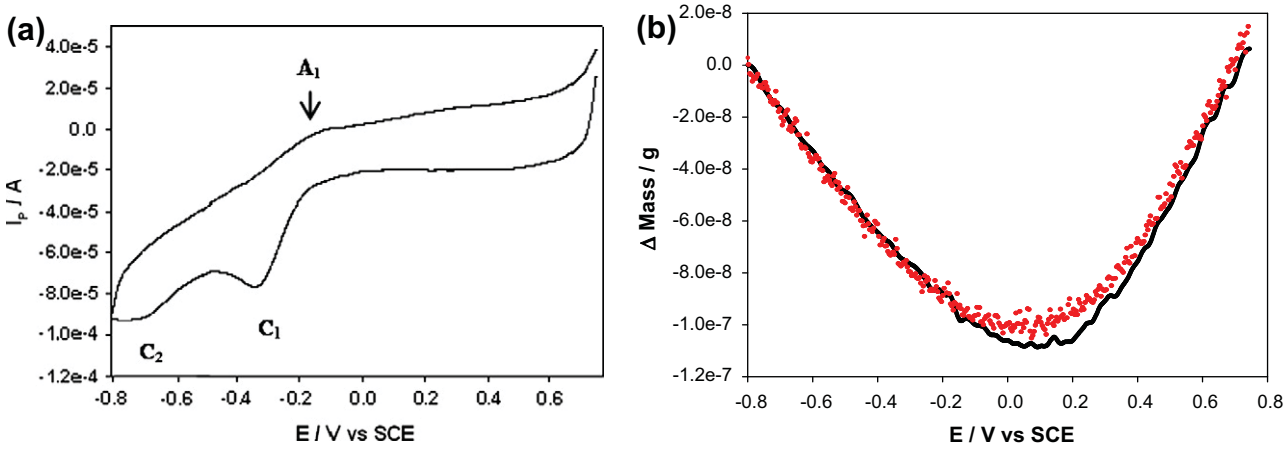


Fig. 1. Cation exchange; PEDOT redox activity with corresponding mass changes for the oxidation sweep. (a) Typical cyclic voltammogram showing the redox activity of a PEDOT/S-β-CD modified gold electrode when cycled in 0.1 M NaCl, pH 5.8, exhibiting an oxidation wave, A_1, and two reduction waves, C_1 and C_2. (b) EQCM mass changes for the oxidation sweep as a function of applied potential for two electrolytes, 0.1 M $NaNO_3$ and 0.1 M NaCl (red). *Note*: mass data was zeroed for clarity. Scan rate was 5 mV s^{-1} and electrode area = 0.204 cm^2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pected. A second possibility is that solvent is the dominant species entering the film. Furthermore, incorporation of water molecules or solvated Na^+ ions should increase capacitance in the polymer film and we do observe this trend in the electrochemical impedance spectroscopy (EIS) derived capacitance values.

When cycled from −0.20 to +0.75 V in 0.1 M NaCl yields the voltammetric response shown in Fig. 2a. There are no redox peaks evident in this potential range and the polymer (grown using three potential cycles) has capacitance values, 1.6×10^{-3}–2.5×10^{-2} F cm^{-2}, as determined from CV and EIS, respectively (Fig. S-2, SI). The discrepancy between CV and impedance measured capacitance values, has previously been reported in the literature and is justified elsewhere [49,50].

It should be noted here that the current passing through the polymer film does reduce with cycle number, and steady-state was not attained. An interesting feature of the current profile is the 'trough' region (Fig. 2b). The trough is constant in potential width but does shift anodically with potential cycling in background electrolyte (Fig. S-3, SI). Thus, the position of the trough was controlled so that it lay in a potential region, centred at *ca* +0.45 V. This position of the polymer trough ensured the lowest background currents and, thus, returned the highest peak currents for catecholamine oxidation. The trough shift indicates an increasing irreversibility, in the polymer redox behaviour, with potential cycling. The EQCM data, in Fig. 1b, can be used to justify this assertion.

3.2. Detection of dopamine/epinephrine/norepinephrine using cyclic voltammetry

DA, EP and NEP exhibit similar electrochemical behaviour at the PEDOT/SB-CD electrode but, for simplicity, only DA will be presented here. Adams and co-workers [51] characterised the electrochemical oxidation of the catecholamines in aqueous solution at carbon paste electrodes (Scheme S-1.1, SI). The overall catecholamine redox reaction is assigned an ECE mechanism. Direct electrochemical oxidation of the catecholamine (CA) to the o-quinone (OQ) form at an electrode occurs via the loss of two electrons and two protons. Typical cyclic voltammograms that describe the redox behaviour of DA at a bare gold and at a PEDOT/S-β-CD modified gold electrode are shown in Fig. 3. At the bare electrode a diffusion-like DA oxidation peak is observed at +0.45 V, with the corresponding DA-o-quinone (OQ) reduction peak at +0.29 V. This yields a peak separation of 0.162 V which is indicative of quasi-reversible behaviour [52]. Randles–Sevcik analysis indicates that DA oxidation is diffusion controlled at the bare gold electrode. At the PEDOT/S-β-CD modified electrode, well defined redox behaviour was observed for the DA/OQ couple at 0.434/0.321 V, ΔE_P = 0.113 V, for 1.0×10^{-3} M DA.

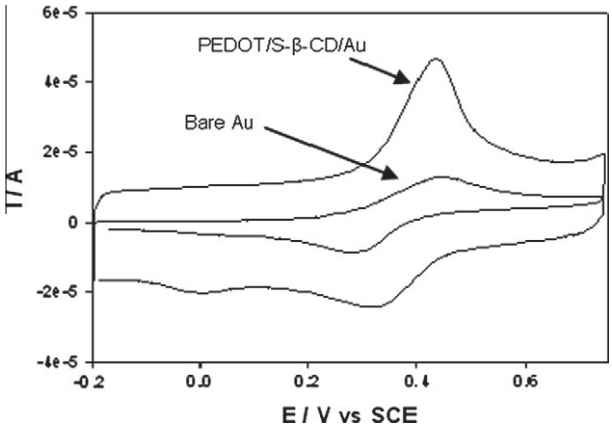


Fig. 3. 1 mM DA redox behaviour at bare and PEDOT/S-β-CD modified gold electrodes. Comparison of the typical voltammetric responses of a bare and PEDOT/S-β-CD gold electrode to a 1.0×10^{-3} M DA solution. Scan rate 100 mV s^{-1}, in supporting electrolyte 0.1 M NaCl, pH ~ 5.8. Electrode area = 0.0314 cm^2.

At lower DA concentrations the redox peaks become less defined (PEDOT/S-β-CD background current exhibits more influence) with an $I_{P,A}/I_{P,C}$ ratio >1.5 for all DA concentrations investigated. The redox reaction of DA at the PEDOT/S-β-CD electrodes, therefore, is quasi-reversible. The shapes of the DA redox peaks are adsorption-like and plots of I_P *vs.* υ are indeed linear. This diagnostic plot reveals that the oxidation of DA at the PEDOT/S-β-CD/Au electrode is under adsorption control [52]. A Randles–Sevcik plot using the same data (Fig. S-4, SI) is linear below scan rates of 60 mV s^{-1} and so DA adsorption at the PEDOT/S-β-CD film can be classified as weak.

The OQ has an electron deficient ring and an electron donating amine group. If the amine group is deprotonated, a 1,4 Michael addition can occur and this cyclisation reaction forms the leucoaminochrome (LAC) species. The apparent rate constants calculated for the catecholamine intracyclisation reactions are; DA = 0.13 ± 0.05 s^{-1}, NEP = 0.98 ± 0.52 s^{-1}, EP = 87 ± 10 s^{-1}, in 0.15 M sodium phosphate buffer, pH 7.4 [53]. The LAC is easily oxidised, *via* a homogeneous electron transfer reaction with the OQ in solution [54], to the aminochrome (AC) form. The reduction of AC back to LAC is driven electrochemically and was observed at 0.0 V as shown in Fig. 3. Therefore, due to experimental timescale, the AC reduction peak is more pronounced at lower scan rates and becomes less prominent as the scan rate reaches 200 mV s^{-1}. This

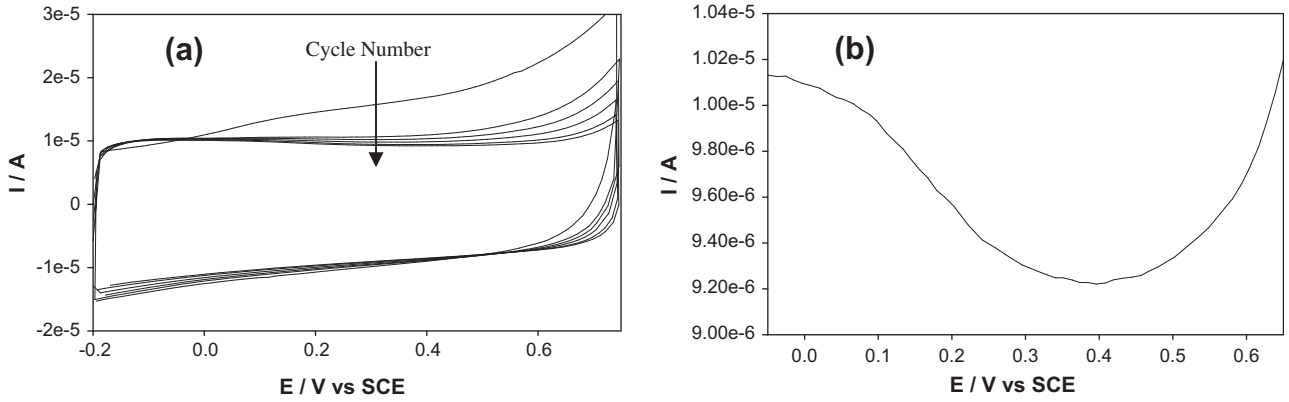


Fig. 2. Voltammetric profile of PEDOT/S-β-CD/Au in 0.1 M NaCl, pH 5.8. (a) Typical voltammetric profile of the PEDOT modified electrode cycled in 0.1 M NaCl electrolyte, pH ~ 5.8. Cycle numbers shown are 1, 2, 3, 5, 10, and 15. (b) Enlarged section of cycle 15 from (a), highlighting the current 'trough' centred at ~0.4 V.

AC to LAC reduction peak was also observed at bare gold electrodes.

PEDOT/S-β-CD promotes more facile oxidation of DA, when compared to the bare gold electrode, with a slight cathodic shift observed in DA oxidation potential. A recent literature publication by Henstridge et al. show, through CV simulation, that a potential shift may not necessarily be a catalytically driven process, but may be caused by a change from planar diffusion to a thin layer diffusion regime at modified surfaces [55]. When comparing bare gold to PEDOT/S-β-CD modified electrode data, garnered using equivalent scan rates, the DA oxidation peak currents are greatly enhanced (∼1.5 times) at the modified electrode. Kumar et al. also report a similar oxidation peak current enhancement (1.7 times) for tetrabutylammonium perchlorate (TBAPC) doped PEDOT films [19]. The authors propose a hydrophobic interaction between DA and 'reduced' regions of the PEDOT/TBAPC film. Another possibility in the mechanism of interaction between the DA and PEDOT is inclusion in the S-β-CD cavity. This interaction may promote greater selectivity of the modified electrode and, consequently, may also enhance signal separation in analyte solution mixtures. In solution DA forms a 1:1 inclusion complex with CD [56,57]. We acknowledge the possibility of the formation of an inclusion complex between the anionic β-CD and DA, where the aromatic ring on DA 'sits' into the S-β-CD cavity. Hydrophobic interactions, between the DA aromatic ring and the S-β-CD cavity, could facilitate this complexation. In a polymer matrix, however, access to the S-β-CD cavity is likely to be hindered or blocked due to the random formation of the polymer strands network. SAM of thiolated-CD's have been used to detect DA, with between 53.5% and 70% CD coverage on the surface [58,59], yielding sensors with a high degree of accessible CD sites. The number of CD active sites accessible to DA in the PEDOT/S-β-CD used here is difficult to determine for two main reasons; (i) the MW of S-β-CD is not accurately known and, (ii) adsorption of analyte at the modified surface is not solely promoted by the S-β-CD.

The third possible interaction mechanism, which we believe to play the most significant role, is the occurrence of electrostatic interaction or ion-pairing between the anionic sulphate groups (degree of substitution ∼7–11) on S-β-CD and the cationic protonated DA. Indeed either mode of DA-S-β-CD association, host–guest or electrostatic, coupled to film hydrophobicity, may be the driving force for the enhanced I_P values for DA oxidation observed in CV.

The PEDOT/S-β-CD/Au electrode displays good sensitivity towards DA with limit of detection ((LoD = $3.29\sigma/m$ [60], where σ is the relative standard deviation of the background current and m, the calibration line slope) calculated as 1.3×10^{-7} M, and limit of quantification (LoQ = $3.04 * \text{LoD}$) of 4×10^{-7} M, when using CV. The DA oxidation peak is very well defined and 'sits' in the aforementioned trough of PEDOT background current profile (Fig. 4).

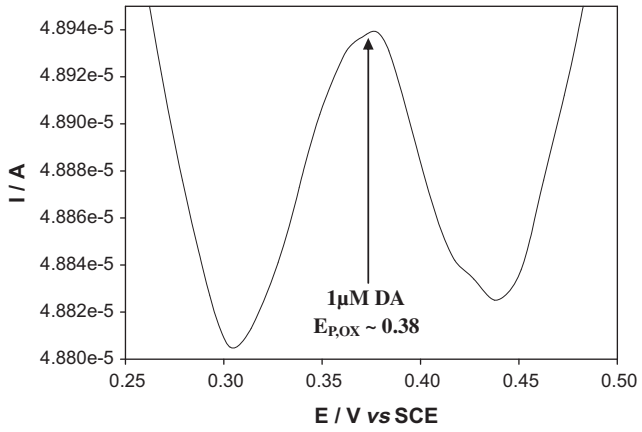


Fig. 4. Detection of 1 μM DA at the PEDOT modified electrode. 1 μM DA 'sitting' in the PEDOT 'trough'. Electrode area = 0.0314 cm^2.

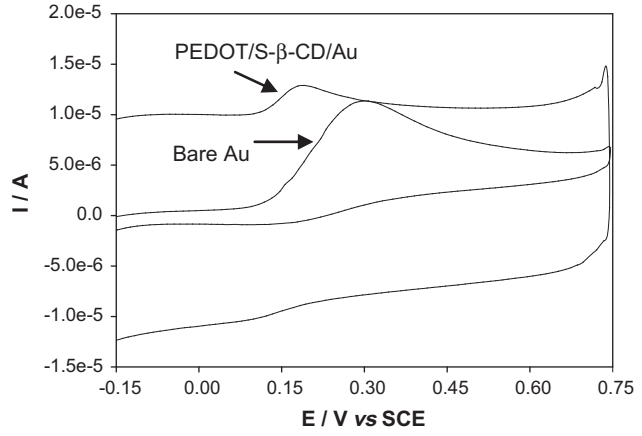


Fig. 5. Ascorbic acid oxidation at bare and PEDOT modified gold electrodes. Typical voltammetric response of a 1.0×10^{-3} M AA solution at a bare gold and at a PEDOT/S-β-CD modified gold electrode. Scan rate 100 mV s^{-1}, supporting electrolyte 0.1 M NaCl, pH 5.8, electrode area 0.0314 cm^2.

3.3. Detection of ascorbic acid using cyclic voltammetry

The electrochemical oxidation of AA involves the net loss of two electrons and two protons [61] and, at a bare gold electrode, this potentially driven oxidation is irreversible (Scheme S-1.2, SI). At pH values above the pK_a value for AA (*ca* 4.5), the oxidation process only involves the loss of one proton as AA exists as AH^- not AH_2 [62]. Typical voltammograms for the irreversible oxidation of 1.0×10^{-3} M AA, at a bare gold and a PEDOT/S-β-CD/Au electrode, are shown in Fig. 5.

The oxidation currents generated at the modified electrode are comparable to those generated at the bare gold. Cathodic shifts in peak potential for AA oxidation at the modified electrode, when compared to bare gold, range from *ca* 80–135 mV, depending on sweep rate utilised. The anionic AA interacts with the polymer matrix which promotes lower ascorbic acid oxidation potentials. These peak shifts have been ascribed, elsewhere, to the affinity that the AA anion has for 'oxidised' regions of the PEDOT film [6]. Interestingly the AA oxidation peak appears in the anodic region after the point where the PEDOT composite is fully oxidised. Positive regions generated along the polymer may also promote interaction with AA.

3.4. Simultaneous DA and AA detection using cyclic voltammetry

The response of a PEDOT/S-β-CD/Au electrode to varying concentrations of DA in the presence of 1.0×10^{-3} M AA is shown in Fig. 6. The response at the bare electrode for a 1.0×10^{-3} M DA concentration is included for comparative purposes. The striking feature is the potential separation of the AA and DA oxidation waves, as observed in the non-mixed AA and DA solutions. Peak separation can be justified by the affinity that the film has for both analytes at different potentials. The current increase for DA oxidation at the modified electrode is consistent with the ∼1.5 times magnitude increase (again after background correction) observed for DA oxidation, when no AA was present. The concentration of DA has no bearing on the AA oxidation peak, which remains at a constant intensity.

We also observe excellent sensitivity towards DA, with the CV linear region extending down to 1.0×10^{-6} M, in the presence of

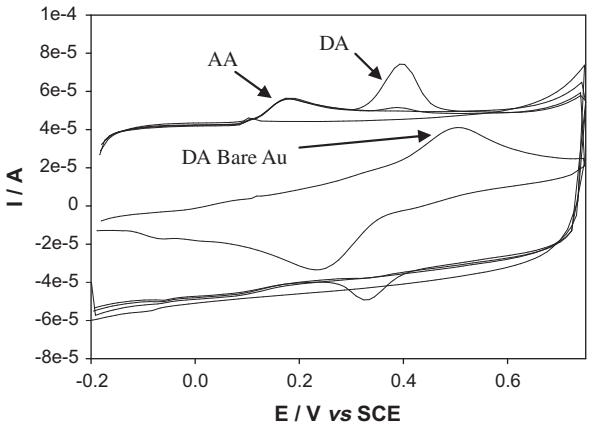


Fig. 6. Simultaneous detection of ascorbic acid and dopamine. Typical voltammogram tracing the response of a PEDOT/S-β-CD electrode to three DA concentrations; 1.0×10^{-4}, 1.0×10^{-5} and 1.0×10^{-6} M, in the presence of 1.0×10^{-3} M AA. The redox behaviour of a 1.0×10^{-3} M DA solution at a bare gold electrode is also shown for comparison. All voltammetry was performed in 0.1 M NaCl electrolyte, pH 5.8, at a scan rate of 100 mV s^{-1}. Electrode area = 0.1257 cm^2.

1.0×10^{-3} M AA. The slopes of the linear region obtained from the highly reproducible CV generated calibration curves, of single analyte and mixed solutions, are very similar in magnitude. The calibration curve slopes for DA only and DA/AA solution mixtures were 0.069 ± 0.001 and 0.071 ± 0.002 A M^{-1}, respectively (Fig. S-5, SI).

3.5. Rotating disc electrode voltammetry

The behaviour of AA, DA and AA & DA mixtures at the PEDOT/S-β-CD gold electrode were investigated at the RDE (RDE derived values in Table S-1, SI). Both AA and DA responses exhibit well defined limiting currents, at all rotation speeds, enabling valid kinetic parameters to be gleaned from the data. Forced solution convection generated by the RDE offsets the weak adsorption regime observed using CV. Thus, the oxidation process is under mass transport control at the RDE, generating well defined limiting currents, and so we can infer that electron transfer between AA, DA and the film is not rate limiting (Fig. S-6, SI).

The data were obtained by monitoring the current response to stepped increments in the rotation speed of the electrode while an oxidative potential was being applied. When analyte oxidation is controlled by mass transport to the electrode surface, the relationship between rotation speed and the limiting current obeys the Levich equation. I_{LIM}, denotes the limiting current; ω, the rotation speed; C, bulk analyte concentration; D is the diffusion coefficient, and n, F, and A, have their usual meanings [52].

$$I_{LIM} = 0.62nFAv^{-1/6}D^{2/3}C^{1/2}$$

Limiting currents for AA and DA oxidation were obtained using rotation speeds 250–3000 rpm, at a scan rate of 5 mV s^{-1}. Levich plots (I_{LIM} *vs.* $\omega^{1/2}$) were constructed for individual solutions and AA/DA solution mixtures, and diffusion coefficients were calculated from the slopes of each plot (Fig. S-7, SI). Typical Levich plots, constructed for oxidation of a range of AA and DA concentrations at PEDOT/S-β-CD/Au, all have zero intercepts. RDE derived diffusion coefficient values are similar in magnitude to the reported literature values which range from 2.7 to 6.9×10^{-6} cm^2 s^{-1} for DA [63–65] and 6.5×10^{-6} cm^2 s^{-1} for AA [66] at bare electrodes.

Kinetic parameters for analyte oxidation at the PEDOT/S-β-CD modified electrodes were determined *via* Koutecky–Levich analysis. Koutecky–Levich plots were constructed from oxidation data, obtained at PEDOT/S-β-CD electrodes, for a range of AA concentrations (Fig. S-8, SI). The condition required for Koutecky–Levich analysis, in the absence of any mass-transfer effects, is that substrate reaction kinetics be first order. Good steady-state responses were recorded at all rotation speeds and analyte concentrations investigated, with excellent reproducibility being achieved. The Koutecky–Levich equation is given with the following symbol meanings; Γ, is the surface coverage and, k_{ME}, is the heterogeneous rate constant in the forward reaction [52].

$$(I_{LIM})^{-1} = (nFAk_{ME}C)^{-1} + \left(0.62nFAv^{-1/6}D^{2/3}C^{1/2}\right)^{-1}$$

We chose to omit surface coverage values in our calculations and let $(\Gamma * k_{ME}) = k'_{ME}$. Derived values for k'_{ME} at the PEDOT/S-β-CD modified electrodes for AA and DA fell within the ranges 0.032–0.059 and 0.042–0.066 cm s^{-1}, respectively. The general feature of the Koutecky–Levich plots, for AA and DA oxidation, is that both the slopes and intercepts varied inversely with respect to analyte concentration. The values are quite similar in magnitude indicating no greater affinity of either analyte towards the PEDOT/S-β-CD composite. This response is indicative of a reaction occurring homogeneously throughout the polymer layer and, as the PEDOT film is thin, it behaves as an extension of the electrode into the bulk solution.

3.6. AA and DA solution mixtures

At rotation speeds ⩽1000 rpm the response at the PEDOT/S-β-CD/Au electrodes to solution mixtures of AA and DA is highly reproducible with separate well defined plateau regions for each analyte (Fig. 7). The limiting currents obtained for each analyte in the solution mixture correspond closely, but not exactly, to those obtained in non-mixed solutions. At rotation speeds ⩽1000 rpm the total current output in the mixed solution Fig. 7c, equates to a simple summation of the individual currents in the non-mixed voltammograms Fig. 7a and b.

At higher rotation speeds we do observe deviation from Levich linearity (Fig. S-9, SI) due to the homogeneous catalytic reaction between AA and dopamine-OQ (Scheme S-1.3, SI). This can be justified in that the solution near the polymer film/solution interface are increasingly better mixed, thus the catalytic reaction is promoted. In effect, the observed limiting currents for AA oxidation decrease, while DA oxidation limiting currents increase, giving rise to deviation from linearity in the Levich plots. Typical Levich plots

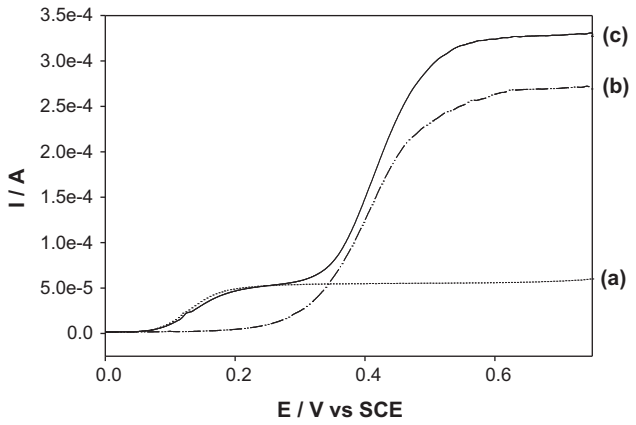


Fig. 7. Simultaneous AA and DA detection at PEDOT modified gold using RDV. RDE voltammetric response of PEDOT/S-β-CD/Au electrodes to; (a) 1.0×10^{-3} M AA, (b) 5.0×10^{-3} M DA, and (c) 1.0×10^{-3} M AA and 5.0×10^{-3} M DA solutions. Rotation speed 250 rpm, scan rate 5 mV s^{-1}. At rotation speeds ⩽1000 rpm I_{LIM} for the solution mixtures are equal to the summation of I_{LIM} of individual DA and AA responses.

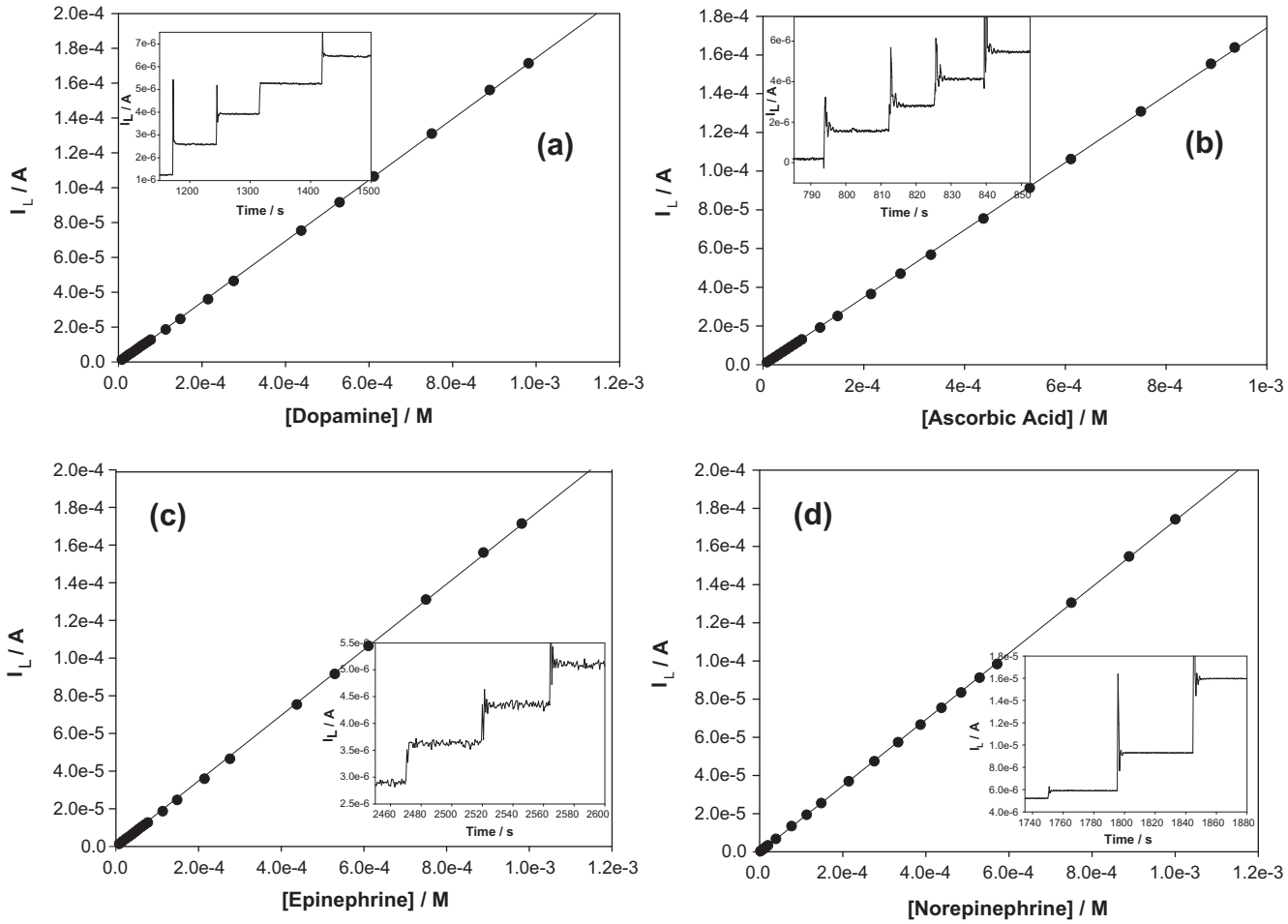


Fig. 8. Amperometry calibration curves for dopamine, ascorbic acid, epinephrine and norepinephrine. Typical amperometry plots (insets) and corresponding calibration curves detailing the response of PEDOT/S-β-CD/Au electrodes to aliquot additions of various analyte concentrations. Aliquots represent the concentration added to the solution and not total solution concentration. Plots represent: (a) DA; 8 μM aliquot additions, E_{app} = +0.75 V, (b) AA; 8 μM aliquot additions, E_{app} = +0.5 V, (c) EP; 4 μM aliquot additions, E_{app} = +0.75 V, and (d) NEP; 4, 20 and 40 μM aliquot additions, respectively, E_{app} = +0.75 V. Rotation speed set to 2500 rpm; gold working electrode area = 0.1257 cm^2. Correlation coefficients: (a–d) $r^2 > 0.999$.

were constructed for a range of DA concentrations in the presence of 1.0×10^{-3} M AA. Limiting currents for AA oxidation and DA oxidation were taken from the RDE data at E = +0.26 V and E = +0.75 V, respectively. The catalytic reaction between AA and dopamine-OQ was clearly observed, in particular and as expected, at lower DA concentrations. Unfortunately determination of k'_{ME} for DA, in DA/AA solution mixtures, was deemed to be futile as the Koutecky–Levich plot intercept values were negative.

The 1.0×10^{-3} M AA traces are all highly comparable at rotation rates ⩽1000 rpm, but subtle differences are observed when the Levich plots are examined in more detail. When the Levich slopes were plotted against DA concentration a linear relationship resulted. Thus, the determination of unknown DA concentrations in solution may be possible by extrapolation of the Levich slopes after additional systematic characterisation/calibration of the sensor response, to AA/DA mixtures, is carried out. This would indeed be a helpful tool if the existing sensor was used to detect AA only. Once the AA concentration was evaluated using rotation speeds up to 1000 rpm, comparison of the newly constructed Levich slope to the AA Levich slopes (Fig. S-10, SI) would allow for the determination of unknown DA concentrations in solution.

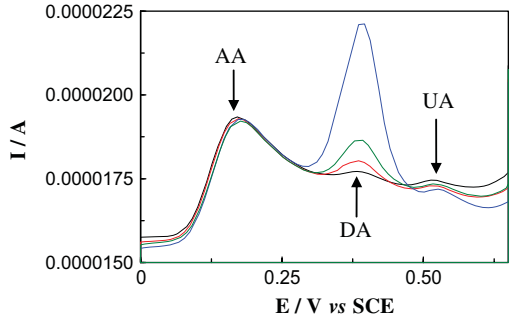
3.7. Amperometry

When the anodic peak current response, for analyte oxidation, was measured under constant reaction conditions, it was found that the amperometric current response varied with the concentration of supplied substrate C^{∞}. The constant conditions referred to are the applied potential, E_{app}, and the rotation speed of the electrode. E_{app}, was held at +0.50 V for AA, and at +0.75 V for the catecholamines, to ensure maximal oxidation of analyte at the modified electrode/solution interface. The substrate flow patterns are governed by the rotating disc speed, which was set at 2500 rpm.

It was found that the steady state current (I_{LIM}) varied with the concentration of supplied substrate, this substrate being the injections of a series of aliquots of analyte to the cell solution. Low concentrations of the analyte were detected amperometrically because PEDOT/S-β-CD/Au electrode background currents were small, being reduced significantly *via* a polarisation period in electrolyte, in the absence of substrate.

Typical amperometric responses obtained at a PEDOT/S-β-CD modified electrode clearly depict the I_{LIM} values obtained after each injection of analyte, Fig. 8, insets (a–d).

The current response to each addition is rapid and sharp, with steady-state behaviour, i.e. 100% I_{LIM}, attained in less than 4 s, irrespective of aliquot concentration, for all of the analytes investigated. We observed clear and reproducible analytical signals for 1 μM aliquot additions of AA and the catecholamines. The constructed calibration curves all exhibit excellent linearity and, thus, oxidation currents of all analytes detected at the PEDOT/S-β-CD/Au electrodes are first order with respect to concentration (Fig. 8a–c). The reproducibility of results was also very high and this is



Urine Sample	Spiked µM	Detected µM	Recovery (%)	RSD (%)
1 DA	1	0.98	98	2.0
AA	1000	1002	100	3.6
2 DA	2.5	2.54	102	1.4
AA	1000	1009	101	2.9
3 DA	10	10.2	102	1.8
AA	1000	990	99	3.1
4 DA	50	51.2	102	3.4
AA	1000	982	98	2.6

Fig. 9. Detection of AA and DA in human urine at the PEDOT modified electrode and summarised analyte recoveries. Typical electrochemical response of PEDOT/CD/Au electrode in Human Urine samples spiked with 1 mM AA and the following DA concentrations; black trace = 1×10^{-6}, red trace = 2.5×10^{-6}, green trace = 1×10^{-5} and blue trace = 5×10^{-5} M. Human Urine samples were diluted, 1:50, with 0.1 M NaCl. Electrode area = 0.0314 cm^2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reflected in the small deviation (all <6%, $n \geqslant 3$) in the calibration curve slopes.

3.8. Detection of ascorbic acid and dopamine in human urine

The presence of twice the normal concentration of catecholamines in urine is used as a diagnostic test for human pheochromocytomas [67] and paragangliomas [68] type neuroendocrine tumours. The performance of the PEDOT/CD sensors was assessed in real human urine samples, obtained from healthy volunteers. Samples of urine were diluted (1:50) using 0.1 M NaCl, spiked with AA and DA standard solutions, and their recoveries were obtained. Uric acid (UA) was tested as an interferent and was found to oxidise at potentials more anodic than that of DA, *ca* +0.52 V (Fig. S-11, SI).

Fig. 9 shows a typical voltammograms of the spiked urine samples with accompanying summary of recovered analyte concentrations.

AA and DA peaks are oxidised at the same potentials as observed in the analyte standards. Uric acid is observed at *ca* +0.52 V and is not an interferent. Very little sensor sensitivity was lost and calibration curves returned regression lines with an average slope ($n = 5$) of 0.072 ± 0.002 A M^{-1}. Using this equation the DA and AA concentration recoveries were determined. In the presence of 1 mM AA the calculated LoD and LoQ for DA, in human urine samples, were 1.8×10^{-7} and 5.7×10^{-7} M, respectively.

4. Conclusions and future work

In conclusion, the results presented in this paper show PEDOT/S-β-CD modified gold electrodes to be effective sensors for the simultaneous detection of AA and the dopamine. We obtained a LoD of 1.8×10^{-7} M for DA in spiked human urine samples. Patients with neuroendocrine tumours excrete elevated levels of catecholamines in urine [69] which can be comfortably detected by the PEDOT/S-β-CD sensor. The LoD reported here compares favourably to those reported previously, for CD modified electrodes, which range from 10^{-7} to 10^{-5} M in non-biological solutions [58,59,70–72], and 10^{-7} M in biological samples [73,74]. Recently graphene-CD composites have shown to be promising new materials in sensor construction, capable of DA detection in the 10^{-8}/10^{-9} M range [75,76] but have yet to be tested in biological samples. Simultaneous quantification of AA and catecholamines in real-time is a technique that is currently highly desirable to help promote our understanding of biological disorders. The PEDOT/S-β-CD sensor offers a cheap, easily fabricated, and reliable electrode for use in DA and AA quantification in human urine samples.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jelechem.2011.12.020.

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