Plumbagin: a natural product for smart materials?†

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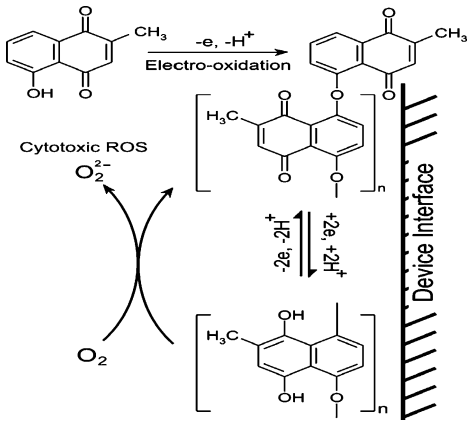
The exploitation of the natural product plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) to produce polymer films capable of serving as controllable source of reactive oxygen species is assessed. The efficacy of the film to catalyse the reduction of oxygen to yield reactive oxygen species has been evaluated through using ascorbate and glutathione redox probes.

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) has been of widespread interest to the medical community in recent years and has long been an integral part of traditional Siddha and Ayurveda practices[1–3] which, although native to the Indian Subcontinent, have been adopted in many complementary health treatments. It has been shown to engage in a multitude of biochemical roles and possesses cytotoxic properties that can be significantly deleterious to normal cellular function. It has however been suggested that if appropriately targeted it could offer a range of therapeutic benefits as it has been shown to modulate cellular proliferation/carcinogenesis[3–5] and possesses antimicrobial properties.[6–8] The primary mode of action in most cases has been ascribed to its ability to redox cycle—the generation of the semiquinone radical resulting in the production of reactive oxygen species (ROS) which subsequently attacks the cellular machinery and ultimately initiates apoptosis.[3,5] The present investigation has sought to examine the possibility of exploiting plumbagin as the basis of a smart material—whereby the electrochemical modulation of a film of the naphthoquinone serves as a controllable source of ROS.

Plumbagin has two core functionalities—the quinone redox centre responsible for the production of ROS and a phenolic group which could allow electropolymerisation and facilitates the production of a thin redox film.[9,10] Electrochemical oxidation of the phenol should result in the production of a radical cation whose head-to-tail coupling—in line with conventional phenol based electropolymerisation—should lead to the deposition of a thin film directly at the electrode surface.[9,10] It would be hoped that the quinone component of the compound would be retained and remain accessible to electrochemical cycling and that upon the imposition of a reducing potential the hydroxyl form should be generated which—in the presence of oxygen—should result in the production of ROS (superoxide anion radical and ultimately peroxide) (Scheme 1).[3] The redox cycling capability of plumbagin and its ability to form an electropolymerised film capable of generating ROS is considered.



Scheme 1 Proposed reaction mechanism.

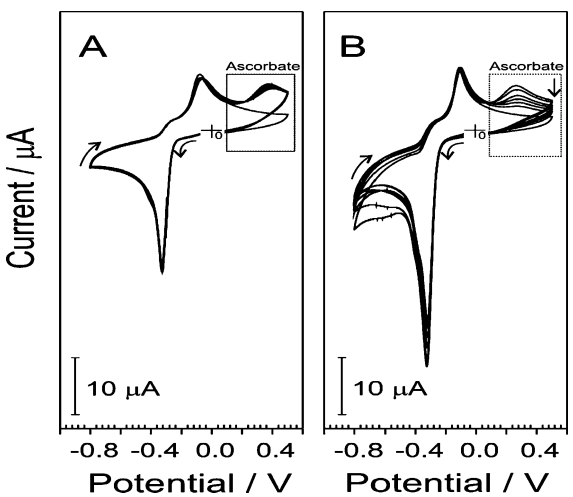


Fig. 1 Cyclic voltammograms detailing the response towards plumbagin (0.8 mM) and ascorbate (0.4 mM) in pH 7 buffer in the absence (A) and presence (B) of oxygen.

Cyclic voltammograms detailing the response of plumbagin (0.8 mM, pH 7) at a glassy carbon electrode in the presence and absence of oxygen are detailed in Fig. 1. The quinone is reduced at −0.28 V with the corresponding oxidation process observed at −0.03 V. In the presence of oxygen, the magnitude of the reduction peak is significantly increased and can be attributed to the catalytic reduction of oxygen by the electro-reduced quinone. Confirmation of the latter was obtained by degassing the solution with nitrogen, where the peak height was found to be significantly decreased.

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The system is clearly capable of catalysing the reduction of oxygen but its effectiveness as an interfacial ROS generator needed to be determined. A novel evaluation strategy was designed in which the interfacial concentration of an anti-oxidant probe would be used to estimate the production of ROS. Ascorbic acid served as our model probe—its free-radical scavenging properties are well established and serves as one of the front line cellular defences against ROS and hence its relevance to the present investigation. It also possesses the key advantage of having a well defined redox signature which is sufficiently distinct from that of plumbagin to allow its unambiguous quantification. It was our hypothesis that the ROS generated as a consequence of the electro-chemically induced plumbagin redox cycling would react with the ascorbate (converting it to the electrochemically invisible dehydroascorbate) and thereby leading to a visible decrease in ascorbate. The advantage of this approach was that it would directly reflect the production of ROS at the electrode interface.

Five repetitive scan cyclic voltammograms detailing the response observed at a glassy carbon electrode to plumbagin (0.8 mM) and ascorbate (0.4 mM) in pH 7 buffer under nitrogen flow are highlighted in Fig. 1A. Three distinct processes can be observed—the redox cycling of the quinone component of plumbagin (at −0.28 V and −0.03 V respectively) and the irreversible oxidation of the ascorbate at +0.37 V. The scans can be seen to be effectively stable but there is a gradual decrease in the magnitude of the ascorbate—this can be attributed to the irreversible oxidation leading to a small but cumulative depletion of ascorbate at the electrode, the scan rate being too fast to allow complete diffusional replenishment of the interfacial concentration between cycles. The experiment was repeated in the presence of oxygen and the corresponding voltammograms are detailed in Fig. 1B. It can be seen that the magnitude of the quinone reduction peak process increases markedly while the ascorbate peak diminishes much more rapidly than that observed under the degassed conditions. Given near identical conditions, bar the presence of oxygen, it is possible to draw the conclusion that the electro-generation of ROS species at the interface results in the marked depletion of the ascorbate probe. There is a depletion under degassed conditions but this can be ascribed to the diffusional artefact noted earlier and results in a 10% decrease over the experiment lifetime. In contrast, in the presence of oxygen the interfacial ascorbate is reduced by 70% and indicates clearly the efficacy of plumbagin redox cycling as a means of generating ROS.

Plumbagin film formation at the carbon substrate was attempted through the direct electro-oxidation of the 5-hydroxy functionality. Poly-plumbagin films have been used in a number of biosensing applications[9] as an entrapment matrix. While a number of oxidation products can arise, it was anticipated that in this instance, electro-oxidation would lead to a predominantly non-conducting polyphenylene oxide film type formation consistent with conventional phenolic electro-oxidation.[10] Cyclic voltammograms detailing the response at a glassy carbon electrode are shown in Fig. 2. The irreversible oxidation of the phenol was observed at +0.95 V and was found to decrease on successive scanning which is again consistent with the formation of a phenol film.

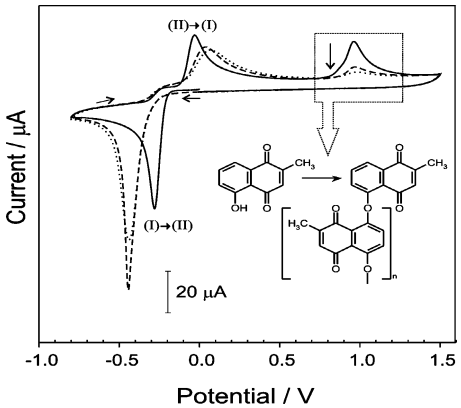

Fig. 2 Repetitive scan cyclic voltammograms detailing the electro-polymerisation of plumbagin (0.8 mM, pH 7).

The profile of the quinone component changed markedly with the scan number. On the first scan—prior to phenol oxidation—the reduction and oxidation processes are consistent with the standard solution based monomer. After oxidation of the phenol had occurred the subsequent scans show a shift in the quinone reduction peak to more negative potentials, along with an increase in the magnitude and the sharpness of this peak. This can be rationalised on the basis of the formation of a surface immobilised redox species—consistent with our main aim.

Removal of the modified electrode and placement within fresh buffer—devoid of plumbagin monomer—revealed that the activity of the quinone component was retained at the electrode surface. Cyclic voltammograms detailing the electrode response are shown in Fig. 3A. The shapes of the redox processes are broad and stand in contrast to the sharp processes observed in the initial polymerisation stages (Fig. 2). This may reflect a more heterogeneous population of different redox species. The magnitude of the peak processes was found to be markedly smaller than expected and this is ascribed to formation and subsequent loss of oligomeric material during polymerisation and transfer. Thus, while sharp surface redox processes were observed in Fig. 2, these are now more likely to reflect particulate/oligomeric structure which are only loosely adhered to the electrode surface. The significance of the latter was corroborated through conducting EQ CM studies (Fig. 3B). The polymerisation process was repeated using a gold patterned crystal (deposited from 10 mM plumbagin dissolved in ethylacetate containing 0.05 M tetrabutyl-ammonium perchlorate as supporting electrolyte, 50 scans, −0.8 V to +1.5 V). A non-aqueous solvent was used in this instance to increase the concentration of monomer and enable the deposition of a thicker and a potentially more active film. Upon removing the crystal and placing in fresh pH 7 buffer devoid of the monomer, it can be seen that there is a gradual loss of material from the electrode surface (expressed as ΔFreq, 0–5 min) ascribed to the oligomers slowly diffusing into the solution.

The stability of the film was briefly assessed through investigating the reaction with common biological agents. An interesting observation was found when glutathione (385 μM) was added to the solution. The latter is liable to

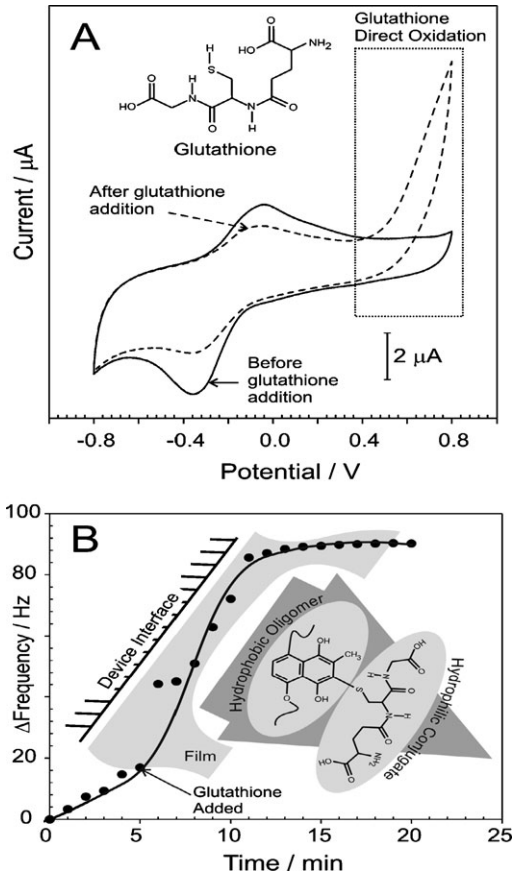

Fig. 3 (A) Response of a plumbagin modified electrode towards glutathione (0.3 mM). (B) EQ CM response of a Au-plumbagin modified electrode towards glutathione (0.385 mM). Data recorded in pH 7 buffer.

present within biological fluids and known to react with quinones to form water soluble conjugates (indicated in the inset schematic within Fig. 3B)[11,12] and a similar effect was found in this instance with a dramatic increase in the rate of oligomer removal before attaining a stable plateau. This is again corroborated in Fig. 3A where the polymer formed at the glassy carbon electrode is shown to degrade in the presence of glutathione (330 μM, pH 7). There is however an underlying layer of insoluble polymer—as the redox processes attributed to the poly-plumbagin are not removed completely, however, the reduction in the population of the quinone redox cycler will inevitably compromise the effectiveness of the films ability to generate ROS. Cyclic voltammograms detailing the response of the plumbagin modified glassy carbon electrode in buffer solution in the presence and absence of oxygen were recorded (not shown). The magnitude of the quinone reduction peak was significantly larger in the scan carried out in oxygen than in the absence of oxygen and was consistent with the electrochemistry seen for the monomer solution. This highlights that the quinone component of the molecule is retained and is accessible within the film enabling it to catalyse the reduction of oxygen. ROS are known to react with glutathione causing a decrease in the concentration. The poly-plumbagin film's ability to produce ROS was examined further with a spectrophotometric technique using Ellmans reagent to monitor the changes in glutathione concentration due to the presence of the ROS produced by the film. The modified electrodes were placed into a solution containing glutathione (167 μM) and held at a reduction potential (−1.2 V) for 5 and 10 minutes. An aliquot of the electrolysis solution was reacted with a solution of Ellmans reagent allowing the glutathione concentration to be determined spectrophotometrically *via* a calibration graph. The glutathione concentration was seen to decrease by 56.9% and 88.9%, respectively, compared to the no change in concentration for the control solution (modified electrode placed in glutathione solution for 10 minutes with no reduction potential applied); this confirms that the poly-plumbagin film is able to catalyse the production of ROS.

The ability of plumbagin monomer and polymer to generate reactive oxygen species at the electrode interface has been demonstrated and has the potential to be used as a smart material in devices opening up a new avenue for exploration and adaptation of other quinone systems.

Experimental details

Electrochemical measurements were conducted using a μAutolab computer controlled potentiostat (Eco-Chemie, Utrecht, The Netherlands) using a three electrode configuration consisting of either a glassy carbon working electrode (3 mm diameter, BAS Technicol, UK), a platinum wire counter electrode and a 3 M NaCl Ag|AgCl half cell reference electrode (BAS Technicol, UK). The scan rate in all cases was 50 mV s^{-1}. All measurements were conducted at 22 °C ± 2 °C in Britton Robinson buffer (pH 7) unless stated otherwise. Electrochemical quartz crystal microbalance (EQ CM, Maxtek INC, USA) studies were obtained using polished 5 MHz titanium/gold crystals (Maxtek INC, USA).

References

1 J. C. Tilak, S. Adhikari and T. P. A. Devasagayam, *Redox Rep.*, 2004, **9**, 219.
2 S. Sowmyalakshmi, M. Nur-e-Alam, M. A. Akbarsha, S. Thirugnanam, J. Rohr and D. Chendil, *Planta*, 2005, **220**, 910.
3 V. SivaKumar, R. Prakash, M. R. Murali, H. Devaraj and S. N. Devaraj, *Drug Chem. Toxicol.*, 2005, **28**, 499.
4 Y. L. Hsu, C. Y. Cho, P. L. Kuo, Y. T. Huang and C. C. Lin, *J. Pharmacol. Exp. Ther.*, 2006, **318**, 484.
5 L. C. Lin, L. L. Yang and C. J. Chou, *Phytochemistry*, 2003, **62**, 619.
6 B. S. Park, H. K. Lee, S. E. Lee, X. L. Piao, G. R. Takeoka, R. Y. Wong and Y. J. Ahn, *J. Ethnopharmacol.*, 2006, **105**, 255.
7 J. W. Chen, C. M. Sun, W. L. Sheng, Y. C. Wang and W. J. Syu, *J. Bacteriol.*, 2006, **188**, 456.
8 J. Q. Gu, T. N. Graf, D. H. Lee, H. B. Chai, Q. W. Mi, L. B. S. Kardono, F. M. Setyowati, R. Ismail, S. Riswan, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, S. M. Swanson, D. J. Kroll, J. O. Falkinham, M. E. Wall, M. C. Wani, A. D. Kinghorn and N. H. Oberlies, *J. Nat. Prod.*, 2004, **67**, 1156.
9 J. Haccoun, B. Piro, L. D. Tran, L. A. Dang and M. C. Pham, *Biosens. Bioelectron.*, 2004, **19**, 1325.
10 M. Ferreira, H. Varela, R. M. Torresi and G. Tremiliosi, *Electrochim. Acta*, 2006, **52**, 434.
11 I. A. Solsona, R. B. Smith, C. Livingstone and J. Davis, *J. Colloid Interface Sci.*, 2006, **302**, 698.
12 P. C. White, N. S. Lawrence, J. Davis and R. G. Compton, *Electroanalysis*, 2002, **14**, 89.