Major differences in the behaviour of carbon paste and carbon fibre electrodes in a protein–lipid matrix: implications for voltammetry *in vivo*

The Analyst

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The widely documented differences in behaviour of carbon fibre electrodes (CFEs) and carbon paste electrodes (CPEs) used for neurochemical analysis *in vivo* were investigated. Differential staircase voltammetry was used to study the electrooxidation of ascorbic acid (AA) at CFEs and CPEs in the presence of major constituents of brain tissue, *viz.*, protein, lipid or a mixture of both. Both electrode types were poisoned by protein, reflected in positive shifts in the AA voltammetric peak potential, and also peak broadening, following exposure of the electrodes to protein solution. In contrast, CFEs and CPEs responded very differently to exposure to lipid suspension: CFEs exhibited poisoning whereas CPEs showed enhanced electron transfer kinetics for AA. This significant difference in the response of the two carbon materials to lipid was further demonstrated by showing that lipid could reverse the poisoning caused by protein for CPEs but not CFEs. It appears, therefore, that proteins adsorb on both CPEs and CFEs, hindering electron transfer from AA to the electrode surface. Surfactant lipid molecules, in contrast, have a cleaning effect on CPEs, removing pasting oil and adsorbed proteins from the CPE surface. These results provide an explanation for the stability of CPEs in brain tissue and for the contrasting instability of CFEs in the same environment. The data also suggest that a lipid–protein matrix represents a valuable *in vitro* chemical model of brain tissue that should allow a truer characterisation *in vitro* of new and existing *in vivo* sensors, reducing the need for animal experiments in these studies.

Introduction

A variety of voltammetric techniques have been developed for neurochemical studies of the living brain in which the chemical composition of the extracellular fluid (ECF) surrounding neurones can be monitored to study chemical communication between cells (for reviews, see refs. 1–5). The most common types of electrodes used to detect electroactive molecules, such as ascorbic acid (AA)[6,7] and dopamine,[8] are carbon paste electrodes (CPEs) and carbon fibre electrodes (CFEs), although there is increasing use of enzyme-based biosensors to detect electroinactive compounds in the ECF.[9–12]

The relative advantages and limitations of CFEs and CPEs have been reviewed recently.[4] Briefly, CFEs, which are usually activated by electrochemical treatments (*a*CFEs) before use *in vivo*, have smaller cross-sections (typically 8–30 μm od) and higher chemical resolution (about 150 mV peak separation between AA and catechols),[13] but are relatively unstable, lasting only a few hours in the tissue.[14] CPEs, on the other hand, have a larger cross-section (typically 150–300 μm od) and lower chemical resolution (about 80 mV separation between AA and catechols),[15] but are very stable, even after weeks of continuous recording in the brain.[16]

As part of our ongoing efforts to produce an electrode that combines the advantages of both electrode types (*viz.,* small, selective and stable), we investigated factors affecting the stability of these electrodes in brain tissue.

Experimental

Carbon paste was prepared by thoroughly mixing 2.83 g of carbon powder (UCP-1-M, Ultra Carbon, Bay City, MI, USA) and 1.0 mL of silicone oil (Aldrich, Milwaukee, WI, USA, Cat. No. 17 563-3). The CPEs were prepared using approximately 4 cm lengths of Teflon-coated platinum wire (Advent Research Materials, Eynsham, UK) of the dimensions usually used *in vivo* (320 μm od, 250 μm id). The Teflon insulation was slid along the wire to create an approximately 1 mm deep cavity. The cavity was tightly packed with the paste mixture using a bare platinum wire as plunger and the disk surface was smoothed by rubbing gently on clean card. CFEs were prepared by sealing carbon fibres (30 μm od, Avco Specialty Materials, Lowell, MA, USA) into glass tubing using a Model 720 vertical pipette puller (Kopf Instruments, Tujunga, CA, USA) and cutting the exposed fibre to a 1.0 mm length.

Experiments were carried out in a phosphate buffered saline (PBS) solution (pH 7.4) consisting of NaCl (Merck, Poole, Dorset, UK, AnalaR grade, 0.15 mol L^{-1}), NaH_2PO_4 (Merck, AnalaR grade, 0.04 mol L^{-1}) and NaOH (Merck, analytical-reagent grade, 0.04 mol L^{-1}). The lipid phosphatidylethanolamine (PEA, sheep brain cephalin, type II-S, Sigma, St. Louis, MO, USA), bovine serum albumin (BSA, fraction V, Sigma) and L-ascorbic acid (AA, Aldrich) were used as supplied. A 100 mM stock standard solution of AA was prepared using 0.01 M HCl. A 10% solution of BSA was prepared by dissolving 0.1 g of BSA in 1 mL of PBS and a 10% PEA suspension was made by vibrating 0.1 g of PEA in 1 mL of PBS.

Most working electrodes were then treated with a variety of media (*e.g.*, BSA or PEA) for 30 min before being transferred to the electrochemical cell containing that medium. The working electrodes, a saturated calomel reference electrode (SCE) and a large silver wire auxiliary electrode were arranged in a 30 mL electrochemical cell thermostated at 25 °C. The working electrodes were housed in a 1 mL vial, linked to the main cell by a small salt bridge, to protect the other cell components from the treatment media. The experiments were controlled using an in-

house potentiostat interfaced to an IBM PS2 Model 90 microcomputer through a Biodata Microlink system.

Linear staircase voltammograms (SCVs) for 1 mM AA in PBS were recorded between −200 and 600 mV *versus* SCE at 50 mV s^{-1} with a 5 mV step size and differentiated by subtracting successive currents (see Fig. 1 and Analysis of voltammogram parameters, below). CFEs were activated electrochemically by applying 2.9 V *versus* SCE for 30 s followed by −2.0 V for 10 s in PBS solution. Voltammograms recorded in previous studies *in vivo*[17] were re-analysed for comparison with those obtained *in vitro* here. Data are reported as means ± *s*, with *n* = number of electrodes. The significance of differences observed was estimated using Student's two-tailed *t*-tests. Paired tests were used for comparing signals recorded with the same electrode (*e.g.*, before and after a treatment); unpaired tests were used for comparing responses obtained with different electrodes.

Results and discussion

Analysis of voltammogram parameters

The shape of a voltammetric wave is determined by a subtle interplay of thermodynamic, kinetic and mass transport phenomena, and as such does not lend itself easily to explanation.[18] Since this statement applies to voltammograms generated at homogeneous electrode surfaces in contact with simple electrolyte solutions, the complete analysis of signals recorded with heterogeneous electrodes, such as carbon paste or fibre, in a complex tissue matrix (where restricted diffusion,[19] thin-layer behaviour[20] and adsorption[21] complicate matters further) is beyond reach at present. Hence, although theoretical analyses have been applied with some success to understand current transients recorded *in vivo*,[19,20,22] a phenomenological approach may be more useful at this stage.

Here we seek to understand the difference in behaviour of the two most commonly used electrode types (CPEs and CFEs) for voltammetry in brain studies, specifically why CPEs are stable even months after implantation, whereas activated CFEs lose their sensitivity and resolution after a few hours in tissue.[14] The electrooxidation of AA was chosen as a suitable probe for detecting changes in the characteristics of the electrode surface for two main reasons. First, AA is one of the main analytes present in brain ECF with a concentration of the order of 500 μM,[19,23,24] and second, the electron transfer reaction for AA displays irreversible behaviour at carbon electrodes. The oxidation of AA has been studied extensively at different electrode materials and is considered to follow an EC mechanism.[25] However, the large overvoltage needed to drive AA oxidation on carbon electrodes[26,27] means that the electron transfer step at these electrodes can be considered irreversible and thus the succeeding chemical reaction has no effect on the stationary electrode voltammogram.[28]

The signals recorded in brain ECF using staircase or linear sweep voltammetry with CPE disks are usually peak-shaped because of both thin-layer behaviour in the tissue[20] and adsorption of substrate on the electrode surface as a result of surfactant modification by lipids present in the brain.[21] Voltammograms for irreversible electron transfer observed under both of these 'trapped substrate' conditions have a number of features in common. Here we note that the peak is asymmetric[20,29] (slowly increasing before E_p and decreasing more rapidly thereafter) and that the peak width, $w_{\frac{1}{2}}$, is given by eqn. (1) below.

In contrast, voltammograms recorded with microcylinder CFEs *in vivo* or *in vitro* consist of peaks because differential techniques, such as differential pulse voltammetry (DPV)[4,13] or differential staircase voltammetry (DSV),[30] are usually used to record the current. Therefore, to produce peak-shaped voltammograms for CPEs and CFEs *in vitro* to model those observed *in vivo*, we used DSV. The DSV peak potential, E_p, corresponds to the potential of maximum slope, $E_{s,max}$, of SCV (see Fig. 1) and is a good index of electron transfer kinetics ($k^{\circ\prime}$).[18] Because of surface heterogeneity of some electrodes studied here (see below), changes in E_p will reflect changes in the average of a $k^{\circ\prime}$ distribution for a given surface.[31,32] Hence E_p measured at the same scan rate and pH was used to compare the effects of different treatments on $k^{\circ\prime}$ for AA electrooxidation, whereas changes in i_p were used as an index of changes in electrode sensitivity (Fig. 1).

Changes in the heterogeneity of the electrode surfaces were monitored using peak width, $w_{\frac{1}{2}}$ (see Fig. 1). This parameter is independent of scan rate and reference electrode so that values can be compared across a wider range of conditions than for E_p. For a homogeneous electrode, $w_{\frac{1}{2}}$ is given by the following equation for both adsorbed substrate[29] and electrolysis in thin-layer cells:[20]

$$w_{1/2} = 2.44\frac{RT}{\alpha n_\alpha F} = \frac{62.5}{\alpha n_\alpha}\text{mV (at 25° C)}$$
$$= \frac{65.0}{\alpha n_\alpha}\text{mV (at 37 ° C)} \quad (1)$$

where the transfer coefficient, α, has been determined as 0.50 ± 0.05 for AA at a range of carbon electrodes both *in vitro*[21] and *in vivo*.[20]

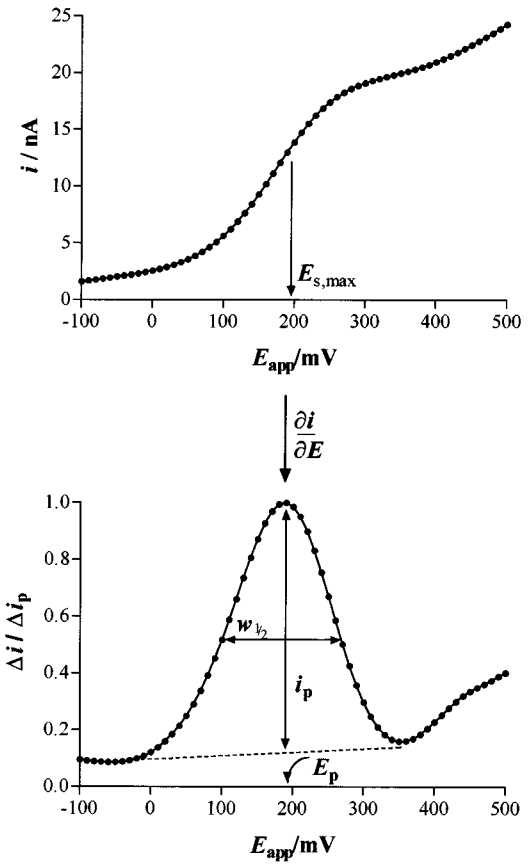

Fig. 1 Top: staircase voltammogram (SCV) recorded with a CPE_{PEA} at 50 mV s^{-1} in PBS (pH 7.4) containing 1 mM ascorbate (AA). Changes in the potential of maximum slope ($E_{s,max}$) recorded under similar conditions (scan rate, pH, *etc.*) can be used as an index of changes in electron transfer kinetics ($k^{\circ\prime}$) following different electrode treatments. Bottom: the SCV (*above*) differentiated by subtracting successive currents and dividing by the corresponding difference in potential (data resolution), $\Delta i/\Delta E$, yielding a DSV. For SCVs recorded at the same resolution (as was done in this investigation), Δi itself can be used to compare the characteristic DSV parameters E_p, i_p and $w_{\frac{1}{2}}$.

Although $w_{\frac{1}{2}}$ for the DSV of AA at untreated CPEs *in vitro* is greater than this ideal (see Fig. 2 and Table 1), presumably because of uneven oil coverage, the value for the lipid-treated CPE (CPE_{PEA}, 140 ± 40 mV, n = 4) approximates ideality [125 mV at 25 °C, $p > 0.5$; see eqn. (1)], presumably because surface oil is removed by the PEA,[21] decreasing surface inhomogeneity. The finding that this value of $w_{\frac{1}{2}}$ for DSV is the same as that recorded using SCV with lipid-treated CPEs under trapped substrate conditions in the brain (130 ± 10 mV, n = 10; see Fig. 2), is evidence that the DSV approach *in vitro* is an adequate model of voltammograms recorded under 'trapped substrate' conditions in general and in tissues *in vivo* in particular. The asymmetry of the DSV peak (Figs. 1 and 2) is further support for this conclusion.

Carbon paste electrodes (CPEs)

Fig. 2 and Table 1 show the effects of protein and lipid treatments on the DSV peak parameters for AA at CPEs. For the untreated CPE (CPE_{UN}) the peak potential, 260 ± 40 mV (n = 10), was considerably higher than the redox potential, which may be as low as −150 mV under these conditions,[33] indicating irreversibility of electron transfer. However, the peak width (200 ± 35 mV, n = 10) was considerably larger than that for classical irreversible reactions at homogeneously active electrode surfaces [125 mV for α = 0.5, $p < 0.001$; see eqn. (1)] and may arise from inhomogeneity in electron transfer sites due to different oil coverage.

Protein treatment of the CPE resulted in a significant change in the AA electrooxidation DSV signal. Paired t-tests on data from the same electrodes before and after treatment (n = 10; see Table 1) showed that BSA caused a significant positive shift in E_p (ΔE_p = 50 mV, $p < 0.02$), a broadening of peak width ($\Delta w_{\frac{1}{2}}$ = 125 mV, $p < 0.001$) and a 43% decrease in i_p ($p < 0.001$). These changes represent, respectively, a decreased average $k^{\circ\prime}$ for electron transfer, increased heterogeneity of the electrode surface and decreased sensitivity, *i.e.*, classical symptoms of poisoning that have been observed for BSA at CPEs[34] and other electrode types.[35,36] These effects may be due to adsorption of the large globular BSA molecules (approximately 70 kDa) on the electrode surface[36] that may either hinder access by AA to electron transfer sites thus affecting the Arrhenius frequency factor, A, and/or giving rise to a range of activation free energies for electron transfer, $\Delta G^{\neq}$:

$$k^{\circ\prime} = A\exp\left(\frac{-\Delta G^{\neq,\circ}}{RT}\right) \quad (2)$$

Subsequent treatment of poisoned CPE_{BSA} electrodes with a 10% PEA suspension completely reversed the fouling effects of BSA (see Fig. 2 and Table 1). Paired t-tests on data from the same electrodes before and after treatment (n = 4) showed that PEA caused a significant negative shift in E_p (ΔE_p = −150 mV, $p < 0.02$), a narrowing of peak width ($\Delta w_{\frac{1}{2}}$ = −200 mV, $p < 0.02$) and a 30% increase in i_p ($p < 0.04$). Indeed, the effects of PEA were so powerful that, not only was the BSA poisoning completely reversed, but also surface pasting oil of the CPE was removed, as observed previously following treatment of CPE_{UN} with lipid[21] and surfactant,[21,37] giving rise to an electrode surface with apparent homogeneous characteristics ($w_{\frac{1}{2}}$ = 140 ± 40 mV). This renewal of the surface of CPEs may be a continuous process, because the reservoir of oil in the bulk carbon paste should persistently permeate through to the electrode surface as it is removed.

Since surfactants have been observed to prevent BSA adsorption on pyrolytic graphite electrodes,[36] we investigated whether the effects of PEA could be attributed to its surfactant

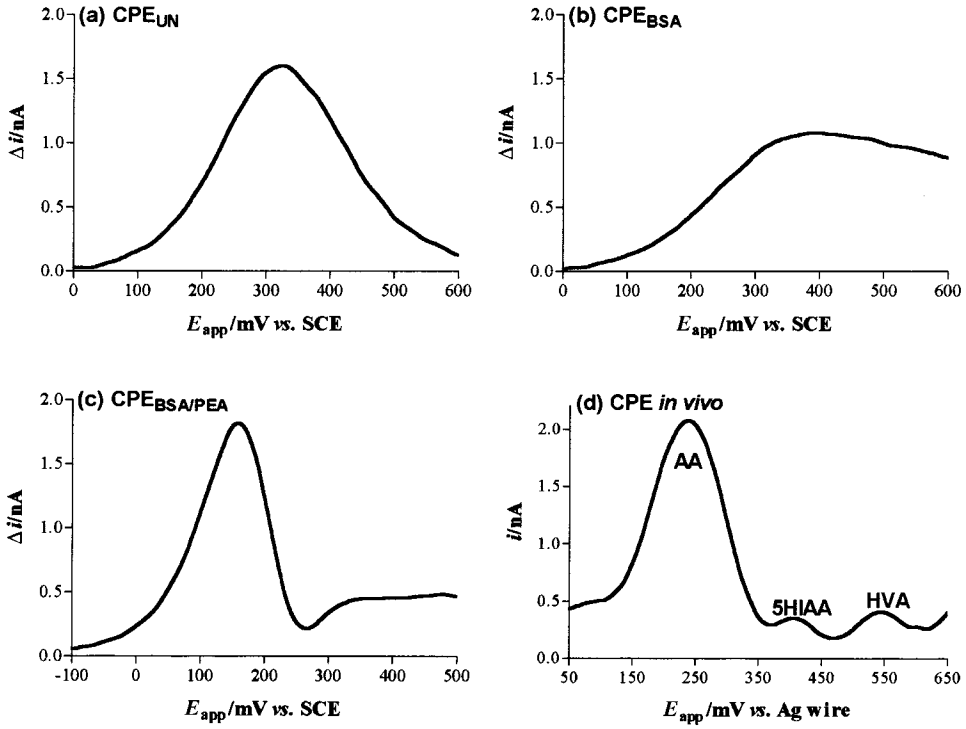

Fig. 2 Ascorbate (AA) voltammograms recorded under different conditions with CPEs. Although the start potential differs because of different E_p values, the voltage range is the same (600 mV) in all four cases so that peak width, $w_{\frac{1}{2}}$, can be compared visually. (a)–(c) Differential staircase voltammograms (DSVs) recorded *in vitro* for 1 mM AA with a CPE in PBS containing various treatment media: (a) PBS only; (b) 10% protein BSA in PBS; and (c) followed by 10% lipid PEA in PBS. The current scales are the same to compare the effects of treatment on electrode sensitivity. See Table 1 for DSV parameters. (d) SCV recorded *in vivo* in rat brain striatum with a CPE several weeks after implantation of the electrode. The chemical identity of these peaks, formed as a result of thin-layer behaviour and adsorption on the electrode, has been reviewed:[4] peak 1, AA; peak 2, a serotonin metabolite (5-hydroxyindoleacetic acid, 5HIAA); and peak 3, methylated metabolites of dopamine, mainly homovanillic acid (HVA). Note that the SCV for AA recorded *in vivo* ($w_{\frac{1}{2}}$ = 130 ± 10 mV, n = 10) is ideal for an irreversible electron transfer [see eqn. (1)] and is very similar in shape to that recorded using DSV *in vitro* (140 ± 40 mV, n = 4) after BSA and PEA treatment of the CPE (c).

nature. The neutral polyether surfactant Triton X-100 (TX) also reversed BSA poisoning of CPEs (Table 1); E_p and $w_{\frac{1}{2}}$ values for $CPE_{BSA/TX}$ were indistinguishable from those of $CPE_{BSA/PEA}$. However the sensitivity (i_p) of the TX-treated electrode was significantly greater ($p < 0.02$) than for all other treatments. The value of i_p, which is a measure of the slope of the SCV at $E_{s,max}$ (see Fig. 1), is affected by a number of parameters, including $k^{\circ\prime}$. However, if DSV peaks recorded with two electrodes under the same conditions have the same E_p, then differences in i_p reflect differences in effective electrode surface area. Thus, from the data for $CPE_{BSA/PEA}$, CPE_{MIX} and $CPE_{BSA/TX}$ for 1 mM AA (anionic) substrate (same E_p but different i_p values; see Table 1), it appears that the zwitterionic PEA adsorbed on the surface is blocking some sites that neutral TX does not. Further studies using a range of neutral, cationic and anionic surfactants of different lipophilic content are needed to ascertain the basis of this difference in sensitivity. Blocking effects of the carboxylate moieties of CPEs on AA electrooxidation have been observed previously.[5]

To determine whether PEA could protect CPEs from fouling by BSA when the protein was present in the medium during electrooxidation of AA, DSVs for AA were recorded in a mixture of 10% BSA and 10% PEA. The data in Table 1 show that PEA can protect CPEs from fouling by BSA, the peak parameters being the same when recorded in PEA or a PEA–BSA mixture. The data in Table 1 and Fig. 2 suggest that a lipid–protein matrix represents a valuable *in vitro* chemical model of brain tissue that should allow a truer characterisation *in vitro* of new and existing *in vivo* sensors, reducing the need for animal experiments in these studies.

Carbon fibre electrodes (CFEs)

Table 2 shows the effects of protein and lipid treatments on the DSV peak parameters for AA at CFEs. Despite differences in the structures of the two types of carbon electrode studied here, the behaviour of AA at CFE_{UN} (E_p, i_p and $w_{\frac{1}{2}}$) was not statistically different from that at CPE_{UN}. Moreover, the fouling effects of BSA were also similar for the two electrode types (Table 2).

The major difference in the property of fibre electrodes, relative to CPEs, lay in their response to lipid. Whereas PEA reversed BSA fouling at CPEs (Table 1), PEA failed to alleviate the symptoms of poisoning of CFEs, and even exacerbated it ($\Delta E_p = 65$ mV, $p < 0.02$).

Table 1 Peak parameters showing the effect of protein (BSA), lipid (PEA), polyether surfactant (Triton X-100, TX) or a mixture of BSA and PEA (MIX) on the CPE response to 1 mM AA in PBS + appropriate treatment medium compared with untreated paste electrodes (CPE_{UN}). Subscript X/Y indicates treatment with Y following treatment with X. Note that there was no significant difference between the response of $CPE_{BSA/PEA}$ and CPE_{MIX}

Treatment	E_p/mV	i_p/nA	$w_{\frac{1}{2}}$/mV
CPE_{UN} ($n = 10$)	260 ± 40	2.8 ± 1.7	200 ± 35
CPE_{BSA} ($n = 10$)	310 ± 45	1.6 ± 1.1	325 ± 75
$CPE_{BSA/PEA}$ ($n = 4$)	175 ± 30	2.6 ± 1.3	140 ± 40
CPE_{MIX} ($n = 3$)	175 ± 15	2.5 ± 0.3	145 ± 15
$CPE_{BSA/TX}$ ($n = 3$)	175 ± 35	7.6 ± 4.3	145 ± 50

Table 2 Peak parameters showing the effect of protein (BSA) and lipid (PEA) on the CFE response to 1 mM AA in PBS + appropriate treatment medium compared with untreated fibre electrodes (CFE_{UN})

Treatment	E_p/mV	i_p/nA	$w_{\frac{1}{2}}$/mV
CFE_{UN} ($n = 3$)	275 ± 40	2.7 ± 0.4	255 ± 45
CFE_{BSA} ($n = 3$)	360 ± 30	3.8 ± 2.2	350 ± 10
$CFE_{BSA/PEA}$ ($n = 3$)	425 ± 5	3.1 ± 0.7	305 ± 55

Activated carbon fibre electrodes (*a*CFEs)

The majority of neurochemical studies that employ CFEs to monitor AA and neurotransmitter activity in the brain use electrochemically pretreated activated forms of the electrode.[4] The untreated *a*CFE was the most active electrode type encountered in this study, with the lowest value of $k^{\circ\prime}$ and the largest i_p. The oxidation of AA at *a*CFEs was still irreversible with an overpotential of about 150 mV and a $w_{\frac{1}{2}}$ value close to ideal for that scenario (Table 3).

Table 3 and Fig. 3 show the effects of protein and lipid treatments on the DSV peak parameters for AA at *a*CFEs. Similarly to CPEs and CFEs, protein treatment of the *a*CFE resulted in a significant change in the AA DSV peak. Paired *t*-tests on data from the same electrodes before and after treatment ($n = 3$; see Table 3) showed that BSA caused a significant positive shift in E_p ($\Delta E_p = 140$ mV, $p < 0.005$), a broadening of peak width ($\Delta w_{\frac{1}{2}} = 90$ mV, $p < 0.01$) and a 50% decrease in i_p. Similarly to the unactivated CFE (Table 2), and in contrast to CPEs (Table 1), PEA treatment failed to reverse BSA fouling of *a*CFEs and, more clearly than for CFEs, led to further poisoning of the electrode: $\Delta E_p = 170$ mV, $p < 0.05$; $\Delta w_{\frac{1}{2}} = 40$ mV, $p < 0.02$; and a 30% decrease in i_p (see Fig. 3).

Conclusions

Table 4 summarises the behaviour of CPEs, CFEs and *a*CFEs in response to exposure to both protein and lipid. There was little difference between the final state of the CFE and *a*CFE, both showing extensive poisoning expressed as high E_p and large $w_{\frac{1}{2}}$. In contrast, the CPE showed no evidence of fouling after contact with the BSA and lipid-rich medium: the mean E_p value was significantly less than that for the *a*CFE (by 145 mV, $p < 0.03$), and the mean $w_{\frac{1}{2}}$, which was close to ideality for irreversible electron transfer at a homogeneous electrode surface, was some

Table 3 Peak parameters showing the effect of protein (BSA) and lipid (PEA) on the *a*CFE response to 1 mM AA in PBS + appropriate treatment medium compared with untreated activated fibre electrodes ($aCFE_{UN}$)

Treatment	E_p/mV	i_p/nA	$w_{\frac{1}{2}}$/mV
$aCFE_{UN}$ ($n = 3$)	10 ± 20	7.7 ± 4.3	150 ± 30
$aCFE_{BSA}$ ($n = 3$)	150 ± 25	3.1 ± 1.3	240 ± 15
$aCFE_{BSA/PEA}$ ($n = 3$)	320 ± 65	2.1 ± 1.4	280 ± 5

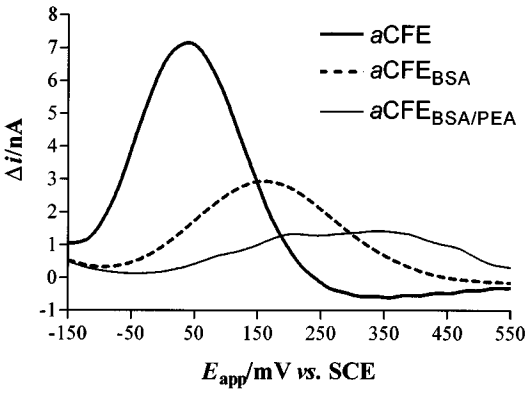

Fig. 3 DSVs recorded in PBS with *a*CFEs: untreated (*a*CFE), treated with protein (BSA, $aCFE_{BSA}$) and treated with BSA followed by lipid (PEA, $aCFE_{BSA/PEA}$); the PBS contained the corresponding treatment medium and 1 mM ascorbate (AA). See Table 3 for the DSV parameters.

Table 4 Peak parameters showing the effect of lipid (PEA) on protein (BSA)-poisoned electrode responses to 1 mM AA

BSA/PEA treatment	E_p/mV	i_p/nA	$w_{\frac{1}{2}}$/mV
CPE (n = 4)	175 ± 30	2.6 ± 1.3	140 ± 40
CFE (n = 3)	425 ± 5	3.1 ± 0.7	305 ± 55
*a*CFE (n = 3)	320 ± 65	2.1 ± 1.4	280 ± 5

140 mV less than for *a*CFEs ($p < 0.01$). Hence AA oxidation at *a*CFEs is more facile than at unmodified CPEs in PBS *in vitro*, but exposure to lipid-rich media *in vitro*, or brain tissue *in vivo*, has opposite effects on the two electrode types, leading to the lipid-treated CPE being much more responsive to AA oxidation than the lipid-treated *a*CFE (or CFE). These results provide an explanation for the loss of *a*CFE signals *in vivo*, and also elucidate the mechanism whereby CPEs can be used over months in brain tissue.

Preliminary data (not shown) suggest that it is the presence of pasting oil in the CPEs that affords them their resistance to poisoning in a BSA–PEA matrix and that incorporation of oil into CFEs does provide some protection from fouling. The main difficulty, at present, is impregnating the CFEs efficiently with the oil, and further work is needed in this area.

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References

1 *Voltammetric Methods in Brain Systems*, ed. A. A. Boulton, G. B. Baker and R. N. Adams, Humana Press, Clifton, NJ, 1995.
2 K. T. Kawagoe, J. B. Zimmerman and R. M. Wightman, *J. Neurosci. Methods*, 1993, **48**, 225.
3 R. D. O'Neill, *Analyst*, 1994, **119**, 767.
4 R. D. O'Neill, J. P. Lowry and M. Mas, *Crit. Rev. Neurobiol.*, 1998, **12**, 69.
5 C. D. Blaha and A. G. Phillips, *Behav. Pharmacol.*, 1996, **7**, 675.
6 G. V. Rebec and R. C. Pierce, *Prog. Neurobiol.*, 1994, **43**, 537.
7 R. D. O'Neill, in *Voltammetric Methods in Brain Systems*, ed. A. A. Boulton, G. B. Baker and R. N. Adams, Humana Press, Clifton, NJ, 1995, p. 221.
8 J. A. Stamford and J. B. Justice, *Anal. Chem.*, 1996, **68**, A359.
9 P. Pantano and W. G. Kuhr, *Electroanalysis*, 1995, **7**, 405.
10 J. P. Lowry, K. McAteer, S. S. El Atrash, A. Duff and R. D. O'Neill, *Anal. Chem.*, 1994, **66**, 1754.
11 S. Poitry, C. Poitry-Yamate, C. Innocent, S. Cosnier and M. Tsacopoulos, *Electrochim. Acta*, 1997, **42**, 3217.
12 S. S. El Atrash and R. D. O'Neill, *Electrochim. Acta,* 1995, **40**, 2791.
13 F. Gonon, M. Buda, R. Cespuglio, M. Jouvet and J. F. Pujol, *Nature* (*London*), 1980, **286**, 902.
14 C. A. Marsden, M. H. Joseph, Z. L. Kruk, N. T. Maidment, R. D. O'Neill, J. O. Schenk and J. A. Stamford, *Neuroscience*, 1988, **25**, 389.
15 R. D. O'Neill, R. A. Grunewald, M. Fillenz and W. J. Albery, *Neuroscience*, 1982, **7**, 1945.
16 M. Fillenz and R. D. O'Neill, *J. Physiol*. (*London*), 1986, **374**, 91.
17 A. Duff and R. D. O'Neill, *J. Neurochem.*, 1994, **62**, 1496.
18 K. B. Oldham, *J. Electroanal. Chem.*, 1985, **184**, 257.
19 C. Amatore, R. S. Kelly, E. W. Kristensen, W. G. Kuhr and R. M. Wightman, *J. Electroanal. Chem.*, 1986, **213**, 31.
20 W. J. Albery, N. J. Goddard, T. W. Beck, M. Fillenz and R. D. O'Neill, *J. Electroanal. Chem.*, 1984, **161**, 221.
21 D. E. Ormonde and R. D. O'Neill, *J. Electroanal. Chem.*, 1990, **279**, 109.
22 M. E. Rice and C. Nicholson, in *Voltammetric Methods in Brain Systems*, ed. A. A. Boulton, G. B. Baker and R. N. Adams, Humana Press, Clifton, NJ, 1995, p. 27.
23 M. Miele and M. Fillenz, *J. Neurosci. Methods*, 1996, **70**, 15.
24 M. W. Lada and R. T. Kennedy, *J. Neurosci. Methods*, 1995, **63**, 147.
25 J. J. Ruiz, J. M. Rodriguez-Mellado, M. Dominguez and A. Aldaz, *J. Chem. Soc., Faraday Trans. 1*, 1989, **85**, 1567.
26 I. F. Hu and T. Kuwana, *Anal. Chem.*, 1986, **58**, 3235.
27 L. A. Coury, Jr. and W. R. Heineman, *J. Electroanal. Chem.*, 1988, **256**, 327.
28 R. S. Nicholson and I. Shain, *Anal. Chem.*, 1964, **36**, 706.
29 A. J. Bard and L. R. Faulkner, *Electrochemical Methods*, Wiley, New York, 1980, p. 523.
30 R. C. Pierce, A. J. Clemens, C. P. Grabner and G. V. Rebec, *J. Neurochem.*, 1994, **63**, 1499.
31 J. Cassidy, W. Breen, A. McGee, T. McCormac and M. E. G. Lyons, *J. Electroanal. Chem.*, 1992, **333**, 313.
32 T. M. Nahir and E. F. Bowden, *J. Electroanal. Chem.*, 1996, **410**, 9.
33 Y. I. Turyan and R. Kohen, *J. Electroanal. Chem.*, 1995, **380**, 273.
34 J. Kulys, L. Gorton, E. Dominguez, J. Emneus and H. Jarskog, *J. Electroanal. Chem*., 1994, **372**, 49.
35 A. J. Downard and A. D. Roddick, *Electroanalysis*, 1994, **6**, 409.
36 A. E. F. Nassar, W. S. Willis and J. F. Rusling, *Anal. Chem.*, 1995, **67**, 2386.
37 F. N. Albahadily and H. A. Mottola, *Anal. Chem.*, 1987, **59**, 958.

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