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Covalent Tethering of Organic Functionality to the Surface of Glassy Carbon Electrodes by Using Electrochemical and Solid-Phase Synthesis Methodologies

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Abstract: Organic linkers such as (*N*-Boc-aminomethyl)phenyl (BocNHCH$_2$C$_6$H$_4$) and *N*-Boc-ethylenediamine (Boc-EDA) have been covalently tethered onto a glassy carbon surface by employing electrochemical reduction of BocNHCH$_2$C$_6$H$_4$ diazonium salt or oxidation of Boc-EDA. After removal of the Boc group, anthraquinone as a redox model was attached to the linker by a solid-phase coupling reaction. Grafting of anthraquinone to electrodes bearing a second spacer such as 4-(*N*-Boc-aminomethyl)benzoic acid or *N*-Boc-β-alanine was also performed by following this methodology. The surface coverage, stability and electron transfer to/from the tethered anthraquinone redox group through the linkers were investigated by cyclic voltammetry. The effects of pH and scan rate were studied, and the electron-transfer coefficient and rate constant were determined by using Laviron's equation for the different types of linker. The combination of electrochemical attachment of protected linkers and subsequent modifications under the conditions of solid-phase synthesis provides a very versatile methodology for tailoring a wide range of organic functional arrangements on a glassy carbon surface.

Keywords: cyclic voltammetry · electrochemistry · quinones · solid-phase synthesis · surface chemistry

Introduction

Covalent attachment of monolayers of specific organic functional groups to conducting surfaces has received great attention because of its benefits for potential application in molecular electronics, bioelectronics, sensors, surface adhesion, and corrosion protection. Two methods, recently reviewed by Downard[1] and by Pinson and Podvorica,[2] are available to covalently bond functional organic monolayers directly to the surfaces of carbon, metal or semiconductor electrodes. The first method is based on the electrochemical generation of primary or secondary amine radical cations, which then form covalent bonds to the carbon surface. A wide range of amine-modified electrodes has been made by this amine electro-oxidation method.[1,3–7] However, this modification method has the disadvantage of forming polymeric chains or bridge structures on the carbon surface when an organic substrate bearing two amino groups is used.[1,3] In the second method, developed by Pinson et al.[3] the covalent bond between the organic molecule and the carbon electrode surface is formed by electrochemical reduction of aryl diazonium salts.[2,3,8] The literature on modified electrodes prepared by this method and bearing a wide variety of aromatic functionality has been reviewed.[1,2] These attachment methods show good reproducibility, and the covalently bonded organic layers produced by them were found to be stable for long-term storage and upon sonication in aggressive solvents.[8] Further chemical modification can be performed on the electrodes through specific substituents (e.g., –COOH,[9] –SO$_3$H,[9] –NMe$_2$[9] or –CH$_2$Cl[10,11]) present on the aromatic ring and used to bond metal complexes,[9] enzymes,[12] or as potential supports for combinatorial chemistry.[10] Of particular relevance to our work described here, Pinson, Savéant et al. attached both *p*-nitrophenol and *p*-acetamidophenyl radicals onto a carbon surface using diazonium salts. Electrochemical reduction of

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the resulting nitro group or acid hydrolysis of the acetamide group provided a route to aminophenyl-modified carbon surfaces which could be further functionalised with epichlorohydrin and characterised by XPS.[13] The major disadvantage of using electrochemical reduction of substituted aryl diazonium salts for surface modification is the low stability and availability of diazonium salts bearing suitable functional groups. In addition, under some circumstances multilayers of substituted aromatic compounds can be formed by electrolysis of diazonium salts, as reported by McDermott and Kariuki[14] and Podvorica et al.[15] Nevertheless, the robust linkage between the carbon surface and the modifier makes the covalently attached monolayer very suitable for further chemical modification.

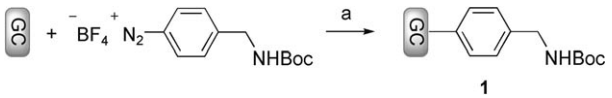
Here we describe a novel approach in which, for the first time, linkers bearing a Boc-protected amino group have been grafted onto carbon surfaces. This avoids the formation of polymeric chains or bridge structures in the oxidation of a diamine linker and solves the problem of stability and difficulties of synthesising diazonium salts with different functional groups. After initial grafting of the protected linker at the carbon surface, the protecting group can be removed to give a free amino group with high surface coverage. Further modification with various organic molecules can be made at this reactive site by using solid-phase synthesis methodology, and modified carbon surfaces can subsequently be characterised by cyclic voltammetry. This therefore provides a more general and flexible approach to the synthesis of modified carbon electrodes than those used hitherto.

Results and Discussion

Two different linkers, (*N*-Boc-aminomethyl)phenyl (BocNH-$CH_2C_6H_4$) and *N*-Boc-ethylenediamine (Boc-EDA), were immobilised by electrochemical reduction of $BocNHCH_2C_6H_4$ diazonium salt and electrochemical oxidation of Boc-EDA, respectively, at glassy carbon (GC) electrodes. After removal of the Boc group, coupling of anthraquinone 2-carboxylic acid directly to the linker or via a second spacer such as 4-(*N*-Boc-aminomethyl)benzoic acid or *N*-Boc-β-alanine, was achieved by using solid-phase synthesis methodologies. Anthraquinone (AQ) was chosen in these model studies because of its application in electroanalytical chemistry and its reversible redox behaviour.[16] In addition, coupling of the anthraquinone redox group through different spacers allows the modification efficiency and yield of each attachment step to be monitored electrochemically. Anthraquinone has previously been directly attached on carbon[13,17,18] or nickel electrodes[19] by electrochemical reduction of the corresponding diazonium salt or electrochemical oxidation of the corresponding carboxylate[20] but, to our knowledge, there are no examples in the literature of grafting of anthraquinone onto a carbon surface by solid-phase coupling reactions.

The surface coverage, stability and electron transfer to/from the anthraquinone redox group tethered to the glassy carbon electrode through the different linkers were explored by cyclic voltammetry (CV). The effects of pH and scan rate were also studied, and the electron-transfer rate constants determined by using Laviron's equation for the different spacers.

Electrochemical immobilisation of $BocNHCH_2C_6H_4$ and Boc-EDA linkers on GC surface: Immobilisation of the $BocNHCH_2C_6H_4$ linker on the GC electrode was carried out by electrochemical reduction of the corresponding diazonium tetrafluoroborate salt to give electrode **1** (Scheme 1).[2]



Scheme 1. Electrochemical immobilisation of $C_6H_4CH_2NHBoc$ on the GC surface (electrode **1**). a) 0.6 to −1.0 V versus Ag/AgCl, CH_3CN.

4-(*N*-Boc-aminomethyl)benzene diazonium tetrafluoroborate salt was obtained in two steps from 4-aminobenzylamine: first, regiospecific Boc protection of the amino group in the benzylic position[21] in 97% yield was carried out, followed by formation of the diazonium salt[22] in 86% yield. Figure 1 shows consecutive cyclic voltammetric scans in

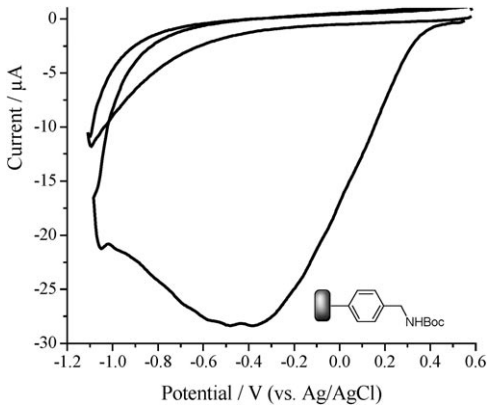
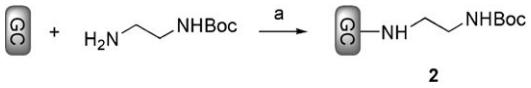


Figure 1. Cyclic voltammogram recorded at a scan rate of 50 mV s^{-1} for 3 mm diameter GC electrode in 3 mM 4-(*N*-Boc-aminomethyl)benzene diazonium tetrafluoroborate salt in acetonitrile with 0.1 M TBATFB.

5 mM $BocNHCH_2C_6H_4$ diazonium salt in acetonitrile at a GC electrode. In the first scan at 50 mV s^{-1} a rather broad irreversible peak is obtained starting from +0.4 V and reaching a peak at −0.4 V versus Ag/AgCl; this was attributed, as shown in Scheme 1, to one-electron reduction of the $BocNHCH_2C_6H_4$ diazonium salt and formation of the aromatic radical, which bonds to the GC surface.[2] Continuing cycling in the same solution leads to complete disappearance of the cathodic peak in the second and subsequent cycles. This indicates blocking of the electrode by the organic layer generated during the first cycle, and this is consistent with the results obtained in the literature for the reduction of diazonium compounds.[1,2] A charge of −2.6 mC cm^{-2}

was obtained by integration of the area under the first scan after baseline correction. However, it is difficult to relate this charge accurately to surface coverage or number of immobilised layers, since the reduction efficiency and molecular orientation are not known.

Electrode **2** was prepared by electrochemical immobilisation of Boc-EDA as linker on the surface of the GC electrode (Scheme 2). Figure 2 shows the cyclic voltammogram



Scheme 2. Electrochemical immobilisation of Boc-EDA on the GC surface (electrode **2**). a) 0.5 to 1.8 V versus Ag/AgCl, CH_3CN.

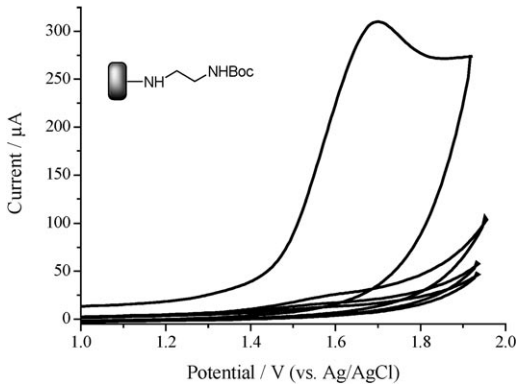
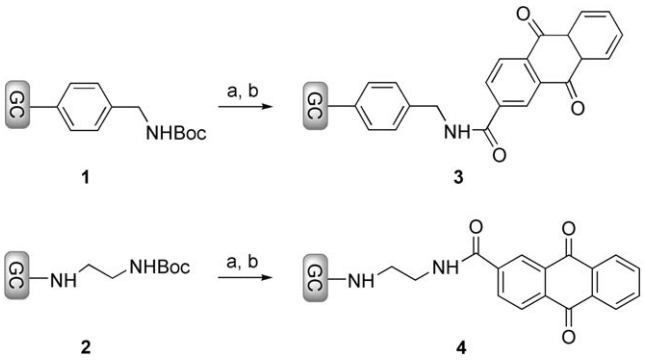


Figure 2. Cyclic voltammogram recorded at a scan rate of 50 mV s^{-1} for 3 mm diameter GC electrode in 10 mM *N*-Boc-ethylenediamine in acetonitrile with 0.1 M TBATFB.

for the electrochemical oxidation of 10 mM Boc-EDA in 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) and acetonitrile. It shows a single broad irreversible peak starting from +1.4 V and reaching a peak at about +1.6 V versus Ag/AgCl. The absence of the corresponding cathodic peak on the reverse scan indicates that the amine radical cation generated during the forward scan undergoes fast chemical reaction, either binding to the GC surface (as shown in Scheme 2) or forming dimers or polymeric species in solution.[23] In subsequent scans, complete disappearance of the oxidation peak indicates that the electrode has been passivated by grafting of a Boc-EDA layer to the GC electrode surface.[3] The charge obtained by integrating the area under the peak (first scan) is about 100 times larger than that required to form a monolayer of Boc-EDA (2.0 nmol cm^{-2}).[4] This could be attributed to the occurrence of side reactions, such as dimerization of the radicals in the solution, which contribute to the overall current.[23]

No attempt was made to measure the surface coverage of GC with the $BocNHCH_2C_6H_4$ or Boc-EDA linker, because the subsequent coupling of anthraquinone allows us to estimate the surface coverage directly using cyclic voltammetry.

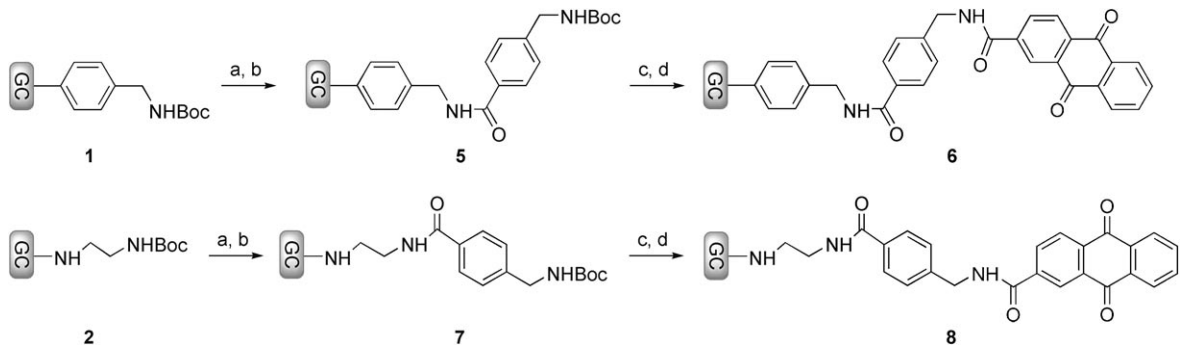
Synthetic modification of glassy carbon by anthraquinone redox groups and cyclic voltammetric characterisation: The Boc groups of electrodes **1** and **2** were removed by a deprotection reaction with a 4.0 M HCl solution in dioxane. Ninhydrin tests (colorimetric test used in solid-phase synthesis to monitor the deprotection of primary amines[25]) on electrodes **1** and **2** were negative before deprotection but positive when performed after removal of the Boc group, that is, the deprotection reaction was successful. This result is only qualitative and the true yield of deprotection was not determined. The deprotected modified carbon surfaces are analogues of aminomethyl resins classically used in solid-phase synthesis, and therefore solid-phase synthetic methodologies can now be applied to further develop the surface modification. The electrodes were coupled with anthraquinone-2-carboxylic acid in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent and *N*,*N*-diisopropylethylamine (DIEA) in *N*,*N*-dimethylformamide (DMF) at room temperature for 16 h to give electrodes **3** and **4**, respectively (Scheme 3).



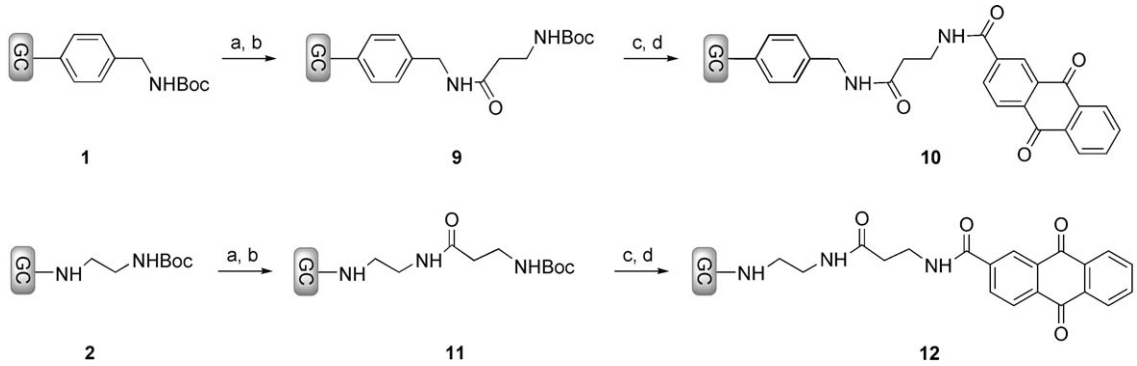
Scheme 3. Synthesis of anthraquinone-modified electrodes **3** and **4**. a) 4.0 M HCl in dioxane, RT, 1 h. b) Anthraquinone-2-carboxylic acid, HBTU, DIEA, DMF, RT, 16 h.

A spacer was added to another set of electrodes **1** and **2** by using solid-phase synthetic methodology. 4-(*N*-Boc-aminomethyl)benzoic acid was obtained in 85% yield by treating di-*tert*-butyl dicarbonate with 4-(aminomethyl)benzoic acid in dioxane/water (1/1) in the presence of 1 M NaOH[26] and, after removal of the Boc groups from electrodes **1** and **2**, was then linked to the surface by a coupling reaction with HBTU and DIEA leading to electrodes **5** and **7**. The Boc group on the spacer was then removed and anthraquinone-2-carboxylic acid was coupled onto the GC surface by using the same conditions as before to give electrodes **6** and **8**, respectively (Scheme 4).

To demonstrate the flexibility of this synthetic strategy, we varied the type of the spacer starting with another set of electrodes **1** and **2**. Thus, *N*-Boc-β-alanine was coupled with the Boc-deprotected electrodes to obtain electrodes **9** and **11**. These electrodes were deprotected and coupled with anthraquinone-2-carboxylic acid to give electrodes **10** and **12**, respectively (Scheme 5).



Scheme 4. Synthesis of anthraquinone-modified electrodes **6** and **8** with $C_6H_4CH_2NHBoc$ as spacer. a) 4.0 M HCl in dioxane, RT, 1 h. b) 4-(*N*-Boc-aminomethyl)benzoic acid, HBTU, DIEA, DMF, RT, 16 h. c) 4.0 M HCl in dioxane, RT, 1 h. d) Anthraquinone-2-carboxylic acid, HBTU, DIEA, DMF, RT, 16 h.



Scheme 5. Synthesis of the anthraquinone-modified electrodes **10** and **12** with Boc-β-Ala as spacer. a) 4.0 M HCl in dioxane, RT, 1 h. b) *N*-Boc-β-alanine, HBTU, DIEA, DMF, RT, 16 h. c) 4.0 M HCl in dioxane, RT, 1 h. d) Anthraquinone-2-carboxylic acid, HBTU, DIEA, DMF, RT, 16 h.

After the chemical modifications, electrodes **3**, **4**, **6**, **8**, **10** and **12** were sonicated in DMF and ethanol for 5 min and then rinsed with deionised water and characterised by electrochemistry. Figure 3 shows the stable cyclic voltammetry in 0.1 M phosphate buffer solution (PBS, pH 7), recorded at a scan rate of 50 mV s^{-1} for the glassy carbon electrodes functionalised with AQ redox groups and various modes of attachment to the electrode surface. All voltammograms show the characteristic redox peaks for AQ around −0.48 V versus SCE. As expected for surface-immobilised redox centres, the peak currents are linearly proportional to scan rate (see Figure 7b below) and the ratio between oxidation and reduction peak currents at any given scan rate is close to unity. However, on the first scans (not shown), the modified GC electrodes showed 8–10% larger reduction peak currents, before remaining stable for more than 100 cycles. This is most likely attributable to desorption of some non-covalently bonded AQ molecules.[18]

Table 1 reports the values of the peak separation ΔE, the midpoint potential E_{mp} [Eq. (1)] and the surface coverage Γ for the different electrodes in 0.1 M PBS (pH 7).

$$E_{mp} = (E_p^a - E_p^c)/2 \quad (1)$$

The AQ redox potential E_{mp} varies slightly for the different linkages (Table 1) with an average value of -475 ± 5 mV in 0.1 M PBS for all the modified electrodes. Thus, the type of linkage does not appear to have a significant effect on the thermodynamics of anthraquinone oxidation and reduction. On the other hand, the type of linkage has a significant effect on the kinetics of the reactions, as shown by the differences in peak separation, ΔE. The EDA linker (electrode **4**) showed the smallest values for ΔE, with 41, 112 and 132 mV in basic, acidic and neutral solution respectively. The diazo linker with the alanine spacer (electrode **10**) showed the largest ΔE value of 232 mV in PBS. Based on the peak-separation data, the electron-transfer kinetics are consistently faster for structures with the EDA linker rather than the diazo linker. The charges passed in oxidation or reduction of the immobilised anthraquinone are given in Table 1 based, in each case, on measurements on three replicate electrodes. The associated surface coverages Γ are calculated from Faraday's law [Eq. (2)]

$$\Gamma = Q/nFA\rho \quad (2)$$

where Q is the charge obtained from integration of the baseline-corrected area under the oxidation or reduction peak, F the Faraday constant, A the geometric area of the electrode (0.071 cm^2), $n = 2$ the number of electrons transferred, and ρ is the roughness of the electrode. Typical values of ρ for polished GC electrodes of the type used here

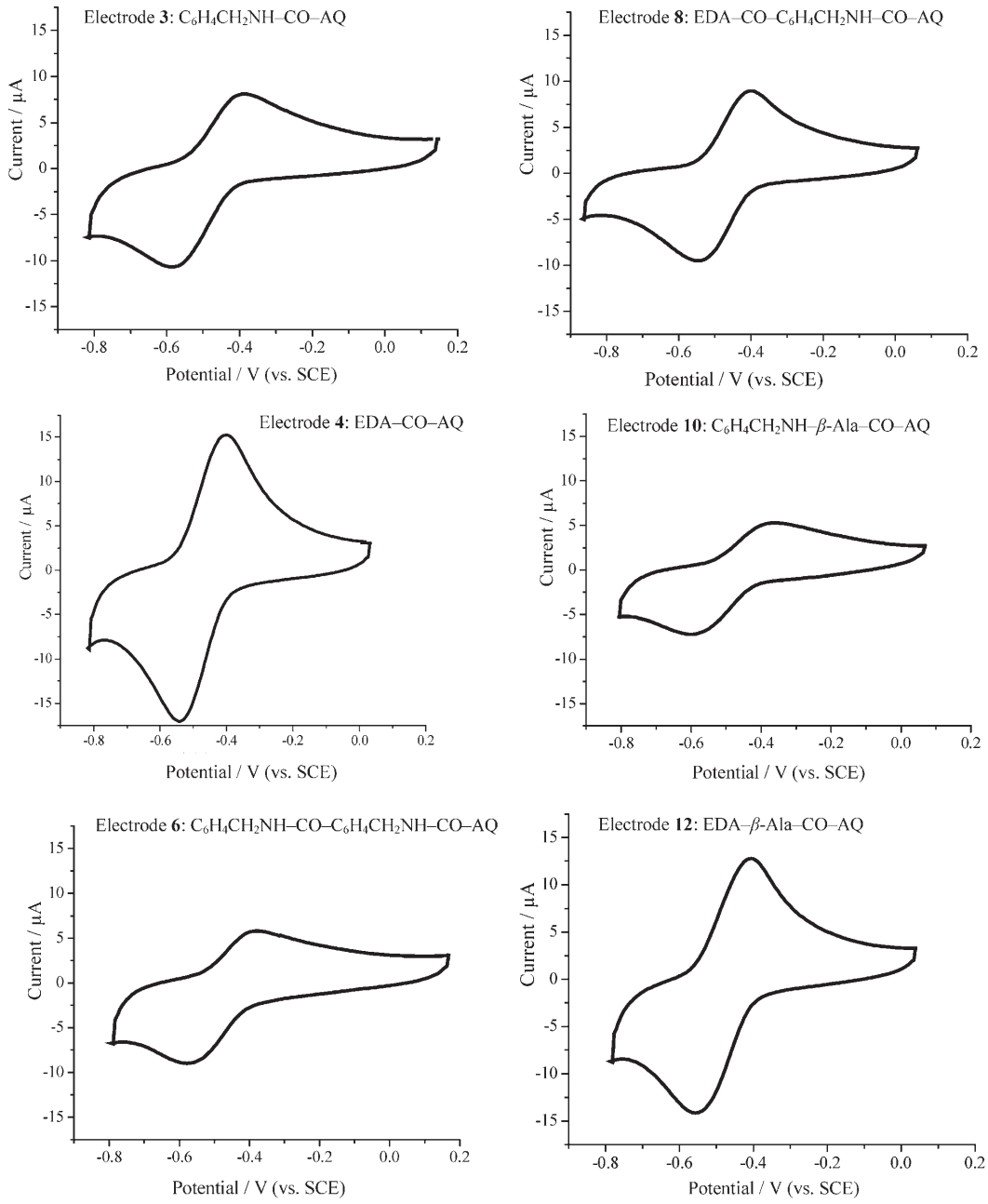


Figure 3. Cyclic voltammograms recorded at a scan rate of 50 mV s^{-1} in 0.1 M PBS, pH 7, for 3 mm diameter GC electrodes modified by AQ through various linkages.

Table 1. AQ separation peak, midpoint potential and surface coverage for modified electrodes in 0.1 M phosphate buffer solution.

Electrode	ΔE [mV]	$E_{mp} \pm 3$ [mV]	$Q \pm 0.01$ [mC cm^{-2}]	$\Gamma \pm 0.1$ [nmol cm^{-2}]
3 ($C_6H_4CH_2NH$-CO-AQ)	186	−485	0.540	0.70
4 (EDA-CO-AQ)	132	−472	0.944	1.22
6 ($C_6H_4CH_2NH$-CO-$C_6H_4CH_2NH$-CO-AQ)	189	−475	0.463	0.60
8 (EDA-CO-$C_6H_4CH_2NH$-CO-AQ)	142	−471	0.494	0.64
10 ($C_6H_4CH_2NH$-β-Ala-CO-AQ)	232	−479	0.386	0.50
12 (EDA-β-Ala-CO-AQ)	146	−483	0.800	1.05

lie between 4 and 8 and a value of 4 was used in our calculations. The coverages in Table 1, given the uncertainty in the roughness of the electrodes, are consistent with approximately monolayer coverage of the AQ groups. The results fall into two groups. For electrodes **4** and **12**, where the linker is an alkyl chain, the coverages are approximately twice as large as for electrodes **3**, **6**, **8**, and **10**, which have a more bulky aryl ring within the linker. The other trend which emerges from Table 1 is that the AQ coverage falls slightly as the number of linking steps increases, so that the coverage of electrode **4** is greater than that of electrode **12**, and that of electrode **3** is greater than those of electrodes **6**

and **10**. This is expected if the coupling efficiency is not 100% at each step. Finally, comparing the coverages for the AQ-modified electrodes with the calculated coverage for a full monolayer of EDA[4] we find, as expected, that our coverages are somewhat smaller, presumably due to the presence of the bulkier AQ group. Pinson et al. reported that, after grafting of EDA onto carbon surfaces, approximately 75% of the diamine molecules were bonded through both nitrogen atoms in a bridge configuration, and only 25% through one nitrogen atom.[3] A subsequent quantification of unprotected EDA-modified carbon fibre by Antoniadou et al. showed that only 1% of free amino groups were available and could be coupled to an electroactive group.[23] Using Boc-EDA means that only the unprotected primary amine is bonded to the GC surface, as the protected amine is both less reactive and the bulky Boc group sterically hinders access to the active sites on the surface of GC.[4]

To investigate the effect of the surface coverage of the linker on the final AQ surface coverage, we altered the surface coverage of the linker by varying the amount of charge passing during immobilisation of EDA-Boc and then coupled the anthraquinone to the different surfaces. Figure 4

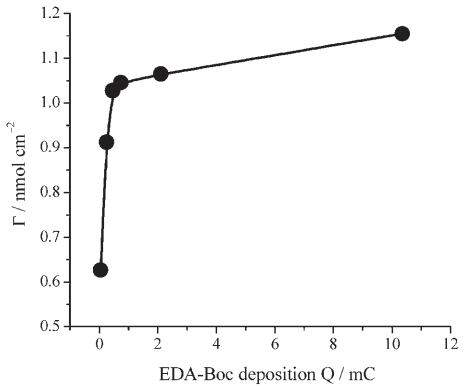


Figure 4. The effect of charge for immobilisation of EDA-Boc on the AQ surface coverage for EDA-CO-AQ-modified electrode **4**.

shows a plot of AQ surface coverage as a function of the charge passed in deposition for EDA-CO-AQ (electrode **4**). The AQ coverage increases with the charge passed in the initial coupling of EDA-Boc to the electrode but then reaches saturation quite sharply after the passage of about 0.2 mC cm^{-2} of charge during EDA-Boc coupling. This charge is consistent with monolayer coverage of EDA on the surface.

The stability of the AQ-modified electrodes was monitored by recording CVs in PBS at pH 7 for different storage times in air at room temperature. Table 2 shows the change in ΔE, E_{mp} and Γ for electrodes modified with $C_6H_4CH_2NH$-CO-AQ (electrode **3**) and EDA-CO-AQ (electrode **4**) over a period of 20 d. The data reveal very small changes in ΔE and E_{mp}, and the surface coverage gradually decreased by 23% after 20 d. There does not appear to be any significant difference in stability for the two linkages.

Table 2. Stability of anthraquinone-modified electrodes **3** and **4** over time at pH 7 (0.1 M PBS).

Electrode	t [days]	ΔE [mV]	$E_{mp} \pm 3$ [mV]	$\Gamma \pm 0.1$ [nmol cm^{-2}]
3 ($C_6H_4CH_2NH$-CO-AQ)	0	186	−485	0.70
3 ($C_6H_4CH_2NH$-CO-AQ)	2	182	−496	0.62
3 ($C_6H_4CH_2NH$-CO-AQ)	10	178	−492	0.58
3 ($C_6H_4CH_2NH$-CO-AQ)	20	180	−500	0.55
4 (EDA-CO-AQ)	0	132	−472	1.22
4 (EDA-CO-AQ)	2	115	−475	0.96
4 (EDA-CO-AQ)	10	109	−480	0.92
4 (EDA-CO-AQ)	20	111	−479	0.89

Effect of pH: The AQ redox couple is known to be pH-dependent because it undergoes two-proton/two-electron reduction to give the corresponding hydroquinone derivative. Figure 5 shows representative voltammograms for a GC electrode modified by EDA-CO-$C_6H_4CH_2NH$-CO-AQ (electrode **8**) in 0.1 M NaOH, 0.1 M PBS (pH 7) and 0.1 M H_2SO_4 solutions.

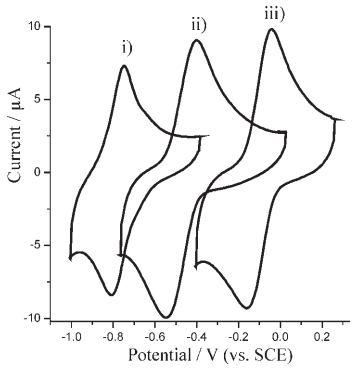


Figure 5. Cyclic voltammograms at a scan rate of 50 mV s^{-1} for EDA-$C_6H_4CH_2NH$-CO-AQ-modified electrode **8** in solutions of i) 0.1 M NaOH, ii) 0.1 M PBS, pH 7 and iii) 0.1 M H_2SO_4.

Each voltammogram shows the AQ redox peaks for all studied pH values, and in strongly acidic solution E_{mp} shifts to more positive potentials (about −106 mV), while in strongly basic solution E_{mp} is about −775 mV. This shift in E_{mp} with pH is consistent with the $2e^-/2H^+$ anthraquinone redox system. In 0.1 M NaOH solution, there is a slight decrease in the peak currents, which is probably due to hydrolysis of the immobilised anthraquinone at higher pH.[28,29] This hydroquinone hydrolysis is not recovered by cycling in acid, as confirmed by our own experiments. Figure 6 shows the relation between E_{mp} of anthraquinone-modified electrode **4** and pH in the range from 1 to 13. In the pH range from 1 to 8, E_{mp} is linearly shifted to more negative potential with a slope of 59 mV per pH unit, which is equal to the anticipated Nernstian value for a two-electron/two-proton process. Above pH 8, the slope changes to 35 mV per pH unit, corresponding to a two-electron/one-proton mechanism. This is consistent with the pK_a of 7.5 for anthraqui-

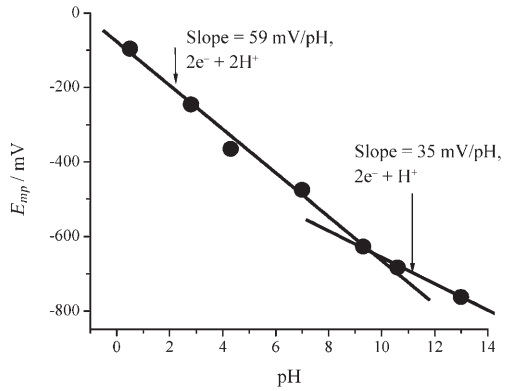


Figure 6. Dependence of AQ midpoint potential E_{mp} on pH for EDA-CO-AQ-modified electrode **4** in 0.1 M PBS solution.

none and indicates that the pK_a of anthraquinone is not changed by immobilisation.

Effect of scan rate: The effect of scan rate on the electrochemical behaviour of the $C_6H_4CH_2NH$-CO-AQ (**3**) and EDA-CO-AQ (**4**) electrodes was studied to investigate the effect of the spacer on the heterogeneous electron-transfer kinetics. Figure 7 shows a series of cyclic voltammograms at different scan rates in PBS (pH 7) for $C_6H_4CH_2NH$-CO-AQ-modified electrode **3**.

A well-defined redox peak can be observed that corresponds to the reversible process of the immobilised anthraquinone group. As shown in Figure 7b, the anodic and cathodic currents increase linearly with scan rate with a small ΔE at slow scan rates. This is consistent with surface immobilisation of the AQ redox centre. The midpoint potential stayed almost unchanged at -795 ± 5 mV versus SCE and the ratio between anodic and cathodic peak was almost unity for all studied scan rates. However, at higher scan rates ($>200\,mV\,s^{-1}$), the anodic–cathodic separation ΔE is significantly increased. The apparent rate constant k_{app} and the transfer coefficient α for a two-electron/two-proton surface-confined anthraquinone redox centre can be determined by plotting the variation of peak potential separation ΔE with the logarithmic scan rate by using the well-known Laviron formulation.[30]

Figure 8a and b show plots of $E_{peak}-E_{mp}$ versus $\log\nu$ for electrodes **3** ($C_6H_4CH_2NH$-CO-AQ) and **4** (EDA-CO-AQ), respectively. At higher scan rate, the slopes of the linear

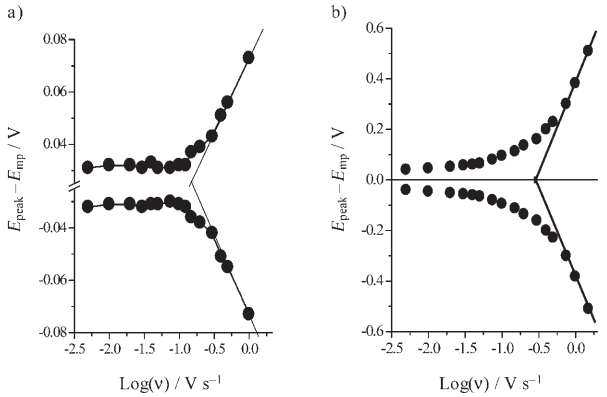


Figure 8. Plot of the variation of $E_{peak}-E_{mp}$ vs the logarithm of the scan rate for GC electrodes modified by a) $C_6H_4CH_2NH$-AQ (electrode **3**) and b) EDA-AQ (electrode **4**).

part of the plot are equal to $-2.3\,RT/\alpha nF$ for the cathodic branch, and $2.3\,RT/(1-\alpha)nF$ for the anodic branch. In our case, we obtain α values of 0.48 and 0.5 and apparent rate constants k_{app} of 1.47 and $2.36\,s^{-1}$ for electrodes **3** ($C_6H_4CH_2NH$-CO-AQ) and **4** (EDA-CO-AQ), respectively. The symmetry of anodic and cathodic branches of the plot in Figure 6 confirms that the transfer coefficient α is about 0.5. These values for k_{app} are consistent with the values obtained for alkylthiol-terminated hydroquinone (C_4 to C_6 chains) self-assembled on a gold electrode.[31]

Conclusion

Glassy carbon electrodes have been modified with anthraquinone redox systems and different spacers. Modification was performed in two steps. Firstly, two different linkers, $C_6H_4CH_2NHBoc$ and EDA-Boc, were electrochemically immobilised on the carbon surface by electrochemical reduction and oxidation, respectively. Secondly, after removal of the Boc group of the linkers, anthraquinone as a model redox centre was covalently attached under solid-phase synthesis conditions. This anthraquinone centre was also at-

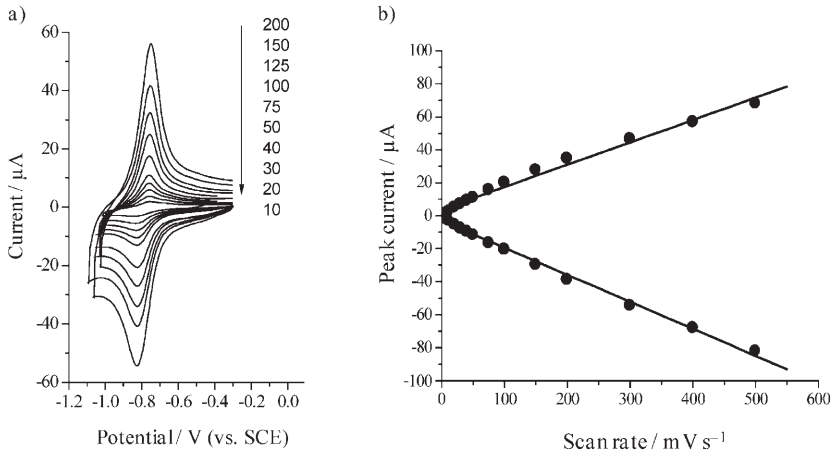


Figure 7. a) Effect of scan rate on $C_6H_4CH_2NH$-CO-AQ-modified electrode **3** in 0.1 M PBS (pH 7). b) Plot of AQ anodic and cathodic peak current and scan rate.

tached to the GC electrodes via other spacers such as 4-(*N*-Boc-aminomethyl)benzoic acid and *N*-Boc-β-alanine. These new AQ-modified electrodes were fully characterised by cyclic voltammetry, and the electrodes with EDA as a spacer were shown to have larger electron-transfer rate constants and have a more tightly packed monolayer surface coverage than electrodes modified with $C_6H_4CH_2NH$ spacers. Furthermore, the AQ-modified electrodes were shown to have long-term storage stability, and no change in thermodynamic potential or pK_a value occurred for any of the studied linker arrangements.

Hence we have shown that it is possible to covalently modify GC surfaces with linkers bearing a Boc-protected amino group. The Boc protecting group appears to prevent the formation of bridged structures or polymeric layers in the case of EDA coupling. In the case of diazonium coupling our approach using the Boc-protected compound allows us to use a single diazonium salt to couple a range of molecules to the electrode surface, avoids the necessity to synthesise a variety of different diazonium salts and overcomes problems of poor stability for some of the salts. Removal of the Boc group from the amine leads to modified carbon surfaces which are analogues of aminomethyl resins used routinely in solid-phase synthesis and provides a reactive site at the carbon surface for further synthetic modifications by various organic molecules. This strategy, which combines electrochemical and solid-phase synthetic methodologies, therefore constitutes a general and flexible approach for the functionalisation of different types of carbon surfaces. Work is currently in progress for the design of libraries of large numbers of organic compounds on different types of carbon surfaces.

Experimental Section

General: *N*,*N*-Dimethylformamide (DMF, synthesis grade) and ethanol (analytical grade) were obtained from Fisher Scientific. *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) was obtained from Novabiochem. All other reagents were obtained from Sigma-Aldrich and were used as received without any further purification. *tert*-Butyl *N*-(4-aminobenzyl)carbamate[20] and 4-(*N*-Boc-aminomethyl)benzoic acid[23] were synthesised by literature procedures.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV300 spectrometer at 300 and 75.5 MHz, respectively. Spectra were referenced to the residual ^{1}H peak of the deuterated solvent.

All solutions for electrochemical experiments were prepared with reagent-grade water (18 MΩ cm) from a Whatman RO80 system coupled to a Whatman "Still Plus". All glassware was cleaned by soaking in a 5% Decon 90 (Sigma-Aldrich) solution for at least 5 days followed by rinsing with deionised water and drying in an oven at 50 °C.

Electrochemical experiments were performed in a conventional three-electrode cell using an Autolab PGSTAT30 Potentiostat/Galvanostat. A home-made saturated calomel electrode (SCE) or Ag/AgCl was used as the reference electrode, and about 1 cm^2 platinum gauze as the counter-electrode. The working electrode was a 3 mm diameter (0.071 cm^2) glassy carbon disc (RA3-100, Tokai Carbon, Japan) sealed in glass tube and wired up with copper wire by using melted indium (Aldrich). Prior to modification, the working electrodes was polished with silicon carbide polishing paper (grade 1200) then with 1 μm alumina and rinsed with deionized water followed by sonication for 5 min in acetonitrile.

4-(*N*-Boc-aminomethyl)benzene diazonium tetrafluoroborate salt: Following a literature procedure,[21] *tert*-butyl *N*-(4-aminobenzyl)carbamate[20] (1.67 g, 7.5 mmol) was dissolved in HBF_4 (40%, 1.65 mL, 7.5 mmol) and water (10 mL). The solution was cooled on ice, and $NaNO_2$ (0.55 g, 8.0 mmol) dissolved in water (2 mL) was added dropwise under an inert atmosphere while the solution was stirred. The reaction mixture was allowed to warm to room temperature and the solution was concentrated to half its original volume. The residue was cooled in ice before filtration to give the diazonium salt as an orange solid, which was washed with diethyl ether and dried in vacuo (2.08 g, 86%); ^{1}H NMR ([D$_6$]DMSO): $\delta = 1.40$ (s, 9H), 4.35 (d, 2H, $J = 5.5$ Hz), 7.65 (br, 1H), 7.79 (d, 2H, $J = 8.8$ Hz), 8.61 ppm (d, 2H, $J = 8.8$ Hz); ^{13}C NMR ([D$_6$]DMSO): $\delta = 41.9$, 115.4, 130.8 (2C), 132.7 (2C), 147.6 ppm; ATR-IR: $\tilde{\nu} = 845$, 1022, 1278, 1368, 1460, 1517, 1585, 1687, 2286, 3361 cm^{-1}.

Electrode 1: Covalent attachment of $C_6H_4CH_2NHBoc$ to the GC surface was performed by electrochemical reduction from a solution containing 5 mM $BocNHCH_2C_6H_4$ diazonium salt and 0.1 M TBATFB in acetonitrile. The modification of three electrodes in parallel was carried out by cycling the electrode potential from 0.6 to −1 V versus Ag/AgCl for three cycles at a scan rate of 50 mV s^{-1}.

Electrode 2: The Boc-EDA spacer was grafted from a solution containing 10 mM Boc-EDA and 0.1 M TBATFB in acetonitrile by threefold cycling of the electrode potential in the potential range from 0.5 to 1.8 V versus Ag/AgCl at a scan rate of 50 mV s^{-1}.

General procedure for Boc deprotection of modified GC electrodes: A Boc-protected modified GC electrode was suspended in a solution of HCl in dioxane (4.0 M, 0.5 mL) at room temperature for 1 h. The electrode was then washed by sonication in DMF (0.5 mL), 1.0 M NaOH (0.5 mL), deionised water (0.5 mL) and absolute EtOH (0.5 mL) for 5 min before electrochemical characterisation.

General procedure for the coupling reaction of anthraquinone-2-carboxylic acid at the GC surface: Anthraquinone-2-carboxylic acid (252 mg, 1.0 mmol), HBTU (450 mg, 1.2 mmol) and DIEA (0.7 mL, 4.2 mmol) were dissolved in DMF (1.0 mL). The mixture was heated at 60 °C for 15 min with magnetic stirring to obtain a homogeneous solution. A Boc-deprotected modified GC electrode was then suspended in this solution, which was allowed to cool to room temperature and stirred for 16 h. The electrode was then washed by sonication in DMF (1.0 mL) and absolute EtOH (1.0 mL) for 5 min before electrochemical characterisation.

General procedure for the coupling reaction at the GC surface: A Boc-deprotected modified GC electrode was suspended in a solution of carboxylic acid (1.0 mmol), HBTU (450 mg, 1.2 mmol) and DIEA (0.7 mL, 4.2 mmol) in DMF (1.0 mL) at room temperature under magnetic stirring for 16 h. The electrode was then washed by sonication in DMF (1.0 mL) and absolute EtOH (1.0 mL) for 5 min before electrochemical characterisation.

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