Electroactive Poly(amino acids). Part 1.[1] Modified Electrodes from Platinum with an Adsorbed Film of Poly(N^{ε}-4-nitrobenzoyl-L-lysine)

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Poly(N^{ε}-4-nitrobenzoyl-L-lysine) is obtained with average molecular weight $(2.0 + 1.3) \times 10^5$ by reaction between the monomer *N*-carboxyanhydride and sodium hydride. Platinum has been dip-coated with this material from dimethylacetamide solution, and the behaviour of the coating has been examined in acetonitrile by cyclic voltammetry and chronoamperometry. Surface coverage was in the range 28—5.4 nmol cm^{-2} of 4-nitrobenzoyl residues. On repeated cyclic voltammetry the development of multiple peaks is observed. On allowing the electrode to rest, the original smooth cyclic voltammogram is restored. This process is ascribed to a reversible conformational change in the film. Under prolonged electrical stress the films age in a characteristic manner which is ascribed to separation between the film and the metal support. The polymer in solution adopts the α-helix structure and it is assumed to be adsorbed in this form onto platinum. A close-packed layer of α-helices has surface coverage of 0.66 nmol cm^{-2} and so we estimate that our films range from *ca.* 40—10 layers thick.

Polymers bearing electroactive groups have recently been the subject of much research aimed at producing coatings which will modify the properties of a solid electrode.[2-6] The most commonly used backbone for these polymers has been the poly(ethene) derived from a vinylarene monomer where the arene contains an electroactive group. We wish to explore the use of poly(amino acids) for the preparation of electroactive films. Amino acids with an additional function group such as amino, hydroxy, or carboxylic acid on the side chain can be modified to carry an electroactive group and then polymerized through the α-amino acid function.

Poly(amino acids) in solution can adopt either a random coil where the conformation of individual amino acid units is constantly changing or a rigid α-helix where the individual units adopt the same conformation. The same situation is found for films of these polymers obtained by evaporation of concentrated solutions. The β-sheet can sometimes be stabilized in these films.[7] Rigid, highly orientated films of these polymers can also be formed at the air–water interface and these orientated films can be transferred to solid supports.[8] Crystalline forms containing ordered arrays of the α-helix are also known.[9] A number of low molecular-weight peptides are known to be adsorbed from solutions as a monolayer at mercury and solid electrode surfaces.[10]

Electrodes modified with a poly(amino acid) film containing electroactive groups are thus an intriguing possibility because the high degree of order than can be built into the film may lead to useful properties. There is a particularly 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. In other approaches towards driving an enzyme with current from a solid electrode, a layer of small adsorbed molecules have been used as electron relay.[11] Electroactive relay groups have recently been attached to the side-chain basic functions of amino acids that form part of the enzyme itself.[12]

A further interesting point is that stereoselective effects in electron transfer between an Fe^{III} chelate ion anchored to polypeptides and dopamine or adrenalin have been demonstrated.[13] Thus electroactive poly(amino acid) films may be useful mediators for stereoselective electrochemical reduction. Work with non-electroactive poly(amino acid) films indicates that asymmetric reduction can be achieved at electrodes coated with such films.[14]

Other workers have examined the properties of an electroactive poly(amino acid) film obtained by transesterification of poly(methyl L-glutamate) with ferrocenylmethanol and with an alcohol to which the *N,N'*-dialkyl-4,4'-dipyridyl group was attached.[15] The ferrocene-doped film has been shown to have a permeability by ions which depends markedly on its redox state. The dipyridyl-doped film has been shown to transport electrons between redox solution kept on either side of the film. We have prepared poly(N^{ε}-4-nitrobenzoyl-L-lysine) and, in a preliminary communication, demonstrated the diffusion of charge through films of this material prepared on platinum.[1]

Preparation of the Monomer.—N^{ε}-Acyl and N^{ε}-aroyl derivatives of lysine are usually obtained by reaction of the appropriate acid chloride with the copper(II) complex of lysine and then removal of the copper ion by precipitation with hydrogen sulphide. This route has been reported for the N^{ε}-4-nitrobenzoyl derivative.[16] Repetition of this procedure gave a yellow product whose ^1H n.m.r. spectrum indicated the presence of two different A_2B_2 systems in the aromatic region. One A_2B_2 system was identified as due to the 4-nitrobenzoyl compound, the other, centred at δ 7.14 and 7.82, is probably due to the 4-aminobenzoyl compound. Approximately 12% of the amino compound was present. The two lysine derivatives could be separated by t.l.c. on silica, eluting with ethanol–water (35:20) and both gave a positive ninhydrin reaction. Clearly hydrogen sulphide is behaving as a reducing agent in this preparation.

Salicyl aldoxime proved a useful reagent for sequestering copper(II) from the 4-nitrobenzoyl-L-lysine complex. Reaction occurred in cold ethanol to give a precipitate of the copper(II) oxinate and the free amino acid. The amino acid was then extracted from this mixture with hot water and crystallized as colourless prisms. The yellow colour previously reported for this compound is due to a charge-transfer band from a mixture of the nitro- and the 4-amino-benzoyl derivatives.

Table 1. I.r.-adsorption bands (/cm^{-1}) of poly(N^{ε}-4-nitrobenzoyl-L-lysine) and related compounds in the amide region

Solids in KBr disc				Films from DMA		Solids in KBr disc			
(1)	(2)	(3) Pptd. from DCA	(4) Pptd. from DCA	(3)	(4)	(5)	(6)	(7)	
1 635	1 635	1 640	1 650	1 650	1 650	1 640	1 635	1 620	Amide I band (strong)
1 600	1 600	1 600	1 600	1 600	1 600	1 600	1 600	1 600	Benzene C=C stretch (weak)
1 577	1 572	1 575		1 575		1 577			Benzene C=C stretch (weak)
1 535	1 530	1 530	1 520	1 540	1 540	1 540	1 540		Amide II band (strong)[a]
	b		*b*		1 525		1 515	1 520	NO_2 band (weak)[a]
1 520	1 510								NH_3^+ deformation
1 490	1 435	1 490	1 485	1 490	1 485	1 490	1 480		CH_2 chain vibration (weak)

[a] The NO_2 band occurs in the same region as the amide II band. It is easily distinguished, being weak relative to the strong and broad amide II and amide I bands. [b] Probably submerged in the amide II band.

Condensation Polymer.—Poly(amino acids) can be obtained by reaction of the amino acid *N*-carboxyanhydride (NCA) with a catalytic amount of a strong base. Previous work suggests that dioxane is a good solvent for obtaining high-molecular-weight polymers.[17] The NCA must be of high purity and free from chloride ions. Some comparative studies of amines,[18] sodium hydroxide, sodium methoxide,[18] or sodium hydride[19] as initiators have been made.

In preliminary experiments, N^{ε}-(4-nitrobenzoyl)-N^{α}-benzyloxycarbonyl-L-lysine was converted into the NCA by reaction with phosphorus pentachloride in dioxane.[20] The product was polymerized in dioxane using diethylamine as the catalyst. This procedure gave erratic results and the highest molecular weight obtained for the polymer was 1.3×10^4.

High-purity samples of the NCA were obtained by treating a suspension of N^{ε}-(4-nitrobenzoyl)-L-lysine in dioxane with a solution of phosgene in toluene.[21] The first-formed product was the carbamoyl chloride. Addition of ethyl acetate promoted cyclisation of this intermediate to the NCA. The NCA was precipitated by addition of hexane and the recrystallized product proved relatively stable in air. Polymerization of this NCA proceeded better with sodium hydride than with amines as the catalyst. Dioxane was expected to be the best solvent in which to obtain a high-molecular-weight polymer. However, the polymeric material was rather insoluble, thus limiting the extent of polymerization that could be obtained. A compromise solvent mixture of dimethylacetamide and dioxane was used for polymerizations. Samples of poly(N^{ε}-benzoyl-L-lysine) were also prepared in the same way.

Polymer molecular weights were estimated from viscosity measurements in either dichloroacetic acid (DCA) or dimethylformamide (DMF). For calibration we used the results of two groups for poly(L-γ-benzyl glutamate) in DCA.[22,23] A more satisfactory model is poly(N^{ε}-benzyloxycarbonyl-L-lysine) but only a limited number of calibration points are available for this compound in DCA. For calibration in DMF we used the results obtained by two groups for poly(N^{ε}-benzyloxycarbonyl-L-lysine).[24,25] In DCA these polymers adopt a flexible random coil conformation while in DMF they adopt the rod structure of the α-helix.

The following relationships between intrinsic viscosity and molecular weight were used and are obtained by the method of least squares from the combined literature data indicated above:

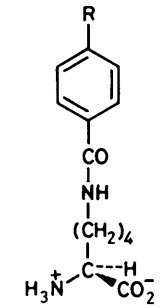
in DCA

$$\log \{[\eta]/\mathrm{d}l\,g^{-1}\} = -(4.09 \pm 0.21) + (0.77 \pm 0.04) \log M$$

in DMF

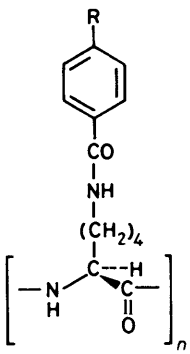
$$\log \{[\eta]/\mathrm{d}l\,g^{-1}\} = -(6.19 \pm 0.56) + (1.16 \pm 0.11) \log M$$

Infrared spectra of the polymers were obtained in two ways. First the polymer was dissolved in DCA and precipitated by pouring it into water, collected, dried, and the i.r. spectrum was taken in a KBr disc. Alternatively, a solution of the polymer in dimethylacetamide (DMA) was poured onto a KBr plate and the solvent was evaporated in a slow stream of dry air under reduced pressure to leave a skin of the polymer. Absorption bands in the amide region are recorded in Table 1 for the polymers (**3**) and (**4**) as well as for related compounds chosen so as to assist confirmation of the assignments made. The data are consistent with the material from DMA being largely in the α-helix form (expected for amide bands: 1 650 and 1 546 cm^{-1}) and the material from DCA being more disordered (expected amide bands for random coil: 1 655 and 1 535 cm^{-1}).[26]



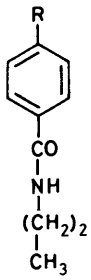
(**1**) R = H

(**2**) R = NO_2



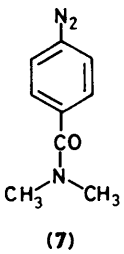
(**3**) R = H

(**4**) R = NO_2



(**5**) R = H

(**6**) R = NO_2



(**7**)

Preparation of Electrodes and their Normal Behaviour.—Poly(N^{ε}-4-nitrobenzoyl-L-lysine) (PNBL) swells in DMA to form a viscous solution. Platinum spheres (diameter *ca.* 2 mm)

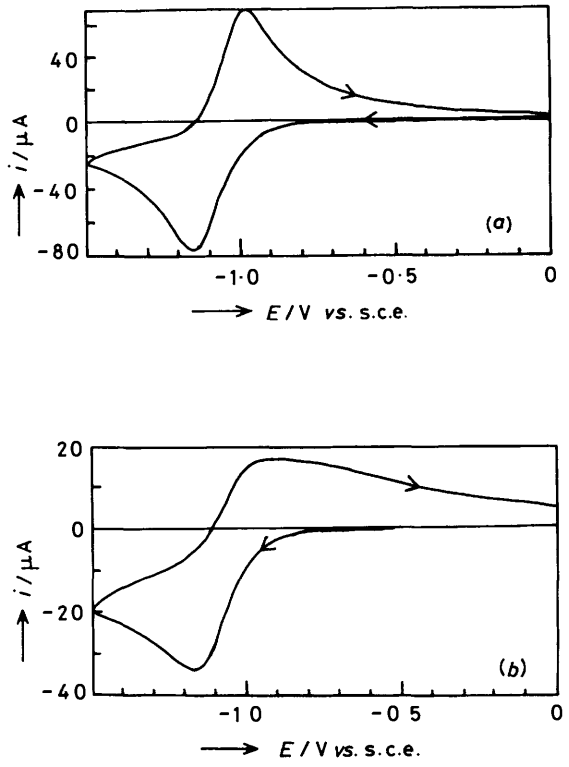


Figure 1. Cyclic voltammogram of a poly(N^{ε}-4-nitrobenzoyl-L-lysine) thick-film-modified Pt electrode: film area 0.087 cm^2, Γ 21.4 nmol cm^{-2}, scan rate 0.1 V s^{-1}, solvent acetonitrile containing 0.1M-Pr_4NBF_4: (*a*) a freshly 'broken-in' film, (*b*) the same film after several runs at slower scan rates. Film prepared from 0.3% polymer solution

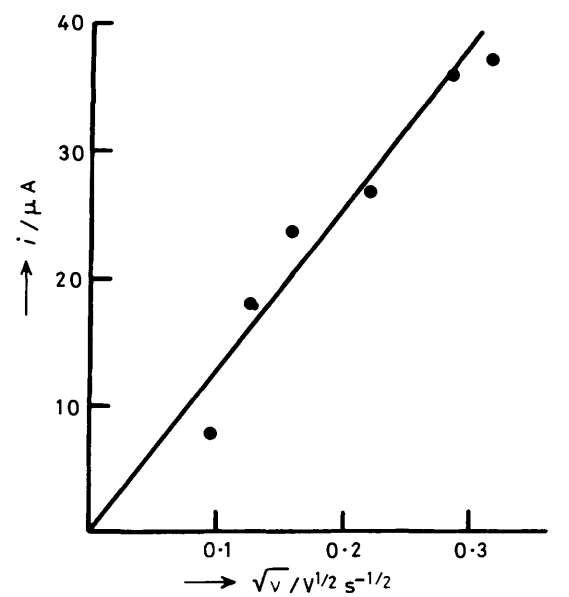


Figure 2. Data from cyclic voltammetry using a thick film of poly(N^{ε}-4-nitrobenzoyl-L-lysine) on Pt; film area 0.087 cm^2, Γ 21.4 nmol cm^{-2}, solvent acetonitrile containing 0.1M-Pr_4NBF_4. Peak current is proportional to $v^{\frac{1}{2}}$. Film prepared from 0.3% polymer solution

* The presence of carbon-containing material on a platinum surface, originally ultra-clean but left to stand in air for some time, was demonstrated using ESCA spectroscopy through the kindness of Professor D. R. Lloyd, Trinity College, Dublin.

supported on glass were dip-coated from such solutions (0.3% w/v). Experience showed that better coatings were obtained when the platinum support was cleaned in a hydrogen flame and then kept immersed and undisturbed in the polymer solution for 15—20 min. Used coatings were also removed by flaming after we found that the PNBL coating was resistant to oxidative removal by nitric acid. The electrode was then withdrawn and dried under vacuum. Examination of the coating for electrochemical activity was carried out in acetonitrile in which the polymer is relatively insoluble. Similar results were obtained with either tetramethyl-, tetraethyl-, or tetrapropyl-ammonium fluoroborate as the supporting electrolyte. The change in film properties which results from repeated cyclic voltammetry or chronoamperometry (the 'ageing' process) is, however, faster in the presence of tetramethylammonium fluoroborate. The electrolyte concentration was usually 0.1M but in some chronoamperometry experiments this was raised to 1.0M because the initial transient currents were in the region of 400 μA. Reproducible results were obtained with coatings obtained from the same solution and on the same day. However as the stock polymer solution aged it gave less satisfactory results. Some solutions gave thinner coatings than others and this may reflect an inhomogeneous distribution of molecular weight between individual solid polymer aggregates in the bulk of material.

Adsorption[10] can play a part in attaching the polymer to the platinum surface. The 'clean' surface of our platinum was obtained by heating the metal to dull red heat in a hydrogen flame and allowing the material to cool in air with no special precautions. Platinum surfaces cleaned in this way quickly become covered with a layer of organic material* which can assist adsorption of hydrophobic polymers.

The 'breaking-in' phenomenon noted with other film electrodes[4,5] was observed with PNBL. It is associated with swelling of the polymer coat as solvated ions are forced to migrate into the film as it is charged or discharged. A freshly prepared film was subjected to repeated cyclic voltammetry, sweep speed 100 mV s^{-1}, and was allowed to rest for a few minutes between scans. During 'breaking-in' the cyclic voltammogram changed from an irregular shape to the normal response of Figure 1(*a*). With tetramethylammonium fluoroborate as the electrolyte this required only one cycle. About three cycles were usually required with tetraethyl- and tetrapropyl-ammonium fluoroborates. After this a series of normal cyclic voltammograms could be obtained. Chronoamperometry was carried out on other films conditioned by cyclic voltammetry. When the ageing process developed in the film, the cathodic peak of the cyclic voltammogram was progressively displaced to more negative potentials and the anodic peak became progressively flatter towards the shape of Figure 1(*b*).

Most preparations resulted in films that were sufficiently thick when swollen for the charging process to be diffusion-limited (associated diffusion coefficient D). This was demonstrated by successive cyclic voltammograms at different sweep rates (v) (Figure 2) where the value of $i_{pc}/v^{\frac{1}{2}}$ is approximately constant over the range. Diffusion-limited transport is found[4] when the distance of charge transport during the experiment [approximately $2(Dt)^{\frac{1}{2}}$] is less than the film thickness (d), that is when the value of d^2/D (/s) is considerably greater than 0.25. A value for the surface coverage of active redox sites was obtained by integration under the cathodic branch of cyclic voltammograms obtained at slow sweep rates.

Some preparations had a low surface coverage of electroactive sites and showed the cyclic voltammetric response expected when d^2/D is small so that the concentration of electroactive sites remains uniform across the thickness of the film.[4,27,28] Low surface coverage is best obtained by using

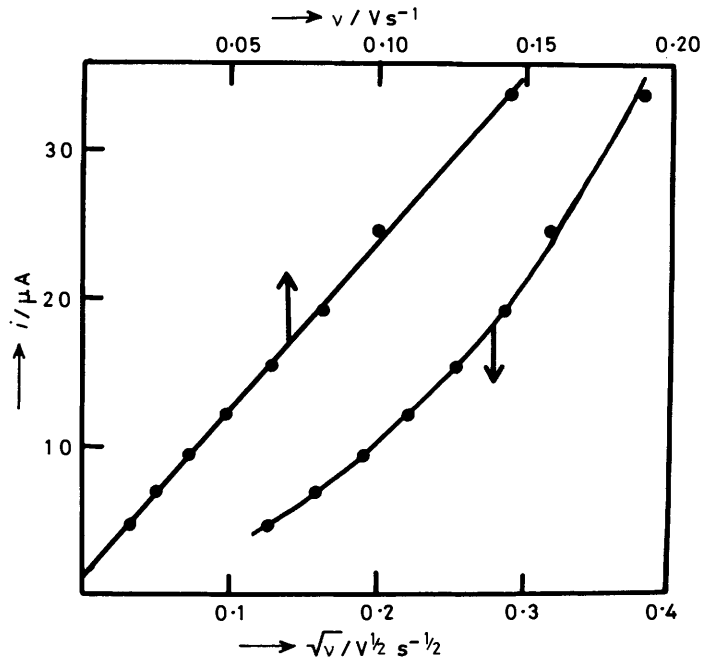


Figure 3. Data from cyclic voltammetry using a thin film of poly(N^{ε}-4-nitrobenzoyl-L-lysine) on Pt; film area 0.106 cm^2, Γ 5.4 nmol cm^{-2}, solvent acetonitrile containing 0.1M-Pr_4NBF_4. Peak current is proportional to v. Film prepared from 0.05% polymer solution

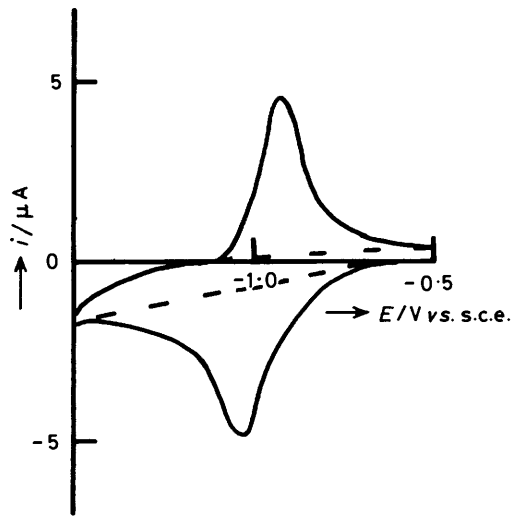


Figure 4. Cyclic voltammogram of a thin poly(N^{ε}-4-nitrobenzoyl-L-lysine) film on Pt; film area 0.106 cm^2, scan rate 16 mV s^{-1}, solvent acetonitrile containing 0.1M-Me_4NBF_4. Taking the background current as indicated by the dashed line, cathodic area = 55.2 μCoulomb and anodic area = 53.1 μCoulomb. Film prepared from 0.05% polymer solution

lower concentrations (0.05%) of the polymer in DMA. In these cases a good linear relationship was found between i_{pc} and v on cyclic voltammetry (Figure 3) represented by

$$i_{pc}\ (/\mu A) = (1.2 \pm 0.2) + (229 \pm 3)v\ (V\ s^{-1})$$

with correlation coefficient 0.9994. A typical cyclic voltammogram is shown in Figure 4. When the small background current indicated by the relationship between i_{pc} and v is allowed for then the cathodic and anodic branches of the cyclic voltammogram fall close to the base line. The observed peak separation of 115—150 mV, depending on scan rate, diverges considerably from the expected value of zero which indicates an electrical resistance within the film.

The ideal behaviour of such thin films has been well established in the literature[27] and

$$\Delta i_p/\Delta v = n^2F^2\Gamma_{total}/4RT.$$

Thus for our film $\Gamma_{total} = 2.4 \times 10^{-10}$ mol (see the Experimental section for a definition of the molar unit used here). Integration of the cathodic branch of the cyclic voltammogram with v = 0.016 V s^{-1} gives $\Gamma_{total} = 5.7 \times 10^{-10}$ mol ($\Gamma = 5.4 \times 10^{-9}$ mol cm^{-2}). Other workers have investigated the consequences of activity corrections to the concentration of electroactive species in the film and have shown that such activity effects decrease the value of i_{pc} and broaden the cyclic voltammetry curve.[28] This non-ideal behaviour is to be expected with the PNBL film and may account for some of the discrepancy between the values of surface coverage obtained by the two different methods.

The redox potential of the films [$= \frac{1}{2}(E_{pc} + E_{pa})$] is in the region expected for the 4-nitrobenzoyl group. Values are obtained in the range −0.99 to −1.06 V *vs.* s.c.e.

Chronoamperometry with a potential step from 0 to −1.5 V *vs.* s.c.e. was carried out on freshly broken-in films. Current transients were fitted to the Cottrell equation for bounded diffusion where the film is treated as a plane sheet of infinite area.[4,5] In fact we have a sheet moulded to the surface of a sphere, but since d is negligible in comparison with the radius of this sphere, the sheet can be considered as a plane. In the Cottrell equation the current i at time t is given by

$$i = (nFAD^{\frac{1}{2}}C_0/\pi^{\frac{1}{2}}t^{\frac{1}{2}})[1 + 2\sum_{k=1}^{\infty}(-1)^k \exp(-kd^2/Dt)]$$

where n is the number of electrons per active site, A is surface area, C_0 surface concentration of active sites per unit volume, and k is an integer. The other symbols have already been defined.

The data obtained by reduction of a number of separately prepared films are collected in Table 2. Values for $D^{\frac{1}{2}}C_0$ and d^2/D are obtained by fitting the Cottrell equation. Examples of the fit of i *vs.* $t^{-\frac{1}{2}}$ obtained for 'thick' and for 'thin' films are given in Figures 5 and 8. It was possible to fit only the early sections of the current–time transient. We could not carry out both slow-scan cyclic voltammetry and chronoamperometry on the same film without markedly changing its properties after the first of this pair of experiments. Surface coverage ($\Gamma = C_0d$) estimates for films subjected to chronoamperometry were obtained by integrating the chronoamperometry curves. We chose to integrate the current over 4 s. Over longer times the background current becomes important.

The values obtained for d^2/D agree reasonably well with values calculated by combining the limiting slope of the Cottrell equation and the surface coverage. Independent measurements of d and D have not been obtained. Later in this paper we will proceed to estimate C_0.

The Ageing Process.—This term conveniently describes the effects which are found when a PNBL film is subjected to prolonged electrical stress. The changes occur over a shorter time span when tetramethylammonium fluoroborate is used as the supporting electrolyte. The ageing process under continuous cyclic voltammetry is illustrated in Figure 6. (Some of the sequence of cyclic voltammograms has been removed for clarity.) Features of the process are (*a*) the cathodic peak potential slowly moves towards more negative values, (*b*) multiple charging peaks appear on the cathodic cycle, and (*c*) the anodic cycle deteriorates to a broad hump. Feature (*c*) is illustrated also in Figure 1(*b*) which is the final one of a long series of individual cyclic voltammograms.

Table 2. Chronoamperometry experiments with PNBL films on Pt. The solvent is acetonitrile, charging potential −1.5 V *vs.* s.c.e., discharging potential 0.0 V *vs.* s.c.e. Current transient was fitted to the Cottrell equation to obtain $D^{\frac{1}{2}}C_0$ and d^2/D. Γ is derived from the charge passed during 4 s. Films prepared from a 0.3% polymer solution in DMA

Experiment no.	$D^{\frac{1}{2}}C_0$[a]/nmol cm^{-2} s$^{-\frac{1}{2}}$	$\Gamma = C_0 d$/nmol cm^{-2}	$(\Gamma/D^{\frac{1}{2}}C_0)^2$[b]/s	d^2/D[a]/s	Comments
AM 61-16	17.5	28.0	2.6	3.5	Pr_4NBF_4 (0.1M)
AM 61-17	9.4	14.9	2.5	1.4	Discharge of AM 61-16
AM 61-19	16.0	27.9	3.0	3.5	Pr_4NBF_4 (0.1M)
AM 61-20	8.3	13.9	2.8	1.4	Discharge of Am 61-19
SD 37/2	8.4	17.0	4.4		Et_4NBF_4 (1M)
SD 78/2	10.6	16.2	2.3		Et_4NBF_4 (1M)
SD 71/3	10.2	15.1	2.2		Et_4NBF_4 (1M)
PNBL 60/3	16.9	15.2	1.1		Et_4NBF_4 (0.1M)
SD 70/2	7.87	14.0	3.2		Et_4NBF_4 (1M)
SD 77/2	7.26	13.0	3.2		Et_4NBF_4 (1M)
SD 113/2	11.1	12.5	1.3	0.3	Me_4NBF_4 (0.1M)

[a] Obtained by fitting the Cottrell equation to the data. [b] Calculated from the two previous values, $(\Gamma/D^{\frac{1}{2}}C_0)^2 = d^2/D$.

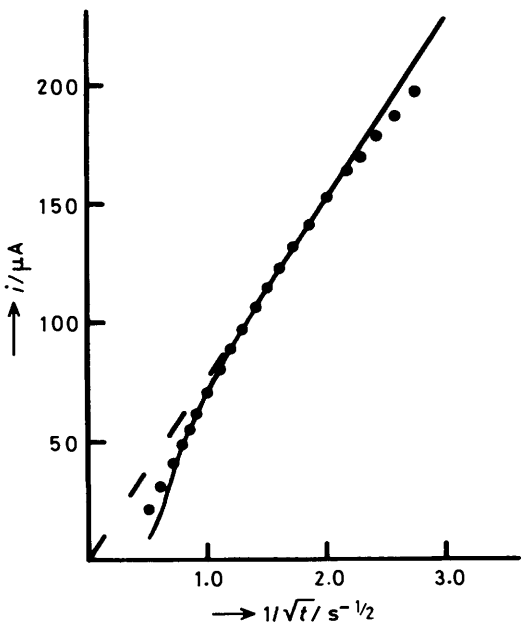


Figure 5. Chronoamperometry on a freshly 'broken-in' thick film of poly(N^{ε}-4-nitrobenzoyl-L-lysine) on Pt; film area 0.087 cm^2, potential step 0 to −1.5 V *vs.* s.c.e., charge passed over 4 s is 242.1 μCoulomb, solvent acetonitrile containing 0.1M-Pr_4NBF_4. Film prepared from 0.3% polymer solution.

Experiments showed that the film is not completely discharged during the anodic sweep in cyclic voltammetry. After one run at 100 mV s^{-1}, the film had a residual potential of −1.03 V *vs.* s.c.e. which discharged slowly through an external resistance. The decrease in charge passed during successive reduction cycles of Figure 6 is in part due to an accumulation of residual charge after the previous oxidation cycle. The shift in cathodic peak potential and the deterioration in the anodic peak shape can be ascribed either to the development within the film of an impediment to ion diffusion or to electrical-resistance effects.

With a fresh film, we carried out a sequence of one cyclic voltammogram at 100 mV s^{-1}, then chronoamperometry, repeated several times and allowing 10 min between each event for the film to discharge. Data from this sequence are collected in Table 3. The cyclic voltammograms show a gradual deterioration along the sequence, initial and final examples are given in Figure 7. By contrast, values of $D^{\frac{1}{2}}C_0$ and surface coverage obtained from chronoamperometry change very little, plots of i *vs.* $t^{-\frac{1}{2}}$ for initial and final runs are given in Figure 8. Thus diffusion through the bulk of the film is not influenced by the 'ageing' process. The most likely explanation for the ageing process is the development of areas of separation between the film and the supporting metal surface, resulting in a decrease in the electron transfer rate across these regions. A slow surface electron-transfer rate has a large effect on the cyclic voltammograms but is less important in chronoamperometry when a substantial negative potential is placed on the metal surface.

Continuous cyclic voltammetry of PNBL films leads to the development of waves (multiple peaks) on the charging and discharging curves. Examples of these multiple peaks are seen on some of the cyclic voltammograms already presented. The effect can best be demonstrated using a 'broken-in' thick film ($d^2/D \sim 3.5$ s) with tetrapropylammonium fluoroborate as the supporting electrolyte. Three successive cyclic voltammograms are presented in Figure 9. After the electrode has been allowed to rest for a few minutes, the next cyclic voltammogram shows one cathodic and one anodic peak only. This pattern of behaviour can be repeated until, eventually, the irreversible ageing process discussed in the previous paragraph becomes predominant.

Structure of the Film.—PNBL films show two types of behaviour which must be explained in terms of the structure of the film. Continuous cyclic voltammetry causes the reversible development of multiple peaks in the current–voltage response (using Pr_4NBF_4 supporting electrolyte) which are due to reversible conformational charges in the film. Prolonged charging of the film causes irreversible deterioration due to separation of the film from the electrode surface. The deterioration is faster with Me_4NBF_4 as the supporting electrolyte.

The existence of electrochemically non-equivalent sites in polymer films, leading to multiple cyclic voltammetry peaks, has been noted before.[5,6] We are not however aware of another electrochemical system which can be restored to the original state after perturbation simply by resting and without chemical treatment. Anson's electrode[5] was coated with poly(vinylpyridine) bearing Fe^{II} centres. It displayed a gradual transition from one pair of anodic, cathodic peaks to a second pair on continuous cyclic voltammetry. This was due to a conformational change in the film, the result of protonation at uncoordinated pyridine sites. The change could not be reversed except by neutralizing the film with a base. Reversible conformational changes in polypeptide membranes have also been induced photochemically.[29]

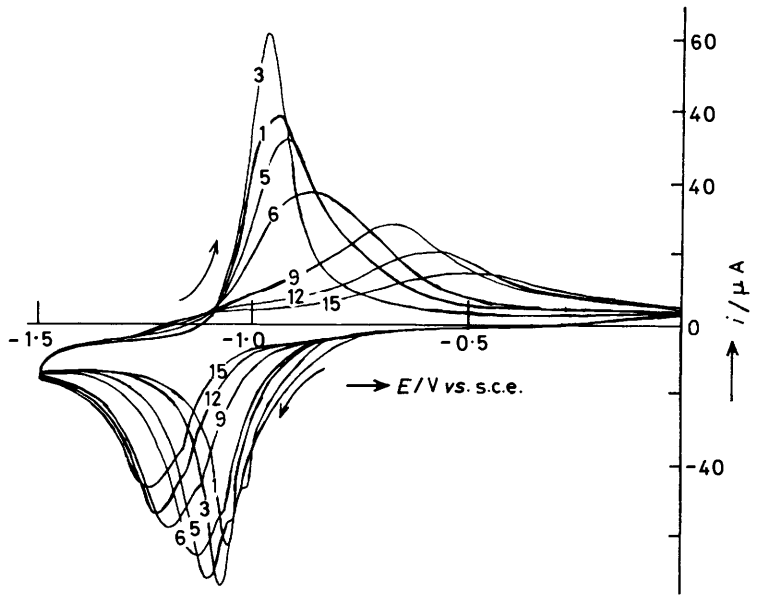


Figure 6. Continuous cyclic voltammetry of a poly(N^{ε}-4-nitrobenzoyl-L-lysine) film on Pt. The cycles are numbered in sequence, some are omitted for clarity; film area 0.106 cm^2, solvent acetonitrile containing 0.1M-Me_4NBF_4. Film prepared from 0.3% polymer solution

Table 3. PNBL film on Pt in acetonitrile (0.1M-Me_4NBF_4). Data from a sequence of cyclic voltammetry and chronoamperometry with a wait of 10 min between each experiment. Experiment SD 113/1 is the first cyclic voltammetry run (not recorded here) to swell the film

	Cyclic voltammetry		Chronoamperometry	
Experiment no.	E_{pc}/V *vs.* s.c.e.	i_{pc}/μA	$D^{\frac{1}{2}}C_0$/nmol cm^{-2} $s^{-\frac{1}{2}}$	Γ/nmol cm^{-2}
SD 113/2[a]			11.1	12.5
SD 113/3[b]	−1.10	57		
SD 113/4			12.0	12.3
SD 113/5	−1.12	50		
SD 113/6			12.0	11.8
SD 113/7	−1.14	47		
SD 113/8[a]			11.6	11.2
SD 113/9[b]	−1.15	40		

[a] Illustrated in Figure 7. [b] Illustrated in Figure 8.

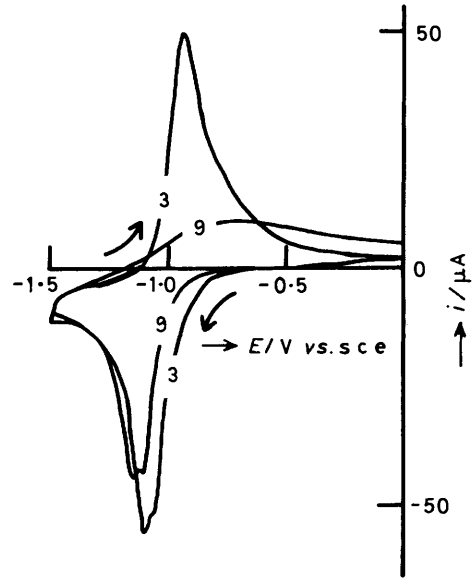


Figure 7. Cyclic voltammograms from the sequence of cyclic voltammetry, chronoamperometry summarized in Table 3. Film of poly(N^{ε}-4-nitrobenzoyl-L-lysine) on Pt; film area 0.106 cm^2, scan rate 0.1 V s^{-1}, solvent acetonitrile containing 0.1M-Me_4NBF_4. Film prepared from 0.3% polymer solution

PNBL is present in dimethylacetamide solution as the α-helix and we assume it is adsorbed as such onto platinum. Charging the film on reduction introduces a repulsive force between side chains which will destabilise the helix. A similar situation arises with poly(L-lysine) where the α-helix is stable in alkaline solution but the polymer reverts to a random coil in acid solution when the N^{ε}-groups are protonated.[30] Thus we expect that when PNBL is in the radical-ion form a conformational change occurs over some sections of the polymer that is a helix-to-random-coil process. These conformational changes cause islands to appear in the polymer where the electrical resistance and apparent diffusion rate are changed. Multiple peaks on cyclic voltammetry result from these changes. Extensive changes in polymer conformation give rise to separation of areas of polymer from the metallic surface and in these circumstances the irreversible 'ageing' process observed.

The dimensions of poly(N^{ε}-benzyloxycarbonyl-L-lysine) in the solid state are known from *X*-ray crystallography.[31] We can take these dimensions as an approximate model for PNBL. The α-helix is hexagonal close packed with a distance of 16.69 Å between axes of adjacent helices and a repeat length along the helix containing 18 amino acid residues within 27 Å. A close packed monolayer with these dimensions has

$$\Gamma = 0.663 \text{ nmol cm}^{-2}$$

Thus the thinnest film we prepared with the cyclic voltammogram of Figure 4 has *ca.* 10 layers of adsorbed helices whilst the thickest films with Γ = 28 nmol cm^{-2} have *ca.* 40 layers of adsorbed helices. A film of close-packed helices would have

$$C_0 = 4.59 \times 10^{-3} \text{ mol cm}^{-3}$$

but this value is unrealistic for the film swollen by solvent and ions. No other more relevant dimensions for the film are available so far.

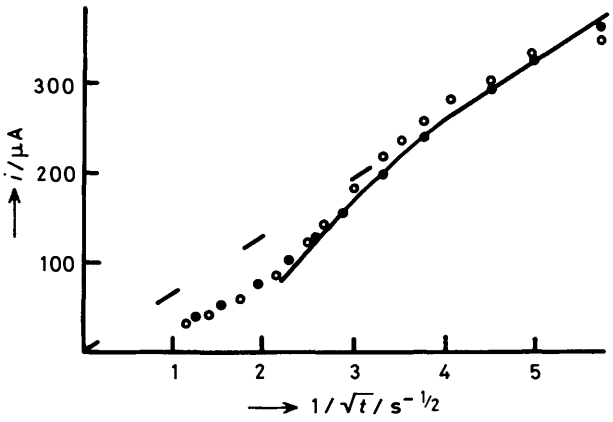


Figure 8. Chronoamperometry results from the sequence of cyclic voltammetry, chronoamperometry summarized in Table 3. Film of poly(N^{ε}-4-nitrobenzoyl-L-lysine) on Pt; film area 0.106 cm^2, Γ 12.5 nmol cm^{-2}, potential step 0 to −1.5 V *vs.* s.c.e., solvent: acetonitrile containing 0.1M-Me_4NBF_4; ●, experiment no. 2; ○, experiment no. 8. Film prepared from 0.3% polymer solution

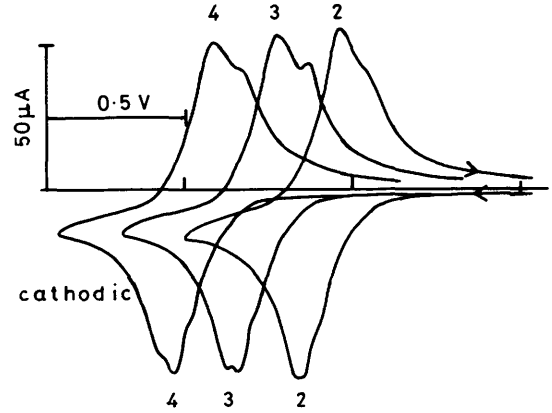


Figure 9. Scans 2, 3, and 4 of repetitive cyclic voltammetry of a 'broken-in' thick film of poly(N^{ε}-4-nitrobenzoyl-L-lysine) on Pt; film area 0.087 cm^2, Γ 12.5 nmol cm^{-2}, scan rate 0.1 V s^{-1}, solvent: acetonitrile containing 0.1M-Pr_4NBF_4. Each scan begins at 0 V and has a maximum excursion to −1.5 V *vs.* s.c.e. Consecutive scans are displaced by −0.2 V for clarity. Film prepared from 0.3% polymer solution.

Experimental

^1H N.m.r. spectra were recorded at 250 MHz in 0.1M-DCl/D_2O relative to DSS or in either $CDCl_3$ or CD_3SOCD_3 relative to $SiMe_4$. All solvents used for the preparation and polymerization of oxazolidine-2,5-diones were rigorously purified and dried according to literature methods.

Non-S.I. units of surface concentration (mol cm^{-3}), surface coverage (mol cm^{-2}), and diffusion constant (cm^2 s^{-1}) are common in the literature and have been retained here. In this context 1 mole of electroactive species refers to 6.02×10^{23} electroactive units that are attached to the polymer chains.

N^{ε}-(4-*Nitrobenzoyl*)-L-*lysine*.—L-Lysine monohydrochloride (18.3 g, 0.10 mol) was heated with an excess of basic copper carbonate [$CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$] in water (300 ml). When the initial effervescence had subsided, reflux was maintained for 15 min. The resulting blue solution was cooled and the excess of copper carbonate was filtered off. To this stirred solution of the copper complex of lysine, at 0 °C, was added dropwise one equivalent of 4-nitrobenzoyl chloride (18.6 g, 0.1 mol) in ether (75 ml) while simultaneously maintaining the solution alkaline by the dropwise addition of 5M-sodium hydroxide. Stirring was continued for 2 h after all the acid chloride had been added and the mixture was maintained alkaline throughout. The copper complex which precipitated was filtered off and washed with water (3 × 200 ml), ethanol (2 × 150 ml), and ether (2 × 150 ml).

The resulting blue precipitate was suspended in ethanol (1 200 ml) and an excess of salicyl aldoxime (16 g, 0.117 mol) was added. After having been vigorously stirred for 24 h, the mixture was filtered to leave a green solid containing the copper(II) oxinate and the required amino acid. This mixture was extracted with boiling water and the filtrate containing the amino acid was evaporated under reduced pressure to leave N^{ε}-(4-nitrobenzoyl)-L-lysine (9.2 g, 30%) which was recrystallized from water as fine colourless prisms, m.p. 210—216 °C (decomp.) (Found: C, 52.7; H, 5.8; N, 14.0. $C_{13}H_{17}N_3O_5$ requires C, 52.9; H, 5.8; N, 14.2%); $[\alpha]_D^{20}$ +15.2° (*c* 0.1 in 2M-HCl); $\lambda_{max.}$ (10% EtOH) 270 nm (ε 1.29 × 10^4); δ (DCl) 1.52—1.76 (4 H, m, γ- + δ-H_2), 2.00—2.10 (2 H, m, β-H_2), 3.44 (2, H, t, *J* 7 Hz, ε-H_2), 4.20 (1 H, t, *J* 6.2 Hz, α-*H*), 7.87 (2 H, d, ArH), and 8.31 (2 H, d, ArH).

A specimen prepared according to the literature method, using hydrogen sulphide,[16] showed additional aromatic resonances δ 7.14 (d) and 7.82 (d) due to a 4-aminobenzoyl group.

N^{α}-(*Benzyloxycarbonyl*)-N^{ε}-(4-*nitrobenzoyl*)-L-*lysine*.—A solution of N^{ε}-(4-nitrobenzoyl)lysine (0.6 g) in sodium hydroxide (2M; 4 ml) was cooled in ice and stirred during the simultaneous addition of benzyloxycarbonyl chloride (0.6 ml) and sodium hydroxide (4M; 4 ml). After 30 min the mixture was acidified with concentrated hydrochloric acid and the oily product was isolated with ethyl acetate. The solution was evaporated under reduced pressure and diluted with light petroleum (b.p. 60—80 °C) to yield a colourless solid. Recrystallization of this from ethyl acetate–light petroleum (b.p. 60—80 °C) yielded colourless needles of N^{α}-(*benzyloxycarbonyl*)-N^{ε}-(4-*nitrobenzoyl*)-L-*lysine* (0.53 g, 61%), m.p. 148—150 °C (Found: C, 59.0; H, 5.3; N, 9.8. $C_{21}H_{23}N_3O_7$ requires C, 58.7; H, 5.4; N, 9.8%); $[\alpha]_D^{20}$ −8.7° (*c* 0.1 in MeOH); $\lambda_{max.}$(MeOH) 264 (ε 1.54 × 10^4); δ($CDCl_3$) 1.4—1.7 (6 H, m, β- + γ- + δ-H_2), 3.40 (2 H, m, ε-H_2), 3.95 (1 H, m, α-H), 5.03 (2 H, s, benzylic-H_2), 7.36 (5 H, s, Ph), 8.07 (2 H, d, *J* 9 Hz, ArH), and 8.31 (2 H, d, *J* 9 Hz, ArH).

N^{α}-(*Benzyloxycarbonyl*)-N^{ε}-(*benzoyl*)-L-*lysine*.—N^{ε}-(Benzoyl)lysine (1.0 g) was dissolved in sodium hydroxide (2M; 7 ml) and treated with benzyloxycarbonyl chloride (1.0 ml) and sodium hydroxide (2M; 7 ml) as for the previous example. The product was obtained from ethyl acetate–light petroleum (b.p. 60—80 °C) as a microcrystalline powder (1.0 g, 76%), m.p. 98—100 °C (Found: C, 65.4; H, 6.3; N, 7.4. Calc. for $C_{21}H_{24}N_2O_5$: C, 65.6; H, 6.3; N, 7.4%); δ (CD_3SOCD_3) 1.4—1.7 (6 H, m, β- + γ- + δ-H_2), 3.25 (2 H, m, ε-H_2), 3.95 (1 H, m, α-H), 5.03 (2 H, s, benzylic H_2), 7.36 (5 H, s, Ph), 7.46 (3 H, m, ArH), and 7.85 (2 H, d, *J* 6 Hz, ArH).

4-(4-*Benzoylaminobutyl*)*oxazolidine*-2,5-*dione*.—(*a*) *Preferred procedure* [*cf.* ref. (21)]. N^{ε}-Benzoyl-L-lysine (10mM; 2.5 g) was stirred with dioxane (50 ml), under a nitrogen atmosphere, in a three-necked flask. A glass-sintered filtration tube was attached to the flask. The mixture was heated to 65 °C and an excess of phosgene (12.5% solution in toluene; 31.6mM; 25 ml) was added, maintaining a static nitrogen atmosphere. After 1.5 h a clear solution resulted and this was filtered hot, under a pressure of nitrogen into another flask. A stream of nitrogen was bubbled through the hot filtrate for 0.5 h to remove the excess of phosgene. The product oxazolidinedione crystallized on being cooled and was filtered under nitrogen. The filtrate was heated

to 70 °C and hexane was added so as to obtain a second crop of the product on cooling. A third crop was obtained giving a total yield of 2.0 g (72%). The *oxazolidinedione* recrystallized from ethyl acetate–hexane as colourless needles, m.p. 148—154 °C (decomp.) (Found: C, 60.9; H, 5.8; N, 10.1. $C_{14}H_{16}N_2O_4$ requires C, 60.9; H, 5.8; N, 10.1%); ν_{CO}(KBr) 1 850 and 1 780 cm^{-1}; *m/z* 277 (M^+, 2%), 105 (20), 92 (18), 91 (21), and 88 (26) (Found: M^+, 276.1119. $C_{14}H_{16}N_2O_4$ requires *M*, 276.1110).

(*b*) A solution of N^{ε}-benzoyl-N^{α}-(benzyloxycarbonyl)-lysine (1.0 g) in dioxane (25 ml) was cooled to the freezing point, powdered phosphorus pentachloride (1.1 g) added, and the mixture was stirred for 10 min. The excess of phosphorus pentachloride was removed by filtration and the filtrate was heated at 50 °C for 2 h. The solvent was removed under reduced pressure and the residue triturated with pentane to leave the oxazolidinedione as a yellow solid. The product was collected and crystallized from dioxane–pentane as an off-white solid, m.p. 145—152 °C (0.46 g, 64%), ν_{CO}(KBr) 1 850 and 1 780 cm^{-1} (due to NCA group).

Poly(N$^{\varepsilon}$-*benzoyl*-L-*lysine*).—4-(4-Benzoylaminobutyl)oxazolidine-2,5-dione (0.50 g) was dissolved in a mixture of dimethylacetamide (1.5 ml) and dioxane (15 ml) and sodium hydride was added (50% dispersion in oil; 0.009 g) (monomer:initiator = 10:1). After 20 h at room temperature the i.r. spectrum of the mixture showed the disappearance of the characteristic NCA carbonyl bands. The solution was poured into ether (200 ml) and the gel-like precipitate obtained was collected, washed with ethyl acetate and ether, and dried under vacuum. This product was purified by dissolution into dichloroacetic acid and dilution of the solution with 15 volumes of water. The polymer was obtained as a fibrous precipitate (0.3 g) which was washed with water and ether and then dried under vacuum.

A solution of this polymer in dichloroacetic acid gave intrinsic viscosity $[\eta/dl\ g^{-1}] = 0.86$ from which $M = (1.7 \pm 0.7) \times 10^5$, see Table 1 for the i.r. absorption frequencies.

Poly(N$^{\varepsilon}$-4-*nitrobenzoyl*-L-*lysine*).—*Preferred procedure.* N^{ε}-4-Nitrobenzoyl-lysine (1.0 g. 3.4 mmol) was suspended in ethyl acetate (20 ml) and dioxane (20 ml) under an atmosphere of nitrogen in a three-necked flask fitted with a sintered-glass filtration tube. The mixture was heated to 65 °C and an excess of phosgene (12.5% solution in toluene; 25mM; 20 ml) added. After 4.5 h a cloudy solution resulted. Nitrogen was bubbled through the hot solution to remove the excess of phosgene. The solution was then filtered into hexane (400 ml) and the whole was allowed to stand for 12 h at 0 °C. The oxazolidinedione crystallized and was collected and recrystallized twice from ethyl acetate–hexane. It was obtained as large colourless rods (0.51 g, 47%), ν_{CO}(KBr) 1 850 and 1 780 cm^{-1} (due to NCA group).

A solution of the oxazolidinedione (0.51 g) in a mixture of dimethylacetamide (6 ml) and dioxane (12 ml) was treated with sodium hydride (50% dispersion in oil; 0.009 g) (monomer:initiator = 8.3:1). After 20 h at room temperature the i.r. spectrum of the solution showed disappearance of the characteristic NCA carbonyl bands. The solution was poured into dichloromethane (400 ml) to give the polymer as a fibrous precipitate. This was collected and purified by dissolution in dichloroacetic acid and precipitation by pouring into water. The polymer (0.31 g) formed a pale cream fibrous solid which was dried under vacuum.

A solution of this polymer in dimethylformamide showed intrinsic viscosity $[\eta/dl\ g^{-1}) = 1.045$ from which $M = (2.0 \pm 1.3) \times 10^5$, see Table 1 for the i.r. absorption frequencies.

Electrochemical Measurements.—Poly(N^{ε}-4-nitrobenzoyl-L-lysine) was allowed to swell in dimethylacetamide so as to make a 0.3% solution. The solution was somewhat viscous. 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 platinum was exposed. Using a micromanipulator, the platinum sphere was lowered into the polymer solution so that the sphere and shank of platinum wire were immersed, but not the glass support. After 15—20 min, the electrode was slowly removed from the polymer solution, then dried at 50 °C and 0.1 mmHg for 20 min. The electrode was then ready for use.

Old electrodes were cleaned by burning off the coating in a hydrogen–oxygen flame, then recoated as above. This process could be repeated *ca.* 25 times before the surface of the metal began to deteriorate.

A coated electrode was immersed in a solution of the electrolyte (usually 0.1M-tetrapropylammonium fluoroborate, but see Tables for individual examples) in acetonitrile. A platinum wire formed the counter electrode. The reference electrode was saturated aqueous potassium chloride–calomel dipping into 1.0M-sodium nitrate, then connected through a bridge of the supporting electrolyte in acetonitrile. The cell was deoxygenated by a stream of nitrogen.

Cyclic voltammetry was performed sweeping between 0 and −1.5 V *vs.* s.c.e. Chronoamperometry was carried out stepping the potential to −1.5 V *vs.* s.c.e. and collecting the current transient over 4 s. The potentiostat was a PAR 176 model with 274 digital interface and controlled through an Apple IIe computer.

Acknowledgements

We thank the S.E.R.C. and Queens University for support. A. M. A. and K. S. D. P. are grateful for leave of absence from Sri Jayewardenepura University, Sri Lanka.

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Received 21*st December* 1987; *Paper* 7/2227