Synthesis and Anodic Polymerisation of an L-Cystine derivatised Pyrrole; Copolymerisation with a Tetraalkylammonium Pyrrole allows Reduction of the Cystinyl Film to a Cysteinyl State that Binds Electroactive $\{Fe_4S_4\}^{2+}$ Centres

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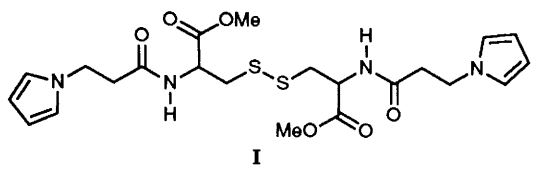
The cystine derivatised pyrrole **I** is synthesised and stable neutral polymers or cationic copolymers are produced by anodic oxidation at Pt and glassy carbon electrodes; chemical reduction of cystine–tetraalkylammonium co-functionalised films gives ion-exchange polymers with pendant cysteinyl groups and these thiolate films tightly bind $\{Fe_4S_4\}^{2+}$ centres giving an electrode–polymer assembly with electroactive, cysteinyl ligated, ferredoxin-like units.

The first examples of cystinyl and cysteinyl derivatised poly(pyrrole) electrodes are described: we show that chemical reduction of cystine and tetraalkylammonium co-functionalised poly(pyrrole) films give co-polymers with pendant cysteinyl groups; these groups bind $\{Fe_4S_4\}^{2+}$ centres thereby giving electrode–polymer assemblies with electroactive, cysteinyl S ligated, ferredoxin-like units.

The cystine derivatised monomer **I** was synthesised from 3-(pyrrol-1-yl) propanoic acid and the methyl ester of L-cystine, Scheme 1. ^1H and ^{13}C NMR spectra with 2D chemical shift correlation, infrared and C, H, N and S microanalytical data confirmed the identity of the product (white crystals, m.p. 146 °C, yield *ca.* 15%). The cystine monomer **I** is optically active with $[\alpha]_D^{30}$ −98. ^1H and ^{13}C NMR spectra of the cysteinyl derivative **II** (see below) in the presence of the chiral paramagnetic shift reagent $[Eu(fod)_3]$† showed that **II**, and by inference **I**, were at least 90% optically pure.



I

Electropolymerisation of **I** is facile on platinum or vitreous carbon electrodes and stable, conducting films are readily produced by oxidation at +0.56 V *vs.* the ferrocinium–ferrocene couple (fc^+/fc) in MeCN–0.1 mol dm^{-3} $[NBu_4][BF_4]$, Fig. 1. The films are transparent, golden in the

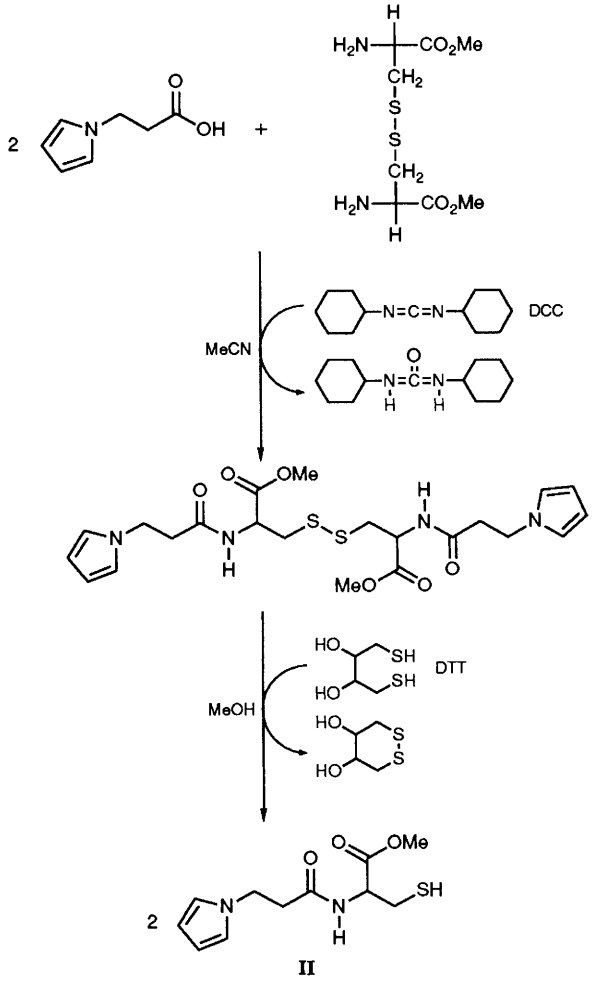
† $Eu(fod)_3$ = tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium.

reduced state and blue–purple in the oxidised state. Examination of the polymer by diffuse reflectance Fourier transform infrared (FTIR) spectroscopy confirmed that the esterified amino acid group had been retained during electropolymerisation, Fig. 2 and Scheme 2. Since the electropolymerisation occurs at a position remote from the asymmetric centres, it is likely that chirality is retained in the polymer.

The monomer **I** reacts with *threo*-1,4-dimercapto-2,3-butanediol [Cleland's reagent, dithiothreitol (DTT)] to give the cysteinyl monomer **II**, Scheme 2. Although **II** electropolymerises, it does so with concomitant oxidation of the thiol group: ν(SH) is absent in the diffuse reflectance FTIR spectrum of the film. We, therefore, sought a method to convert the cystine derivatised polymer to the cysteinyl form.

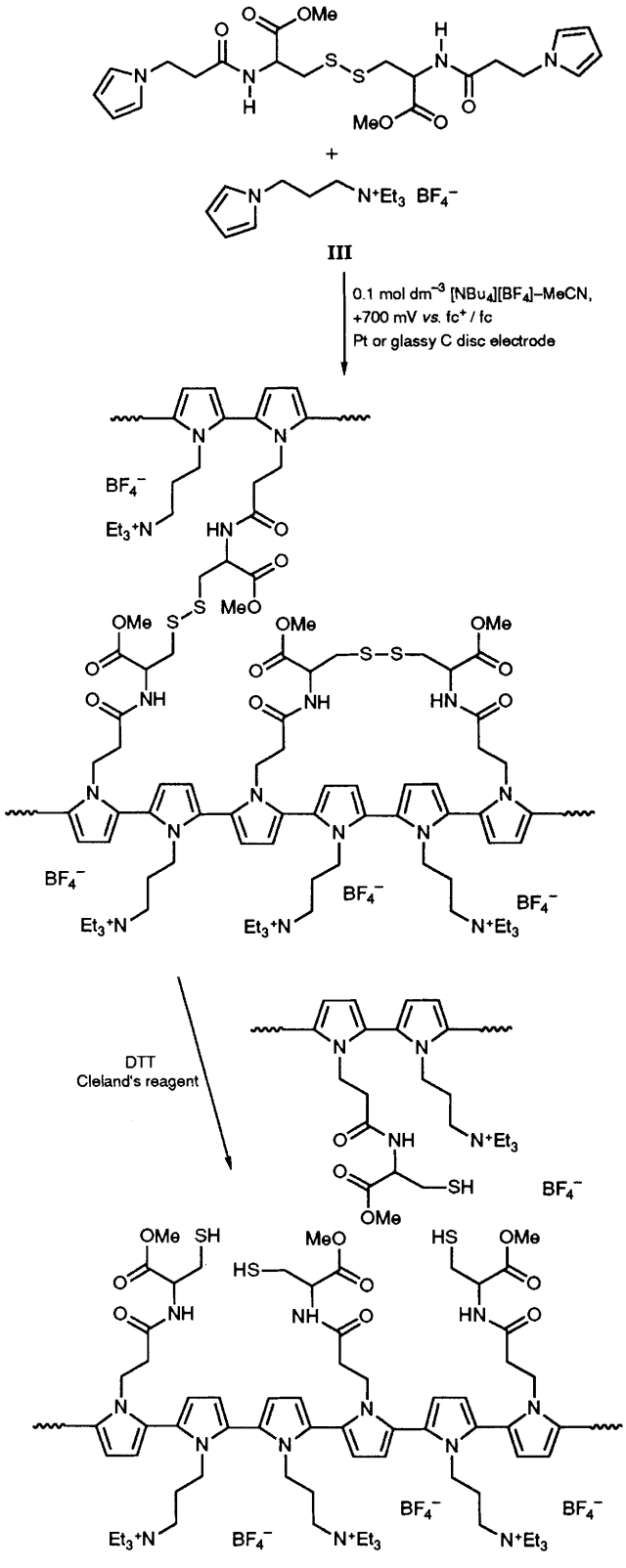
Chemical modification of poly(pyrrole) or its derivatives, is generally difficult because penetration of the film by reagents is often poor.[1] This was found to be the case when direct cleavage of the S–S bonds of the cystine polymer by DTT was attempted. However, copolymerisation of **I** with the alkylammonium pyrrole **III**‡ gives a cystine derivatised film with anion-exchange properties and this readily reacts with basic solutions of DTT to produce a cysteinyl copolymer. Presumably the thiolate form of the reagent permeates the film by an anion-exchange mechanism. Fig. 2(*a*) shows the diffuse reflectance FTIR spectrum of the unreacted cystine copolymer and Fig. 2(*b*) shows the appearance of ν(S–H) at 2570 cm^{-1} after cleavage of the S–S bonds: notably absorption at the ν(OH) frequency of the reagent is absent, this implies that it is not present in either a covalently bound or unreacted form in the polymer after work-up.

Films prepared by copolymerisation of **I** and **III** in the concentration ratio 1 : 10 [2] and converted to the cysteinyl SH form by DTT were exposed to MeCN solutions of



II

Scheme 1 Synthesis of pyrrole derivatives of cystine and cysteine; DCC = 1,3-dicyclohexylcarbodiimide, DTT = dithiothreitol



Scheme 2 Copolymerisation of the cystine and alkylammonium pyrroles **I** and **III** and cleavage to give the cysteinyl copolymer: it is assumed that a random copolymer is produced

‡ The relative rate of electropolymerisation of the neutral monomer **I** is greater than that for the alkylammonium monomer **III**, in order to achieve an appropriate composition of the copolymer it was necessary to employ an excess of **III** in the copolymerisation experiments.

$[Fe_4S_4(SPh)_4]^{2-}$. The FTIR spectra of these showed the absence of the ν(SH) band, the absence of aromatic C–H bending vibrations, and an attenuation of the intensity of ν(BF). The cluster modified films displayed a well-defined reversible redox response which we attribute to the cysteinyl ligated $\{Fe_4S_4\}^{2+/1+}$-couple, Figs. 3 and 4. Coulometric measurements of charge injected for cluster reduction and of charge removed for the oxidation of the pyrrole backbone, gave the ratios of total pyrrole groups to cluster units as 7 ± 1 : 1. We estimate the concentration of the cluster within the film to be in the order of 1 mol dm^{-3} and that the composition of the assembly approximates to that shown in Fig. 4.

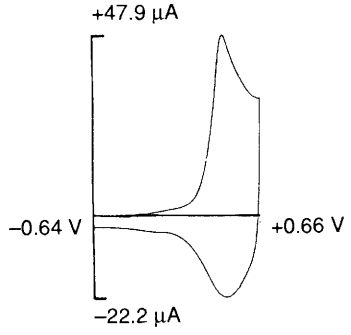


Fig. 1 Cyclic voltammogram of cystine/tetraalkylammonium copolymer film on a glassy carbon disc electrode of area 0.07 cm^2 in meCN–0.1 mol dm^{-3} $[NBu_4][BF_4]$. The scan-rate was 150 mV s^{-1} and all potentials are relative to the fc^+/fc couple. Electropolymerisation was initiated at 0.83 V in the same electrolyte with a concentration ratio of **III** : **I** of 10 : 1. The charge passed during electropolymerisation was 21.2 mC cm^{-2} and the polymerisation efficiency was *ca.* 70%. The voltammetric behaviour of homopolymers of **I** and **III** is analogous to that of the copolymer.

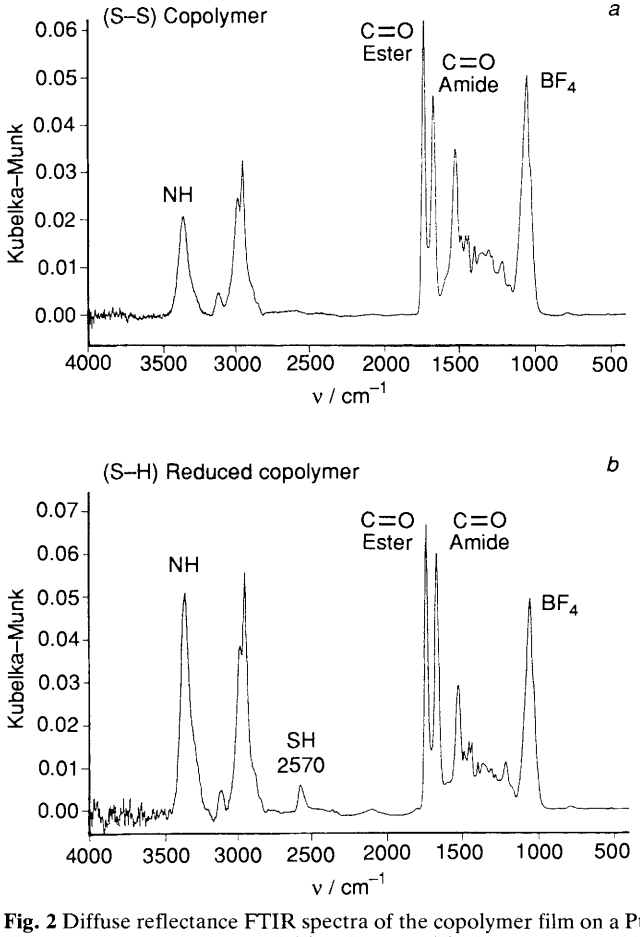


Fig. 2 Diffuse reflectance FTIR spectra of the copolymer film on a Pt disc electrode of area 0.64 cm^2 (*a*) before and (*b*) after reduction with Cleland's Reagent. Electropolymerisation conditions were as above with a total charge passed of 160 mC cm^{-2}. The homopolymer produced from **I** shows the same amide and ester bands as the copolymer but the absorption due to ν(B–F) is absent in the neutral film.

In MeCN–0.1M $[NBu_4][BF_4]$, the E° for the cysteinyl ligated $\{Fe_4S_4\}^{2+/1+}$-couple occurs at −1.48 V *vs.* fc^+/fc. In an aqueous electrolyte containing $Li[ClO_4]$ it occurs at −800 mV *vs.* SHE (SHE = standard hydrogen electrode), a value just beyond the negative limit of the range of reduction potentials observed for ferredoxin proteins [3].

The $[BF_4]^-$ anions in cystine copolymers of **I** and **III** (untreated with DTT) are displaced by $[Fe_4S_4(SPh)_4]^{2-}$, as we have previously shown for ion-exchange polymers based on **III** alone.[4] The FTIR spectra of the ionically bound clusters show aromatic ν(C–H) and voltammetry shows E° for the $[Fe_4S_4(SPh)_4]^{2-/3-}$ couple at −1.32 V *vs.* fc^+/fc. The ionic binding of $[Fe_4S_4[SPh)_4]^{2-}$ in cystine copolymers is readily reversed on exposure of the film to MeCN–0.2 mol dm^{-3} $[NBu_4][BF_4]$, the cluster is rapidly leached from the film and $[BF_4]^-$ re-incorporated. In contrast, the covalently bound $\{Fe_4S_4\}^{2+}$ centres in the cysteinyl copolymers are indefinitely stable under the same conditions and can be redox cycled through the 2+/1+ levels without measurable leaching or decomposition. It is likely that ion-exchange provides the transport mechanism by which $[Fe_4S_4(SPh)_4]^{2-}$ permeates the

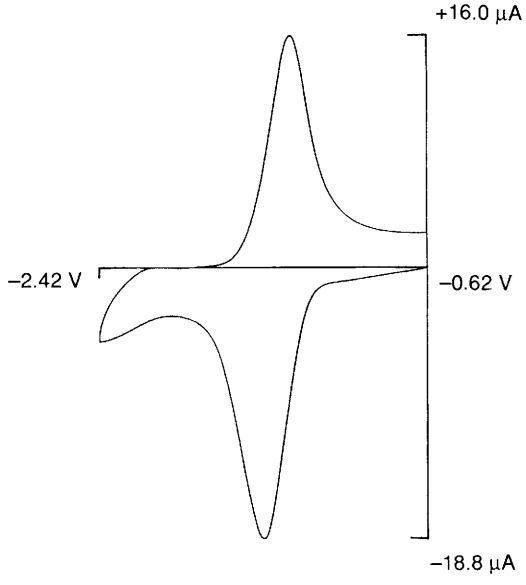


Fig. 3 Cyclic voltammogram (7th scan at 100 mV s^{-1}) of the cysteinyl ligated $\{Fe_4S_4\}^{2+/+}$-couple in the reduced copolymer film at a glassy carbon electrode (area 0.07 cm^2, scan-rate 100 mV s^{-1})

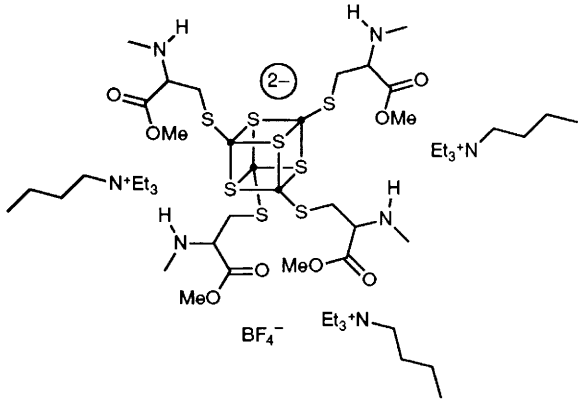


Fig. 4 Schematic representation of cluster–polymer assembly with stoichiometry of seven pyrrole units per cluster

copolymer film and can thence undergo thiolate ligand exchange with the appended cysteinyl SH groups.

We have shown how one type of prosthetic group, an iron–sulphur cluster, can be incorporated in a cysteinyl derivatised poly(pyrrole) on an electrode surface: in principle, it should be possible to bind other inorganic or bioinorganic groups. Although preliminary attempts to entrap the FeMo cofactor of nitrogenase within such cysteinyl polymers have so far failed, the general methodology offers the prospect of constructing polymer modified electrodes with nitrogen fixation or other capabilities: to this end we are exploring the synthesis and electropolymerisation of oligopeptide derivatised pyrroles and metallo-derivatives of the chiral monomers **I** and **II**.

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References

1 A. Deronzier and J.-C. Moutet, *Acc. Chem. Res.*, 1989, **22**, 249.
2 S. Cosnier, A. Deronzier, J.-C. Moutet and J. F. Roland, *J. Electroanal. Chem.*, 1989, **271**, 69.
3 G. Backes, Y. Mino, T. M. Loehr, T. E. Meyer, M. A. Cusanovich, W. V. Sweeny, E. T. Adman and J. Sanders-Loehr, *J. Am. Chem. Soc.*, 1991, **113**, 2055 and references cited therein.
4 J.-C. Moutet and C. J. Pickett, *J. Chem. Soc., Chem. Commun.*, 1989, 188.