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Preparation and characterisation of a quinone-functionalised polythiophene film on a modified electrode. Application to the potentiometric determination of glutathione and cysteine concentrations

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ABSTRACT

The new compound 3-((2,5-dimethoxyphenyl)ethynyl)thiophene has been synthesised by Sonogashira coupling. A modified electrode coated with a polythiophene film bearing a quinone moiety was obtained by electropolymerisation of the thienyl group followed by anodic oxidation of *para*-dimethoxyphenyl group. The cyclovoltammetric response resulting from the reaction of glutathione with the benzoquinone moiety was investigated. The responses of the modified electrode as a new potentiometric sensor of reduced thiols are proposed.

1. Introduction

Reduced thiols are essential in biological systems, on account of their crucial role in the maintenance of redox homeostasis. Their concentration and bioavailability are of major importance to balance the accumulation of reactive oxygen and nitrogen species and to prevent subsequent deleterious oxidative stress associated with the pathogenesis of cancer, cardiovascular diseases, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis and ageing.[1]

Over the past three decades, intensive studies have been devoted to the detection of biological thiols as cysteine, homocysteine and more particularly glutathione.[2] Ellman's test is the classical method used for quantification of thiols based on a spectrophotometric method laying an exchange reaction between the thiol and the disulfide DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)).[3] The sulfide produced shows a characteristic yellow colour. However, this test and more generally spectrophotometric methods require a pre-treatment when thiols have to be assayed in coloured samples and their results may be influenced by variable levels of specific enzyme activities such as glutathione *S*-transferase or γ-glutamyltransferase.

High-performance liquid chromatography and capillary electrophoresis are used as separative techniques to determine glutathione and congeners in several bio-matrices.[4] Besides, fluorescence methods have been recently developed for the measurements of glutathione in yeast.[5]

In contrast with most of these techniques, the electrochemical methods provide the possibility of being used with coloured samples, such as blood, without tedious pre-treatment. Two types of electrochemical sensors for the determination of thiol concentrations are commonly studied.

- With an amperometric sensor, the thiol detection is generally based on chemically-modified electrodes that induce an electrocatalytic oxidation of another thiol obtained after an exchange reaction as in the Ellman's test.[6–9] Another amperometric approach is the use of organic redox cofactors like pyrroloquinoline quinone, glutathione peroxidase or inorganic redox mediators as transition metal complexes immobilised in a matrix.[10–13] In these systems, intermediate

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steps are necessary because the direct oxidation of thiols is slow at conventional electrodes and consequently require an important overpotential due to electrode passivation.

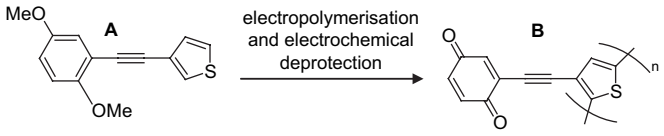
- In potentiometric methods, the sensors are usually based on the reaction between thiols and quinone indicators, as described by Davis et al.[14]Among the electrochemical methods, the potentiometric method can be implemented very easily.

Lau et al. have shown that the reaction of 1,4 benzoquinone (BQ) with the reduced form of glutathione (GSH) results in the formation of adducts that exhibit increasing degrees of glutathione substitution.[15] Moreover, as preliminary studies, the interaction of GSH with BQ by cyclic voltammetry on a vitreous carbon electrode was investigated (data not shown). The cyclic voltammogram of BQ was significantly modified when this compound was added to a solution of GSH. The principal changes consisted in the oxidation process that became irreversible and in the significant decrease in intensity of the oxidation peak. The latter modification characterised the diffusion of a bigger species than BQ. Consequently, in agreement with Lau's work, we postulated that the modification of the electrochemical behaviour of the couple BQ/BHQ (BHQ=1,4 hydroquinone) was due to the formation of adducts between GSH and BQ.

In this work, we describe a new potentiometric sensor for the detection of glutathione (GSH) and cysteine (CSH). This system is based on the reaction between the BQ moiety borne by a polymeric film and the thiol.

2. Results and discussion

The indicating modified electrode was obtained by anodic electropolymerisation (Scheme 1) of the thiophene group of 3-((2,5-dimethoxyphenyl)ethynyl)thiophene **A** where the 1,4-dimethoxyphenyl moiety is the precursor of quinone **B**.



Scheme 1. Electropolymerisation of 1-(3-(1,4-dimethoxyphenyl)-2-(3-thienyl))ethyne and deprotection of methoxy groups.

2.1. Synthesis of 3-((2,5-dimethoxyphenyl)ethynyl)thiophene monomer A

After different attempts, the more efficient route to obtain **A** is described below (Scheme 2).

By bromination of *para*-dimethoxybenzene with *N*-bromosuccinimide in acetonitrile, 2-bromo-1,4-dimethoxybenzene (**I**) was obtained with a yield of 90%. Two routes were tested (step 1, Scheme 2). In the first one, we used a water/acetonitrile mixture (1:10) as solvent, room temperature, without copper(I) in presence of a quaternary ammonium salt to improve the efficiency.[16] However, the yield was no better than 34%. In the second route, with CuI in triethylamine at 80 °C after refluxing for one night, compound **II**, 2-methyl-4-(2,5-dimethoxyphenyl)-3-butyn-2-ol, was isolated with a yield of 40%. By a retro-Favorskii reaction, the protective group R_1 was removed by heating compound **II** in a solution of sodium hydroxide in toluene.[17] The expected product **A** was prepared by a second coupling between **III** and 3-bromothiophene. To try to improve the yield in this last step, we also used the conditions described by Thorand and Krause,[18] but the efficiency was no better than 38%. The quinonic product corresponding to the chemical oxidation of **A** by cerium(IV) ammonium nitrate (CAN) appeared to be very unstable. Consequently, we attempted to obtain the quinone group after electropolymerisation of monomer **A**.

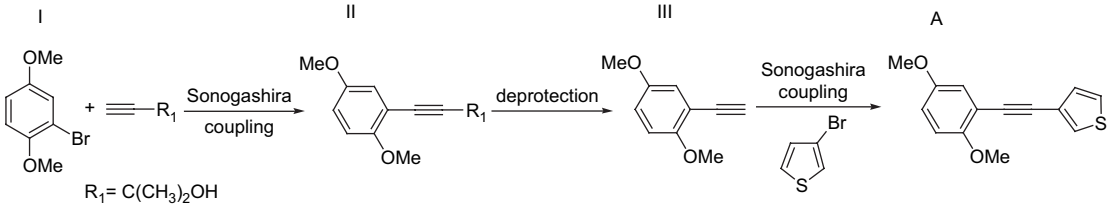
2.2. Electrochemical preparation of a polythiophene film bearing a quinone moiety

The electrodeposition of the polythiophene film was performed by successive scans in the positive potential part (0.5–1.5 V). The coverage of deposited polymer can be adjusted by varying the number of cyclical potential scans. Typically, after 20 scans corresponding to the exchange of 34 mC, the thickness of the dark green polymeric film was estimated at 0.2 μm.[19]

By chronoamperometry at 1.6 V/SCE for 5 min, the methoxy groups were oxidised to the corresponding quinonic forms. The presence of the quinone moiety was confirmed by the cyclic voltammetry experiments performed in the monomer-free buffer electrolyte (pH 7), the resulting yellow-brown film being washed with the solvent and water before this experiment. The voltammogram of the corresponding coated electrode (Fig. 1A) indeed clearly shows a reversible peak at 0.0 V/SCE (ΔE_p=40 mV) corresponding to the BQ/BHQ couple immobilised at the modified electrode. Moreover, the formation of the quinone after electrochemical oxidation of the methoxy group was confirmed by FTIR in diffuse reflectance mode (DRIFT). The DRIFT analysis of the film after oxidation shows a strong band at 1659 cm^{-1} corresponding to the presence of the quinone group. The efficiency of the electrochemical oxidation is confirmed by a large decrease of the 1221 cm^{-1} band attributed to the methoxy function observed on the film before the oxidation step.

2.3. Cyclic voltammetric response in the presence of glutathione

The voltammograms of the modified electrode bearing the quinone moiety are modified in presence of GSH in pH 7 buffer. Indeed, with 1 mM of GSH, the intensity of the current peak corresponding to the BQ/BHQ couple is time-dependent (Fig. 1B, C and D) and decreases over time. The signal corresponding to the redox couple completely disappeared after about 1 h. There are two possible different pathways through which quinone moieties could interact with GSH. In the first one, in agreement with the apparent redox potential ($E^{0'}_{GSSG/GSH}$=−0.47 V/SCE at pH 7), the quinone form could oxidise GSH to its corresponding disulfide form (GSSG) and be reduced in hydroquinone.[20] In the second pathway, the modification of the electrochemical behaviour could be due to



Scheme 2. Synthesis of 1-(3-(1,4-dimethoxyphenyl)-2-(3-thienyl))ethyne.

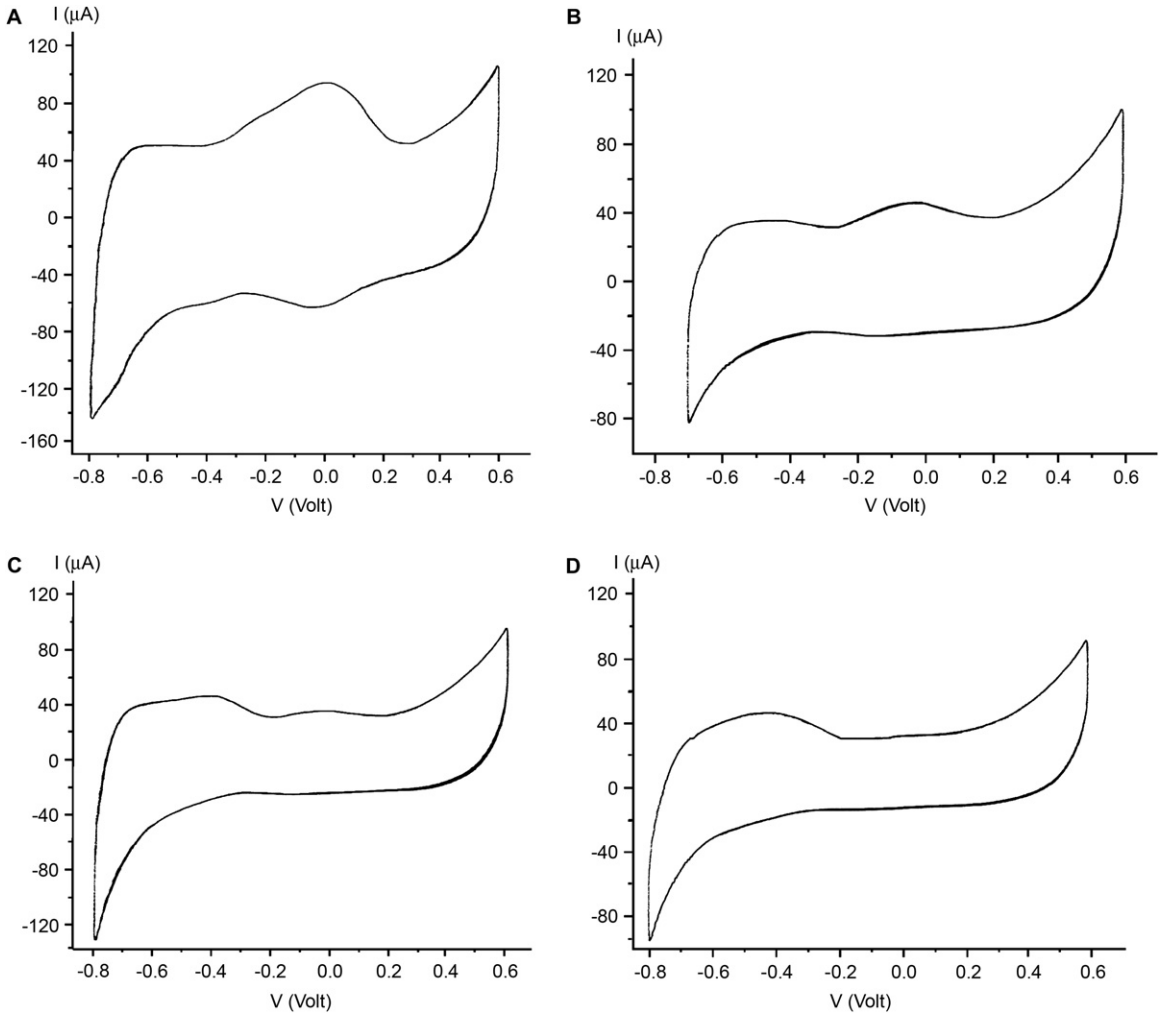


Figure 1. Cyclic voltammetric responses without or with glutathione. A: phosphate buffer pH=7; B, C and D: in presence of GSH (1 mM in phosphate buffer) after 7 min, 14 min and 60 min of reaction time, respectively. Magnetic stirring (100 rpm) between each measurement.

a nucleophilic addition as a reductive 1,4-Michael addition of GSH to BQ to form a reduced adduct.[14a,15] The second pathway is predominant as the peak corresponding to the redox couple BQ/BHQ disappeared after 1 h of reaction between GSH and the quinone. This hypothesis agrees with our preliminary results and the work of Lau et al.[15] A redox mechanism corresponding to the oxidation of GSH to GSSG by BQ should induce the formation of BHQ that can be reoxidised in quinone and consequently detected by cyclovoltammetry.

These results allowed us to consider the assay of two biologically significant reduced thiols, GSH and cysteine (CSH), by potentiometry, using this new modified electrode as an indicating sensor.

2.4. Potentiometric measurements of GSH and CSH in a pH 7 buffer solution

The potentiometric response of the modified electrode in pH 7 buffer is shown in Figure 2. Upon adding aliquots of GSH to the solution (3 to 30 μmol), the electrode potential decreases significantly. This fact is attributed to the reductive 1,4-Michael addition of GSH to the quinone moiety borne by the polythiophene film to form reduced adducts. With a higher concentration of thiol, the amount of adducts formed increases, hence, the surface concentration in the quinonic moiety decreases and the electrode potential is modified. The modified electrode potential stabilised at a value dictated by the relative surface concentrations of oxidised and reduced forms, which can be quinone, hydroquinone and adducts also in their oxidised and reduced forms because the measurements were performed with O_2 present in the solution, which can oxidise the adducts. The potential was recorded 4 min after addition of an aliquot of thiol in order to obtain a steady state

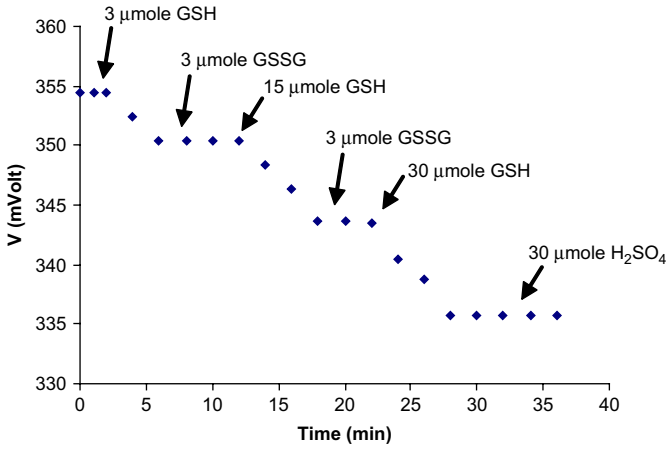


Figure 2. Response of the modified electrode after addition of several quantities of GSH, GSSG and H_2SO_4.

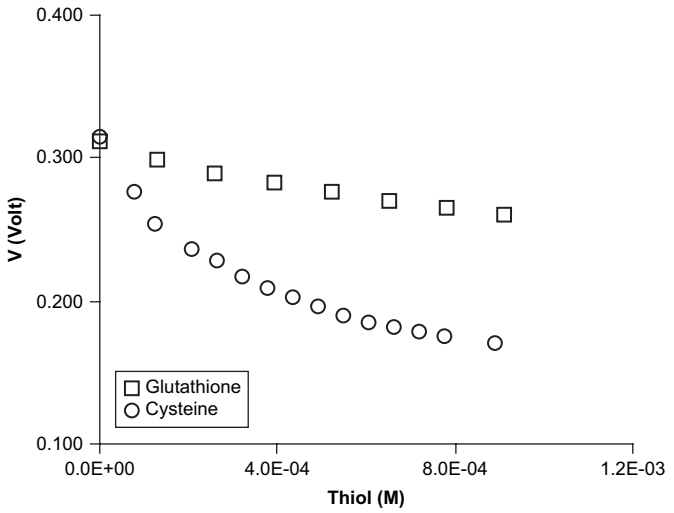


Figure 3. Effect of addition of thiol (GSH □, CSH ○) on potential electrode response in pH 7 buffer.

between the concentration of the quinone at the electrode surface and the concentration of the thiol in the solution.

It should be pointed out (Fig. 2) that the potential remains constant after the addition aliquot of GSSG. Likewise, adding acid (H_2SO_4, 0.6 mM) does not change the potential. The addition of GSH has also no effect on the potential of a polythiophene electrode prepared without quinone moiety. These experiments show clearly that the decrease of the potential is due to a reaction between GSH and the quinone on the polythiophene film. To confirm this, we studied the response of the modified electrode with the addition of CSH.

Figure 3 shows the potential responses increasing concentrations of GSH and CSH. A plateau appears, giving a maximal detection of 0.8 mM for GSH and 1 mM for CSH, respectively. This plateau is attributed to the saturation of the surface by the thiol. It appears that the modified electrode gives a highest response factor in the case of CSH than for GSH. Figure 4 shows the logarithmic dependence where a linear response is obtained with a good correlation, the slope being $-0.100\ V\ log^{-1}$[CSH] and $-0.045\ V\ log^{-1}$[GSH]. This behaviour can be explained by the steric hindrance of the analytes, CSH being far less bulky than GSH.

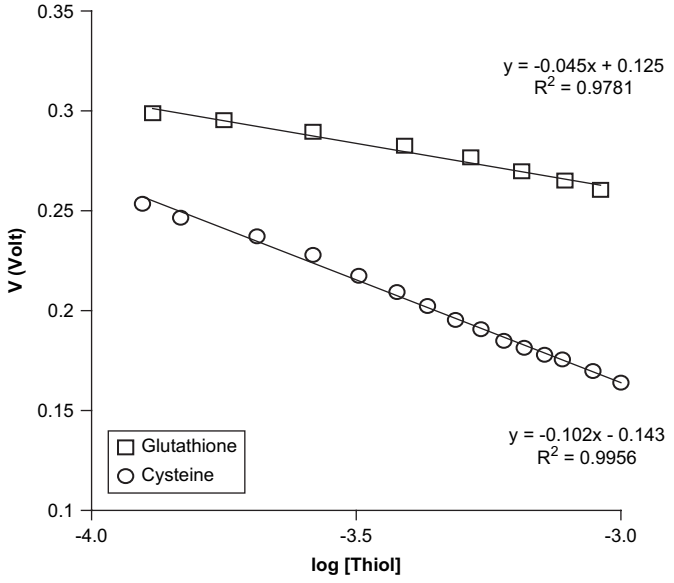


Figure 4. Calibration curve for GSH and CSH in pH 7 buffer.

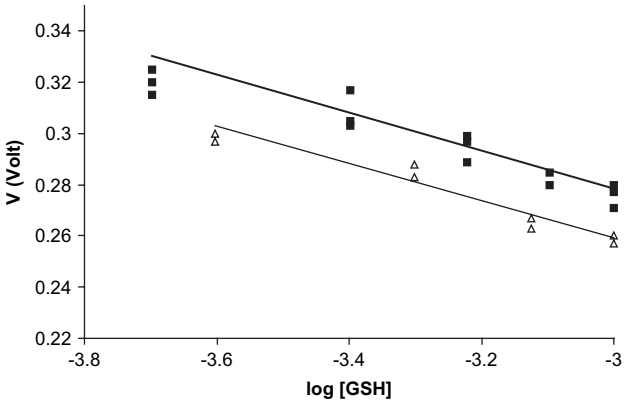


Figure 5. Comparison of electrode response of GSH in pH 7 buffer in the presence (△) and absence (■) of bovine serum albumin (10 g/L).

Therefore for the same concentration and the same electrode surface, the amount of adducts is more significant with CSH than with GSH.

2.5. Potentiometric measurements of GSH and CSH in complex media

The modified electrode was also evaluated in complex media as culture media in order to determine thiol concentration in biological samples. Because oxidative stress is increasingly involved in more and more pathological situations, it is necessary to assess the advantage of the electrode in the clinical measurements of thiols, especially with respect to the possible influence of albumin and other proteins abundant in most biological fluids.

The presence of serum albumin (10 g/L) in the potassium buffer (Fig. 5) does not affect the sensitivity with GSH concentrations up to 1 mM, where the value of potential is decreased. Then, the concentration can be estimated with respect to the log equation of a standard curve established with 0.2–1 mM GSH standards in the biological medium of interest, yielding a linear response (regression coefficient of at least 0.98) with pretty good repeatability (variation coefficient lower than 2.5%) and reproducibility (variation coefficient lower than 7%).

We compared the levels of reduced thiols (mainly GSH and CSH) in a standard 5%-foetal calf serum-completed Dulbecco's Modified Eagle Medium (DMEM), used for in vitro cell cultures of numerous mammalian cell types. As expected from previously reported data obtained after HPLC and fluorometric detection of GSH adducts,[21] the potential response measured by our modified electrode was roughly 50% lower in the medium in which the cells grew for 4 days compared with a fresh medium (data not shown). In addition, the potentials differ proportionally in successive dilutions of human and mouse serum samples in the potassium buffer. The data are in agreement with thiol uptake by the cells along culture and also suggest that our modified electrode could be a valuable tool in the control of cell culture medium in various biotechnology processes.

3. Conclusions

The electropolymerisation of the thienyl group of the new compound 1-(3-(1,4-dimethoxyphenyl)-2-(3-thienyl))ethyne allows us to obtain a modified electrode bearing quinone and hydroquinone moieties. The reactivity of the quinone group with thiols shows that this electrode appears to be a promising potentiometric sensor for thiols' analysis in biological media. It is worth emphasising that the method presented here also offers

many substantial advantages that could make it very helpful for assaying GSH and thiols as redox status markers. First, this method can be applied directly to biological sample solutions, since it does not request complicated pre-analytical steps that are susceptible to inappropriately modify the balance between the reduced and oxidised forms of the thiols,[22] which also means that the data could be expected within a few minutes. Besides, this technique could easily fit in most research and clinical chemistry laboratories since it does require neither high technical experience nor expensive equipment and reagents to acquire. This could lead to further investigations aimed at establishing the significance of oxidative stress in the clinical status of many diseases and that could be measured as a follow-up marker of therapeutic efficiency. Additional experiments are now required with the view to improve the use of our modified electrode and to conciliate the whole technique with the requests of these laboratories and the restrained sample volumes usually available.

4. Experimental

4.1. Reagents

All chemicals from commercial sources were of analytical grade. All aqueous solutions were prepared daily using deionised water with a resistivity superior to 18 MΩ.

4.2. Syntheses

4.2.1. Synthesis of 2-bromo-1,4-dimethoxybenzene

para-Dimethoxybenzene (5.0 g, 36.2 mmol, 1 equiv) in acetonitrile (150 mL) was stirred at room temperature until complete dissolution. *N*-Bromosuccinimide (6.44 g, 36.2 mmol, 1 equiv) was added and the reaction mixture was stirred at room temperature for 6 h. The mixture was concentrated under vacuum. The residue was washed with water (50 mL) and extracted with diethylether (3×30 mL). The organic layer was dried and concentrated under vacuum. The residue was purified by distillation (100 °C, 4×10^{-2} mbar) giving an orange oil. Yield was 90%.

^{1}H NMR: (250 MHz, $CDCl_3$): δ=7.13 (d, J=2.2 Hz, 1H), 6.84–6.82 (m, 2H), 3.84 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=153.9, 150.1, 118.9, 112.8, 111.8, 56.8, 55.6.

4.2.2. Synthesis of 2-methyl-4-(2,5-dimethoxyphenyl)-3-butyne-2-ol

Palladium(II) acetate (25 mg, 2 mol %) was added under argon to a solution of triphenylphosphine (73 mg, 5 mol %) in 25 mL of triethylamine. 2-Bromo-1,4-dimethoxybenzene (1.23 g, 5.6 mmol, 1 equiv) was incorporated and then copper(I) iodide (32 mg, 3 mol %). 2-Methyl-3-butyn-2-ol (0.7 mL, 6.7 mmol, 1.2 equiv) dissolved in 20 mL of triethylamine was then added and the reaction mixture heated overnight at 80 °C. After returning to room temperature, the triethylamine bromohydrate was filtered and rinsed several times with diethylether. The filtrate was concentrated under vacuum and the coupling product purified by column chromatography on silica gel using chloroform as eluent. Orange oil. Yield was 40%.

^{1}H NMR (250 MHz, $CDCl_3$): δ=6.92 (d, J=3.0 Hz, 1H), 6.82–6.80 (m, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 1.64 (s, 6H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=154.1, 152.9, 115.8, 112.4, 112.1, 111.8, 83.9, 77.9, 65.2, 56.3, 55.5, 31.0, 30.8.

4.2.3. Synthesis of (2,5-dimethoxyphenyl)ethyne

Sodium hydroxide powder (80 mg, 2 mmol, 0.85 equiv) was added to a solution of 2-methyl-4-(2,5-dimethoxyphenyl)-3-butyne-2-ol (514 mg, 2.34 mmol) in 9 mL of toluene. The reaction mixture was heated at reflux under argon for 4 h. After returning to room temperature, the mixture was hydrolysed in 15 mL of water and extracted with diethylether. The organic layer was dried with sulfate magnesium, filtered and concentrated under vacuum giving brown oil. Yield was 90%.

^{1}H NMR (250 MHz, $CDCl_3$): δ=7.0 (d, J=2.9 Hz, 1H), 6.91–6.88 (m, 2H), 3.85 (s, 3H), 3.76 (s, 3H), 3.30 (s, 1H).

4.2.4. Synthesis of 3-((2,5-dimethoxyphenyl)ethynyl)thiophene

To a solution of triphenylphosphine (189 mg, 0.72 mmol, 4 mol %) in 20 mL of anhydrous THF, palladium(II) chloride (64 mg, 0.36 mmol, 2 mol %) was added under argon. Then, copper(I) iodide (137 mg, 0.72 mmol, 4 mol %), 3-bromothiophene (2.9 g, 18 mmol, 1 equiv) and triethylamine (3.6 mL, 27 mmol, 1.5 equiv) were incorporated. A solution of alkyne (2.5 g, 15.4 mmol, 0.86 equiv) dissolved in 5 mL of anhydrous THF was added over a 1 h period. The reaction mixture was stirred overnight at room temperature. Triethylamine bromohydrate then formed was filtered and rinsed several times with diethylether. The filtrate was concentrated under vacuum and the coupling product purified by column chromatography on silica gel using chloroform as eluent. Brown oil. Yield was 40%.

^{1}H NMR (250 MHz, $CDCl_3$): δ=7.57–7.54 (m, 1H), 7.24–7.33 (m, 2H), 7.0 (m, 1H), 6.86–6.84 (m, 1H), 3.9 (s, 3H), 3.8 (s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=154.5, 153.3, 131.6, 128.2, 123.4, 118.1, 115.8, 113.0, 112.2, 112.1, 85.7, 85.1, 56.5, 55.8.

4.3. Electrochemical experiments

All cyclic voltammograms were carried out in a conventional three-electrode electrochemical cell under argon with a PGP 201 Radiometer potentiostat.

All potentials in the text were quoted versus a saturated calomel electrode (SCE). The working electrode was a 10 mm Pt wire (0.1 cm^2) and the counter-electrode was a 1 cm^2 vitreous carbon rod.

4.3.1. Formation of film by electropolymerisation

The electropolymerisation of **A** in CH_3CN with 0.1 M $LiClO_4$ as supported electrolyte was performed by repeatedly scanning the potential region from 0.5 to 1.5 V at a scan rate of 100 mV/s. The thickness of the polythiophene film was controlled by monitoring the amount of charge passed during the polymerisation.

4.3.2. Electrochemical preparation of the quinone active form

The electrochemical deprotection of the methoxy groups to obtain the quinone active form was obtained by holding the potential at 1.6 V/SCE for about 5 min until the current was constant and near zero. The formation of a surface-bound quinone was evidenced by cyclic voltammetry.

4.3.3. Potentiometric measurements

Measurements were carried out at room temperature (21±2 °C) in a cell volume of 50 mL using a PHM 210 Radiometer voltammeter operating under zero current. Without other specification, the thiols and biological samples were added at appropriate concentrations in 50 mL of potassium phosphate buffer (0.05 M pH 7). Measurements in biological media were performed using bovine serum albumin (10 g/L) in pH 7 buffer or in a standard 5%-foetal calf serum-completed DMEM.

Both modified electrode and reference electrode were immersed in solutions stirred magnetically. The potentials were recorded 4 min after thiol addition, i.e., when the electrode response was stable.

4.4. DRIFT characterisation

DRIFT spectra were recorded with a Mattson 3000 spectrometer by adding 100 scans from 4000 to 400 cm^{-1} with 2 cm^{-1} resolution using anhydrous KBr as background.

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References and notes

1. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T.; Mazur, M.; Telser, J. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44–84.
2. Kleinman, W. A.; Richie, J. P., Jr. *Biochem. Pharmacol.* **2000**, *60*, 19–29.
3. Dickinson, D. A.; Forman, H. J. *Ann. N.Y. Acad. Sci.* **2002**, *973*, 488–504.
4. Parmentier, C.; Leroy, P.; Wellman, M.; Nicolas, A. *J. Chromatogr., B* **1998**, *719*, 37–46.
5. Diez, L.; Martenka, E.; Dabrowska, A.; Coulon, J.; Leroy, P. *J. Chromatogr., B* **2005**, *827*, 44–50.
6. Kruusma, J.; Beham, A. M.; Williams, J. A. G.; Kataky, R. *Analyst* **2006**, *131*, 459–473.
7. Ricci, F.; Arduini, F.; Tuta, C. S.; Sozzo, U.; Moscone, D.; Amine, A.; Palleschi, G. *Anal. Chim. Acta* **2006**, *558*, 164–170.
8. Pereira-Rodrigues, N.; Cofre, R.; Zagal, J. H.; Bedioui, F. *Bioelectrochemistry* **2007**, *70*, 147–154.
9. Sehlotho, N.; Nyokong, T.; Zagal, J. H.; Bedioui, F. *Electrochim. Acta* **2006**, *51*, 5125–5132.
10. Rover, L.; Kubota, L. T.; Hoehr, N. F. *Clin. Chim. Acta* **2001**, *308*, 55–67.
11. Inoue, T.; Kirchoff, J. R. *Anal. Chem.* **2000**, *72*, 5755–5760.
12. Brunmark, A.; Cadenas, E. *Free Radical Biol. Med.* **1989**, *6*, 149–165.
13. Cox, J. A.; Gray, T. J. *Electroanalysis* **1990**, *2*, 107–111.
14. (a) Gracheva, S.; Livingstone, C.; Davis, J. *Anal. Chem.* **2004**, *76*, 3833–3836; (b) Yonge, L.; Gracheva, S.; Wilkins, S. J.; Livingstone, C.; Davis, J. *J. Am. Chem. Soc.* **2004**, *126*, 7732–7733.
15. Lau, S. S.; Hill, B. A.; Highet, R. J.; Monks, T. J. *Mol. Pharmacol.* **1988**, *34*, 829–836.
16. Jeffery, T. *Tetrahedron Lett.* **1994**, *35*, 3051–3054.
17. Greene, T. W.; Wuts, P. G. M. Protective Groups in Organic Synthesis, 3rd ed.; John Wiley and Sons: New York, NY, 1999, pp 246–292.
18. Thorand, S.; Krause, N. *J. Org. Chem.* **1998**, *63*, 8551–8553.
19. Diaz, F. A.; Castillo, J. I. *J. Chem. Soc., Chem. Commun.* **1980**, 397–398.
20. *Lehninger Principles of Biochemistry*; Nelson, D. L., Cox, M. M., Eds.; W. H. Freeman: New York, NY, 2005.
21. Thioudellet, C.; Oster, T.; Wellman, M.; Siest, G. *Eur. J. Biochem.* **1994**, *222*, 1009–1016.
22. Thioudellet, C.; Oster, T.; Leroy, P.; Nicolas, A.; Wellman, M. *Cell Biol. Toxicol.* **1995**, *11*, 103–111.