Electroactive Poly(amino acids). Part 3.[1] Ferrocene-doped Poly(L-lysine) as an Electroactive Layer on Platinum

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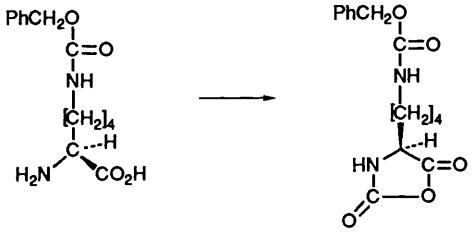
Poly(L-lysine) hydrobromide in water was coated onto a platinum electrode and then doped with ferrocene residues by reaction with ferrocenecarbonyl chloride in benzene containing pyridine. The behaviour of this coating to cyclic voltammetry in acetonitrile was examined. There is no evidence of the ageing phenomenon previously reported for poly(N^{ε}-4-nitrobenzoyl-L-lysine) films. In the unchanged state, ferrocene residues are associated. In the oxidised state, the ferrocinium residues appear evenly distributed through the film. Reduction of the oxidised state yields a film in which the ferrocene residues are evenly distributed but which relaxes within a few minutes to the state where ferrocene residues are associated. Charging can be repeated a large number of times with no deterioration in response.

Polymers bearing electroactive groups are the subject of much research aimed at producing coatings which will modify the properties of a solid electrode.[2,3] We have proposed the use of modified poly(amino acids).[2] So far poly(N^{ε}-4-nitrobenzoyl-L-lysine)[2] and copolymers of N^{ε}-4-nitrobenzoyl-L-lysine and electro-inactive amino acids have been examined.[1] These materials formed films by dip-coating onto metal electrodes from solutions in dimethylacetamide (DMA). Adsorbed films are formed which show reversible redox activity in acetonitrile. However, the films deteriorated on continuous charging, due to electrostatic repulsion between the nitrobenzoyl radical-anions. An α-helix to random coil transition was triggered over sections of the charged polymer and this caused separation between the film and the metal support.

Electrodes modified with a poly(amino acid) film are an attractive possibility for the mediation of electron transfer between the electrode and the active site of a redox enzyme, due to the compatibility of the peptide-like modified surface and the enzyme.[2] The ideal coated electrode for this purpose will have a redox centre which is stable in water. In a step towards this goal we have examined electroactive films formed from poly(L-lysine) by reaction of the side-chain amino groups with ferrocenecarbonyl chloride. Ferrocenecarboxylic acid undergoes a one-electron reversible oxidation at $E^{\circ} = 0.555$ V *vs.* saturated aqueous sodium chloride–calomel electrode[4] in acetonitrile. Anson[5] has used poly(lysine) films to incorporate electroactive anions by electrostatic attraction to the side-chain $-\overset{+}{N}H_3$ groups.

Ferrocene-doped Poly(L-lysine).—N^{ε}-Benzyloxycarbonyl-L-lysine (**1**) was obtained from the reaction of benzyloxycarbonyl chloride with the copper(II) complex of lysine followed by removal of copper ions by precipitation with hydrogen sulphide. Treatment of a suspension of compound (**1**) in 1,4-dioxane with a solution of phosgene in toluene gave the *N*-carboxyanhydride (**2**). A pure sample of compound (**2**) was treated with a catalytic amount of sodium hydride in 1,4-dioxane to give poly(N^{ε}-benzoyloxycarbonyl-L-lysine). The molecular weight of this material was estimated from viscosity measurements in dichloroacetic acid (DCA). For calibration we used the results of Applequist and Doty[6] on the same system. Our material showed an intrinsic viscosity [η] of 5.3 dl g^{-1} from which a molecular weight of 1.6×10^6 was derived.

Removal of the benzyloxycarbonyl protecting groups was



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achieved by passage of hydrogen bromide through a solution of the polymer in DCA. Poly(L-lysine) hydrobromide was precipitated by addition of diethyl ether. The salt could be swollen into water to form a viscous gel.

Reaction between an aqueous solution of poly(lysine) hydrobromide and ferrocenecarbonyl chloride contained in an upper layer of diethyl ether was promoted by the addition of sodium hydroxide. A brownish yellow, insoluble polymer precipitated out. This material did not dissolve in any of the common solvents such as DMA, dimethyl sulphoxide, or DCA, and could not be used to form a film. A reaction between poly(lysine) and 4-nitrobenzoyl chloride, carried out in the same way, also resulted in an insoluble polymer. We know, however, that the homopolymer of N^{ε}-4-nitrobenzoyl-L-lysine is soluble in DMA and in DCA.[2] Thus, the insolubility of the materials prepared by acylation of poly(lysine) must, in large part, be due to incomplete reaction of the amino groups, giving a heteropolymer that is no longer soluble in organic solvents or in water.

A film of ferrocenylated poly(L-lysine) was obtained in the following manner. A platinum electrode was dipped into an aqueous solution of poly(L-lysine) hydrobromide and was then withdrawn. The polymer solution was very viscous, resulting in a layer clinging to the electrode as it was withdrawn from the solution. In some experiments an obvious layer of solution was held on the platinum close to the glass support. An electrode coated with poly(lysine) hydrobromide was then dipped into a solution of ferrocenecarbonyl chloride in benzene containing some pyridine. Finally, the electrode was withdrawn, washed successively with benzene and diethyl ether, and dried.

Electrodes prepared in this way could be transferred to fresh solutions, and they retained their electrochemical response due

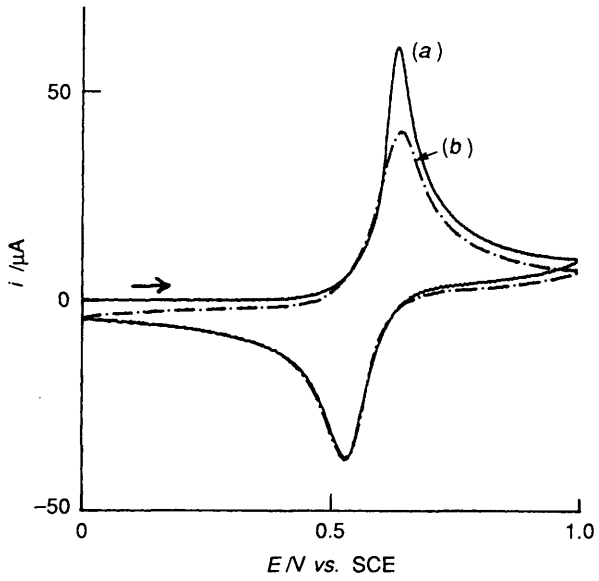

Fig. 1. Electrode of ferrocene-doped poly(L-lysine) on Pt (area 0.088 cm^2, Γ 2.1 nmol cm^{-2}). Cyclic voltammetry in acetonitrile containing 0.1 mol dm^{-3} Pr_4NBF_4, v 0.100 V s^{-1}: (*a*) first scan on a rested electrode; (*b*) third scan of a continuous series.

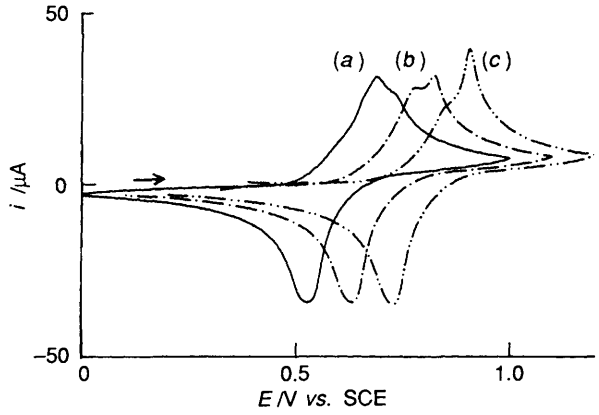

Fig. 2. Electrode of ferrocene-doped poly(L-lysine) on Pt (area 0.088 cm^2, Γ 2.1 nmol cm^{-2}). Cyclic voltammetry in acetonitrile containing 0.1 mol dm^{-3} Pr_4NBF_4, v 0.100 V s^{-1}. One cyclic voltammogram was run and a second recorded here (*a*) after 0 s rest; (*b*) after 40 s rest; (*c*) after 160 s rest. Curves (*b*) and (*c*) have been displaced by 0.1 V and 0.2 V, respectively.

to ferrocene groups. Ferrocene groups are covalently bonded to the polymeric film around the electrode.

Electrochemical Behaviour of Ferrocene-doped Poly(L-lysine) Films.—The redox properties of these films were examined by cyclic voltammetry in acetonitrile containing 0.1 mol dm^{-3} tetrapropylammonium fluoroborate. A potential scan from 0 to +1.0 V *vs.* SCE was used. One or two scans at 0.1 V s^{-1} were sufficient to achieve 'breaking-in', after which the film was swollen with solvent and ions. Redox behaviour is due to oxidation of the iron(II) centre in ferrocene to iron(III), giving a monopositive ferrocinium ion, and then reduction of the ferrocinium ion species back to the ferrocene.

The films showed characteristic time-dependent behaviour on cyclic voltammetry. A film which had been allowed to rest at 0 V *vs.* SCE for 10 min or more showed a characteristic, narrow anodic peak on the first scan and a broader, cathodic peak on reverse sweep. Sequential scans showed broad peaks on both the anodic and cathodic branches. This behaviour is illustrated

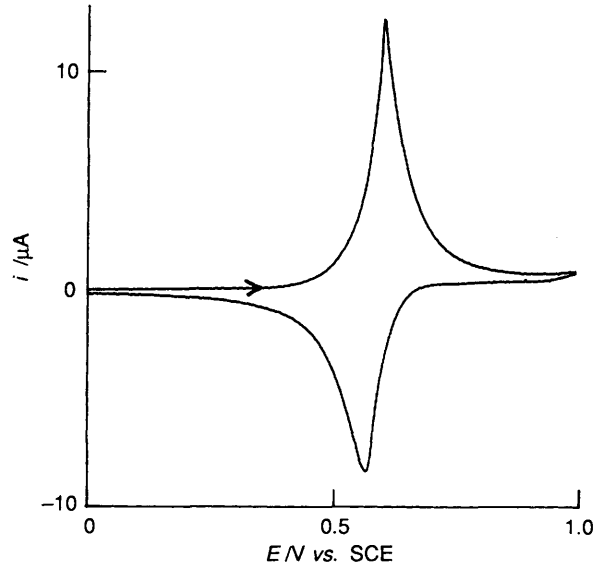

Fig. 3. Electrode of ferrocene-doped poly(L-lysine) on Pt (area 0.088 cm^2, Γ 2.1 nmol cm^{-2}). Cyclic voltammetry in acetonitrile containing 0.1 mol dm^{-3} Pr_4NBF_4, v 0.009 V s^{-1}.

in Fig. 1. Immediately following a cyclic voltammetry experiment, the electrode showed a rest potental of *ca.* 0.5 V *vs.* SCE, indicating incomplete discharge of the iron(III) sites. The charge slowly dissipated. After a 10 min rest the electrode showed the cyclic voltammetry behaviour of Fig. 1(*a*) on the first sweep, reverting to Fig. 1(*b*) on subsequent sweeps.

Slow relaxation of the film after cyclic voltammetry was demonstrated in the following manner. A cyclic voltammogram was run at 0.1 V s^{-1}. The film was then left on open circuit for a pre-selected length of time after which a second cyclic voltammogram was recorded. The film was then allowed to relax completely for 10 min before the sequence was repeated. Fig. 2 illustrates the results obtained after various rest times. For clarity, two of the curves have been displaced along the potential axis. When all three curves are superimposed, the cathodic branches of the curves coincide and the anodic peaks are found in a range of 0.70 ± 0.03 V *vs.* SCE. Immediately following the charging sequence the film state is characterised by broad anodic and cathodic peaks. This state slowly relaxes to a final rest state characterised by a sharp anodic peak. Intermediate situations are characterised by mixed behaviour of the film on cyclic voltammetry.

This variation in peak shape with the history of the electrode makes it impossible for us to draw conclusions on film thickness from the variation of peak height with the scan rate. At a scan rate of 0.009 V s^{-1}, illustrated in Fig. 3, the film is essentially fully charged on the anodic sweep so that the current falls to zero near completion of the anodic sweep. Integration of this curve indicates that the same amount of charge, 118 C, is required for charging and discharging sections, and gives a surface coverage, Γ, of 2.1 nmol cm^{-2}, of active sites.

Interpretation of the Film's Behaviour.—The film was obtained from an aqueous solution of poly(L-lysine) hydrobromide. This polymer is known [7] to form a random coil in aqueous solution because the repulsive force between the side-chains with protonated amino functions is sufficient to destabilise the helix. After treatment with ferrocenecarbonyl chloride, the film was not brought into solution but was dried at 40 °C. The product may still be the random coil but drying may induce a change to the β-form.

Unprotonated poly(lysine) is present in aqueous solution as the α-helix but undergoes a helix to β-sheet transition on mild

Table 1. Cyclic voltammetry experiments with a ferrocene-doped poly(L-lysine) film on Pt. The solvent is acetonitrile containing 0.1 mol dm ^{3} Pr_4NBF_4. Scan 0 ⟶ 1.0 V *vs.* SCE.

Experiment No.	v/V s^{-1}	E_{pa}/V *vs.* SCE	E_{pc}/V *vs.* SCE	i_{pa}/μA	i_{pc}/μA	$E^{\circ a}$/V *vs.* SCE	ΔE^b/mV	Comments
1	0.100	0.634	0.525	61.0	37.1	0.580	109	Illustrated in Fig. 1(*a*)
2	0.100	0.636	0.526	41.8	37.6	0.581	110	Like Fig. 1(*b*)
3	0.100	0.638	0.528	40.6	37.5	0.583	110	Illustrated in Fig. 1(*b*)
6	0.025	0.616	0.552	25.8	17.3	0.584	64	Like Fig. 3
7	0.009	0.603	0.562	12.5	8.4	0.583	41	Illustrated in Fig. 3
9	0.100	0.657	0.529	46.6	39.4	0.593	128	Like Fig. 1(*a*)

^{a} $E^{\circ} = \frac{1}{2}(E_{pa} + E_{pc})$. ^{b} $\Delta E = E_{pa} - E_{pc}$.

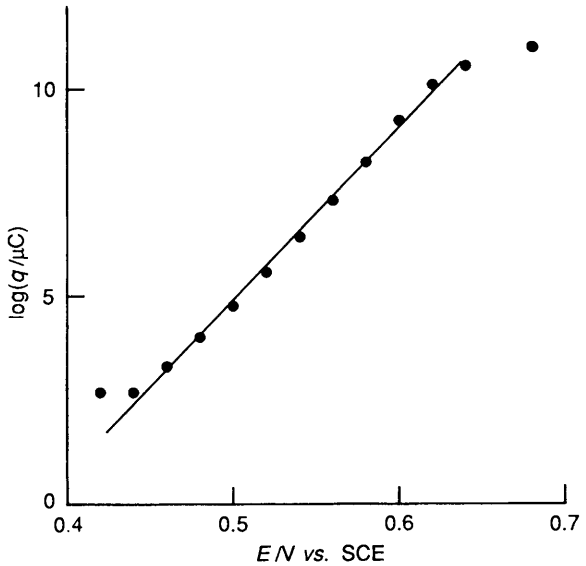

Fig. 4. Results from anodic cyclic voltammetry scan of ferrocene-doped poly(L-lysine) on Pt at v 0.009 V s^{-1} shown in Fig. 3. ●, experimental points. The line has the equation $E = 0.382 + 0.024 \log (q)$, where q is the total charge passed from 0 V to E V *vs.* SCE.

heating.[7] Crystals of protonated poly(lysine) with HPO_4^{2-} counter-ion have been grown in the α-helix form and are transformed into the β-form either by heating or by drying.[8]

Electrodes modified with a film of ferrocene-doped poly(lysine) are considerably more stable than the electrodes modified with poly(N^{ε}-nitrobenzoyl-lysine). They could be cycled up to 100 times without showing the ageing process noted for poly(N^{ε}-4-nitrobenzoyl-lysine). This is in accord with our interpretation that the ageing process is due to the transformation of the α-helix to a random coil over sections of the polymer chain, causing a separation between film and metal support. Ferrocene-doped poly(lysine) is already in either the random coil conformation or the β-form. We have already noted[1] that the copolymer of N^{ε}-4-nitrobenzoyl-L-lysine and glycine (2:1) does not show the ageing phenomenon associated with poly(N^{ε}-4-nitrobenzoyl-L-lysine) itself. This is attributed to the large blocks of poly(glycine) present as the β-sheet, which modify the properties of the α-helix in the homopoly(N^{ε}-4-nitrobenzoyl-L-lysine).

The reversible changes in the cyclic voltammetry response of ferrocene-doped poly(lysine) can be interpreted as being due to a change in the distance between iron(II) sites in the uncharged polymer and iron(III) sites in the charged polymer. The uncharged ferrocene sites are strongly hydrophobic and will tend to form localised areas of high density. Charged ferricinium sites will repel each other and adopt a random distribution throughout the polymer. Conversion of iron(III) into iron(II) gives rise to a normal response to cyclic voltammetry, as does immediate reconversion of iron(II) into iron(III). Here the electroactive sites are randomly distributed throughout the polymer. When the uncharged polymer is allowed to rest, the ferrocene groups re-associate to form localised areas of high density. These areas will charge rapidly and give rise to a sharp peak on cyclic voltammetry.

The behaviour we observed is similar to that reported by Daum and Murray[9] for films of poly(vinylferrocene) in water. Our experiments 1, 6 and 7 (see Table 1) are first scans of a rested electrode. They show a sharp anodic peak and a peak potential that vary with scan rate in a manner similar to that for poly(vinyl ferrocene). The response of poly(vinylferrocene) is explained in terms of a phase-like behaviour of ferrocene sites in the film, so that the activity of ferrocene remains constant during oxidation of the film. In contrast, the iron(III) sites are thought to be mobile and to show an activity dependent upon concentration. This explanation is essentially the same as ours for the response of ferrocene-doped poly(lysine).

Daum and Murray[9] indicated that a graph of log (*i*) *vs.* *E* for the rising part of the anodic peak, at slow scan rates, should be linear with a slope of 59 mV (at 25 °C). The results in Fig. 3, where the scan rate is 9 mV s^{-1}, give a linear plot but with a slope of 21 mV.

If we accept this model for the film, the activity of ferrocene residues will remain constant during oxidation, due to the phase-like behaviour. Then we can assume equilibration of iron(III) sites across the thickness of the film during slow oxidation, so that the concentration of iron(III) sites is given by the amount of charge passed (q). Taking the activity coefficient of iron(III) sites as unity, a graph of log (q) *vs.* E should be linear with a slope of 59 mV (at 25 °C) over a large section of the charging curve. Fig. 4 shows that this plot is linear over the central region of the charging curve, but with a slope of only 24 mV. Thus, whilst we feel that the model for the film's behaviour is qualitatively correct, a quantitative description of the results is still lacking.

Conclusions.—A useful method for the preparation of electroactive poly(amino acid) films is to cast a film of poly(lysine) hydrobromide from aqueous solution and then to dope some of the amino groups by converting them into amides by reaction with a suitable acid. These films are essentially in either the random coil or β-sheet form. In parallel work we have reported the doping of poly(γ-ethyl glutamate) with electroactive transition metal centres to form a stable electroactive film.[10]

Experimental

Non-SI units of surface coverage (mol cm^{-2}) and intrinsic viscosity [η] (dl g^{-1}) are common in the literature and have been retained here. In this context 1 mol of electroactive species refers to 6.02×10^{23} electroactive units which are attached to polymer chains.

All solvents were purified and dried according to standard

methods. Gaseous effluents containing phosgene were passed through scrubbing towers containing aq. sodium hydroxide before being released.

4-(4-Benzyloxycarbonylaminobutyl)oxazolidine-2,5-dione (**2**).—*Preferred procedure* (cf. *ref.* 11). N^{ε}-Benzyloxycarbonyl-L-lysine (2.0 g, 7 mmol) was stirred with 1,4-dioxane (40 cm^3) under nitrogen in a three-necked flask. A sintered-glass filtration tube was attached to the flask. The mixture was heated to 65 °C and an excess of phosgene (12.5% solution in toluene; 20 cm^3, 26 mmol) was added, whilst a static nitrogen atmosphere was maintained. After 20 min most of the starting material had passed into solution. Further phosgene (12.5% solution in toluene; 10 cm^3) was added together with ethyl acetate (40 cm^3) and the mixture was maintained at 65 °C for a further 4 h. The solution was filtered under a pressure of nitrogen into another flask. A stream of nitrogen was bubbled through the hot filtrate for 30 min to remove the excess of phosgene. The solution was poured into hexane (400 cm^3), causing immediate crystallisation of the product. After 12 h at 0 °C, the 4-(4-benzyloxycarbonylaminobutyl)oxazolidine-2,5-dione (**2**) was collected and recrystallised from ethyl acetate–hexane as prisms (1.68 g, 77%).

Poly(N$^{\varepsilon}$-benzyloxycarbonyl-L-lysine).—Preferred procedure using sodium hydride (cf. *ref.* 12). The oxazolidine-2,5-dione (**2**) (1.6 g) was dissolved in 1,4-dioxane (50 cm^3) and sodium hydride (50% dispersion in oil; 24 mg) was added. The mixture was stirred at room temperature for 22 h under a static nitrogen atmosphere. The slow disappearance of the oxazolidine-2,5-dione (**2**) was followed by the disappearance of the characteristic carbonyl bands in the IR spectrum of successive aliquots of reaction mixture evaporated onto an NaCl disc.

The resulting very viscous liquid was poured into water and the polymer was precipitated as a solid (1.4 g), which was filtered off and washed with 50% aq. methanol. A solution of this polymer in DCA gave an intrinsic viscosity [η/dl g^{-1}] 5.3, from which M was calculated to be 1.2×10^6.

Poly(L-lysine) Hydrobromide.—Poly(N^{ε}-benzyloxycarbonyl-L-lysine) (0.37 g) was dissolved in stirred DCA (20 cm^3) over 2 days. Dry hydrogen bromide was passed through the stirred solution for 1 h during which time poly(L-lysine) hydrobromide slowly separated as a ball. The product was separated by decantation and washed with diethyl ether. Poly(L-lysine) hydrobromide was dissolved in the minimum volume of water and precipitated by addition of ethanol and diethyl ether. The purified product was dried at 70 °C/0.1 mmHg overnight to yield a flaky solid (0.27 g).

Electrochemical Measurements.—Platinum wire was melted in a hydrogen–oxygen flame to form a sphere 1–2 mm in diameter. The wire was silver-soldered to a copper lead and then fused into a glass support so that only the platinum was exposed. The platinum was coated with polymer as described below. A coated electrode was immersed in a solution of tetrapropylammonium fluoroborate (0.1 mol dm^{-3}) in acetonitrile. A platinum wire formed the counter-electrode. The reference electrode was saturated aq. potassium chloride–calomel (SCE) dipping into 1.0 mol dm^{-3} sodium nitrate, then connected through a bridge of the supporting electrolyte in acetonitrile. The cell was deoxygenated by a stream of nitrogen.

Cyclic voltammetry on coated electrodes was performed with a sweep between 0 and +1.0 V *vs.* SCE. The potentiostat was a PAR 176 model with 274 digital interface and was controlled through an Apple IIe computer. Data were transferred to an IBM personal computer for processing.

Preparation of Ferrocene-doped Poly(L-lysine)-modified Electrodes.—To a stirred solution of phosphorus pentachloride (2.5 g, 12 mmol) in benzene (25 cm^3) under nitrogen was added ferrocenecarboxylic acid (2.3 g, 10 mmol). The mixture was stirred for 2 h and was then filtered, and the filtrate was concentrated under reduced pressure at 25 °C. The resulting syrup was dissolved in pentane (70 cm^3), and the solution was washed successively with 2 mol dm^{-3} sodium hydroxide and water, dried (Na_2SO_4), and evaporated under reduced pressure to give ferrocenecarbonyl chloride[13] as a dark red solid (1.18 g, 48%).

Poly(L-lysine) hydrobromide (3 mg) was swollen into water (3 cm^3) to yield a viscous gel. A platinum sphere was dipped into this gel at room temperature for 15 min and was then slowly withdrawn. It was next dipped into a stirred solution of ferrocenecarbonyl chloride (20 mg) in benzene (1 cm^3)–pyridine (0.05 cm^3) under nitrogen for 30 min. The electrode was then withdrawn and washed by being shaken in benzene for 5 min, then in diethyl ether for 5 min. Finally, the electrode was dried *in vacuo* for 20 min at 40 °C.

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References

1 Part 2, J. Grimshaw and S. D. Perera, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1711.
2 A. M. Abeysekera, J. Grimshaw, S. D. Perera and D. Vipond, *J. Chem. Soc., Perkin Trans. 2*, 1989, 43.
3 J. Audebert and G. Bidan, *J. Electroanal. Chem. Interfacial Electrochem.*, 1987, **238**, 183; F. Bedioui, A. Meino, J. Devynck, C. E. Mestres and C. B. Charreton, *J. Electroanal. Chem. Interfacial Electrochem.*, 1988, **239**, 433; S. Cosnier, A. Deronzier and N. Vlachopulos, *J. Chem. Soc., Chem. Commun.*, 1989, 1259. For a review see: A. Deronzier and J.-C. Mouter, *Acc. Chem. Res.*, 1989, **22**, 249; see also earlier work quoted in ref. 2.
4 H. Hennig and O. Gürtler, *J. Organomet. Chem.*, 1968, **11**, 307.
5 F. C. Anson, T. Ohsaka and J. M. Savéant, *J. Phys. Chem.*, 1983, **87**, 640; F. C. Anson, J. M. Savéant and K. Shigehara, *J. Am. Chem. Soc.*, 1983, **105**, 1096.
6 J. Applequist and P. Doty, in *Polyaminoacids, Polypeptides and Proteins*, ed. M. A. Stahmann, University of Wisconsin Press, Madison, 1962, p. 161.
7 Y.-W. Tseng and J. T. Yang, *Biopolymers*, 1977, **16**, 921, and references therein.
8 F. J. Padden, H. D. Keith and G. Giannoni, *Biopolymers*, 1969, **7**, 793.
9 P. Daum and R. W. Murray, *J. Phys. Chem.*, 1981, **85**, 389.
10 J. Grimshaw and J. Trocha-Grimshaw, *J. Chem. Soc., Chem. Commun.*, 1990, 157.
11 W. D. Fuller, M. S. Verlander and M. Goodman, *Biopolymers*, 1976, **15**, 1869.
12 M. Goodman, E. Peggion, M. Szwarc and C. H. Bamford, *Macromolecules*, 1977, **10**, 1299.
13 E. M. Acton and R. M. Silverstein, *J. Org. Chem.*, 1959, **24**, 1487; H. H. Lau and H. Hart, *J. Org. Chem.*, 1959, **24**, 280.

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