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Electroanalytical properties of chlorophenol red at disposable carbon electrodes: Implications for *Escherichia coli* detection

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ABSTRACT

The use of coliforms and *Escherichia coli* as indicator species for assessing the quality of water is well established and a large variety of methods based on β-galactosidase (B-GAL) activity, inherent to the microbes within this classification, have arisen to enable their detection and enumeration. Chlorophenol red (CPR) is widely used as a chromogenic label, but its capacity for translation to electroanalytical devices has yet to be fully explored. The CPR moiety is capable of undergoing oxidation at carbon substrates (+0.7 V) giving rise to a variety of phenolic intermediates. Electrochemical, XPS and enzymatic techniques were employed to characterise the underpinning chemistry and the intermediate identified as a 1,2-quinone derivative in which the chlorine substituent is retained. The latter was found to accumulate at the electrode and, in contrast to the parent CPR, was found to be detected at a significantly less positive potential (+0.3 V). Bacterial hydrolysis of a CPR labelled substrate was demonstrated with the 1,2-quinone oxidation product found to accumulate at the electrode and detected using square wave voltammetry. Proof of concept for the efficacy of the alternative electrode pathway was established through the detection of *E.coli* after an incubation time of 2.5 h with no interference from the labelled substrates.

1. Introduction

Electroanalytical techniques which target the detection of molecular biomarkers have a long history and there have been many technological advances in past decades [1–6]. It is only recently however that attention has shifted from small molecular targets to larger moieties such as bacteria [7–10]. The β-galactosidase (B-GAL) enzyme common to most coliforms offers a versatile route through which small molecule detection can be still be harnessed to detect the presence of these larger targets. The B-GAL enzyme, which is encoded by the *lacZ* gene, catalyzes the hydrolysis of β-galactosides (such as lactose) into monosaccharides (such as glucose and galactose) and has been the subject of extensive investigations with a history dating back to the mid 1900's. Monitoring the enzyme activity has enabled insights into the genetic regulation of enteric bacteria [11,12] and has since found use in a host of applications [13]. The prevalence of B-GAL in coliforms and *E. coli*, however, has seen the enzyme's significance move from fundamental science to the development of low cost field diagnostics for water quality measurements in low and middle income countries. Coliforms are used as the principal indicator organisms that highlight faecal contamination of water sources and, at present, B-GAL forms the foundation of a diverse range of assay systems [14–17]. Bigham et al. (2019) recently demonstrated the potential of voltammetric approach with a carbon fibre system to quantify pH for microbial coliform water quality assessment [18]. Irrespective of the assay format, the underpinning methodology relies upon the ability of the enzyme to hydrolyse labelled galactopyranoside substrates yielding a colorimetric, fluorescent, luminescent or electrochemical marker [19]. A large variety of molecular species have been used as markers to enable quantification but chlorophenol red (CPR) has emerged as a more convenient and highly sensitive substrate from both spectroscopic [13] and electrochemical perspective [20] as indicated in Scheme 1.

Electrochemically, CPR has been shown to be capable of direct oxidation at carbon electrodes and Wutor and colleagues (2007) demonstrated detection limits as low as 1 colony-forming unit (CFU)/100 mL [20]. This is widely regarded as the threshold for microbial drinking water quality required by the Sustainable Developments Goals (SDGs),

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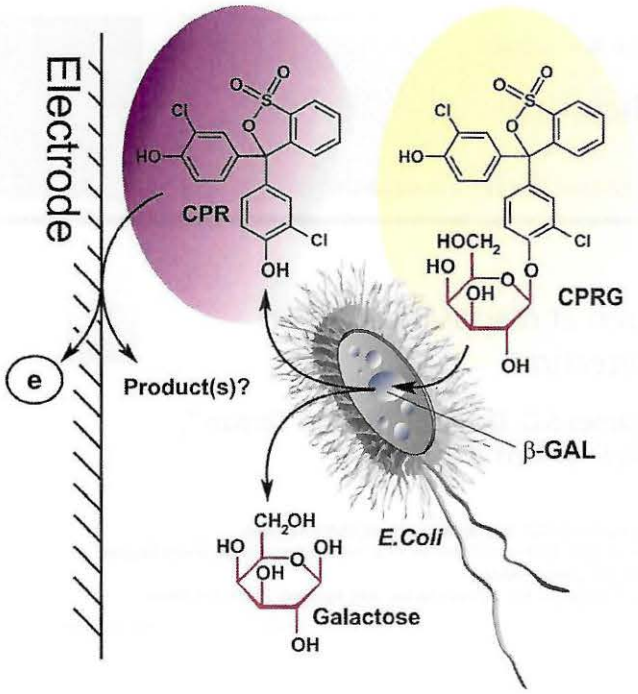

Scheme 1. Spectroscopic or electrochemical detection of *Escherichia.coli* through the β-galactosidase (β-GAL) mediated hydrolysis of chlorophenol red - galactopyranosidase (CPRG).

which were ratified by the United Nations [21]. Inspection of the CPR molecular structure (highlighted in Scheme 1) reveals two discrete 2-chlorophenol functionalities, the oxidation of which are attributed to the electrochemical peak process observed by Wutor and colleagues. The products of the oxidation process however were unascribed, but it is inevitable that a range of intermediates could arise, and some tentative pathways are highlighted in Fig. 1.

For the purposes of mechanistic considerations, the CPR molecule could be simplified to a core consisting of two 2-chlorophenol (2CP) groups with the remainder of the molecule being a relatively inert spectator. While there is no mechanistic information on the oxidation of CPR in the literature, there is an abundance of studies on substituted phenols [22–27]. As indicated in Fig. 1, electrochemical oxidation would be expected to lead to the generation of the radical cation (**I** → **II**) and therein to a spectrum of products (**II** → **VI**). In simple systems with low steric hindrance, this tends to result in coupling to form oligomeric and polymeric species directly at the electrode surface. While similar reactions are inevitable in the oxidation of CPR, the molecular bulk could be a limiting factor allowing competing process to prevail. The CPR structure, by virtue of the blocked 4-position and the 2-chloro substituent, is sterically less likely to form polymeric films upon oxidation, however, the vacant 5-position would be susceptible to attack leading to oligomeric CPR-like aggregations or the production of discrete 1,2-dihydroxy species (**III** and **V**). Given that the potential required to oxidise these intermediates is less than that required to oxidise the parent CPR, it could be anticipated that their conversion to the corresponding 1,2-quinone (**III** → **IV**, **V** → **VI**) should be immediate upon the imposition of a large positive potential.

Electrochemical signatures pertaining to the intermediates were not reported by Wutor et al., instead their focus was solely on the direct oxidation of the phenol (**I** → **II**) [20]. As such, a number of questions remain in terms of the possible pathways highlighted in Fig. 1. The oxidation of CPR was observed at +0.72 V which can be problematic in complex media where the oxidation of other components (i.e. humic substances) may lead to ambiguities in ascribing the peak currents and could give

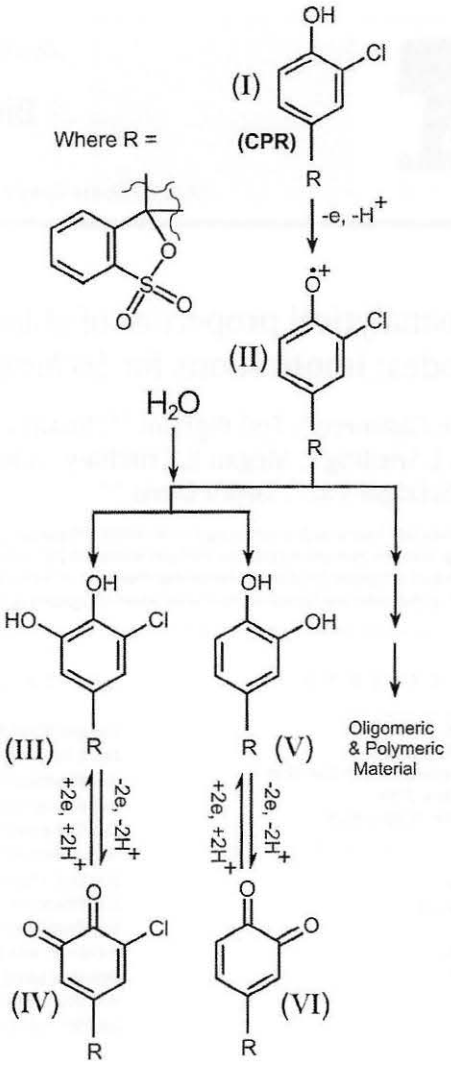

Fig. 1. Possible reaction pathways following the oxidation of chlorophenol red (CPR).

rise to false positives. In contrast to the direct oxidation of CPR, the electrogenerated 1,2-dihydroxy intermediates could be expected to have redox peak processes at much lower potentials. Exploiting their redox signature as the analytical signal for B-GAL activity could potentially avoid interference issues from other species present in the sample. From a more fundamental perspective, there are two competing pathways in Fig. 1 where the chloro substituent is either retained throughout the process or is lost through nucleophilic attack during the initial oxidation. As yet, no investigations into the nature of the electrode products from CPR have been reported. The present study has attempted to counter this issue, but also examine the possibility of exploiting the electrogenerated dihydroxy products as a viable diagnostic tool for detecting B-GAL activity and hence the presence of coliforms.

2. Experimental details

2.1. Materials and instrumentation

All chemicals were obtained from Sigma-Aldrich (Gillingham, UK), were of the highest grade available and were used without further purification. Toray Carbon Fibre Paper (TGP-H-30) was purchased from *E*-TEK (USA) and used as received. Electrochemical analysis was carried

out using an Anapot potentiostat (Zimmer & Peacock, Royston UK) with a standard three-electrode configuration with either a glassy carbon (3 mm diameter) or Toray carbon fibre mat (2.5 × 2.5 mm) as the working electrode. Platinum wire served as the counter electrode and a conventional silver/silver chloride (3 M KCl, BAS Technicol UK) half-cell reference electrode unless otherwise specified. All measurements were conducted at 22 °C ± 2 °C. Carbon fibre electrodes were sealed within a polyester laminate as described previously [28] and pre-anodised in 0.1 M NaOH (+2 V, 5 mins) [29]. Electrochemical measurements were conducted in Britton-Robinson (BR) buffer (acetic, boric and phosphoric acids – each with a concentration of 0.4 M and adjusted to the appropriate pH through the addition of concentrated sodium hydroxide) unless otherwise specified. While the pH of treated water can range from pH 6 to pH 9, the electrochemical investigations were conducted at pH 7 throughout to facilitate comparison of electrode responses. The influence of pH on the peak responses was however assessed and summarised in the following discussion.

NMR spectra were recorded on a Bruker Avance-III 300 MHz spectrometer at ambient temperature. Chemical shifts are reported in ppm relative to residual protic solvent (^{1}H NMR d_6-DMSO, 2.500 ppm; ^{13}C NMR d_6-DMSO, 39.520 ppm). The preparation of 2-chloronaphthoquinone was based on a method by Neufeind et al. (2011) [30] and is detailed in the Electronic Supporting Information (ESI) file along with the procedures followed and spectra obtained from the NMR investigations.

A Kratos Axis Ultra DLD Spectrometer was used to quantify surface composition and acquire X-ray photoelectron spectroscopy (XPS) spectra. Spectra were analysed using monochromated Al Kα X-rays (hν = 1486.6 eV (eV)) with typical operating parameters of 15 kV and 10 mA (150 W). During analysis, a hybrid lens mode was used (electrostatic and magnetic) with a 300 μm × 700 μm analysis area and a take-off angle (TOA) of 90° with respect to the sample surface. Wide energy survey scans (WESS) were collected across a range of −5 to 1200 eV binding energy (BE), with a pass energy of 160 eV and step size of 1 eV. High-resolution spectra were collected with a pass energy of 20 eV with a 0.05 eV step size, a scan width of 25 eV, a dwell time of 150 milliseconds and at least 3 sweeps to reduce the signal noise. A Kratos charge neutraliser system with a filament current between 1.8 and 1.95 A and a charge balance of 3.3–3.6 V and a filament bias of 1.3 V was used for all samples. Charging effects on the BE positions were adjusted by setting the lowest BE for the C1s spectral envelope to 284.8 eV, which is commonly accepted as adventitious carbon surface contamination. Three measurements were analysed per sample, with a Shirley background subtracted from each XPS spectra. The peak areas of the most intense spectral lines for each elemental species were used to determine the percentage atomic concentration. Peak fitting of high-resolution spectra was carried out using Casa XPS software.

2.2. Bacterial culture

Escherichia coli (K12, CETC 4624 (NCTC 12486)) was grown from an existing stock (previously stored at -80 °C, 25% glycerol) by streaking onto a Tryptic Soy Broth (TSB) agar plate containing tryptone (17 g/L), soy extract (3 g/L), NaCl (5 g/L), K_2HPO_4 (2.5 g/L), glucose (2.5 g/L) and agar (15 g/L). A single colony was transferred from the stock plate into 10 mL of LB broth. The latter was supplemented with 500 μM of isopropyl-β-d-thiogalactopyranoside (IPTG) in accordance with previous reports [31,32] and the resulting mixture added to a 50 mL falcon tube and incubated at 37 °C for 18 h at 150 rpm. Overnight cultures were sub-cultured and grown to an OD_{600} of 0.4 before undergoing a 2 fold serial dilution.

2.3. Electrochemical assay

Lysozyme (10 mg/mL) was dissolved within Tris buffer, adjusted to a pH of 8 and added at a 1 mg/mL concentration to each dilution [33]. This was incubated at room temperature for 20 min and then pelleted and the supernatant removed. The pellet was resuspended in Minimal Media (pH 7) consisting of Na_2HPO_4 (25.6 g/L), NaH_2PO_4 (5.28 g/L), KCl (1 g/L), NH_4Cl (2 g/L), $MgCl_2$ (0.19 g/L) and $CaCl_2$ (0.011 g/L). Chlorophenol red galactopyranoside (6 mg/mL) was then added and the mixture incubated at 37 °C over a period of 24 h. Voltammetric readings were taken at 2.5 h and 24 h. Electrochemical investigations within the resulting *E.coli* cultures were conducted through placing the three electrodes (carbon fibre working, 3 M Ag/AgCl reference and Pt counter) within the bacterial culture. Square wave voltammograms (−0.2 V to +1.2 V, Step = 2 mV, Pulse = 20 mV, Frequency 25 Hz) were scanned.

3. Results and discussion

Cyclic voltammograms detailing the response of an anodised carbon fibre electrode towards 100 μM CPR in pH 7 Britton-Robinson (BR) buffer are shown in Fig. 2A. A broad oxidation process (+0.6 V) is seen on the first scan with the successive decrease in the peak magnitude characteristic of phenol oxidation. The oxidation peak is slightly less positive than that observed by Wutor et al. [20] and could be attributed to the increased edge plane/interfacial oxygen functional group population associated with anodised carbon fibre [29]. The initial oxidation is irreversible and can be attributed to the generation of the radical cation which is then expected to undergo the various structural transformations as indicated in Fig. 1. Rather than being characteristic of a single electrode process, the breadth and multicomponent structure of the "peak" highlights a multitude of secondary processes attributed to the consequent oxidation of reaction by-products. Importantly, a series of new electrode processes are observed on the second scan covering the range: +0.1 V to +0.4 V and suggests multiple overlapping peak processes. It is important to note that restricting the scan range on the first scan to +0.5 V - a potential limit insufficient to induce CPR oxidation - does not lead to the appearance of any secondary redox peak processes. Voltammograms were also recorded in BR buffers covering the range pH 6 to pH 9 and it was observed that the secondary peak process exhibited a shift in peak position of 57 mV/pH consistent with a 2 proton/2 electron transition.

As the magnitude of the peak process at +0.3 V was found to increase with additional scanning, irrespective of any time delay between scans, it was hypothesised that the products were adsorbed onto the electrode surface and this was tested through removing the electrode, rinsing and placing in fresh pH 7 BR buffer devoid of any solution CPR. The resulting voltammograms are highlighted in the Inset in Fig. 2A, where it is clear that the electro-oxidation product is retained and exhibited near-reversible behaviour which was found to be relatively stable with only a 9% diminution in the peak current after 20 voltammetric cycles. The response to scan rate was also investigated (**Fig. 2B** and **C**) with the linearity of the peak current vs scan rate being consistent with a surface-confined species. Surface coverage (Γ_c) after 8 scans was estimated from:

$$I_P = \frac{n^2 F^2 A \Gamma_c}{4RT} v \quad (1)$$

where I_p is the peak height, v is the sweep rate, A is the effective surface area (0.0625 cm^2) of the carbon fibre electrode and the other symbols have their usual meaning. From the slope of cathodic peak currents vs. scan rate, the calculated surface concentration of the CPR quinone compound was found to be 2.49×10^{-5} mol cm^{-2}.

Surface characterisation of the adsorbed intermediate highlighted in the inset within Fig. 2A was conducted using high resolution X-ray photoelectron spectroscopy (XPS). Fresh carbon fibre electrode samples were prepared and cyclic voltammetry performed on 100 μM CPR under the same conditions as those detailed above. A total of 10 scans were recorded and the electrode then removed and rinsed thoroughly

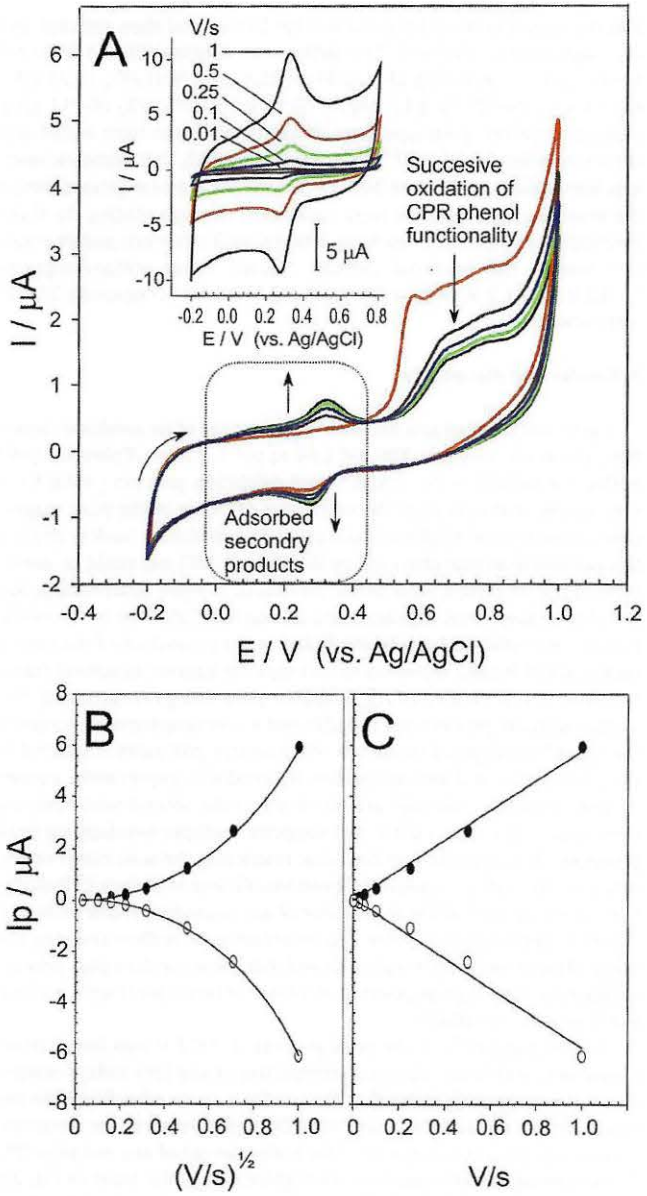

Fig. 2. A) Cyclic voltammograms detailing the oxidation of 100 µM chlorophenol red (CPR) at a carbon fibre electrode in pH 7 Britton-Robinson buffer (Scan rate: 50 mV/s). Inset: response of the same electrode in fresh pH 7 buffer. B) Variation of peak heights with square root of scan rate (B) and scan rate (C).

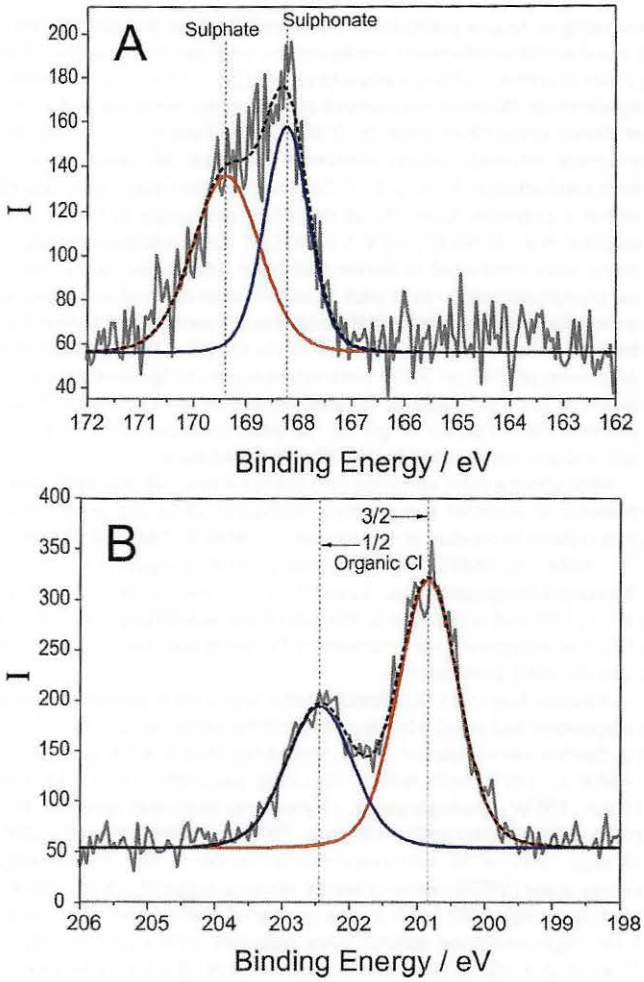

Fig. 3. X-ray photoelectron spectra of the S 2p (A) and Cl 2p (B) regions of a carbon fibre electrode confirming the presence of adsorbed CPR oxidation products.

to remove residual/unoxidized CPR. Representative XPS spectra detailing the S 2p and Cl 2p regions are shown in Fig. 3. While it could be expected that the oxidation process would lead to changes in the carbon framework through the incorporation of new C-OH bonds, the use of the C 1 s XPS profile was not used in this instance as the variety of carbon-oxygen functionality already present in the underlying carbon fibre prevents unambiguous assignment. The unmodified carbon fibre is however devoid of any sulphur or chlorine moieties and hence, it was anticipated that the atomic% ratio of S:Cl at the surface could therefore give some insights into the reactivity of the chlorine substituent. The observed ratio was 0.757 ± 0.098 ($N = 4$). Had the chlorine been unreactive towards attack, a ratio of 0.5 would have been expected (Pathway **I** → **II** → **III** ↔ **IV**, Fig. 1) and conversely, the ratio would be expected to be substantially greater than 1 were the chlorine substituent subject to replacement (Pathway **I** → **II** → **V** ↔ **VI**, Fig. 1). As such, the XPS results indicate that very little of the chlorine is lost (as chloride ion) as a consequence of both the initial oxidation of the CPR and repeated redox cycling of the adsorbed intermediates.

Corroborating evidence for the retention of the chlorine substituent was obtained through examining the electrochemical responses of 2-chloro-1,4-naphthoquinone, 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone as models for the intermediates highlighted in Fig. 1. Repetitive cycling of the chloro-naphthoquinone-hydroquinone system in pH 7 BR buffer did not lead to any significant change in the peak profiles and no transition to the 2-hydroxy analogue was observed, indicating that attack by water does not occur to any appreciable extent. Similarly, a range of NMR experiments (1H, 1H1H COSY, 13C and 135-DEPT) were undertaken to determine if nucleophilic substitution occurs at the 2-chloro-1,4-naphthoquinone in aqueous solution. Detailed spectra for the 2-chloro-naphthoquinone and the 2-hydroxynaphthoquinone are detailed in Fig. S1 along with the investigative protocol followed. In summary, the substitution can be judged by the proton singlet at the 3rd position on both naphthoquinone molecules which occurs at 6.16 ppm for the 2-hydroxy-1,4-naphthoquinone and 7.50 ppm on the 2-chloro-1,4-naphthoquinone. It could be anticipated that were nucleophilic substitution to occur at the 2-chloro derivative as a consequence of attack from water, the 6.16 ppm singlet, characteristic of the 2-hydroxy product, would be observed. The emergence of the latter was not observed after 10 min exposure of the 2-chloro-naphthoquinone to water.

Evidence supporting the proposition that the intermediate is, in fact, a 1,2-quinone type redox species was acquired through employing a tyrosinase assay with CPR as the enzyme substrate. Tyrosinase is widely used for the quantitative analysis of phenols where it first converts the latter to the 1,2-dihydroxy analogue and then oxidises it to the corresponding 1,2-quinone. The enzyme has been used in a number assays for the detection of coliforms where phenol-galactopyranoside was employed as the bacterial/B-GAL substrate [34]. In the present case, tyrosinase (23.5 kU/mL) was employed to convert 25 μM CPR directly to its 1,2-dihydroxy variant as indicated in Fig. 4A. Square wave voltammetry was used to periodically monitor the reaction in the expectation that the enzymatically produced 1,2-dihydroxy analogue of the CPR molecule would adsorb onto the carbon fibre electrode. The switch to the square wave format was employed to harness the greater discrimination of Faradaic processes from the background thereby improving both detection sensitivity and peak definition. Voltammograms comparing the response of carbon fibre electrodes to the enzymatically generated 1,2-quinone and the adsorbed intermediate arising from the electro-oxidation of CPR are shown in Fig. 4B.

While the responses indicated in Fig. 4B are not definitive, the close similarity of the peak positions combined with the knowledge of both the enzyme mechanism and the XPS data would suggest that the predominant product in the electro-oxidation of CPR is the 1,2-dihydroxy species in which the chlorine substituent is retained.

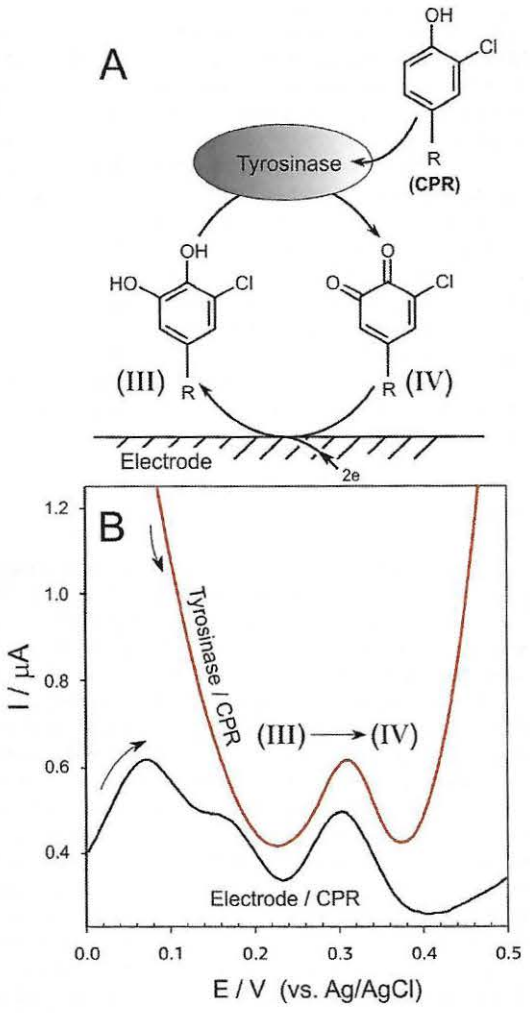

Fig. 4. A) Tyrosinase – CPR reaction scheme and B) Square wave voltammograms comparing the response of a carbon fibre electrode towards the products arising from the electrochemical and enzymatic oxidation of CPR in pH 7 Britton-Robinson buffer.

3.1. Preliminary bacterial assays

The overarching aim was to determine if the adsorbed electrode products arising from the CPR oxidation could themselves be used as a diagnostic handle through which to detect B-GAL activity. The standard coliform assay involves incubating the bacteria in the presence of a labelled galactose substrate. It is commonplace to introduce polymyxin B and lysozyme to enable the release of the B-GAL enzyme into the extracellular assay medium and thereby enhancing the rate at which the labelled substrate is hydrolysed [35]. In the case of chlorophenol red galactopyranoside (CPRG), there is a distinct colorimetric signal upon the B-GAL enzyme cleaving the CPR label with the latter providing a magenta colouration (λ_{max} 575 nm). Achieving electrochemical distinction between the labelled substrate and the hydrolysed label however is much more difficult as the oxidation potentials of the two can be similar. Inspection of the structure of CPR in Schematic 1 reveals that the galactose is tethered to only one of the two phenolic functional groups. The remaining phenol is therefore capable of being oxidised at the electrode before any interaction with the B-GAL enzyme and could give rise to a false positive. This possibility was confirmed when comparing the square wave voltammograms highlighted in Fig. 5.

Both CPR and CPRG exhibit similar oxidation profiles on the first sweep and would, ordinarily, negate the possibility of exploiting the direct electrochemical oxidation of CPR as a diagnostic for B-GAL activity. The CPRG is normally supplied in excess as the enzyme substrate and thus differentiation between the hydrolysed CPR and its parent is not possible under these conditions. Wutor and colleagues used various metallophthallocyanine modified glassy carbon electrodes to discriminate between the CPR and CPRG, though the mechanism through

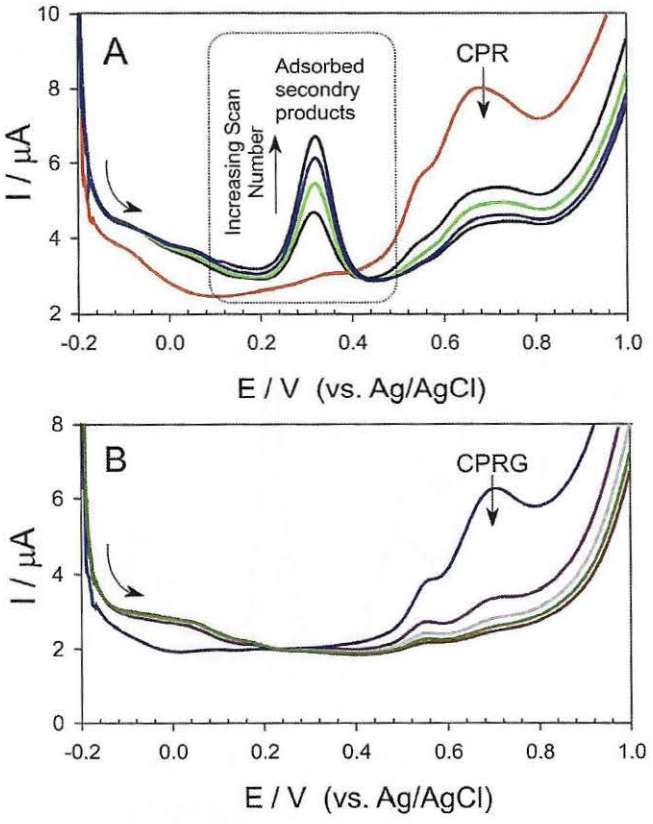

Fig. 5. Square wave voltammograms comparing the consecutive scanning response of carbon fibre electrodes towards (A) 10 μM chlorophenol red (CPR) and (B) its galactopyranoside derivative (CPRG) in pH 7 Britton-Robinson buffer.

which the selectivity was achieved is unclear. Examination of Fig. 5 however, reveals a possible solution where repetitive scanning of CPR leads to the generation of 1,2-quinone species which adsorb to the electrode. This is consistent with the responses discussed earlier but is of particular significance here when compared to the results obtained with CPRG where no accumulation was observed.

It is possible that upon oxidising CPRG, it too follows the transition pathway **I → II → III** highlighted in Fig. 1. It is possible that the oxidation of the free phenolic group in the CPRG molecule gives rise to a 1,2-quinone system not unlike that proposed for the CPR molecule. The absence of any accumulation at the electrode could be attributed to the solubilising effect of the attached galactose substituent – allowing diffusion away from the electrode and hence prevents detection. Thus, while direct oxidation is clearly impractical as a diagnostic marker at simple carbon electrodes in the presence of both CPR and CPRG, the products of the oxidation could offer an alternative approach and enhanced selectivity.

The applicability of the approach was tested through following a conventional galactopyranosidase assay format in which *E. coli* were incubated in combination with isopropyl-β-d-thiogalactopyranoside (IPTG) to increase expression of the β-GAL enzyme [31,32]. Thereafter, the bacteria were harvested (10^8 CFU/mL), lysozyme (1 mg/mL) and (500 μM) CPRG added and the mixture incubated at 37 °C. Square wave voltammograms were recorded after 2.5 h and 24 h and the responses obtained at the carbon fibre electrode are detailed in Fig. 6. After 24 h, repetitive scanning (−0.2 V to +1 V) leads to the gradual accumulation of the adsorbed electrogenerated product. It is noteworthy that there is no peak at +0.3 V on the first scan but the 1,2-quinone product emerges on the 2nd and subsequent scans. A critical point to note is that the adsorbed quinone, after 5 scans, presents a peak magnitude that is greater than the CPR/CPRG oxidation process initially observed on scan 1. The main challenge to coliform detection is the acquisition of a test result in as short a time as possible. Square wave voltammograms detailing the response obtained after 2.5 h are shown in the Inset within Fig. 6. Again, the accumulation of the electrogenerated quinone can be seen and follows much the same trend as that observed with the 24 h sample.

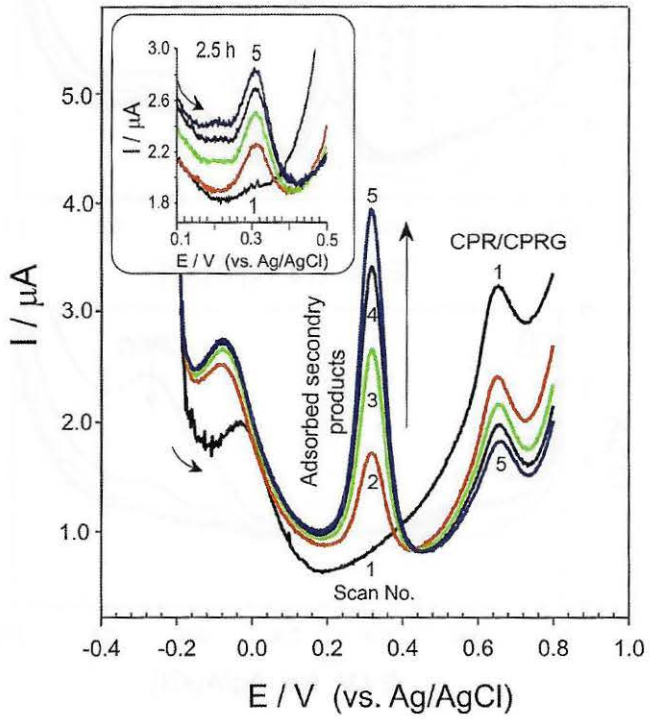

Fig. 6. Square wave voltammograms comparing the consecutive scanning response of carbon fibre electrodes in a lysed *E.coli* (10^8 CFU/mL) solution containing 500 μM CPRG following incubation for 24 h. Inset: Scans obtained after 2.5 h. Scans conducted in pH 7 Minimal Media buffer.

A large number of electrochemical assays exploiting B-GAL have been developed in recent years and have exploited: p-nitrophenol (PNP) [36], p-aminophenol (PAP), 1-aminonaphthol (AN) [37], 8-hydroxyquinoline [38] and chlorophenol red (CPR) [20]. The use of PAP labelled galactose is the most common approach and can be attributed to the low oxidation potential (typically +0.1 V to +0.3 V) required to detect the PAP released [31,39–41]. The detection of the PAP is not however without problems as the low oxidation potential can also create issues over the stability where the presence of oxygen, particularly at prolonged incubation periods, will inevitably lead to the oxidation of both PAP and can result in the degradation of the target marker. In contrast, CPRG is much more stable as indicated by the more positive potential required for its direct oxidation but it is the ability to accumulate the oxidation product that could inform future designs.

The carbon fibre substrate has served as a conductive matrix through which to monitor the various molecular transformations and, being relatively inexpensive, could point the way towards simple disposable electrode systems. Attempting to detect 1 CFU/100 mL however presents a considerable challenge – especially where the emphasis is on short incubation periods. It is inevitable that some form of preconcentration will be required, followed by incubation with a labelled galactose substrate. In such scenarios it is still critical to preconcentrate the released label in order to improve detection limits. It could be envisaged that the macro porous nature of the carbon fibre employed here could serve as a conductive filter acting as both generator-collector of the 1,2-quinone species. The passage of the initially preconcentrated bacterial solution through the carbon fibre mesh would enable the oxidation of the CPR released by the action of B-GAL to the quinone (generation) which subsequently accumulates at the same fibre (collection). The key advantage here would be that rather than relying on diffusion limited transport of CPR to the electrode and hence a slow accumulation of the product, the imposition of flow through the conductive fibre would enable rapid accumulation of most of the available CPR and greatly improve detection response times and sensitivity. Pursuit of such a system is beyond the scope of the present investigation, but the responses highlighted here effectively demonstrate the proof of principle and highlight an avenue for further exploration.

4. Conclusions

Chlorophenol red has been used extensively in the development of colorimetric assays but has been largely ignored in electrochemical assays. This could be attributed to the fact that the large positive potentials needed to acquire a signal are unattractive from an analytical perspective where interference from other matrix constituents can be problematic and where the electrode fouling commonly associated with phenol oxidation can compromise reproducibility. The results presented here highlight an innovative approach to the detection of the label where the exploitation of quinone type intermediates offer routes through which enhanced selectivity and sensitivity can be obtained with inexpensive substrates. The electrochemical generation of the 1,2-quinone intermediate and its subsequent accumulation at the surface of the carbon electrode is readily detected at +0.3 V which stands in marked contrast to the conventional oxidation potential required for the parent phenol (+0.7 V). No interference was observed when conducting the assay in bacterial culture containing *E.coli* with a clear, unambiguous oxidation peak process. A critical advantage is that the accumulation of the quinone reaction product with successive scanning offers a means of amplifying the signal which could significantly reduce the time required for incubation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2019.06.006.

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