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Selective determination of thiols: a novel electroanalytical approach

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The two electron electro-oxidation of *N*,*N*-dimethylphenylene-1,4-diamine and related compounds in aqueous solution leads to the formation of the corresponding 1,4-diimine which reacts with a range of thiol compounds. The resulting ring substituted (R–S–) diamine is further oxidised leading to an increase in the oxidative current which is proportional to the concentration of the thiol species. The electrode responses were found to be selective towards species including cysteine and homocysteine containing sulfhydryl groups (RSH) with no reaction observed with either methionine ($CH_3SCH_2CH(COOH)NH_2$) or cystine (cysteine–S–S–cysteine). The potential required to oxidise the ring substituted diamine is found to be dependent upon the nature of the thiol constituent and may allow some scope for speciation studies.

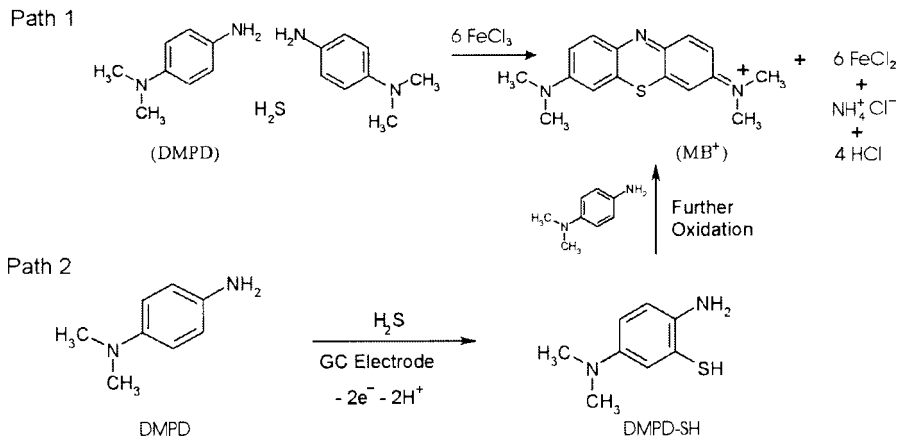
Introduction

The electrochemical properties of thiol species (RSH) has long held the interest of the analytical community with numerous applications particularly in biomedical applications[1] and surface modifications[2,3]. The use of electrochemical techniques for their detection, however is often severely hampered through a combination of electrode fouling and the poor voltammetric response of the target analyte at bare, unmodified electrodes. A number of strategies for improving the electrode response have been investigated and are typically based on redox species capable of catalysing the oxidation of the thiol moieties.[4–8] While these species can significantly improve the electrode response, the oxidation potential is often limited to that of the redox species irrespective of the thiol and thereby effectively rules out any potential for speciation.

One observation that may facilitate the enhanced detection of thiols whilst allowing some opportunities for potentially selective speciation is based upon a modification of the classic hydrogen sulfide–Methylene Blue reaction.[9] This typically involves the reaction of H_2S with the oxidised diamine in the presence of a suitable oxidant (typically ferric ion) resulting in the production of the heterocyclic thiazine dye, Methylene Blue.[10] The standard reaction is shown by Path 1 in Scheme 1. A recent modification to this system has involved the substitution of the chemical oxidant with a suitable electrode material (Path 2, Scheme 1). The oxidation current recorded at the electrode can enable electrochemical detection through its correlation with the concentration of sulfide.

Mechanistic evidence for the reaction of hydrogen sulfide with *N*,*N*-dimethylphenylenediamine (DMPD) under electrochemical control has been compiled[11] but the generic nature and applicability of the reaction has yet to be considered. Given that the sulfide reacts through nucleophilic attack it is possible to envisage species containing sulfhydryl thiol groups (RSH) reacting through an analogous mechanism and hence proffer a novel approach to the detection and speciation of thiols. This is of particular significance given the dearth of applicable electroanalytical techniques for this task. It is also evident that variation of the phenylenediamine component is possible thereby providing considerable experimental flexibility.

An apt demonstration of the electroanalytical promise held by this approach is provided through the electrochemically initiated reactions of DMPD with cysteine ($HSCH_2CH(COOH)NH_2$) and homocysteine ($HS(CH_2)_2CH(COOH)NH_2$). There is considerable interest in the speciation of these biologically relevant thiols[1] but their



Scheme 1

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close structural similarity is matched by equally similar voltammetric profiles in aqueous solution at bare, unmodified electrodes. The situation can often be further complicated through the use of mercaptoethanol ($HSCH_2CH_2OH$) as the reagent of choice for the release of bound homocysteine.

Experimental

All reagents were obtained from Aldrich and were of the highest grade available and used without further purification. All solutions and subsequent dilutions were prepared using deionised water from an Elgastat (Elga, UK) UHQ grade water system with a resistivity of 18 MΩ cm. Solutions were prepared by dissolving the appropriate analyte in pH 4 acetate buffer. In general, all the solutions were degassed and stored under argon with the DMPD being kept in the dark due to its photosensitivity. Stock thiol solutions (0.01 M) were prepared by dissolving the appropriate species in previously degassed buffer (25 mM) and were used within 1 h of preparation to minimise losses due to aerial oxidation. All experiments were conducted at a temperature of 21 ± 2 °C

The electrochemical measurements were recorded using an Autolab PGSTAT 30 computer controlled potentiostat (Eco-Chemie, Netherlands) with a standard three electrode configuration and a typical cell volume of 20 cm^3. Glassy carbon (GC, 0.0707 cm^2, BAS Technicol, UK) served as the working electrode, platinum wire wound into a spiral provided the counter electrode with a saturated calomel reference electrode (SCE, Radiometer, Copenhagen) completing the cell assembly. The glassy carbon electrode was polished between each set of experiments with diamond pastes (Kemet, UK) of decreasing particle size (6 to 0.1 μm).

Results and discussion

The cyclic voltammetric profile of 180 μM DMPD (10 mV s^{-1} in pH 4 acetate at a glassy carbon electrode) is shown in Fig. 1 (solid line) and highlights the electrochemically reversible nature of the system. Upon the introduction of 50 μM cysteine (dotted line) or 330 μM homocysteine (dashed line) into the solution, the magnitude of the oxidation peak is increased. The product resulting from the initial electrochemical oxidation of DMPD undergoes reaction with the appropriate thiol to form the corresponding ring substituted diamine derivative. This species is then subsequently re-oxidised at the electrode leading to the increase in the oxidation current. A probable reaction mechanism is detailed in Scheme 2.

This electrochemical process not only gives rise to an increase in the oxidative current but also results in a small shift in the peak position. The electrochemical properties of the resulting ring substituted diamine species will depend on the chemical nature of the attached thiol (electron withdrawing/releasing characteristics amongst other factors) and thus its oxidation potential may be significantly different from that of the parent DMPD. In both cases examined here, the thiol addition product leads to the production of species with higher oxidation potentials than DMPD, hence new peaks emerge at more anodic potentials. Confirmation of the reaction between the electrogenerated $DMPD^+$ and the thiol is provided through examination of the reverse voltammetric sweep. The height of the reduction peak attributed to DMPD (+0.22 V) is markedly reduced with the appearance of a subtle shoulder attributed to the new redox processes observed at +0.32 and +0.38 V. These correspond to the reduction of the cysteine–DMPD and homocysteine–DMPD derivatives respectively and are suggestive of an ECE type reaction. The responses of 50 μM cysteine and 300 μM homocysteine at the electrode in the absence of DMPD are also shown in Fig. 1 (solid lines) and exhibit little response within the given potential window. The response to a number of structurally different thiols was investigated and the electrochemical details compared in Table 1. There is no reaction to either methionine or cystine and confirms the requirement for a free sulfhydryl group (RSH).

Exploitation of the electrode behaviour observed in the presence of homocysteine for analytical purposes has been assessed at a relatively simple level through relating the oxidation peak height with homocysteine concentration. This was found to give linear responses over the range 38 to 170 μM homocysteine (*i.e.* $I_{p_{ox}} = 3.77 \times 10^{-9}$ [homocysteine (μM)] + 1.34×10^{-6}, $N = 25$, $R^2 = 0.996$, standard error = 9.29×10^{-9}). Whilst the reaction is relatively specific to thiols possessing a labile proton, differentiation of thiol mixtures will depend on the voltammetric profile of each species and matrix

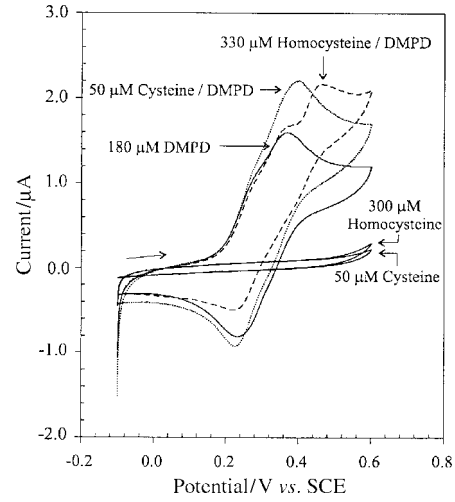
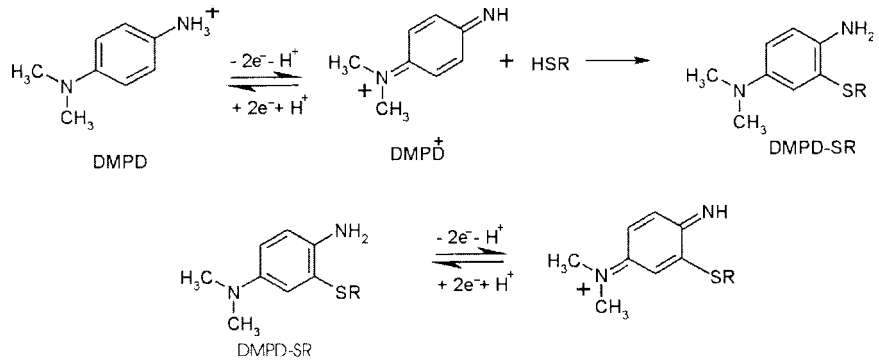


Fig. 1 Cyclic voltammograms detailing the electrode response to 180 μM DMPD (solid line) and in the presence of 50 μM cysteine (dotted line) and 330 μM homocysteine (dashed line). The electrode response to these amino acids in the absence of DMPD is included for comparison (dot–dash line). Scans recorded in pH 4 acetate buffer at 10 mV s^{-1} using a 3 mm glassy carbon electrode.



Scheme 2

composition. The extent to which adequate resolution can be obtained remains speculative though a more comprehensive assessment is beyond the scope of this initial communication. Whilst the analysis of thiol mixtures has not been investigated, the potential for speciation is however demonstrated in Fig. 1 for cysteine and homocysteine ($E_{p_{homocys}} - E_{p_{cys}} = 50$ mV). The system would undoubtedly require further optimisation for the analysis of mixtures in a given matrix but it is likely that increased resolution of the voltammetric peaks could be achieved through the application of pulse techniques such as square wave voltammetry.

The scope of the reaction can be substantially broadened through exchange of DMPD for another electro-oxidisable amine. This need not be restricted to diamines with the reaction found to operate with aromatic systems containing a single amino group. Diphenylamine was initially examined as an alternative system, however it was observed that fouling of the electrode through the formation of oligomers becomes problematic in the absence of a 'blocking' substituent *para* to the nitrogen. A more appropriate reagent was diphenylamine-4-sulfonic acid (DPS) as the possession of a functional group in the 4-position serves to reduce the effects of such fouling. The electrode response of 1 mM diphenylamine-4-sulfonic acid (1 mM, pH 4 acetate buffer) is shown in Fig. 2. This reagent contains a single oxidisable amine group that demonstrates irreversible behaviour. An oxidation peak is shown to occur at +0.58 V and the corresponding reduction peak is observable at +0.39 V. These processes can be tentatively attributed to oligomeric species arising from the oxidation of the monomeric DPS. The potential range has been narrowed to the onset of oxidation rather than proceeding past the peak, as in the case of DMPD, in order to minimise fouling and enable the collection of reproducible voltammograms.

The irreversible nature of such systems often precludes the use of the oxidation peak current as an indicator for thiol concentration. However, the reaction of the oxidised form of DPS with the thiol species can still be utilised in an analytical context through monitoring the diminution in the height of the voltammetric peak associated with the reduction of DPS. The reaction of oxidised DPS with the thiol compound means that on the reverse scan there is less DPS available to reduce and hence the magnitude of the reduction peak is correspondingly smaller. This is demonstrated through the sequential addition of aliquots of homocysteine (50 μL, 0.01 M) detailed in Fig. 2. The relation between the height of the reduction peak and added homocysteine was also found to give a linear response over the range 25 to 125 μM homocysteine ($I_{p_{ox}} = 1.65 \times 10^{-9}$ [homocysteine (μM)] $- 1.2 \times 10^{-6}$, $N = 5$, $R^2 = 0.992$, standard error $= 7.98 \times 10^{-9}$). While this method is undoubtedly less attractive than measuring the oxidation peak height it may prove valuable in instances where matrix effects interfere with the latter (*e.g.* where other electroactive species are present).

Table 1 Electrochemical parameters of the reaction of electrogenerated $DMPD^+$ with various thiol compounds

Thiol	Structure	$E_{p_{ox}}{}^a$	$E_{p_{red}}{}^a$
DMPD	$NH_2C_6H_4N(CH_3)_2$	+0.36 V	+0.24 V
Hydrogen sulfide	H_2S	+0.36 V	+0.12 V
Cysteine	$HSCH_2CH(COOH)NH_2$	+0.41 V	+0.33 V
Homocysteine	$HS(CH_2)_2CH(COOH)NH_2$	+0.46 V	+0.38 V
2-Mercaptoethanol	$HSCH_2CH_2OH$	+0.44 V	+0.35 V
2-Mercaptopyridine	HSC_5H_4N	+0.36 V	+0.16 V
Methionine	$CH_3SCH_2CH(COOH)NH_2$	NR[b]	NR[b]
Cystine	cysteine–S–S–Cysteine	NR[b]	NR[b]

[a] *vs.* saturated calomel electrode. [b] NR ≡ no reaction.

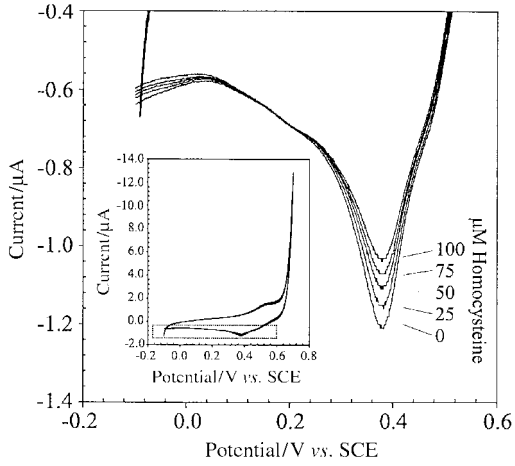


Fig. 2 Cyclic voltammograms detailing the electrode response to 1 mM diphenylamine-4-sulfonic acid (1 mM pH 4 acetate buffer, 100 mV s^{-1}) with increasing aliquots of homocysteine (50 μL, 0.01 M).

Conclusions

The reactions described above highlight the facile nature by which reduced thiols can be introduced into aromatic systems with the ability to simultaneously initiate and monitor the reaction using the same electrochemical assembly a major advantage. A brief exploration of the analytical applicability of the approach for the detection of biologically relevant thiols has been reported and routes for the optimisation of the approach highlighted.

Acknowledgement

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