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Rapid assessment of the latent hazard posed by dissolved mercaptans within aqueous effluent

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Abstract

The presence of mercaptans (RSH) can usually be detected by their inherent noxious odour but there is a need to quantify the concentration within effluent and hence allow an assessment of the latent hazard to be made prior to disposal. The versatility of using naphthoquinone as a rapid derivatising agent through which to trap such species has been evaluated. The quinone moiety provides a label that can be quantified using colorimetric, electrochemical and chromatographic means and offers a significant advantage over conventional thiol labelling agents. The analytical characteristics of each approach have been investigated and the selectivity, sensitivity and applicability of the reaction system critically assessed for a range of model compounds. The naphthoquinone system has a detection limit in the low micromolar range with little interference from other components common to discharge water with 96% recovery of mercaptopropionate. The reaction to sulfide (HS^-) has also been assessed and a disparity in response between the detection methods observed and a possible reaction pathway outlined.

Keywords: Sulfur; Sulfide; Mercaptan; Naphthoquinone; Chromatography; Analysis

1. Introduction

Reduced sulfur compounds (RSH) are routinely found in a wide spectrum of effluents resulting from various industrial processes—particularly those associated with paper pulp and petroleum feedstock [1–4]. Their presence within such fluids and the associated effluent presents an obvious environmental risk to ecosystems but also a considerable occupational hazard to those involved in handling such material both pre and post discharge [5,6]. While they tend to be characterised by particularly noxious odours—cumulative de-sensitisation of those workers directly exposed will inevitably compromise the perception of the latent hazard possessed by the effluent. There is a clear need to able to characterise the sulfur composition in a more robust and quantitative process. Much effort has been expended in the development of technologies to monitor hydrogen sulfide [6–9] but little attention has been paid to other reduced sulfur components that can arise in the processing liquors. Such measurements can be problematic due to selectivity issues and the inherent reactivity of the reduced sulfur functionality where delays in sampling can lead to significant degradation in the more reactive moieties. The present communication has sought to investigate the efficacy of labelling the latter—effectively trapping and thereby preserving the compound prior to analysis such that more accurate assessments of hazard could be made and false negatives minimised.

Numerous chemical derivatisation strategies have been developed that target the sulfhydryl (RSH) group and the more common reagents are summarised in Table 1. These have been reviewed and the various aspects of their chemistry has been discussed elsewhere [10]. The key points however are that they can vary widely in their ease of use, selectivity, expense and, in many cases, are designed for use in more specialised biochemical applications. The reaction of quinones with reduced sulfur functionalities are well established within synthetic contexts [11,12]. The analytical exploitation of the reaction as a possible label however has only recently been investigated and has been used for the detection of sulfide and various thiols of

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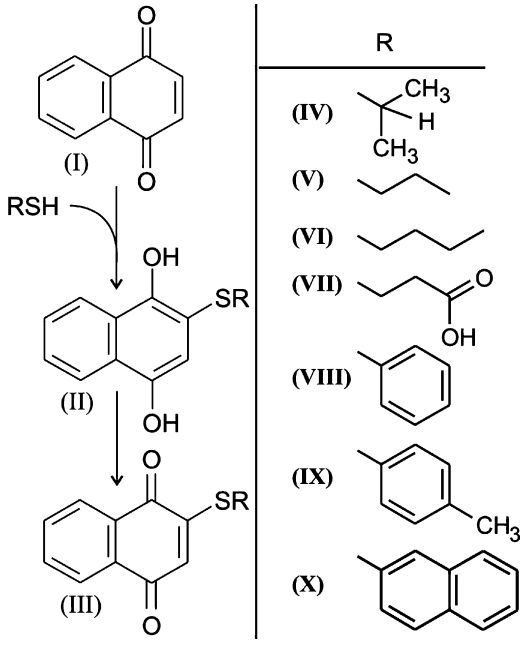
Table 1
Thiol labels and detection methodology

Haloacetamides	FL
Maleimides	FL, ED
Benzoxadizoles	FL
Isoindoles	FL
Disulphide exchange	UV–vis
Dansylaziridines	FL

UV–vis: colorimetry; FL: fluorescence; ED: electrochemical detection.

biomedical significance (principally cysteine, glutathione and albumin) [13–15]. The potential merits posed by the quinone label over the others outlined in Table 1 lies in the fact that it imparts a versatile functionality to the target that can serve as a chromophore and as a redox centre and, as such, is of considerable flexibility for spectroscopic or electrochemical detection. This is particularly true for the determination of alkyl sulfides which would otherwise be largely invisible to either methodology.

The approach advocated here has been to exploit the reaction of naphthoquinone (NQ) with reactive sulfur species (detailed in Scheme 1) to provide a liquid chromatographic label capable of either UV–vis or electrochemical detection. The basic reaction usually proceeds through a conventional 1,4-nucleophilic addition mechanistic pathway [11,12]. The outcome is the covalent bond formation between quinone (I) and reduced thiol leading to the corresponding reduced form of the quinone–thiol conjugate (II) which, through a combination of intermolecular redox transition and aerobic oxidation, will be readily converted to the oxidised form (III). The potential benefits associated with the use of NQ relate to fact that it is readily available, inexpensive, stable and soluble in both aqueous as well as non-protic solvents. The investigation focused on elucidating the nature of the reaction between label and target within a predominantly aqueous effluent environment and to explore the boundaries that define its applicability. As such, a number of novel quinone–thiol conjugates were prepared (Scheme 1) to serve as model reagents for the fundamental studies and the subsequent analytical assessment. A key aim was to develop a simple assay system that could be used for the speciation of a range of functionally disparate thiol targets and hence proffer a generic detection protocol for use in chromatographic detection.



Scheme 1. Proposed assay reaction scheme and model conjugates.

2. Experimental details

2.1. Methods and materials

Electrochemical measurements were conducted using a μAutolab type III computer controlled potentiostat (Eco-Chemie, Utrecht, The Netherlands) using a three electrode configuration consisting of 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). All measurements were done under nitrogen. NMR spectra were measured on a JEOL ECX 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethysilane (TMS). Mass spectra were recorded on a Micromass Platform LC–ESI-MS and a Micromass Quattro II. Flash chromatography was performed on 40–63 silica gel (Merck). All chemicals and solvents were bought from either Acros Organics or Sigma Aldrich and used without further purification. Chromatographic analysis was carried out in isocratic mode with an Agilent 1100 series HPLC system. An ODS 150 mm × 4.6 mm Sphereclone 3 μm column (Phenomenex) was used throughout with a mobile phase consisting of 65/35 methanol/phosphate buffer (0.1 M, pH 7) at a flow rate of 1 mL/min. The detection wavelength in all cases was 250 nm. The typical assay procedure was based on the addition of 0–500 μL of the appropriate thiol (10 mM dissolved in methanol or water) and 500 μL of naphthoquinone (5 mM dissolved in methanol) to 5.0 mL of pH 7 buffer. Direct injection/analysis of the resulting assay being conducted at appropriate time intervals (typically 5–30 min) thereafter.

2.2. Chemical syntheses

2.2.1. 2-(Isopropylthio)-1,4-naphthoquinone (IV)

To a stirred solution of 1,4-naphthoquinone (1.58 g, 10 mmol) in ethanol (40 mL) is added 2-propanethiol (0.93 mL, 10 mmol). The solution was stirred for 4 h at room temperature. The reaction mixture was taken to dryness under reduced pressure and the crude product purified by column chromatography using silica gel as adsorbent and chloroform as eluent. The pure product dried *in vacuo* to yield 2-(isopropylthio)-1,4-naphthoquinone (0.91 g, 39.2%) as a brown solid.

^{1}H NMR (d-$CDCl_3$) δ 1.44 (s, 6H), 3.35–3.39 (m, 1H), 6.64 (s, 1H), 7.74–7.80 (m, 2H), 8.06–8.09 (m, 2H); ^{13}C NMR (d-$CDCl_3$) δ 22.05, 34.49, 118.72, 126.43, 126.83, 127.41, 131.93, 133.20, 134.23, 154.45, 181.62, 182.27; CI-MS (*m*/*z*) 233 $[M - H]^+$.

2.2.2. 2-Propylthio-1,4-naphthoquinone (V) and 2-butylthio-1,4-naphthoquinone (VI)

To a solution of 1,4-naphthoquinone (1.58 g, 10 mmol) in ethanol (40 mL) is added either 1-propanethiol (0.90 mL, 10 mmol) or 1-butanethiol (1.07 mL, 10 mmol). The solution was refluxed at 100 °C for 4 h. The reaction mixture was poured into ice and filtered to leave a brown solid. This was recrystallised from boiling methanol with activated charcoal to yield 2-propylthio-1,4-naphthoquinone (0.51 g, 22%) as orange crystals or 2-butyl-1,4-naphthoquinone (0.53 g, 21.5%) as yellow crystals.

2.2.3. 2-Propylthio-1,4-naphthoquinone

^{1}H NMR (d-$CDCl_3$) δ 1.08–1.12 (t, 3H), 1.77–1.81 (m, 2H), 2.79–2.82 (t, 2H), 6.59 (s, 1H), 7.69–7.73 (m, 2H), 8.06–8.08 (m, 2H); ^{13}C NMR (d-$CDCl_3$) δ 13.62, 20.82, 32.50, 126.45, 126.79, 126.91, 131.85, 132.12, 133.18, 134.24, 155.23, 181.51, 182.10; ESI-MS (*m*/*z*) 233 $[M+H]^+$.

2.2.4. 2-Butylthio-1,4-naphthoquinone

^{1}H NMR (d-$CDCl_3$) δ 0.94–0.98 (t, 3H), 1.50–1.52 (m, 2H), 1.72–1.76 (m, 2H) 2.79–2.83 (t, 2H), 6.58 (s, 1H), 7.68–7.72 (m, 2H), 8.04–8.06 (m, 2H); ^{13}C NMR (d-$CDCl_3$) δ 13.50, 22.14, 29.26, 30.29, 126.42, 126.76, 126.89, 131.83, 132.11, 133.15, 134.20, 155.23, 181.44, 182.05; ESI-MS (*m*/*z*) 247 $[M+H]^+$.

2.2.5. (1,4-naphthoquinone-2-yl)-mercaptopropionic acid (VII)

A solution of 1,4-naphthoquinone (1.58 g, 10 mmol) was in ethanol (40 mL) warmed until a clear yellow solution was obtained. 3-Mercaptopropionic acid (0.90 mL, 10 mmol) was added to the warm solution and the reaction proceeded with stirring for 4 h at room temperature. The solution was removed under reduced pressure to leave a brown solid. Ethanol (20 mL) was added to the solution with hexane (100 mL) and the solution was placed in the fridge overnight. The reaction mixture was stirred at room temperature for 1 h then filtered to yield (1,4-naphthoquinone-2-yl)-mercaptopropionic acid (1.98 g, 75.5%) as yellow crystals.

^{1}H NMR (d_6-DMSO) δ 2.65–2.69 (t, 2H), 3.11–3.12 (t, 2H), 6.77 (s, 1H), 7.81–7.86 (m, 2H), 7.95–7.97 (m, 2H); ^{13}C NMR (d_6-DMSO) δ 24.76, 31.95, 126.03, 126.34, 127.28, 131.43, 131.68, 133.65, 134.74, 153.40, 172.48, 180.95, 181.83; ESI-MS (*m*/*z*) 262 $[M-H]$.

2.2.6. 2-(Phenylthio)-1,4-naphthoquinone (VIII)

To a solution of 1,4-naphthoquinone (1.58 g, 10 mmol) in ethanol (40 mL) is added 4-thiophenol (1.02 g, 10 mmol). The solution was taken to 100 °C with stirring for 4 h. The reaction was poured into water and extracted into chloroform. The organic layer removed, dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. The red solid was recrystallised from ethyl acetate to yield 2-(phenylthio)-1,4-naphthoquinone (0.65 g, 24.7%) as red cubic crystals.

^{1}H NMR (d_6-DMSO) δ 5.86 (s, 1H), 7.61–7.63 (m, 6H), 7.84–7.86 (m, 2H), 7.87–7.91 (d, 1H), 8.03–8.06 (d, 1H); ^{13}C NMR (d_6-DMSO) δ 126.07, 126.45, 126.91, 127.54, 130.60, 130.77, 131.30, 131.60, 133.88, 134.86, 135.49, 155.54, 181.13, 181.55; CI-MS (*m*/*z*) 266 $[M-H]$.

2.2.7. 2-[(4-Methylphenyl)thio]-1,4-naphthoquinone (IX)

To a solution of 1,4-naphthoquinone (1.58 g, 10 mmol) in ethanol (40 mL) is added 4-thiocresol (1.26 g, 10 mmol). The solution was taken to 100 °C with stirring for 4 h. The reaction mixture was taken to dryness under reduced pressure and the crude product purified by column chromatography using silica gel as adsorbent and chloroform as eluent. The pure product was dried *in vacuo* to yield of 2-[(4-methylphenyl)thio]-1,4-naphthaquinone (1.60 g, 57.1%) as a brown solid.

^{1}H NMR (d-$CDCl_3$) δ 2.79 (s, 3H), 6.47 (s, 1H), 7.65–7.67 (d, 2H), 7.76–7.78 (d, 2H) 8.06–8.08 (m, 2H), 8.36–8.38 (d, 1H), 8.47–8.49 (d, 1H); ^{13}C NMR (d-$CDCl_3$) δ 21.49, 123.79, 126.59, 126.89, 128.19, 128.57, 129.87, 131.27, 131.84, 132.33, 133.39, 134.41, 135.66, 157.17, 182.07; CI-MS (*m*/*z*) 280 $[M-H]$.

2.2.8. 2-(2-Naphthylthio)-1,4-naphthoquinone (X)

To a solution of 1,4-naphthoquinone (1.58 g, 10 mmol) in ethanol (40 mL) is added 2-naphthalenethiol (1.60 g, 10 mmol). The solution was taken to 100 °C with stirring for 4 h. The solution was evaporated to dryness under reduced pressure and recrystallised from ethyl acetate and hexane with activated charcoal to yield 2-(2-naphthylthio)-1,4-naphthoquinone (2.02 g, 64%) as orange shards.

^{1}H NMR (d-$CDCl_3$) δ 6.13 (s, 1H,), 7.50–7.76 (m, 5H), 7,84–8.03 (m, 4H), 8.11 (s, 1H), 8.14–8.17 (m, 1H); ^{13}C NMR (d-$CDCl_3$) δ 124.41, 126.52, 126.84, 127.09, 127.87, 127.91, 127.95, 128.37, 130.24, 131.01, 131.71, 132.22, 133.32, 133.72, 133.96, 134.35, 136.17, 156.63, 181.88, 182.10; CI-MS (*m*/*z*) 316 $[M-H]$.

2.2.9. (1,4-Naphthoquinone-2,3-yl)-dimercaptopropionic acid (XI)

To a solution of (1,4-naphthoquinone-2-yl)-mercaptopropionic acid (0.80 g, 3 mmol) in ethanol (20 mL) heated with 3-mercaptopropionic acid (0.90 mL, 10 mmol) at reflux for 2 h until the solution was clear brown. The solution was allowed to cool to room temperature and a brown liquid was observed with no precipitation. The solution was removed under reduced pressure to leave a brown solid. Ethanol (20 mL) was added to the solution with hexane (100 mL) and the solution was stirred overnight. The reaction mixture was filtered to yield 3,3′-(1,4-dihydro-1,4-dioxo-2,3-naphthylenedithio)di-propionic acid (0.48 g, 42.8%) as a yellow powder.

^{1}H NMR (d_6-DMSO) δ 2.65–2.69 (t, 2H), 3.11–3.12 (t, 2H), 6.77 (s, 1H), 7.81–7.86 (m, 2H), 7.95–7.97 (m, 2H); ^{13}C NMR (d_6-DMSO) δ 24.76, 31.95, 126.03, 126.34, 127.28, 131.43, 131.68, 133.65, 134.74, 153.40, 172.48, 180.95, 181.83; ESI-MS (*m*/*z*) 366 $[M-H]^+$

3. Results and discussion

First, the reactivity of NQ has been studied with mercaptopropionic acid (MPA). MPA is a good model because it can arise

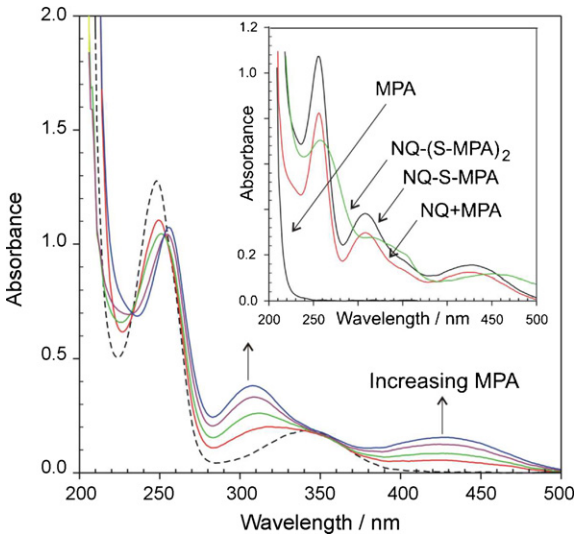


Fig. 1. Absorption spectra detailing the reaction of 58 μM naphthoquinone (NQ) with increasing additions of mercaptopropionic acid (MPA, 14 μM). Inset: comparison of assay products with the spectra from synthetic mono and di-thiol conjugates.

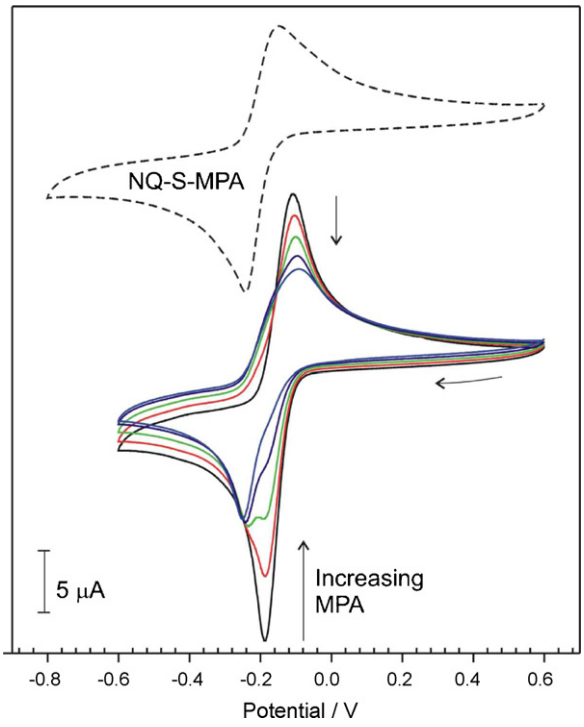


Fig. 2. Cyclic voltammograms detailing the response of 0.45 mM naphthoquinone (NQ) to increasing additions of mercaptopropionic acid (MPA, 90 μM) in pH 7. The response to the synthetic mono-thiol analogue is included for comparison (dashed line). Scan rate: 0.1 V/s.

through numerous chemical and biochemical pathways and is often the prominent thiol within pore water and organic rich sediments where anoxic environments predominate [16–18]. The proposed detection system has been studied by UV–vis spectroscopy, cyclic voltammetry and chromatography in order to assess the reactivity of NQ towards thiols and to evaluate the selectivity and sensitivity of the various methodologies. Fig. 1 details the spectroscopic profile of NQ (58 μM, pH 7). Upon the introduction of a reduced thiol—a new absorption process typically emerges at 425 nm and corresponds to the formation of the thiol-NQ conjugate (III) indicated in Scheme 1. This process is exemplified in Fig. 1 through the addition (14 μM) of mercaptopropionic acid (MPA) which although lacking a native chromophore, is easily identifiable subsequent to reaction with NQ. As such, it has been used as the principal model thiol throughout the subsequent experiments.

The identity of the conjugate was initially corroborated through comparing the synthetically produced mono and di-thiol conjugate absorption spectra (inset diagram, Fig. 1) with that resulting from the assay. The spectral profiles of the monothiol conjugate and assay mixture are in close agreement and it would suggest that the predominant product is indeed the former. Similar responses were recorded for the other thiols but, while it is clear that the use of NQ provides a quick and simple approach to attaching a visible label to otherwise invisible targets, there are a number of significant limitations. The sensitivity of the NQ-S-conjugate chromophore is relatively weak (2586 L mol^{-1} cm^{-1}) especially when compared with the commercially available ligands such as Ellman's reagent (~13,000 L mol^{-1} cm^{-1}) [19] found in Table 1. The peak process also lies within a spectral region (λ_{max} 425 nm) that is liable to be obscured, at least to some extent, by the coloured nature of the sample—whether natural or effluent. The common peak process also prevents direct speciation of the various components.

The reaction between NQ and thiols can also be detected using electrochemical techniques. This is where the system offers potential advantages over the predominantly spectroscopic systems detailed in Table 1. Cyclic voltammograms detailing the response of a conventional glassy carbon electrode to NQ (0.45 mM, pH 7) in the presence of increasing additions of MPA (90 μM) are shown in Fig. 2. The reduction of the quinone is observed as a sharp peak at −0.2 V with the corresponding oxidation at −0.1 V. Upon the addition of the MPA to the solution, the magnitude of the NQ reduction process diminishes and a second peak process emerges at −0.25 V. The latter corresponds to the cumulative formation of the conjugate. Confirmation of the latter was again provided by comparing the voltammetric response of the assay product to the purely synthetic conjugate (dotted line) where it can be seen that the peak positions are essentially identical. The main problem in attempting to exploit the reaction as an electroanalytical method for quantifying thiols within real samples however relates to the poor voltammetric resolution between the NQ indicator and the resulting conjugate. As in the case with direct spectroscopic analysis, similar voltammetric responses were obtained with the different thiols with only minor shifts in peak position compromising the prospect of speciation. It could however provide a simple method of measuring total reduced thiol content.

The NQ label clearly provides a versatile handle for both spectroscopic and electrochemical investigation but their scope is somewhat limited for the direct detection and identification of thiols in complex media. The true value of the NQ label is

liable to lie in its incorporation within liquid chromatographic systems whereby column resolution of the various thiols can be complimented by post column detection that exploits either the NQ chromophore or its redox centre properties. The chromatographic profile detailing the reaction of MPA (32 μM) with NQ (0.8 mM) to yield the conjugate NQ-S-MPA is shown in Fig. 3A. A linear response was obtained with increasing concentrations of MPA (peak area = 21,484 [MPA/mol L^{-1}] – 29.68; $N = 6$; $R^2 = 0.994$). The identity of the peak was again confirmed by examining the response to the synthetic NQ-S-MPA conjugate (82 μM, dotted line).

A small, transient peak was observed at 2.2 min in Fig. 3A and has some significance in the elucidation of the reaction pathway. The peak is attributed to the formation of reduced naphthoquinone (naphthol, NQH_2) and arises as an intermediate in the conversion of the initial, reduced, conjugate to the more stable oxidised form (Scheme 1, II → III). This was again confirmed by comparison with commercially available *p*-naphthol (Sigma Aldrich). In the presence of excess NQ, the predominant product is the single substituted conjugate. It must be noted that while di-substitution is possible, it is likely that the kinetics of formation are significantly slower as a consequence of the steric hindrance imposed by the initial derivatisation.

There is a second influence on the nature of the substitution which leads on from the previous identification of reduced NQH_2 moieties within the assay. The initial reaction with the sulfur nucleophile results in the formation of the reduced form of the conjugate (Scheme 1, II). This is unreactive towards further addition until it is re-converted back to the oxidised form (Scheme 1, III) through interaction with dissolved oxygen or NQ electron acceptors. Excess NQ should therefore have a scavenging effect on the target thiol minimising the occurrence of a multi-product distribution. That the di-substituent was not formed under the assay conditions was confirmed by the bulk preparation of the derivative and its subsequent chromatographic characterisation. The peak (Fig. 3A, dashed line) displays a shorter retention time than that of the mono substituted derivative, as expected, given the greater polarity afforded by the additional carboxyl group.

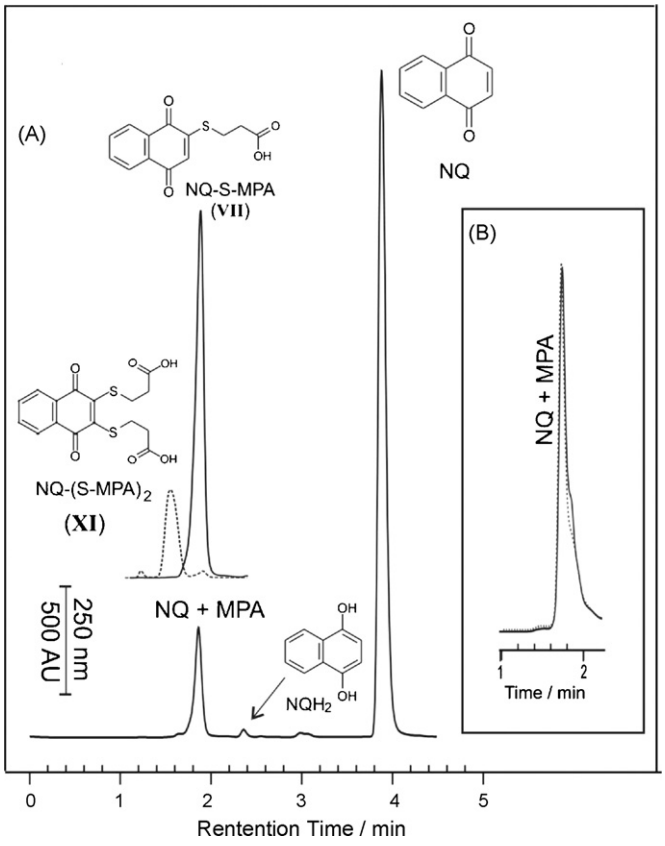


Fig. 3. (A) Chromatograms comparing the naphthoquinone (0.8 mM)—mercaptopropionate (32 μM) assay conjugates to the synthetic analogues. (B) MPA conjugate response after 24 h (solid line) and 48 h (dotted line).

The stoichiometry, kinetics and stability of the proposed system were examined through comparing the responses over different quinone:thiol concentration ratios and over different time periods (0–48 h). The reaction stoichiometry was found to follow the pathway proposed in Scheme 1. Providing the quinone is in massive excess (as expected for a test indicator reagent), then the thiol will be the limiting reagent. Hence, for ratios of quinone:thiol greater than 5:1, the reaction kinetics were found to be extremely fast such that the maximum peak was observed less than 5 min after commencing the assay. The thiol levels will, for the most part, be trace (low micromolar) and hence neither stoichiometry nor kinetics should impede the performance of the assay. However, should more concentrated waste products be handled then it is simply a matter of either increasing the concentration of the quinone or decreasing the test sample volume that is added to the assay mixture. The stability of the conjugate was also assessed and the assay peak response observed after standing for 24 and 48 h (shown in Fig. 3B) found to differ by less than 5%.

Similar reaction profiles were obtained for the other thiol derivatives (IV → X) outlined in Scheme 1 but each possesses a markedly different retention time in keeping with the nature of the individual sulfur substituents. The variation in retention characteristic for each derivative is highlighted in Table 2. Speciation of the individual components is clearly possible. It can be argued that such resolution would have been obtained irrespective of whether or not the NQ label had been attached. The principal advantage of utilising NQ relates to the fact that, with the exception of the aryl derivatives, the majority will be invisible to conventional UV detectors.

The assay selectivity was assessed through examining the response to a range of organic functionalities. The nucleophilic potential of amino groups was thought to be the more likely

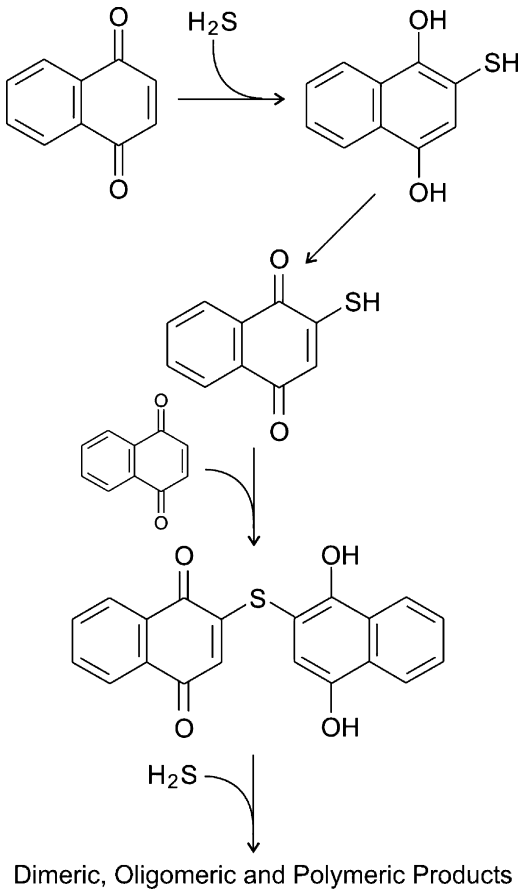
Table 2
Retention times (T_r) of the NQ-S-R derivatives

	R	T_r (min)
(IV)	$CH(CH_3)_2$	8.45
(V)	$CH_2CH_2CH_3$	9.79
(VI)	$CH_2CH_2CH_2CH_3$	15.18
(VII)	CH_2CH_2COOH	1.9
(VIII)	C_6H_5	13.35
(IX)	*p*-$C_6H_4CH_3$	20.99
(X)	$C_{10}H_7$	36.79

interference given that reaction of amines with quinones is well established and known to occur through a common reaction pathway to that outlined in Scheme 1 [11,12]. Providing the solutions are neutral (or subsequently buffered to pH 7), the nucleophilic character of the amino group should be significantly reduced through protonation as the pK_a for most alkyl amines lies within the range of 10–11. Aryl amines, in contrast, should be more of a concern as their pK_a can be significantly below that of the sample pH. A range of amines were tested covering both alkyl and aryl moieties (various amino acids, imidazoles and aniline derivatives in 100-fold excess, millimolar concentration) but were not found to illicit any appreciable reaction under the conditions and the initial timescale of the investigations [20]. Prolonged standing however is obviously problematic allowing the possibility of gradual reaction [21,22].

The degree to which this could compromise the detection sensitivity of the target thiol will obviously depend on the nature of the interfering group and their associated retention characteristics. In the event of peak overlap, acidification of the solution directly after commencing the assay will significantly impede the reaction of both aryl and alkyl amino functionalities. Acidification also prevents reaction with thiols through the same process. It is important that the initial reaction solution is neutral and then acidified. The rationale is that such an approach will exploit the speed through which the reduced sulfur group attacks the quinone allowing the introduction of a terminating acidic shot before any appreciable reaction with an amine occurs.

As the assay is selective for the reduced sulfur nucleophile, there should, in principle, be a reaction with hydrogen sulfide and its dissociated anion (HS^-). The reaction characteristics were however significantly different from that of the other thiols outlined in Scheme 1. A sequential decrease in the NQ peak was observed as expected yet no new peaks were observed in the chromatograms. Extending the run time to over 40 min did not uncover any new peaks. The discrepancy lies not in the fact that the product is not being detected in the UV range but rather that the product simply does not reach the detector. Upon the reaction of the sulfide with the quinone—secondary reactions occur that lead to the formation of oligomeric or polymeric products. A possible reaction pathway is highlighted in Scheme 2. Examination of the assay solution yielded evidence of a precipitate which, when collected and analysed by NMR spectroscopy, confirms the presence of oligomeric products. The simple single addition product is not observed. NMR analysis in d6-DMSO indicated the formation of two types of naphthoquinone substitution product. The first being the direct addition of the thiol species which links two 1,4-naphthoquinone molecules in symmetrical fashion, this was confirmed by the appearance (not shown) of a perfect doublet at 8.04 ppm and a triplet at 7.61 ppm with no proton present around 6–7 ppm; indicating that a substitution reaction has occurred at both the 2nd and 3rd position of the naphthoquinone ring system. The second type of product present confirmed the formation of a 1,4-naphthoquinone in an oligomeric arrangement. 1H NMR assignments for these molecules are confirmed by the loss of a peak at 6–7 ppm which indicates a reaction at the 2nd and



Scheme 2. Possible polymerisation pathway.

3rd positional proton on the naphthoquinone molecule. However, the formation of two quinol peaks was also found at 10.2 ppm and 10.8 ppm respectively. The broadness of the peak at 10.8 ppm and the difference in integration in comparison to the peak at 10.2 ppm confirms the presence of species with varying symmetry and supports the formation of a non-symmetrical oligomer.

3.1. Analytical application

The applicability of the overall approach was evaluated through recovery experiments of MPA from industrial receiving river water samples extracted from sources local to Nottingham. The recovery of 100 μM MPA using the HPLC NQ assay outlined above with detection at 250 nm was conducted. The quantification of recovery was achieved through comparison of peak area of the conjugate (Scheme 1, III) with the standard curve (peak area = 21,484 [MPA/mol L^{-1}] – 29.68; $N = 6$; $R^2 = 0.994$). The recovery was found to be 96.3%. Five replicates were conducted to assess the reproducibility of the method with the relative standard deviation of 5.0% highlighting the robust nature of the assay. There was no appreciable loss over the initial 24 h following derivatisation effectively preserving the reduced sulfur components and minimising losses that would otherwise be attributable to air oxidation or volatilisation.

4. Conclusions

Naphthoquinone has been shown to be a selective and highly versatile label for the trapping and subsequent analysis of reduced thiol functionalities. The reaction pathway has been investigated and the identity of the products and intermediates confirmed through comparison with their synthetic analogues. The assay has comparable detection sensitivity (sub micromolar concentrations in aqueous solution across spectroscopic, electrochemical and chromatographic assays) with a more elaborate mercuric trapping system [4] but possesses greater method flexibility, is inexpensive, avoids issues over reagent handling and can be conducted using conventional analytical systems. The analytical characteristics of the system have been critically appraised and the applicability of the approach demonstrated through the analysis of receiving water samples.

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