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Electrochemical characterisation of poly(3,4-ethylenedioxythiophene) film modified glassy carbon electrodes prepared in deep eutectic solvents for simultaneous sensing of biomarkers

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ABSTRACT

Electrodeposition of the conducting polymer, poly(3,4-ethylenedioxythiophene) (PEDOT) in deep eutectic solvents (DES), prepared by the simple mixing and heating of an H-bond acceptor, choline chloride and H-bond donors such as urea, ethylene glycol and glycerol, is described. The PEDOT modified glassy carbon electrodes prepared in different DES and in the presence of conventional aqueous surfactants or $HClO_4$ media were characterized during and after growth by electrochemical and quartz crystal microbalance studies. PEDOT synthesized in the best performing DES medium, choline chloride – urea (Reline) in the presence of $HClO_4$, was used for the sensing of the biologically relevant molecules ascorbic acid, dopamine and uric acid. The sensing characteristics were compared with those of PEDOT-modified electrodes prepared in aqueous media.

1. Introduction

Innovative research on conducting polymers since their discovery by MacDiarmid, Heeger and Shirakawa [1,2] in 1977 has been directed towards development of materials with specific physico-chemical and mechanical characteristics such as high conductivity, higher capacity to accumulate charge, higher elasticity and stability [3,4]. Electrodeposition of π-conjugated polymers to give conducting polymer modified electrodes has led to application in diverse areas such as in supercapacitors, mechanical actuators, sensors, light emitting diodes, solar cells, electrochromic displays and switching materials [4–6]. Among these, poly(3,4-ethylenedioxythiophene) (PEDOT) exhibits good electrochemical stability, a wide potential window, low band gap and distinct spectral absorption for oxidized and reduced states [7]. Interesting properties exhibited by these materials is mainly due to their doping/dedoping as a function of applied potential [8].

The electrochemical synthesis of conducting polymers in the presence of different additives such as surfactants, acids and ionic liquids influences the properties of the electrodeposited material in terms of conductivity, redox behaviour and doping levels [9–12]. Room temperature ionic liquids (RTIL) are alternative solvents utilized for the electrosynthesis of conducting polymers due to their high conductivity, low volatility and wide potential windows compared to conventional aqueous and organic solvents [12]. Although conventional RTIL possess such advantageous properties, they are costlier, involve tedious synthetic protocols and are non-biodegradable [13].

In the present study, we have carried out the electropolymerisation of 3,4-ethylenedioxythiophene (EDOT) in deep eutectic solvents (DES), which are formed by the interaction between suitable hydrogen bond donors and acceptors [13,14]. These solvents can be easily prepared by mixing a quaternary ammonium salt, which acts as H-bond acceptor, with an H-bond donor (HBD), in specific mole ratios to form a eutectic mixture, with melting point much lower than the two components and which are liquid at room temperature. Commonly used H-bond acceptors are choline chloride, proline, alanine, betaine etc., while a large variety of hydrogen bond donors comes from the family of alcohols, carboxylic acids or amides such as ethylene glycol, glycerol, succinic acid or urea [13–15]. Advantages of deep eutectic solvents include ease of preparation, low cost, easily available components and biodegradability, in addition to the properties exhibited by conventional ionic liquids. Hence, these solvents possess great potential as electrolytic media for the electrodeposition of metals and polymers [16]. The electrochemical synthesis and characterization of conducting polymers such as polyaniline and polypyrrole

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in deep eutectic solvents has been reported elsewhere [11,17]. Hillman et al. conducted studies on the ion transfer mechanism of p-doped PEDOT films prepared in acetonitrile [18]. Recently, we reported the preparation of PEDOT films for highly sensitive electrochemical sensors by electropolymerisation in DES, for the first time [19]. Electrochemical and surface microscopy characterisation of the PEDOT films formed in ethaline, reline and glyceline were carried out.

We now report detailed studies on the electrodeposition of PEDOT in various DES and characterization of the films using quartz crystal microbalance (QCM) gravimetry as well as electrochemical techniques. A gravimetric study using an electrochemical QCM is a powerful tool to detect and quantify the mass change, ion dynamics and polymer film characteristics. Detailed study of the polymerisation parameters, its sequential optimization and comparative evaluation of the sensing characteristics of PEDOT modified electrodes prepared in different DES is carried out using ascorbic acid (AA) as model analyte. Furthermore, the developed modified electrodes were used for the simultaneous detection and quantification of the biomarkers ascorbic acid (AA), dopamine (DA) and uric acid (UA) [20,21]. Techniques often used to detect and quantify these biomolecules are chemiluminescence [22], UV–vis spectroscopy [23] and spectrofluorimetry [24]. However, these techniques are complicated, expensive, time consuming and usually require prior separation of components by chromatography [25] or electrophoresis [26]. For this reason, voltammetric electrochemical methods have been employed exploiting modified electrodes, which are able to separate the potentials for oxidation of these analytes sufficiently well. The voltammetric oxidation peaks of these compounds are in the range 0.2 to 0.7 V at bare glassy carbon electrodes (GCE) within one broad peak [27]. Modifiers which give selective electrocatalysis such as polypyrrole/poly(4-vinyl pyridine) films [28,29], carbon based nanomaterials [30,31], enzymes [23], or semiconductor quantum dots [22] have been employed to obtain well-separated oxidation peaks and high peak currents. Here, we have successfully used PEDOT modified GCE prepared in deep eutectic solvents for the simultaneous detection and quantification of AA, DA and UA.

2. Experimental

2.1. Reagents and buffer electrolyte solutions

Ethylene glycol, urea, glycerol, choline chloride, 3,4-ethylenedioxythiophene (EDOT), ascorbic acid, dopamine, uric acid, monobasic and dibasic potassium phosphate, sodium chloride and sodium poly(styrene sulfonate) (NaPSS) were from Sigma-Aldrich, Germany. Perchloric acid (70%), potassium chloride and monobasic sodium phosphate were obtained from Fluka, Switzerland. For electrochemical sensing studies, the supporting electrolyte was sodium phosphate buffer saline (NaPBS) (0.1 M phosphate buffer + 0.05 M NaCl, pH = 7.0). Millipore Milli-Q nanopure water (resistivity $\geq$18 MΩ cm) was used for the preparation of all solutions. All experiments were performed at room temperature (25$\pm$1 °C).

2.2. Instrumentation

Electrochemical experiments were performed in a three-electrode cell, containing a glassy carbon disc electrode (GCE), area 0.0314 cm^2, as working electrode, a Pt wire counter electrode and an Ag/AgCl (3.0 M KCl) reference electrode, together with a μ-Autolab potentiostat/galvanostat (Metrohm-Autolab, Netherlands). For electropolymerisation in eutectic mixtures, an Ag wire was used as the pseudo-reference electrode (Ag/AgCl vs Ag wire $\sim$ + 90 mV). The electrochemical quartz crystal microbalance (EQCM) used was an eQCM 10 M from Gamry, with 10 MHz carbon-coated crystals. The pH measurements were carried out with a CRISON 2001 micro pH-meter (Crison Instruments SA, Barcelona, Spain) at room temperature.

2.3. Preparation of PEDOT films in deep eutectic solvents

Deep eutectic solvents were prepared by mixing the quaternary ammonium salt, choline chloride, with the HBDs ethylene glycol (to give ethaline), urea (to give reline) or glycerol (to give glyceline) in a 1:2 molar ratio, heating at 60 °C until a homogeneous solution was obtained and then allowing the DES to cool down to room temperature. Thin PEDOT film modified GCEs were prepared potentiodynamically by cycling in a solution of 10 mM EDOT in DES in the presence of 4 M $HClO_4$ in the potential range -0.6–+1.2 V at 50 mV s^{-1}. A uniform, reproducible film was obtained after 15 potential cycles. In order to compare the efficacy of electropolymerisation in eutectic medium, PEDOT films were also prepared in aqueous media, 0.1 M PSS and 4 M $HClO_4$, in the presence of 10 mM EDOT.

2.4. Amperometric sensing

Fixed potential amperometric sensing of ascorbate was conducted in pH 7.0 phosphate buffer saline, PBS (0.1 M phosphate buffer + 0.05 M NaCl) at 0.0 V vs Ag/AgCl. Sensing and quantification of ascorbic acid, dopamine and uric acid was carried out by differential pulse voltammetry in the region -0.2 to +0.5 V with a potential increment of 1 mV, pulse amplitude 25 mV and a scan rate of 5 mV s^{-1} in PBS buffer medium.

3. Results and discussion

Fig. 1 shows typical cyclic voltammograms of the electrochemical synthesis of PEDOT in the eutectic mixtures ethaline, reline and glyceline in the presence of 10 mM EDOT and 4 M $HClO_4$. In order to compare the voltammetric features of PEDOT prepared in DES and other media, the electrochemical synthesis of PEDOT was also carried out in aqueous surfactant medium, 0.1 M PSS, and in aqueous 4 M $HClO_4$.

Characterisation of these PEDOT-modified GCE by cyclic voltammetry, electrochemical impedance and scanning electron microscopy was reported previously [19]. The voltammograms obtained during the potentiodynamic synthesis of PEDOT in various media were analysed, and characteristic peaks were identified. Briefly, the anodic peaks correspond to oxidation of adsorbed monomeric species on the electrode surface and, at 0.70 V, to diffusion-controlled oxidation of radical monomers, dimers or oligomers formed during polymerisation. On the negative scan, the peaks correspond to diffusion-controlled reduction of dimeric/oligomeric species and to reduction of adsorbed polymer. For ethaline and glyceline only, a small shoulder around 0.80 V probably corresponds to oligomer oxidation (only seen after 4 or 5 cycles). More details may be found in [19].

These studies are now complemented by carrying out quartz crystal microbalance gravimetric studies during electropolymerisation for better understanding of the film characteristics and for discussing the doping/dedoping mechanism. Optimisation of sensing film preparation, using ascorbic acid as model analyte, is reported in Section 3.3 and application to simultaneous sensing of ascorbic acid, dopamine and uric acid in Section 3.4.

3.1. Electrochemical QCM studies

An electrochemical quartz crystal microbalance (EQCM) with a carbon-coated quartz crystal, was used to monitor the variation in

frequency during potential cycling electropolymerisation between -0.6 V and +1.2 V in order to investigate both polymer growth and the intercalation/expulsion of anions/cations into and out of the film during cycling. Thus, both growth of the polymer and its redox cycling (with associated mass changes due to ion incorporation/expulsion) were simultaneously probed.

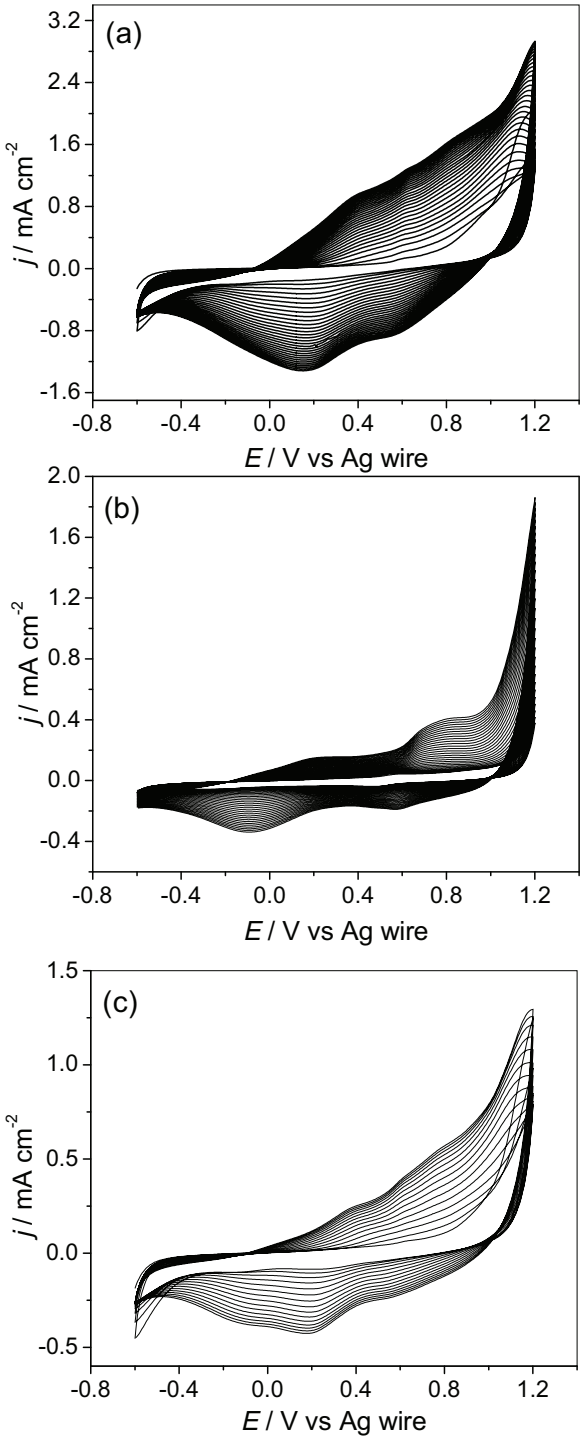


Fig. 1. Electropolymerisation of 10 mM EDOT by potential cycling in the range -0.6 to 1.2 V at 50 mV s^{-1} in (a) Ethaline – 4 M $HClO_4$ (b) Reline – 4 M $HClO_4$ (c) Glyceline – 4 M $HClO_4$.

For rigid films, the frequency variation with time can be used to determine the deposited mass via the Sauerbrey equation [32]:

$$\Delta f = \frac{2f_0^2}{A\sqrt{\mu_q \rho_q}}\Delta m \quad (1)$$

where f_0 is the resonant frequency (Hz), Δf the frequency change (Hz), Δm the mass change (g), A the piezoelectrically active crystal area (cm^2), ρ_q the density of quartz (g cm^{-3}) and μ_q the shear modulus of quartz for AT-cut crystals (g cm^{-1} s^{-2}). In the specific case of the carbon-coated QCM employed in this study, the conversion factor, $-\Delta f/\Delta m$, is 226.0 Hz per μg.

In Fig. 2, the frequency change is shown as a function of time as well as the anodic charge transferred to the electrode during electropolymerisation in the four media studied. The decrease in frequency with the (transferred) charge increase or time reflects the deposition of the PEDOT film on the electrode. The profiles obtained for the different eutectic solvents show that the frequency decrease during electrodeposition of PEDOT is very similar in the three eutectic solvents studied (equivalent to ~1 μg increase in mass assuming a rigid film), whereas in aqueous PSS, the change is much greater (equivalent to 11 μg).

Fig. 3 depicts the mass variation during PEDOT deposition in reline together with the current response during potential cycling. It can be seen that the mass of the PEDOT film increases during oxidation (positive scan) since the positive charge generated at the growing polymer extracts anions (and solvent) to the film, causing swelling. The film continues to increase in mass after the oxidation peak, probably as a result of continuing electro-migration [33]. Swelling continues for a short time during the reverse scan due to accumulation of anions at the oxidised sites until the reduction processes occur with de-intercalation of anions or incorporation of cations, associated with a small decrease of mass.

It may be noted that there is considerable dissipation of acoustic energy, due to the high viscosity of the eutectic media studied. The solvent molecules tend to accumulate on the unoxidised polymer substrate and thereby cause the polymer to swell. The species expected to be present are Ch^+, $Urea^+$, $EG_2\ Cl^-$, PG_2Cl^- and $UR_2{}^+Cl^-$, plus ClO_4^- either free or in bound form with the HBDs. The solvent will accumulate in such a way that electroneutrality will be maintained by cationic and anionic solvent species, without the need for solvent ion pair formation. In

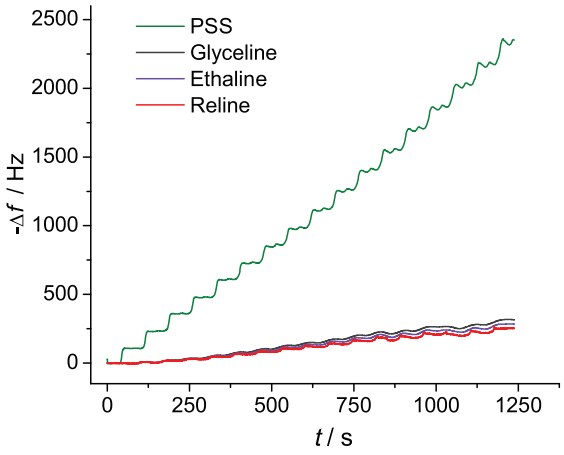


Fig. 2. EQCM profiles of frequency variation with time for electropolymerisation of 10 mM EDOT in the three DES (all with 4 M $HClO_4$) and in aqueous 0.1 M PSS. Potential cycling between -0.6 and +1.2 V vs Ag wire; scan rate 50 mV s^{-1}.

the case of reline, electroneutrality will be maintained by Ch^+, $Urea^+$, $UR_2{}^+Cl^-$, $ClO_4{}^-$ and $UR_2{}^+ClO_4{}^-$.

From Fig. 3a and 3b it can be observed that as the potential is scanned in a positive direction, upon oxidation of the polymer, an increase in mass is observed, which indicates anion injection and during the negative scan, the mass slowly increases instead of decreasing, which rules out anion ejection. Instead, the reductive scan is accompanied by cation injection, to maintain electrical neutrality, which is confirmed by an increase in mass. Cation injection could be of Ch^+, of $Urea^+$ or a combination of both, which of them not being clear from the slope. Further investigation is necessary to identify the predominant species incorporated.

In addition, as mentioned previously, the ionic solvent species present in the bulk medium, could also become trapped in the voids of the non-rigid polymer film. The incorporation of these solvent species will maintain electroneutrality by the entrapment of equivalent cationic solvent molecules. However, polymer swelling will retard further growth upon potential cycling, unlike in molecular solvents such as water, acetonitrile etc., by hindering electron transfer to the conducting substrate. Moreover, injection of the slow moving bulkier anion to the swelled polymer will also be retarded. This could be a plausible reason for the lower film growth in the case of PEDOT in eutectic media (~1 μg) compared to that in aqueous surfactant media (~11 μg). Incidentally, this has an advantageous effect since the thin, non-rigid and porous film shows better sensing characteristics. In Fig. 3 (a), during the 6th and 7th scan in the reverse cycle the mass remains nearly constant after cation injection, whereas in the 8th scan there is a gradual decrease in mass and on further cycling it remains constant. The decrease could be due to ejection of solvent molecules, rather than anion ejection, as there is no sharp decrease in the mass.

In the case of PEDOT prepared in aqueous PSS media, polymer growth is accompanied by PSS^- counteranion injection (oxidation) and expulsion (reduction), in tandem with the polymer redox process. This insertion-removal of anion continues until the rate of the electron transfer process decreases with increase in polymer thickness and distance to the substrate. The incorporation of solvent is less and hence there is negligible acoustic dissipation observed during electrochemical QCM studies. Thus, PEDOT prepared in aqueous PSS media is rigid as evident from Fig. 4a and considerably more polymer growth occurs (~11 μg) in comparison with that in eutectic media since the charge transfer path length is the only limiting factor.

The EQCM used in this investigation is able to measure both mass and energy dissipation properties of deposited films. For some polymerisations, the Sauerbrey relationship greatly underestimates the mass adsorbed on the QCM electrode, since the shear wave of the oscillating quartz is dampened. The frequency penetration depth depends on the material and is typically of the order of 250 nm in water [34] (rigid materials may strongly

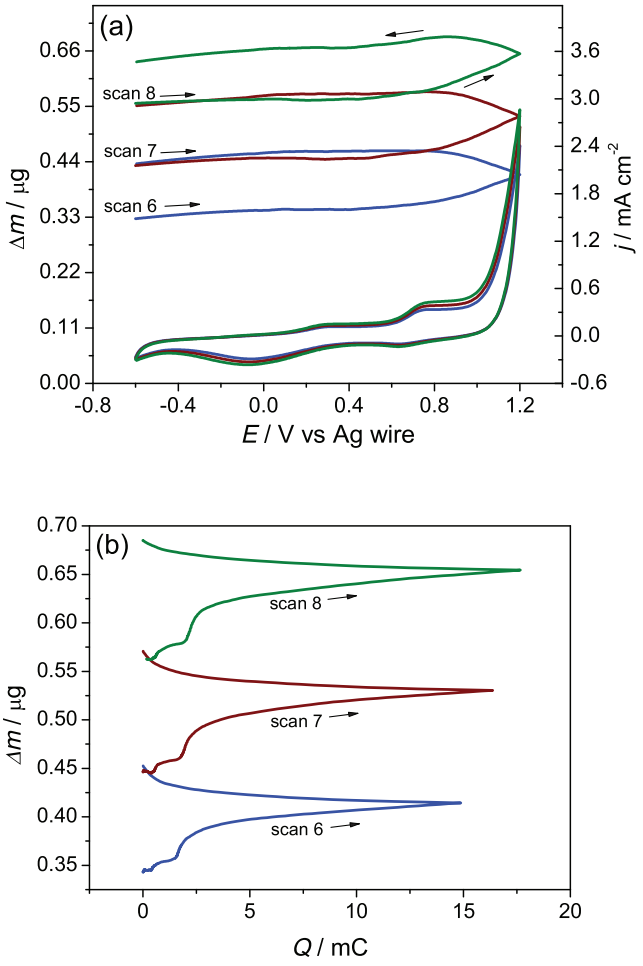


Fig. 3. (a):Variation of frequency and cyclic voltammogram during scans 6 to 8 of the electropolymerisation of 10 mM EDOT in Reline – 4 M $HClO_4$; scan rate 50 mV s^{-1}. (b) corresponding plot of mass change vs charge.

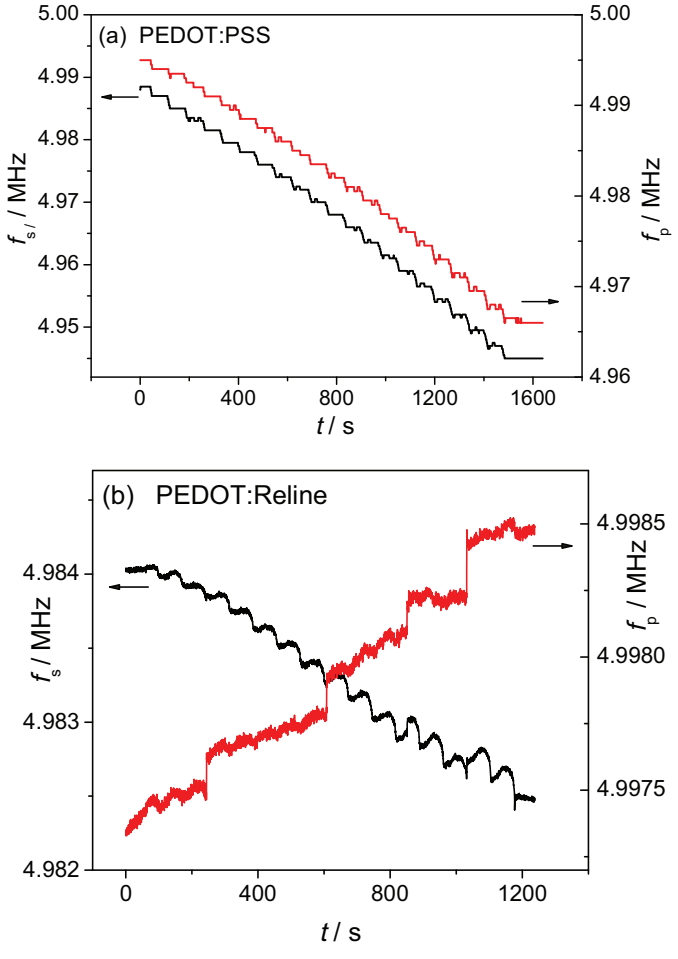


Fig. 4. EQCM response in terms of series-resonant frequency (f_s) and parallel-resonance frequency (f_p) with time for PEDOT deposition in (a) aqueous 0.1 M PSS and (b) Reline – 4 M $HClO_4$.

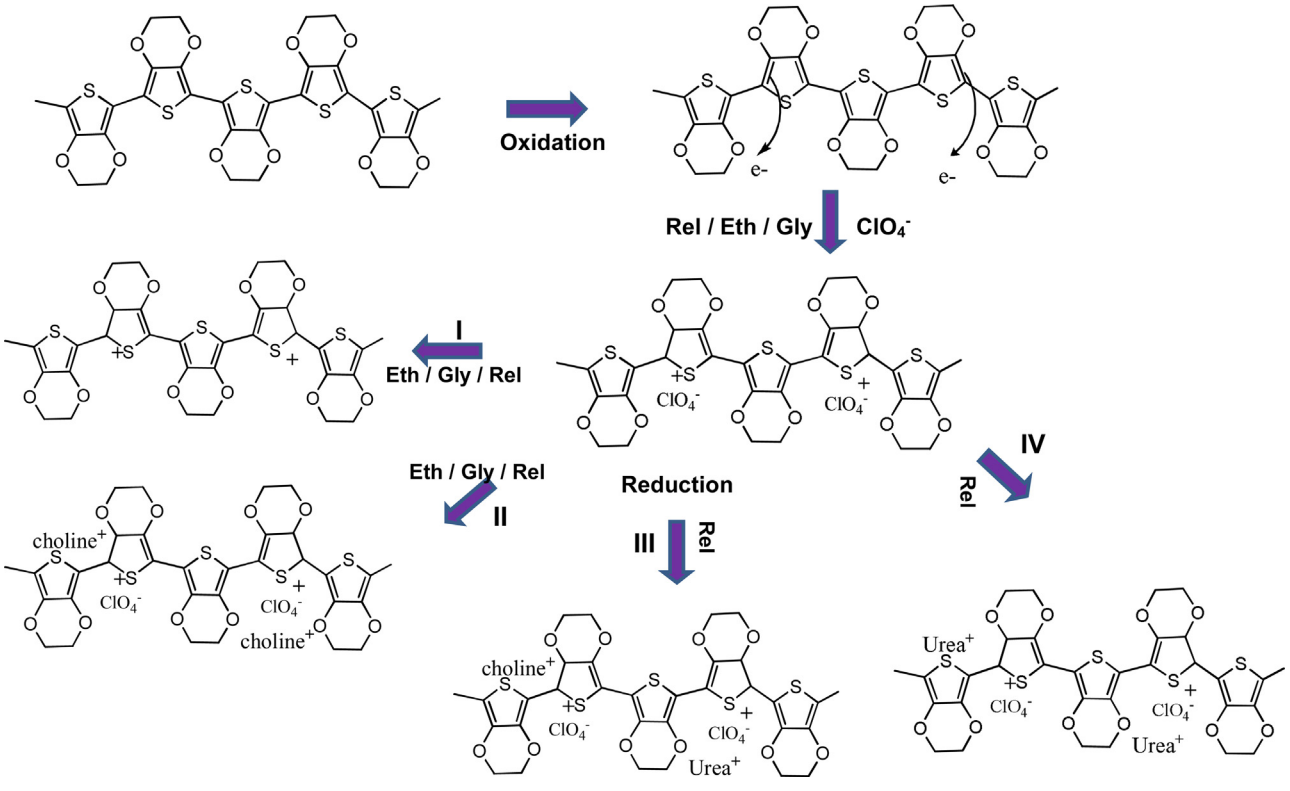
couple to the sensor surface and thus permit monitoring of thicker films, but viscoelastic materials will be limited to within this range). When the adsorbate is viscous and sufficiently soft that it does not follow the sensor oscillation perfectly, this leads to internal friction (due to the deformation) in the adlayer and thus to dissipation. Fig. 4a and 4b show the response in terms of series-resonant frequency (f_s) and parallel-resonance frequency (f_p). The advantage of scanning through the two resonant frequencies is that it is no longer necessary to cancel the parasitic capacitance in order to maintain oscillation and this also allows monitoring of the basic dissipation [35]. When f_s and f_p respond similarly, as can be observed in Fig. 4a for PEDOT:PSS, we can infer that a rigid film is formed. However, as observed in Fig. 4b for electropolymerisation in reline, when f_s and f_p respond differently the film is not rigid.

The dissimilarity of series and parallel resonance frequencies (f_s and f_p) curves obtained from the QCM studies is evidence for the formation of a thin and non-rigid film in DES medium. Hitherto, we reported the lower charge transferred during electrodeposition of PEDOT in DES (~22 mC cm^{-2}) compared to that in aqueous media (27 and 202 mC cm^{-2} respectively). This can be corroborated with the lower capacitance values obtained from the equivalent circuit fitting of the impedance data of the PEDOT modified electrodes prepared in DES compared with aqueous media [19]. Moreover, the previously reported scanning electron micrographs of the PEDOT modified electrodes from DES and aqueous media also showed subtle differences in their morphological characteristics, the former exhibiting non-rigid structures while the latter has a rigid aspect [19]. The above studies suggest a different mechanistic pathway for EDOT polymerisation in the $HClO_4$–eutectic system which needs to be further probed, the plausible pathways being explained below.

3.2. Doping and dedoping mechanism

Normally, polymer doping and dedoping occur during oxidation and reduction of the polymer, when anions are incorporated in the polymer matrix during oxidation with their expulsion occurring during the negative potential sweep. Evidence for the incorporation of cations into the PEDOT polymer matrix during dedoping instead of expulsion of anions to compensate charge has been reported [36]. The common anionic and cationic species present in reline, ethaline and glyceline are ClO_4^- anions and choline cations, respectively. Unlike ethaline/glyceline, reline-based eutectics contain urea cations in addition to choline cations. Hence, during electropolymerisation in ethaline or glyceline based eutectic in the presence of $HClO_4$, there are two possibilities (i) ClO_4^- species are incorporated during oxidation (doping) and expelled upon reduction (dedoping) (ii) ClO_4^- species are incorporated during doping and choline cations are incorporated during dedoping to compensate charge (Scheme 1).

In the case of reline there is the additional possibility of incorporation of urea cationic species as well as choline cations or, alternatively, there is a competition between them. The better redox characteristics during electrodeposition of PEDOT in reline – $HClO_4$ media and high sensitivity towards ascorbate oxidation (see below) demonstrates that the doping/dedoping mechanism is different in reline than in ethaline/glyceline. Moreover, previously we reported a small differences in colour and nature of the pre-polymer mixtures of EDOT and $HClO_4$ in ethaline/glyceline and reline [19]. These indicate that, presumably, in PEDOT (reline), urea cations are also incorporated, which leads to better-defined redox peaks compared to polymers prepared in ethaline or glyceline.



Scheme 1. Plausible pathways for doping/dedoping in ethaline, glyceline and reline based eutectics; (i) Pathways I and II applicable for ethaline/glyceline (ii) pathways I, II, III, IV applicable for reline.

3.3. Optimization studies based on ascorbate sensitivity

Initially, the most widely used and least viscous of the eutectic mixtures, i.e. ethaline, was selected for optimising sensing characteristics, using ascorbic acid as model analyte, such as concentration of $HClO_4$, concentration of EDOT, number of potential cycles and effect of temperature. The optimized conditions were adopted for the other eutectic mixtures and the sensing characteristics were compared with PEDOT prepared in ethaline-based eutectic solvents.

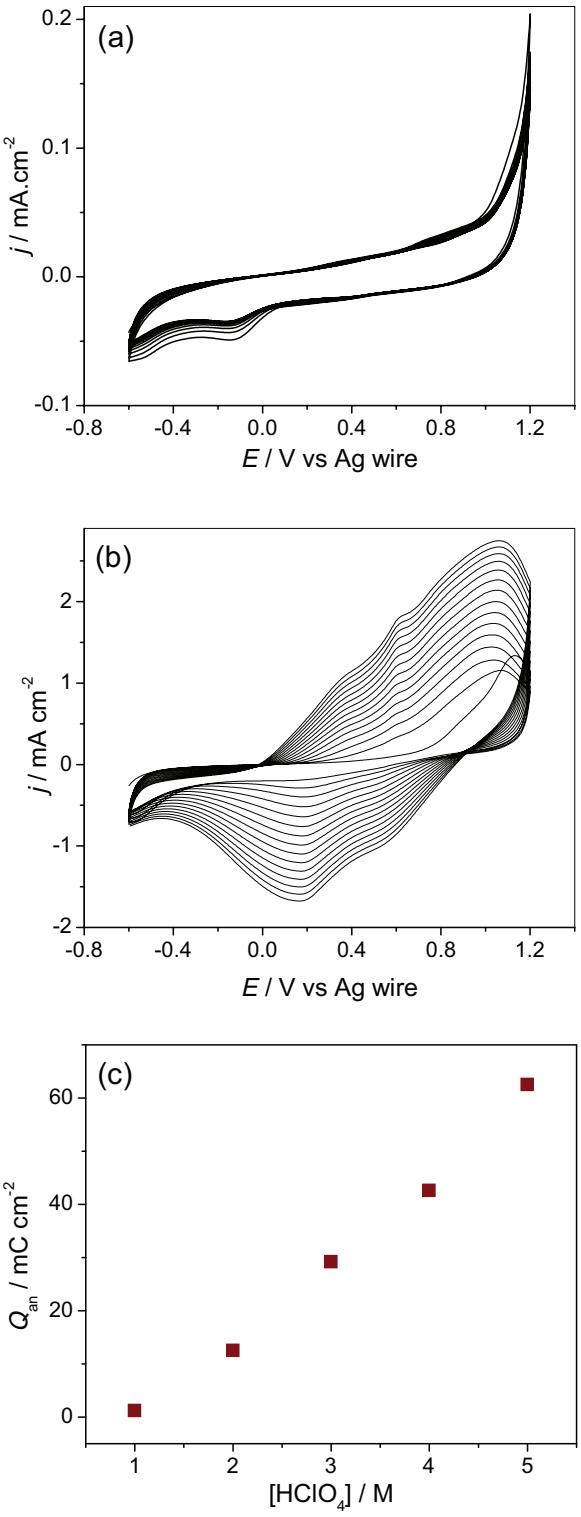


Fig. 5. Potentiodynamic electropolymerisation of 20 mM EDOT in the range -0.6 to 1.2 V for 15 cycles at 50 mVs^{-1} from ethaline eutectic containing (a) 1 M $HClO_4$ (b) 4 M $HClO_4$. (c) Plot of total anodic charge passed during electropolymerisation vs. concentration of $HClO_4$.

3.3.1. Influence of $HClO_4$ and EDOT concentration

Fig. 5a and 5b show typical voltammograms of electropolymerisation of 20 mM EDOT in ethaline in the presence of 1 M and 4 M $HClO_4$. Oxidation of monomers was not observed in the absence of $HClO_4$ (i.e. in pure ethaline medium), the lack of appearance of current peaks with consecutive cycles indicating no radical species in the vicinity of the electrode surface, attributable to the high viscosity. The rate of formation of radicals and subsequent polymerisation increased with addition of perchloric acid, varied from 1 up to 5 M. In the presence of 1 M $HClO_4$, radical formation begins at +1.0 V, and two small broad anodic peaks at +0.38 and +0.80 V appear, which increase slightly in height with each cycle, plus two small cathodic peaks around +0.23 and -0.12 V. On increasing the concentration of $HClO_4$, as seen in Fig. 5b, more radicals are formed with correspondingly higher anodic and cathodic currents although well-defined redox peaks were not observed. Fig. 5c shows a plot of anodic charge, Q_{an}, transferred vs concentration of perchloric acid, demonstrating that the rate of polymerisation increases with increase in perchloric acid concentration. The charge passed during electropolymerisation, Table 1, gives an indication of the quantity of polymer deposited on the electrode surface.

Previously, the greater electrocatalytic effect of PEDOT-modified GCE prepared in deep eutectic solvents towards ascorbate oxidation in aqueous media was reported [19]. An oxidation current peak was observed at 0.0 V using PEDOT (reline/ethaline)/GCE, while it occurred at more positive potentials at PEDOT prepared in other media. Table 1 shows the sensitivities to ascorbate oxidation at PEDOT modified electrodes prepared in media with varying concentrations of $HClO_4$. An increase of sensitivity with increase in $HClO_4$ concentration is observed up to 4 M, a further increase in concentration having only a slight effect. This may be due to excess polymer deposition at higher concentrations of $HClO_4$, as deduced from higher charge transferred during polymerisation, emphasizing that thin polymer films are usually superior in sensing studies due to superior conductivity and often as well as easier analyte access [37]. It can be inferred that $HClO_4$ plays an important role in effective polymerisation, so that 4 M $HClO_4$ was selected as the optimized concentration.

The effect of EDOT concentration on the formation of PEDOT and its sensing characteristics was also studied. On decreasing the concentration of EDOT from 20 mM to 10 mM, it was found that efficient polymerisation occurs although the current densities and charge passed were obviously lower (see Table 1). However, there was no significant difference in the sensing characteristics, so 10 mM EDOT was selected for further studies.

3.3.2. Comparison with PEDOT formation in aqueous solution

A PEDOT-modified GCE was prepared in aqueous 4 M $HClO_4$ to investigate whether the formation of PEDOT and the corresponding sensing characteristics were influenced only by $HClO_4$ or whether, and to what extent, using a DES also plays a role. In fact, the sensitivity towards ascorbate oxidation was much lower using PEDOT prepared in 4 M $HClO_4$ than that prepared in (ethaline – 4 M $HClO_4$) (See Table 1) and occurred at a higher overpotential (peak current at ~+0.2 V instead of 0.0 V vs Ag/AgCl) [19]. Thus, preparation of PEDOT-modified electrodes in DES is beneficial for ascorbate sensing properties. In order to investigate whether the formation of a thinner polymer film increases the sensitivity at

Table 1
EDOT polymerization conditions for optimization of analytical parameters for ascorbate sensing at 0.0 V vs Ag/AgCl.

Parameter varied	[EDOT]/mM	Eutectic solvent	[$HClO_4$]/M	Q_{an}/mC cm^{-2}	Sensitivity/μA $cm^{-1}mM^{-1}$	*LoD/μM
[$HClO_4$]	20	Ethaline	1	1.2	**22.0	8.5
	20	Ethaline	2	12.5	60.0	1.4
	20	Ethaline	3	29.2	107	2.6
	20	Ethaline	4	42.6	191	2.0
	20	Ethaline	5	62.5	216	1.5
[EDOT]	10	Ethaline	4	22.6	208	4.0
	20	Ethaline	4	42.6	191	2.0
Solvent	10	Ethaline	4	22.6	208	4.0
	10	– (H_2O)	4	202	90.8	7.0
	10	– (H_2O)	1	133	18.3	12.6
Eutectic solvent	10	Ethaline	4	22.6	208	4.0
	10	Reline	4	19.4	246	0.9
	10	Glyceline	4	12.6	40.1	2.8

* LoD (limit of detection), calculated as (3*SD)/sensitivity [40].
** +0.2 V vs Ag/AgCl (film unstable at 0.0 V) water.

PEDOT-modified GCE prepared in aqueous media, modified electrodes were also prepared in aqueous 1 M $HClO_4$ (Supporting information Fig S1) and in aqueous 0.1 M PSS. The sensitivity of ascorbate oxidation was significantly less in both cases (18.3 and 3.7 μA cm^{-2} mM^{-1} respectively).

3.3.3. Influence of temperature

From the above studies, it was clear that $HClO_4$ is essential for the effective formation of PEDOT polymer. Temperature plays an important role in increasing the rate of diffusion in viscous ionic liquids. Hence, the influence of temperature during preparation of the PEDOT modified GCE for sensing was analysed in order to examine whether a high concentration of $HClO_4$ during polymerisation can be avoided by increasing the temperature and the EDOT concentration.

Experiments were carried out at 60 °C in the presence of 20 mM EDOT and with 1 and 2 M $HClO_4$. CVs during polymerisation are shown in Figs S2 (a) and S2 (b) respectively. From the CVs, it can be seen that at 60 °C, the rate of electropolymerisation in ethaline with either 1 M or 2 M $HClO_4$ is higher than at room temperature. The sensitivity for ascorbate determination is also higher: PEDOT deposited in ethaline - 1 M $HClO_4$ at 25 °C and at 60 °C gives sensitivities of 22 and 130 μA cm^{-2} mM^{-1} (at 0.20 V) while they are 60 and 147 μA cm^{-2} mM^{-1} (both at 0.0 V) for ethaline - 2 M $HClO_4$. The lowering of ascorbate oxidation potential with increase in $HClO_4$ concentration clearly indicates that a minimum surface coverage of PEDOT is essential for obtaining the electrocatalytic effect. Evidence for this comes from the difference in current densities during the electropolymerisation of EDOT (ethaline) at 60 °C in the presence of 1 and 2 M $HClO_4$ (see Fig. S2). However, since these higher sensitivity values were less than those using the previously optimized ethaline – 4 M $HClO_4$ mixture at room temperature, there is not sufficient benefit in increasing the temperature so all further experiments were carried out at room temperature.

3.3.4. Effect of number of polymerisation cycles

The number of potential cycles influences the PEDOT film thickness and sensing characteristics, and was investigated using optimized conditions i.e. 10 mM EDOT and ethaline – 4 M $HClO_4$ electrolyte at room temperature. Thicker polymer film formation was evident from the higher charge densities for 30 cycles (34.8 mC cm^{-2}) (Fig. 1a) compared to 15 cycles (22.6 mC cm^{-2}). There is little influence on the sensitivity to ascorbate oxidation with the thicker film; in fact, there is a slight decrease (208 to 196 μA cm^{-2} mM^{-1}). Hence 15 potential cycles were used for film formation. This demonstrates that a thin and non-rigid film of PEDOT, as evident from the other characterisation techniques used i.e. EQCM, cyclic voltammetric/electrochemical impedance spectral analysis and SEM micrographs [19], is better suited for electrochemical sensing applications.

3.3.5. Comparison with PEDOT prepared in other deep eutectic solvents

The experimental parameters optimized in ethaline for PEDOT formation were utilized for the electrodeposition of PEDOT in reline and glyceline. The highest sensitivity for ascorbate was obtained using PEDOT (reline)/GCE, as well as the lowest detection limits, as seen in Table 1. Moreover, the currents remained unaltered at this modified electrode after 100 potential cycles in 0.1 M aqueous phosphate buffer saline solution, unlike with PEDOT prepared in the other eutectic media. This might be due to the more facile doping and dedoping processes occurring in PEDOT synthesized in reline–$HClO_4$, with the incorporation of urea cations, as discussed above.

3.4. Electroanalysis of AA, DA and UA

3.4.1. Electrocatalytic oxidation of AA, DA and UA

The excellent analytical sensing characteristics of PEDOT (reline)/GCE for ascorbate prompted further studies on the possibility of sequential or simultaneous determination of AA, DA and UA. Fig. 6a shows cyclic voltammograms of 1 mM AA, DA and UA at bare GCE while Fig. 6b shows CVs of 500, 50 and 100 μM AA, DA and UA respectively at PEDOT(reline)/GCE. Direct electron transfer of these species at bare electrodes is irreversible and requires high overpotentials; the oxidation peaks of AA, DA and UA appear at 0.48, 0.37 and 0.47 V, respectively, at bare GCE, which makes the resolution of individual components in a mixture practically impossible. In addition, due to sluggish electron transfer, the electrode surface is prone to passivation and fouling, resulting in poor selectivity and reproducibility [38].

Fig. 6b shows CVs of 500 μM AA, 50 μM DA and 100 μM UA, at PEDOT (reline)/GCE, where the peak potentials are at 0.0, 0.18 and 0.30 V, respectively. The modified electrode shows an excellent electrocatalytic effect with negative peak shifts of 480, 190 and 170 mV for AA, DA and UA respectively compared to the bare GCE, enabling well-resolved peak separation and sequential/simultaneous sensing of the three analytes in a mixture.

Cyclic voltammograms recorded at scan rates from 10 to 200 mV s^{-1} show a linear peak current *vs* square root of scan rate relationship for AA, DA and UA indicating diffusion-controlled electron transfer (not shown). The diffusion coefficient values

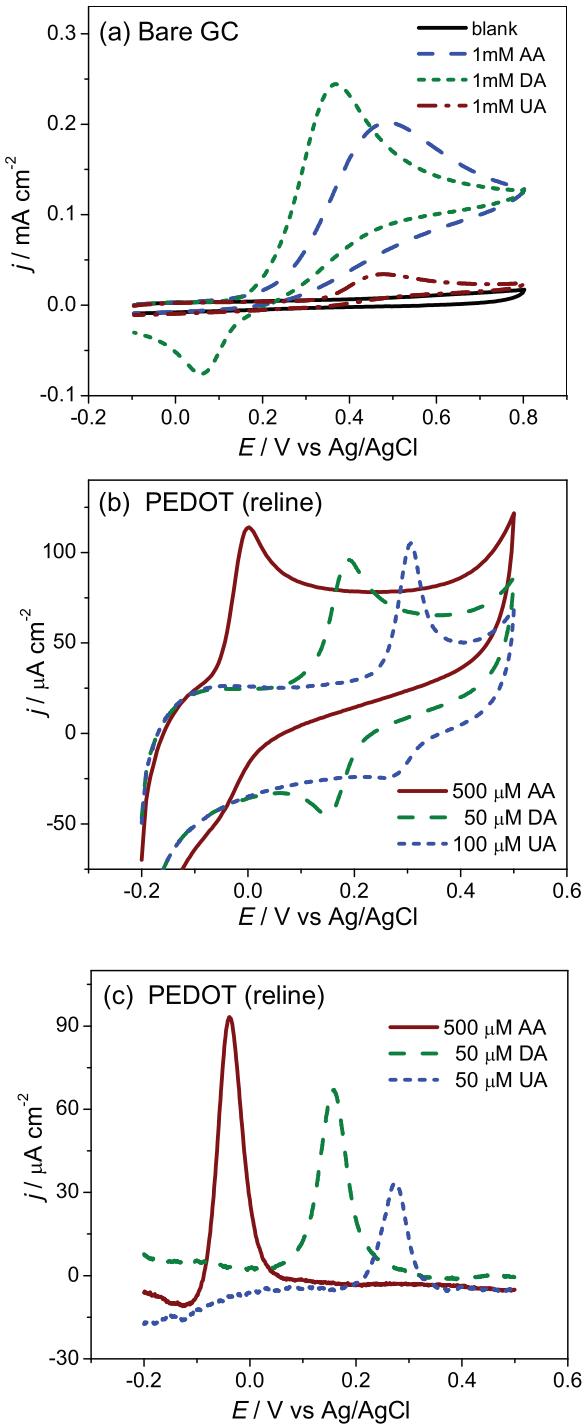


Fig. 6. (a,b) Cyclic voltammograms (scan rate 50 mV s^{-1}) of (a) 1 mM AA, DA and UA at bare GCE and (b) 500 μM AA, 50 μM DA and 100 μM UA at PEDOT/GCE (Reline – 4 M $HClO_4$) (c) Differential pulse voltammograms of 500 μM AA, 50 μM DA and 50 μM UA at PEDOT/GCE (reline – 4 M $HClO_4$). Electrolyte solution 0.1 M PBS (pH 7.0) buffer.

calculated for ascorbic acid, uric acid and dopamine are 4.46×10^{-6} cm^2 s^{-1}, 4.89×10^{-6} cm^2 s^{-1} and 6.42×10^{-6} cm^2 s^{-1} respectively.

The mechanisms of oxidation of AA, DA and UA have been widely studied e.g. [39]. All are two electron oxidation processes, of which ascorbic acid oxidation is irreversible ($2e^-$, $2H^+$) and with a complex mechanism, whereas those of dopamine and uric acid are reversible ($2e^-$, $2H^+$) under ideal conditions. However, it can be observed in Fig. 6a that the redox process of dopamine is quasi-reversible while those of ascorbic acid and uric acid are irreversible at bare GCE. On the contrary, at the PEDOT (reline) modified GCE, the redox process of dopamine is nearly reversible with a peak separation of 37 mV while that of uric acid is quasi-reversible, (Fig. 6b), indicating faster electron transfer.

Fig. 6c shows differential pulse voltammograms of 500 μM AA, 50 μM DA and 50 μM UA at PEDOT (reline)/GCE, where oxidation of AA, DA and UA occurs at -0.03, 0.16 and 0.28 V, respectively. The peak separations between AA-DA, DA-UA and AA-UA were 191, 120 and 311 mV by DPV, which are adequate for selective, simultaneous determination of these species. In addition, the peak currents were all greater than at the bare GCE.

3.4.2. Simultaneous determination of ascorbic acid, dopamine and uric acid

The studies above indicate that the oxidation peaks for AA, DA and UA are well separated for individual or simultaneous two/three component quantification using a sensitive and high resolution electroanalytical technique such as DPV or SWV. The individual determinations of AA, DA and UA in the presence of fixed concentrations of the other two species in 0.1 M phosphate buffered saline medium (pH 7.0) are given in supporting information Fig. S3. Sequential additions of one of the analytes have little influence on the peak currents of the other species, making the system highly selective. Calibration curves of each species are given in the insets.

Simultaneous determination with variation of the concentration of two or all three components was also carried out. For two-component concentration variation, the concentration of the third species was fixed. Results are shown in supporting information Fig. S4(i) – S4 (iii). Although the peak currents were slightly less than those for each component by itself, there was negligible effect on the peak currents of the other two components in the analyte solution. The average sensitivities obtained for the three components under these conditions were 86.0 ± 5.1 μA cm^{-2} mM^{-1}, 1.46 ± 0.13 and 0.54 ± 0.04 mA cm^{-2} mM^{-1} for AA, DA and UA, respectively (Table 2). There were no significant variations in the sensitivities of the three components compared to individual sensing.

Simultaneous three component variation studies are shown in Fig. 7a with calibration plots in Fig. 7b; sensitivities for triplicate measurements are given in Table 2. The dynamic linear concentration ranges for AA, DA and UA were found to be 50-1600, 5-180 and 5-180 μM with LODs of 57, 1.3 and 3.6 μM respectively. Besides the significant lowering of overpotential for AA, DA and UA oxidation at PEDOT-reline, higher sensitivities were obtained than at PEDOT-modified GCEs prepared in aqueous surfactant media [41]. Furthermore, it can be inferred from electrochemical QCM studies that the thinner (and non-rigid) polymer film formed on the electrode surface in eutectic solvents compared with aqueous media, facilitates easier mass and charge transfer, which possibly explains the better electrochemical performance of the PEDOT (reline) film modified electrode.

4. Conclusions

Procedures for the electrodeposition of thin PEDOT films on glassy carbon electrodes in deep eutectic solvents were developed, and a comparative evaluation of their sensing characteristics was carried out. PEDOT electrodeposited in urea (reline) based DES was found to be better in terms of sensitivity, selectivity, stability and reproducibility compared to that deposited in ethaline or glyceline based DES, using ascorbic acid as model analyte. The plausible reasons and mechanistic pathways for the better performance of

Table 2
Sensitivities for ascorbate, dopamine and uric acid in 0.1 M PBS solutions (pH 7.0) containing all three analytes at PEDOT/GCE (reline- 4 M $HClO_4$), n = 3, varying the concentration of one, two or all three analytes simultaneously. Data from Figs. S3 and S4.

Analyte concen-trations varied	Ascorbic acid($\mu A\ cm^{-2}\ mM^{-1}$)	Dopamine($mA\ cm^{-2}\ mM^{-1}$)	Uric acid($mA\ cm^{-2}\ mM^{-1}$)
One	89.2 ± 3.9	1.50 ± 0.08	0.55 ± 0.02
Two	87.5 ± 4.4	1.54 ± 0.10	0.53 ± 0.03
Three	86.0 ± 5.1	1.46 ± 0.13	0.54 ± 0.04

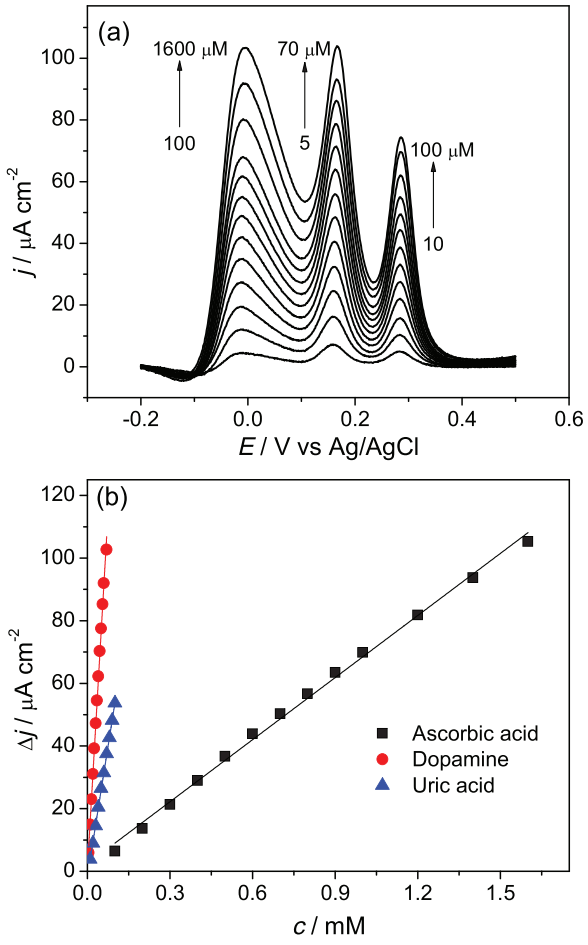


Fig. 7. (a) Simultaneous determination of AA, DA and UA by DPV in 0.1 M PBS (pH 7.0) buffer solutions containing 100 to 1600 μM AA; 5 to 70 μM DA and 10 to 100 μM UA (b) Corresponding calibration curves.

PEDOT synthesized in reline eutectics were discussed. The observations were substantiated by quartz crystal microbalance based dynamic gravimetric studies. Analytical application of the reline based PEDOT modified electrodes was carried out for the sequential/simultaneous sensing of ascorbic acid, dopamine and uric acid with good sensitivities and LODs. These studies show that the hydrogen bond donor plays a major role in determining the properties of the electrodeposited thin films and judicious selection of the better H-bond donor –acceptor combination can influence the size, shape and surface characteristics of polymer nanomaterials deposited from DES. Further work will focus on the extending the studies using other combinations of H-bond donor – acceptor systems and their possible applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.electacta.2015.11.092.

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Supporting information

Electrochemical characterisation of poly(3,4-ethylenedioxythiophene) film modified glassy carbon electrodes prepared in deep eutectic solvents for simultaneous sensing of biomarkers

Krishna P. Prathish, Ricardo C. Carvalho, Christopher M.A. Brett

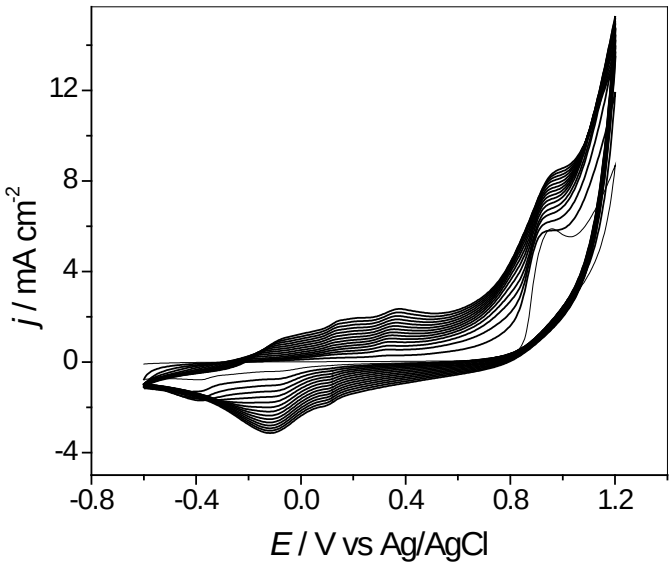

Fig. S1: Electropolymerisation of 10 mM EDOT in aqueous 1 M $HClO_4$ by potential cycling in the range -0.6 – 1.2 V at 50 mVs^{-1} for 15 cycles.

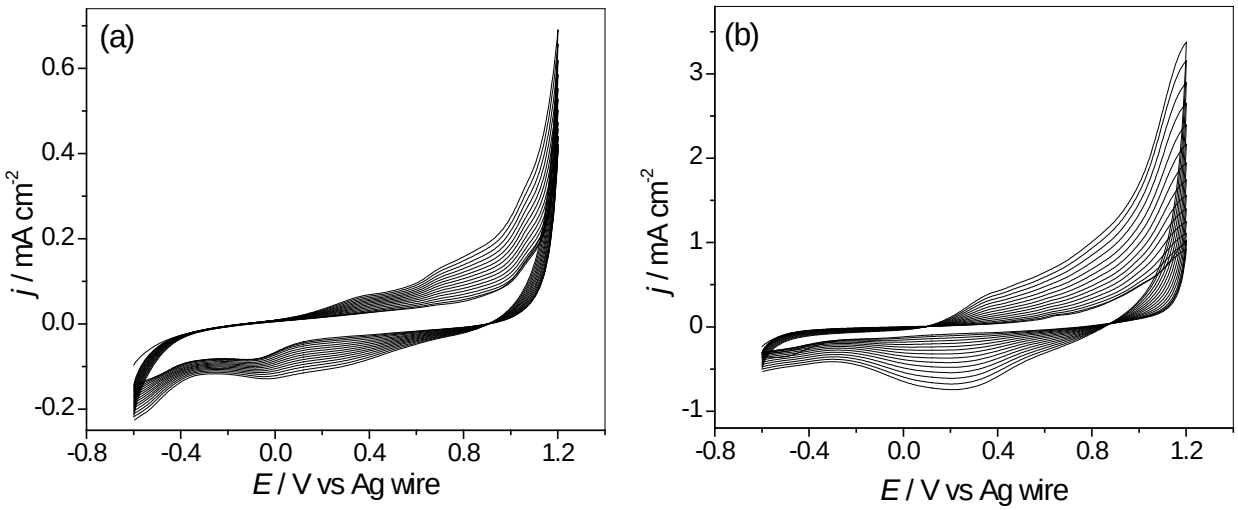

Fig. S2: Electropolymerisation of 10 mM EDOT at 60 ºC by potential cycling in the range -0.6 – 1.2 V at 50 mV s^{-1} for 15 cycles in (a) ethaline - 1M $HClO_4$ and (b) ethaline - 2M $HClO_4$.

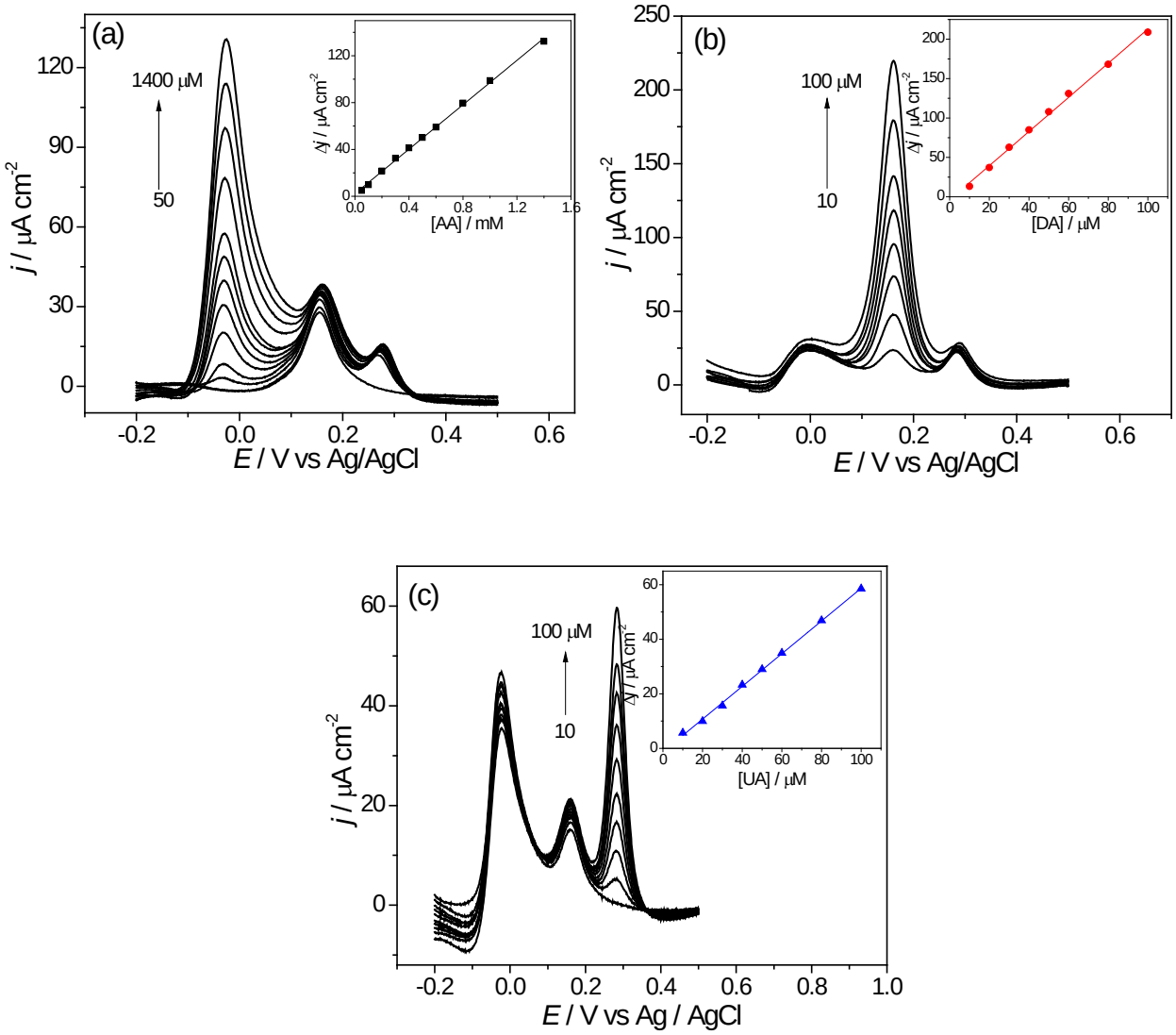

Fig. S3: Differential pulse voltammograms in 0.1M PBS (pH 7) buffer solutions of: (a) 50 to 1400 µM AA in the presence of 20 µM DA and 20 µM UA; (b) 10 to 100 µM DA in the presence of 500 µM AA and 30 µM UA; (c) 10 to 100 µM UA in the presence of 500 µM AA and 10 µM DA. Insets show calibration curves.

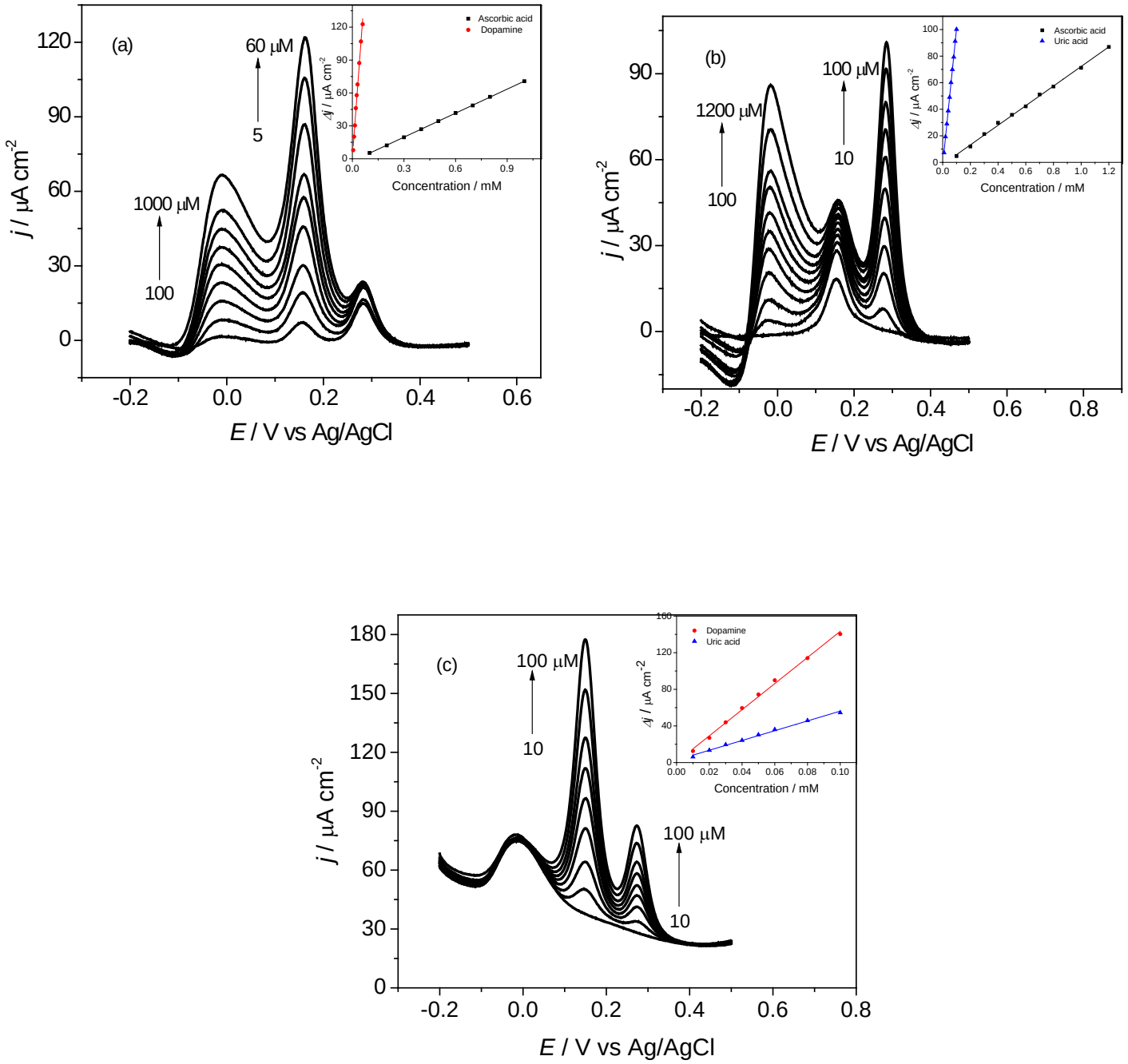

Fig. S4: Differential pulse voltammograms in 0.1 M PBS (pH 7.0) buffer solutions of: (a) 100 to 1000 µM AA and 5 to 60 µM DA in the presence of 20 µM UA; (b) 100 to 1200 µM AA and 10 to 100 µM UA in the presence of 10 µM DA (c) 10 to 100 µM DA and 10 to 100 µM UA in the presence of 500 µM AA. Insets show calibration curves.