Release of the Neurotransmitters Glutamate and γ-Aminobutyric Acid from an Electrode. Catalysis of Slow Redox Propagation through a Polymer Film

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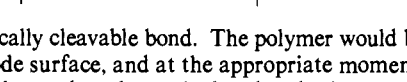
Abstract: Two functionalized polystyrenes were synthesized which held cationic isonicotinamide groups. The amine portions of these polymers corresponded to the neurotransmitters, γ-aminobutyric acid and glutamic acid, respectively. The polymers were individually coated onto glassy carbon disks and used as electrodes in aqueous electrolytes. It was shown, using a thin-layer cell and HPLC, that small amounts of glutamate or γ-aminobutyrate were released in response to a pulse of cathodic current at ca. −0.9 V (SCE). The propagation of the reduction process through these polymer films was not rapid. It could be accelerated by placing methylviologen, MV^{2+}, in the solution. The viologen was reduced at the electrode, and the reduction products, MV^{+} and MV, acted to reduce the isonicotinamide units and thereby increased the current and the amount of neurotransmitter released into the solution. Cathodic current enhancements were also seen when the films were treated with $Cr(ClO_4)_3$ or $FeCl_3$.

In this work we set out to demonstrate that it was possible to use a pulse of current to release organic compounds from an electrode surface. A suitably designed electrode of this kind might have utility for the delivery of biologically active compounds. A specific application of interest is the delivery of neurotransmitters. In neuroscience it is important to be able to deliver neurotransmitters or drugs to specific locations at specific times. In this way the effect of these compounds on single neurons can be studied. At present, two methods exist for drug delivery at the single neuron level.[1] One is pressure injection of a solution of the compound from a micropipet; the other is iontophoresis. In iontophoresis a solution of the active material, in an ionic form, is placed in a micropipet and then "phoresed" out with a small current. Although widely used, each of these techniques has inherent problems.

Our proposal is that a polymer-coated electrode could be used advantageously in this application. Thus, it was envisaged that the active compound would be attached to a polymer backbone



by a cathodically cleavable bond. The polymer would be attached to an electrode surface, and at the appropriate moment a current pulse would be used to cleave the bond and release the chemical.

In our initial studies we attempted to release the neurotransmitters dopamine,[2] glutamate (Glu), and γ-aminobutyric acid (GABA) Since many of the compounds of interest to neuroscientists are amines, the approach chosen was one which could be generalized for any amine. The polymers **4a** and **4b** have been prepared. In these polymers, the polystyrene serves to insolubilize the material on an electrode surface and the cationic isonicotinate unit serves as an electron acceptor which can undergo a cathodic, amide bond cleavage. In this paper are described the electrochemical properties of electrodes with polymers **4a** and **4b** on their surface. It was shown that these electrodes did release Glu and GABA in response to a cathodic current pulse. Application of this technology to single neuron studies will require the use of coated microelectrodes. Preliminary experiments suggest that coated carbon fiber microelectrodes will be useful.

It will be recognized that the properties of polymer modified electrodes play a central role in this study. These properties have been of interest to this group and others for several years. A particular issue of general importance which we have explored here is the propagation of redox reactions from the underlying electrode surface through the polymer layer.[3] Studies in which viologens were used to accelerate the propagation of the cathodic reaction through the polymer film and the effects of some metal ions on the electrochemistry are described.

Experimental Section

Dibenzyl L-glutamate benzenesulfonate salt (1a) was prepared in a manner similar to that of Shields, McGregor, and Carpenter.[4] L-Glutamic acid (8.05 g, 54.73 mmol), benzenesulfonic acid monohydrate (58 mmol), benzyl alcohol (67 mmol), and benzene (150 mL) were refluxed overnight using a Dean-Stark apparatus for the removal of water. The solvent was then reduced to half its original volume under reduced pressure. Dry ether was added after which precipitation occurred. Recrystallization from alcohol/ether gave 19.66 g (74%) of **1a**, mp 115-116 °C (lit.[4] 115-117 °C): IR (KBr) 3000 (b), 1740 (s), 1530, 1500, 1449, 1200 (s), 1125, 1040, 1020, 755, 730, 690 cm^{-1}; NMR (Me_2SO-d_6) δ 8.47 (s, 3 H), 7.61-7.73 (m, 5 H), 7.35 (s, 10 H), 5.21 (s, 2 H), 5.09 (s, 2 H), 4.17 (t, 1 H), 2.55 (t, 2 H), 2.11 (q, 2 H).

***N*-(Dibenzyl glutamate)isonicotinamide (2a)** was prepared by addition of 1.025 g (8.33 mmol) of isonicotinic acid to 15 mL of thionyl chloride. The mixture was refluxed for 8 h. The excess of thionyl chloride was removed under reduced pressure. To the residue, 4.15 g of **1a** (10 mmol) was added with 20 mL of dry dioxane. The stirred mixture was chilled with an ice bath, while 4 mL (26 mmol) of triethylamine was added dropwise. After the addition was completed, the mixture was stirred for 1 h at 0 °C and then stirred for 20 h at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in 30 mL of ether and washed with 20 mL of 1 N NaOH solution and two 20-mL portions of water, successively. An oily product, which was obtained after drying, solidified upon cooling and was washed with anhydrous ether. The yield was 2.21 g (64%), mp 87-88.5 °C: IR (KBr) 3440, 3300, 1645, 1535 cm^{-1}. The ^{13}C NMR data are in Table I.

Anal. Calcd for $C_{25}H_{24}N_2O_5$: C, 69.43; H, 5.59; N, 6.48; O, 18.50. Found: C, 69.51; H, 5.66; N, 6.42; O, 18.66.

Chloromethylated polystyrene was prepared by polymerization of chloromethylstyrene. A solution of 5 mL of chloromethylstyrene (40% para, 60% meta from Polysciences) in 75 mL of toluene was washed successively with three 10-mL portions of 5% sodium hydroxide and two 10-mL portions of water to remove the polymerization inhibitor. After drying over anhydrous magnesium sulfate the solution was degassed with dry nitrogen and a catalytic amount (0.1 g) of benzoyl peroxide was added. After 3 h of reflux, the solvent was removed under reduced pressure and the residue was dissolved in 2 mL of chloroform. Methanol was then added dropwise with vigorous stirring until a milky suspension

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(2) (a) Miller, L. L.; Lau, A. K. N.; Miller, E. *J. Am. Chem. Sco.* **1982**, *104*, 5242. (b) Lau, A. K. N.; Zinger, B.; Miller, L. L. *Neurosci. Lett.* **1983**, *35*, 101. (c) Lau, A. K. N.; Miller, L. L. *J. Am. Chem. Soc.*, preceding paper in this issue.

(3) For leading references, see: (a) Buttry, D. A.; Anson, F. C. *J. Am. Chem. Soc.* **1982**, *104*, 4824. (b) White, H. S.; Leddy, J.; Bard, A. J. *Ibid.* **1982**, *104*, 4811. (c) Henning, T. P.; White, H. S.; Bard, A. J. *Ibid.* **1982**, *104*, 5862. (d) Burgmayer, P.; Murray, R. W. *J. Electroanal. Chem.* **1982**, *135*, 335. (e) Andrieux, C. P.; Dumas-Bouchiat, J.-M.; Saveant, J.-M. *Ibid.* **1982**, *131*, 1. (f) Laviron, E. *Ibid.* **1982**, *131*, 61.

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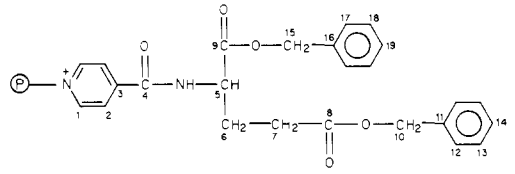
Table I. ^{13}C NMR Data[a] for **2a,b-4a,b**



carbon no.	2a	3a	4a	2b	3b	4b
1	150.119	149.78	149.03	150.18	148.43	148.53
2	121.304	131.21	131.06	121.26	126.03	126.03
3	140.527	147.80	147.74	141.49	145.48	161.38
4	165.293	164.39	164.44	164.80	161.38	161.54
5	52.144	55.15	55.03	*b*	*b*	*b*
6	25.267	27.73	28.18	24.34	24.18	24.80
7	30.059	32.52	32.68	31.04	30.98	31.03
8	171.083	172.66	174.08	172.54	172.35	174.05
9	171.892	173.93	175.56			
10	65.502	67.66		65.42	65.35	
11	135.771	137.91		186.25	136.06	
12	128.289	130.41		128.41	128.28	
13	127.839	129.93		127.93	127.73	
14	127.648	128.57		120.56	122.00	
15	66.072	68.43				
16	135.981	138.27				
17	128.298	131.21				
18	129.938	130.01				
19	127.782	128.83				

[a] In Me_2SO-d_6 vs. Me_4Si. [b] Overlapped with the Me_2SO-d_6 bands. Exact values not known.

was obtained. This suspension was added dropwise to 200 mL of methanol with stirring. The precipitate was filtered, washed with methanol, and air-dried. This precipitation procedure was repeated and the final white precipitate was washed with 5% sodium bicarbonate, water, and methanol. The yield after drying was 1.84 g. Anal. Calcd for $(C_9H_9Cl)_n$: C, 70.83; H, 5.94; Cl, 23.23. Found: C, 70.66; H, 6.07; Cl, 23.43.

Polymer 3a was prepared by adding 0.474 g (11 mmol) of **2a** and 0.159 g (1.04 mmol) of chloromethylated polystyrene to 30 mL of dry toluene and heating the mixture at 90 °C with constant stirring over a period of 3 days. The precipitates were filtered, washed with dry toluene, and air-dried, yield 0.340 g (56%), softening temperature 150 °C. Anal. Calcd for $C_{34}H_{33}N_2O_5Cl\cdot 1/2H_2O$: C, 68.74; H, 5.77; N, 4.72; O, 14.81. Found: C, 68.59; H, 5.95; N, 4.49; O 14.51. Loading of the polymer was calculated to be 95% (7.6 mmol of amino acid per 1 g of polymer). ^{13}C NMR are summed in Table I.

Polymer 4a was prepared by dissolving 0.31 g (0.53 mmol) of **3a** in 15 mL of glacial acetic acid. Through this solution, hydrogen bromide gas was bubbled gently at 65 °C over a period of 3 h. The acetic acid was removed under reduced pressure. The residue was dissolved in 4 mL of DMF, and 5 mL of methanol was added. Anhydrous ether was added dropwise with constant stirring until a milky suspension was obtained. This suspension was added dropwise to 150 mL of vigorously stirred anhydrous ether. The precipitate was filtered and air-dried, yielding 0.21 g (86%), softening temperature 180 °C. Analysis showed 1.65 mmol of isonicotinamide units per 1 g of polymer (96% loading). ^{13}C NMR data are shown in Table I.

Benzyl γ-aminobutyrate sulfonate salt (1b) was prepared in a similar procedure to that used for **1a**. The yield was 99%, mp 86–87 °C: IR (neat on NaCl) 3000 (b), 1740 (b), 1530, 1460, 1440, 1435, 1190 (b), 780, 755, 725, 690 cm^{-1}; NMR ($CDCl_3$) δ 7.92–7.60 (m, 5 H), 7.28 (s, 5 H), 5.00 (s, 2 H), 2.88 (t, 2 H), 2.30 (t, 2 H), 1.89 (q, 2 H). Anal. Calcd for $C_{17}H_{21}NO_5S$: C, 58.10; H, 6.02; N, 3.99; O, 22.76. Found: C, 58.22; H, 6.02; N, 4.01; O, 22.89.

γ-(4-Pyridinecarboxyamido)butanoic acid benzyl ester (2b) was prepared by refluxing 2.0 g (76 mmol) of isonicotinic acid in 10 mL of thionyl chloride for 4 h. The excess of the thionyl chloride was removed under reduced pressure, and the residue was dissolved in 50 mL of chloroform. To this suspension, 5.25 g (14.93 mmol) of **1b** and 8 mL of triethylamine were added. The mixture was stirred at 50 °C for 12 h. The solution was washed with two 20-mL portions of 5% $NaHCO_3$ and three 30-mL portions of water successively. The dark brown organic phase was decolorized with charcoal and dried over anhydrous magnesium sulfate. The chloroform was removed and the residue was crystallized from chloroform/ether, yielding 2.92 g (66%), mp 79–81 °C: IR (KBr) 3340, 1760, 1640, 1555, 1535 cm^{-1}. Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. found: C, 68.37; H, 6.03; N, 9.34. ^{13}C NMR data are shown in Table I.

Polymer 3b was prepared by heating a solution of 0.895 g (3.0 mmol) of **2b** and 0.401 g (2.65 mmol) of chloromethylated polystyrene in 50 mL of dry toluene at 100 °C over a period of 3 days with constant stirring. A precipitate appeared. It was filtered, washed with 10 mL of toluene, then 5 mL of anhydrous ether, and air-dried, yielding 1.062 g, softening temperature 75 °C. Anal. Calcd for $C_{26}H_{27}N_2O_3Cl\cdot H_2O$: C, 66.58; H, 6.23; N, 5.97; O, 13.65; Cl, 7.56. Found: C, 67.06; H, 6.02; N, 5.67; O, 13.69; Cl, 7.96. ^{13}C NMR data are shown in Table I.

Polymer 4b was prepared by stirring 1.123 g (2.41 mmol) of **3b** in 15 mL of glacial acetic acid, in which hydrogen bromide gas was bubbled gently for 5 h at 60–65 °C. The acetic acid was removed under reduced pressure and the residue was dissolved in 3 mL of DMF. The solution was diluted with 20 mL of methanol. Ether was added dropwise with vigorous stirring until a milky suspension was obtained. The suspension was poured dropwise into 20 mL of anhydrous ether with stirring. The precipitate was filtered under a nitrogen atmosphere and dried to yield 0.833 g (83%) of **4b**, softening temperature 170 °C. Anal. Calcd for $C_{19}H_{21}N_2O_3Br$: C, 56.31; H, 5.22; N, 6.91, O, 11.85; Br, 19.72. Found: C, 56.19; H, 5.30; N, 6.60; O, 12.07; Br, 20.08. The calculated loading was 96% (2.0 mmol per 1 g of polymer). ^{13}C NMR data are in Table I.

Materials. All other materials were commercially available and were used without further purification. The buffer solutions (Fisher Scientific) were as follows: pH 3 and 4 potassium biphthalate–hydrochloric acid, pH 6–8 potassium phosphate–sodium hydroxide, pH 9 boric acid–potassium chloride–sodium hydroxide, and pH 10 potassium carbonate–potasium borate–potassium hydroxide. For pH 1 and 2 potassium chloride–hydrochloric acid was used. Glassy carbon electrodes were from Normar Industries, Anaheim, CA.

Cyclic Voltammetry Experiments. Cyclic voltammetry (CV) was carried out using a Princeton Applied Research (PAR) potentiostat, Model 173, in conjunction with a PAR universal programmer. The voltammograms were recorded on a Houston Model 2000 recorder. A single compartment cell containing a saturated calomel electrode (SCE) as a reference was used. The electrolyte always contained 0.1 N KCl and was sometimes buffered. The counter electrode was a graphite rod. The working electrode was a glassy carbon disk (area 0.08 cm^2) set in a glass or Teflon tube. It was cleaned on polishing cloth (Fisher) using Magomet polishing compound No. 40-6440AB (obtained from Buehler Ltd., Evanston, IL), before before each use. Stock solutions of various concentrations of polymer in DMF were prepared. A volume of 0.05–0.4 μL was syringed onto an upright working electrode. Slow evaporation of the solvent gave a polymer film electrode holding amino acid units in amounts equivalent to 0.4–300 monolayers. The amount could be controlled by varying the volume and the concentration of the polymer solution.

Preparative Electrolysis of 4a and 4b. A set of 37 electrodes was prepared by dip-coating of glassy carbon rods (0.25 in. in diameter and 2.5 in. in length) with a 9.5 mg/mL solution of **4a** in DMF. The cyclic voltammograms of these dip-coated electrodes showed peak shapes similar to those obtained by using glassy carbon disks. Individually, these electrodes were taken from 0 to −1.2 V (SCE), by means of a single sweep at 100 mV/s, so that glutamic acid could be discharged into a single 10 mL of degassed pH 7 buffer solution, containing 0.1 M KCl used as supporting electrolyte. A divided cell was used to minimize anodic destruction of the released glutamic acid. After the electrolyses were completed, the catholyte was diluted to a known volume and then analyzed on a Beckman amino acid analyzer, Model 118 BL. The column was 34 cm × 0.9 cm Beckman spherical resin, Type W-3H. The column temperature was 49.7 °C; the flow rate was 104 mL/h. The eluant was pH 3.25 citrate buffer. The retention time of the product was 49.2 min, identical with that of an authentic sample of L-glutamic acid under the same conditions.

Similarly, a set of 40 electrodes was prepd. by dip-coating from a 11.1 mg/mL solution of **4b** in DMF. After electrolyses into the 10-mL volume, amino acid assays were done on a Beckman PA35 column (6.8 cm × 0.9 cm) at 49.7 °C column temperature; flow rate 104 mL/h, pH 4.12 citrate buffer eluant. The retention time of the released product was 29.0 min, which is identical with that of an authentic sample of GABA under the same conditions.

Thin-Layer Electrochemistry Experiments. A carbon rod, serving as a counter electrode, was held upright, and 70 μL of 0.1 N KCl buffered solution was placed on its upper, polished 0.89-cm^2 surface. A glassy carbon electrode (area 0.495 cm^-) which had been coated with polymer by the syringe method was lowered using a micromanipulator until the solution was in contact with both electrodes. A micropipet (tip diameter 3 μm) was inserted into the solution. The pipet led to a syringe barrel with a SCE reference electrode. As soon as the cell was assembled, either

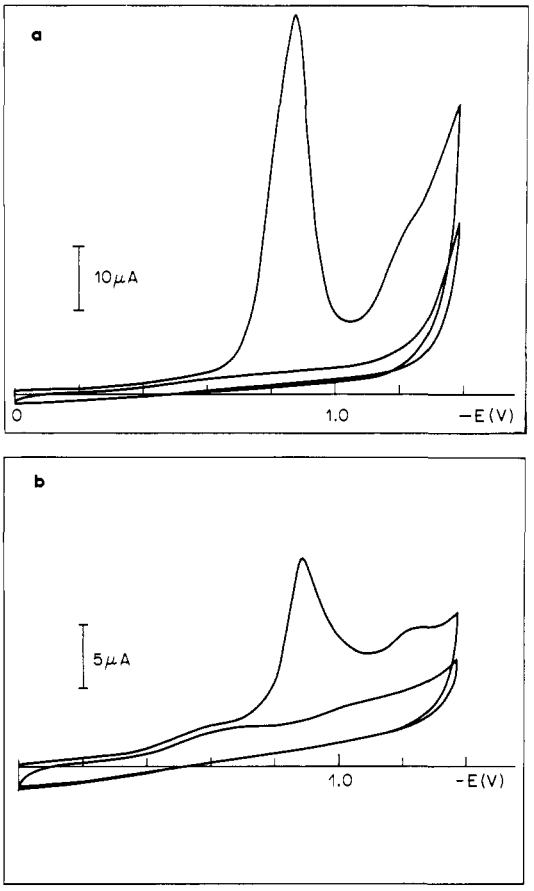


Figure 1. (a) Voltammogram for IVa in unbuffered 0.1 N KCl, $\nu = 0.1$ V s^{-1}, first and second sweeps. (a) $\Gamma_i = 3.96 \times 10^{-8}$ mol cm^{-2}. (b) $\Gamma_i = 1.32 \times 10^{-8}$ mol cm^{-2}.

a constant potential pulse (duration 1 min) or a triangular potential/time sweep (scan rate 50 mV/s) was applied, after which a volume of 50 μL of the solution was removed. Each experiment was carried out twice, and the two volumes were combined to give 100 μL of solution (solution A). The pH of this solution was changed to 9.5 by addition of a known amount of aqueous pH 12.8 solution. The dansylation was performed according to the procedure described by Karger:[5] a solution of 1.5 mg of dansyl chloride (Aldrich Chemical Co.) in 1 mL of dry acetonitrile was prepared. To solution A, 50 μL of dansyl chloride solution was added in the dark, and the mixture was kept at room temperature. After 24 h, the reaction was injected on a Waters HPLC using an Ultrasphere ODS 5-μm Beckman column (4.6 × 150 mm) and Waters Model 440 UV absorbance detector (λ 254 nm). The eluant was a pH 2.3 phosphate buffer–methanol mixture. Usually a 33% methanol solution was used. The presence of MV^{2+} created a separation problem since its retention time overlapped with the retention time of dansyl glutamate. A good separation was achieved, however, by increasing the amount of methanol to 37%. The retention times of dansyl glutamate were 12.0 and 9.48 min in 33 and 37% methanol, respectively.

Results and Discussion

The Polymers. Polymers holding glutamic acid (Glu) and γ-aminobutyric acid (GABA) were prepared as follows: The protected amino acid (**1**) was condensed with isonicotinoyl chloride and the amide product was then reacted with uncross-linked chloromethylated polystyrene to form **3**. The desired polymers were then obtained by removal of the benzyl protecting groups using hydrogen bromide in acetic acid (Scheme I).

All the new compounds, including the polymers, had satisfactory IR spectra and elemental analysis. The elemental analyses for **4a** and **4b** indicated 97 and 96% loading with isonicotinamide units, respectively. Most revealing were the ^{13}C NMR spectra. The carbon atoms of the polystyrene backbone gave broad signals, but the amide portion of the molecule gave sharp lines, which were consistent with the proposed structure. Stock solutions of various concentrations of the polymers in DMF were prepared. The polymers and their solutions were found to be stable, in that the voltammograms did not change over a period of months.

(5) Tapuhi, Y.; Schmidt, D. E.; Linger, W.; Karger, B. L. *Anal. Biochem.* **1981**, *115*, 123.

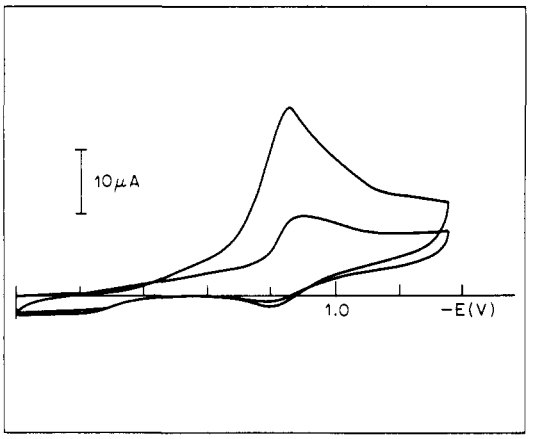


Figure 2. Voltammogram for IVb in 0.1 N KCl, $\Gamma_i = 1.32 \times 10^{-8}$ mol cm^{-2}, $\nu = 0.1$ V s^{-1}.

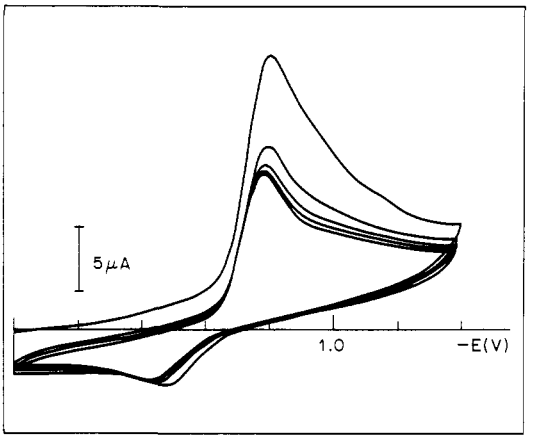


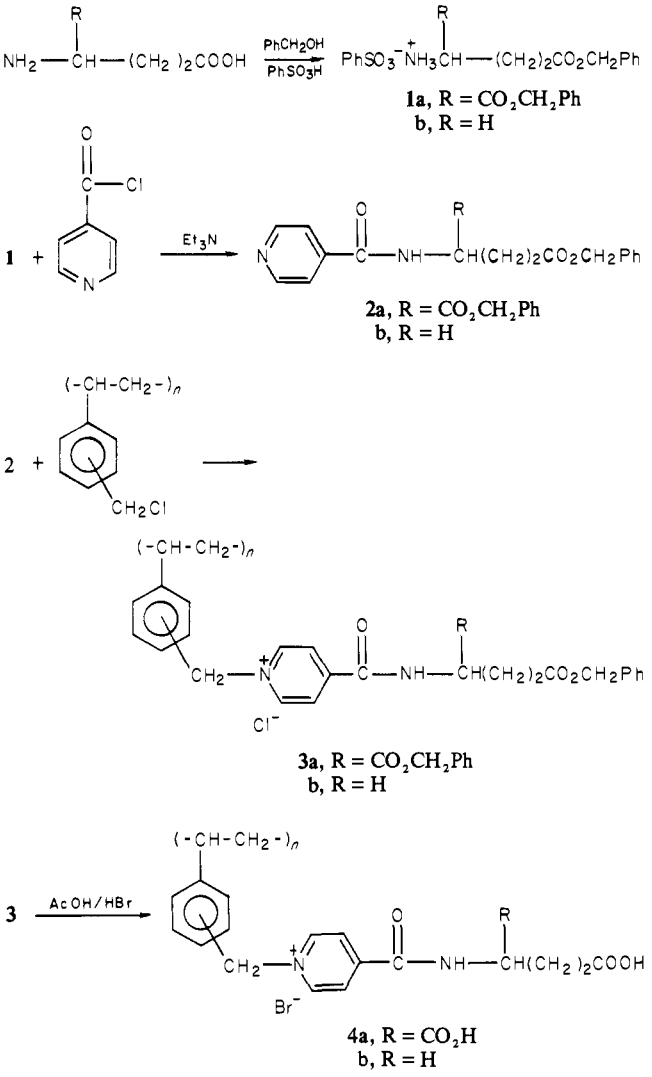
Figure 3. Voltammogram for IIIa in 0.1 N KCl, $\Gamma_i = 4.48 \times 10^{-8}$ mol cm^{-2} $\nu = 0.1$ V s^{-1}.

Electrochemistry of Polymer Modified Electrodes. In a typical experiment an amount of the glutamic acid polymer, **4a**, in DMF equivalent to 3.1 nmol of glutamate units was syringed onto a glassy carbon disk electrode. Slow evaporation gave a electrode, IVa, with an initial surface concentration, $\Gamma_i = 3.96 \times 10^{-8}$ mol/cm^2. In unbuffered aqueous solution, with 0.1 N KCl as a supporting electrolyte, IVa gave the cyclic voltammogram shown in Figure 1a. At a sweep rate $\nu = 0.1$ V s^{-1}, one sharp irreversible reduction peak appeared at −0.88 V (SCE), the shape of which was typical for an adsorbed species. No oxidation peak could be seen on the reverse half-cycle. The reduction waves disappeared after the first scan, and successive cycles did not indicate any peaks.

When GABA polymer, **4b** was coated onto the glassy carbon disk giving electrode IVb, the picture was similar to that for IVa. At 20 mV s^{-1} there was a reduction peak at −0.85 V and no anodic peak on the reverse half-cycle. There were no peaks on the second cycle. At 0.1 V s^{-1} electrode IVb gave a small anodic peak on the return half-cycle and small peaks on the second cycle (Figure 2). Electrode IIIa from polymer **3a**, $\Gamma_i = 4.48 \times 10^{-8}$ mol cm^{-2}, $\nu = 0.1$ V s^{-1} gave a reduction peak at more positive E, −0.71 V. On the reverse half-cycle a prominent anodic peak was visible at −0.43 V and successive sweeps gave substantial peaks (Figure 3).

These data indicate that at slow sweep rates, electrodes IVa and IVb give irreversible cathodic reactions due to the adsorbed polymer. One sweep consumes all of the electroactive sites. At higher sweep rates some intermediate in the cathodic reduction process was detected by its anodic peak on the reverse half-cycle. Voltammograms of electrode IIIa give evidence for an oxidizable intermediate at all sweep rates. These intermediates may be

Scheme I



pyridinyl radicals from one-electron reduction.[6]

It is of interest to estimate the extent of reduction (and, thereby a limit to the amount released) by monitoring the charge that passed during the voltammetric scan. Knowing the amount of polymer syringed onto the electrode allowed calculation of the surface concentration, Γ_i. Integrating the voltammograms gave Q, the charge passed. Comparison of Q and Γ_i allowed an assessment of the relative efficiency for electrodes IVa and IVb (Table II). Comparison of Figures 1a and 1b shows an example of the change with Γ_i. The small "peak" near −0.6 V in Figure 1b is due to traces of oxygen.

It can be seen from Table II that the thickness of the polymer film on the electrode played a certain role. Although thicker films gave larger Q the charge passed did not increase proportionately. Comparing the first and last entries for IVb in Table II, for instance, showed that increasing Γ_i by three orders of magnitude caused only a tenfold increase in Q. A possible explanation is that only those units in the few sublayers in the film near the carbon surface were electroactive. It seems that the slow propagation of charge through the film is responsible for this observation. Similar phenomena have been reported for quinoid polymers.

As previously discussed,[7] two conditions are necessary to prevent the reduction of units in the outer sublayers of the film: (1) "self-exchange" reactions in which a reduced unit near the carbon surface, in turn, reduces a unit in an outer sublayer must be slow; (2) polymer motions, which physically exchange the positions of inner and outer sublayer species, must be slow. Condition 1 may often be met when the units in the layer undergo reactions more complex than one-electron transfer, and one complication which can dramatically decrease the self-exchange rate is protonation. Thus, hydroquinones do not rapidly reduce quinones and propagation is slow in quinoid layers.[7] In the present case, we expected that the reductive cleavage of the amide group would similarly require protonation and this could in part account for slow propagation. Therefore, it was of interest to study the pH dependence.

The potential, E_p, of the cathodic peak, as well as its shape, was sensitive to variations in pH and buffer content. Table III sums the data for E_p, pH variation for electrodes IVa, IVb, and IIIa. Figure 4 shows several voltammograms for IVa. Between pH 1 and 5, there was only one main, sharp reduction peak between 0.0 and −1.0 V. The E_p for this peak was shifted cathodically with increasing pH. The slope of the linear E_p/pH plot was 58 mV for IVa and 49 mV for IVb. At all these pH values

Table II. Comparison of Charge Passed and Surface Concentration for Electrodes IVa and IVb[a]

electrode	$\Gamma_i \times 10^9$ [b] (mol cm^{-2})	$Q \times 10^9$ [c] (F cm^{-2})
IVa	5.1	3.0
	12.8	3.6
	39	9.2
	59	11.2
	125	9.6
IVb	0.24	0.8
	0.47	1.4
	23.7	2.4
	200	9.1

[a] 0.1 N KCl, $\nu = 0.1$ V s^{-1}. [b] Surface concentration calculated by the amount syringed onto the electrode. [c] By the cut and weigh method, subtracting background from cyclic voltammograms.

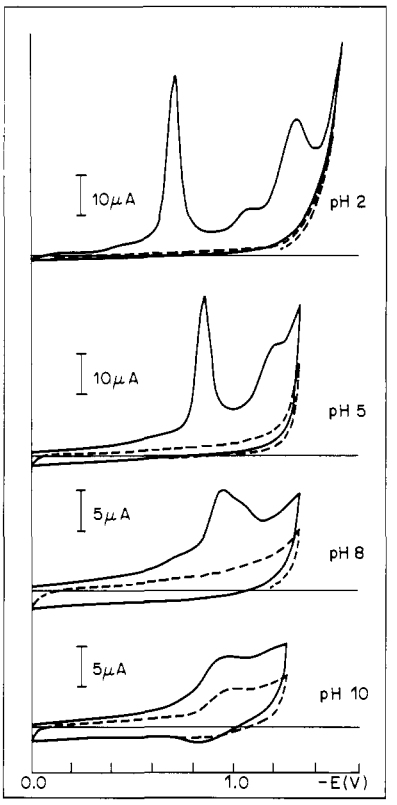


Figure 4. Voltammogram for IVa at pH 2, 5, 8, and 10: $\nu = 0.1$ V s^{-1}, $\Gamma_i = 3.92 \times 10^{-8}$ mol cm^{-2}; first and second sweeps.

(6) Hermolin, J.; Levin, M.; Kosower, E. M. *J. Am. Chem. Soc.* **1981**, *103*, 4808, and references therein to early work by Kosower and co-workers and others on stable isonicotinate radicals.

(7) (a) Fukui, M.; Kitani, A.; Degrand, D.; Miller, L. L. *J. Am. Chem. Soc.* **1982**, *104*, 28. (b) Degrand, C.; Miller, L. L. *Electroanal. Chem* **1982**, *132*, 163.

Table III. E_p Variation with pH for IVa, IVb, and IIIa

pH	E_p (V)[a,b] IVa[c]	IVb[d]	IIIb[e]
1	0.66	0.64	0.735
2	0.705	0.715	0.74
3	0.77	0.747	0.835
4	0.83	0.795	0.825 (0.5)
5	0.85	0.825	0.785 (0.49)
6	0.895	0.81	0.72 (0.49)
		0.90	
7	0.92	0.82	0.75 (0.43)
	0.97 (s)	0.91 (s)	
8	0.95	0.82	0.715 (0.47)
	1.05 (s)	0.92 (s)	
9	0.97	0.85	0.73 (0.43)
	1.14 (s)	0.94	
10	0.99 (0.85)	0.83 (0.76)	0.73 (0.46)

[a] E_p values at 0.1 V s^{-1}, (s) = shoulder. [b] Values in parentheses refer to anodic peaks on the reverse half-cycle. [c] $\Gamma_i = 3.92 \times 10^{-8}$ mol cm^{-2} of **4a**. [d] $\Gamma_i = 6.59 \times 10^{-8}$ mol cm^{-2} of **4b**. [e] $\Gamma_i = 9.09 \times 10^{-9}$ mol cm^{-2} of **3b**.

there was no anodic peak on the reverse half-cycle and no peaks of any kind of the second cycle. Above pH 6 the situation was more complex because two broad, overlapping, or shouldered peaks were observed for IVa and IVb. The peak potentials could not be accurately measured, but in a quantitative sense very little pH dependence was noted. At higher pH, for example, pH 10, both electrodes IVa and IVb showed (1) a small anodic peak on the reverse half-cycle and (2) diminished but real cathodic peaks on the second cycle. Since Q did not change appreciably from pH 4 to 10, it seems clear that at pH 10 and $\nu = 0.1$ V s^{-1} the inner sublayers are being reduced, partially reoxidized, and only fully reduced in a chemically irreversible way after several cycles.

We speculate that at low pH, protonation reactions accelerate the irreversible reduction of the amide group. At high pH these protonations occur to a smaller extent and some species which contain a 4-keto pyridinium group are intact after one cycle at 0.1 V s^{-1}. These could be isonicotinamide units or isonicotinaldehyde units. Whichever, they are localized in the inner sublayers.

A different picture can be seen for electrode IIIa. The peak potential was found to be essentially independent of the pH. The reduction peak was always sharp, and above pH 4 it was accompanied by a broad reoxidation peak. The difference between IIIa and IVa, IVb demonstrates that the carboxyl groups in polymers **4a** and **4b** are somehow important in determining the details of the electrochemistry. The carboxylate or carboxylic acid (depending on pH) groups are much more polar than the benzyl ester groups on polymer **3a**. This could provide a more polar environment in the polymer film and, thereby, accelerate the proton-transfer reactions which are necessary to destroy isonicotinate units. This hypothesis would accommodate all the data cited above.

It was noted above that a condition preventing propagation of redox reactions through the layer was slow polymer chain motion.[7] The rate of chain motion is sensitive to the surrounding environment and a previous study of quinoid polymer elecrodes demonstrated that the rate of redox propagation could be accelerated by using a mixed organic, aqueous electrolyte, rather than aqueous electrolyte.[7a]

Similar results were found in this study for electrode IVa. Using $\Gamma_i = 4 \times 10^{-8}$ mol cm^{-2} and a mixture of methanol and pH 7 phosphate buffer with 0.1 N KCl as electrolyte, the i_p was dramatically increased. Q increased to 5.3×10^{-9} from 2.2×10^{-9} F cm^{-2} when no methanol was present. Addition of methanol did not change E_p and no anodic peak was seen on the reverse half-cycle. Clearly, it was possible to reduce a larger portion of the units in the polymer when a mixed solvent was used. One explanation of this result is that methanol swelled and plasticized the polymer. The increased rate of chain motion allowed more of the film to be reduced.

Table IV. Yield of Glu from Electrode IVa[a]

$\Gamma_i \times 10^9$ (mol cm^{-2})	$-E$ (V)	pH	[Glu] $\times 10^6$[b] (M)
1.6	0.95	7	0.36
16	0.95	7	1.1
16	1.05	4	3.7
16	1.20	9	0.56
16	0.95	7	8.7[c]

[a] E pulsed negative for 1 min. For details see Experimental Section. [b] [Glu] in 70 μL of solution. [c] Electrolyte contained 0.19 mM methylviologen.

In this regard the permeability of the polymer film was of interest. Using aqueous 0.1 N KCl, voltammograms were obtained using electrodes IVa or IVb in the presence of $Ru(NH_3)_6Cl_3$, $Co(NH_3)_3Cl_3$, or ferrocenecarboxylic acid as solutes. In each case the voltammogram was the simple sum of those voltammograms for the modified electrode alone and the organometallic compound on a bare carbon electrode. Thus, the layers are quite permeable to these cationic or anionic species. This implies that slow redox propagation through the layer is not a result of slow counterion diffusion in the film.

The peak shape and the i_p dependence on ν can be informative in the study of polymer electrodes. Studies exist which evaluate these factors both theoretically and experimentally for polymer films undergoing reversible or quasireversible electrochemical reactions. In the present case, the reactions, especially at low pH and small ν, are irreversible. An analogue to this situation is the anodic "stripping" of metals from a surface. Indeed, the sharp peaks, ~75 mV width at half-height, observed at low pH are qualitatively consistent with this analogy.

A more quantitative interpretation of Q and i_p dependence on ν will not be undertaken. The situation is complex and simple treatments, e.g., plots of i_p vs. ν, are not directly meaningful.

Are GABA and Glu Released from Electrodes IVa and IVb? The answer to this question is the central point of this investigation. The Q data cited above demonstrated that if the neurotransmitters GABA and Glu were cathodically released, as hoped, the amounts would be small. For this reason extreme measures had to be used to detect and quantify any released material. HPLC analysis was successful and, indeed, these experiments and similar ones assaying dopamine[2] constitute the first reports of detection of products released from a chemically modified electrode.

In a preliminary experiment a glassy carbon rod was dip-coated with polymer **4a**. After air-drying, the electrode was used in 10 mL of pH 7 buffer in a two-compartment cell. The potential was cycled to −1.2 V. The electrode was removed and cleaned. This procedure was repeated 37 (!) times. The catholyte, which then contained the released material from all 38 electrodes was analyzed on an amino acid analyzer. Glu was present as the only amino acid. Similarly, GABA was obtained when polymer **4b** was tested.

In order to quantitate the released Glu and GABA, a preparative experiment using a thin layer cell[2b] was designed. In this experiment a small volume of aqueous electrolyte was placed on the horizontal surface of an upright glassy carbon disk electrode. A IVa disk electrode was lowered to contact the solution. A micropipet which led to a reference electrode was inserted into the solution. As soon as the cell was assembled, the potential was stepped to an appropriate negative potential and then back to 0.0 V. Part of the solution was removed, derivatized with dansyl chloride, and analyzed using HPLC. The presence of GABA from IVb and Glu from IVa was confirmed. *Control experiments showed that no Glu or GABA was released without the cathodic current pulse.* Quantitative data are in Table IV. At pH 7, E was pulsed to −0.95 V. From electrode IVa containing 1.6×10^{-9} mol cm^{-2}, 70 μL of solution which was 0.36×10^{-6} M in Glu was produced. This corresponds to $0.5 \pm 0.1 \times 10^{-10}$ mol cm^{-2}. A similar experiment using a larger Γ_i electrode gave no increase in the amount released. This is expected since Q limits the amount released and Q does not change appreciably as Γ_i increases. When a pH 4 electrolyte was used, the amount released did increase. At pH 9 less Glu was released. These data are also in agreement

Table V. Cr^{3+} Current Enhancements for Electrode IVa[a]

$[Cr^{3+}]$[b] (mM)	conditions[c]	i_p^*/i_p[d]	Q^*/Q[d]
504	A	5.8	3.9
49		5.2	3.1
4.9		2.3	2.7
0.6		2.1	2.4
504	B	3.6	4.1
0.06		1.5	1.7
504	C	3.9	4.4
0.06		1.2	1.4

[a] IVa dipped for 5 min in $Cr(ClO_4)_3$ solution and dried. Data for $\nu = 0.1$ V s^{-1}. [b] Concentrations of soaking solution. [c] Conditions for voltammetry: (A) $\Gamma_i = 1.0 \times 10^{-8}$ mol cm^{-2} unbuffered 0.1 N KCl; (B) $\Gamma_i = 1.0 \times 10^{-8}$ mol cm^{-2} buffer pH 7, 0.1 N KCl; (C) $\Gamma_i = 3.9 \times 10^{-8}$ mol cm^{-2} buffer pH 7, 0.1 N KCl. [d] For condition A, $i_p = 13.7$ μA, $Q = 2.8 \times 10^{-9}$ F cm^{-2}; for B, $i_p = 11.8$ μA, $Q = 2.2 \times 10^{-9}$ F cm^{-2}; for C, $i_p = 13.5$ μA, $Q = 2.3 \times 10^{-9}$ F cm^{-2}.

with expectations, since amide bond cleavage requires protonation.

In total, the experiments demonstrate that the initial goal of this project was accomplished. For the first time an organic chemical was purposefully released from an electrode in response to a change in potential. The bad news is that the amounts released are very small. In order to understand how to improve this situation, experiments were undertaken with redox catalysts in the solution. Our hope was that larger Q and, therefore, larger yields could be obtained.

Two Approaches to Catalyzing Redox Propagation. It has been previously demonstrated that slow propagation of redox reactions through a polymer layer could be accelerated by redox catalysts with reversible one-electron couples at an appropriate E°.[7a] This approach was attempted for electrodes IVa and IVb with some success. A second method for increasing Q was discovered by accident. It involved the effect of (presumably) electroinactive metal ions, which could complex with units in the film.

A. The Effect of Metal Ions. The discovery came from comparing cyclic voltammograms for electrode IVa alone, for IVa with $Cr(ClO_4)_3$ in solution, and for $Cr(ClO_4)_3$ on a bare carbon disk. On bare carbon, $Cr(ClO_4)_3$ showed no reduction peak in the region 0 to −1.2 V. Even so, the presence of Cr^{3+} in the solution gave a considerable enhancement in the cathodic peak current at −0.9 V when IVa was used. The same phenomenon was observed when an electrode IVa was soaked for 5 min in a aqueous solution of $Cr(ClO_4)_3$, dried, and then studied as usual in aqueous solution containing no $Cr(ClO_4)_3$. Further experiments showed that several minutes were required in the soaking solution to achieve the maximal effect. After this soaking, the electrode could be placed in water for some minutes without changing the voltammetric response.[8] Because the effect was unexpected, some further studies were undertaken using the presoak method. Current enhancements are reported in Table V as ratios of i_p^*/i_p and Q^*/Q, where i_p^* and Q^* refer to Cr^{3+} enhanced values. These ratios are not identical because the peak width changed somewhat. In general, E_p remained constant, except at high Γ_i and high $[Cr^{3+}]$, where it shifted anodically by as much as 150 mV. No anodic peak was seen on the reverse half-cycle and the second sweeps showed only very small peaks. A 5-min soaking time was used in each experiment. The data show that the current enhancement was greatest for the highest $[Cr^{3+}]$. The precise values depended on the presence or absence of phosphate buffer, but these caused only minor differences.

Similar experiments were undertaken using electrodes IVb and IIIa with $Cr(ClO_4)_3$. A IVb electrode with $\Gamma_i = 2.5 \times 10^{-8}$ mol cm^{-2} gave enhanced currents after dipping in 0.6 mM $Cr(ClO_4)_3$, as above; Q^*/Q was 1.8. Using 49 mM $Cr(ClO_4)_3$, Q^*/Q was 2.1. In contrast, electrode IIIa was unaffected by soaking in

(8) Many examples have been reported in which electroactive metal ions are sequestered into ionic polymer films on electrodes. See, for example, ref 3a–c.

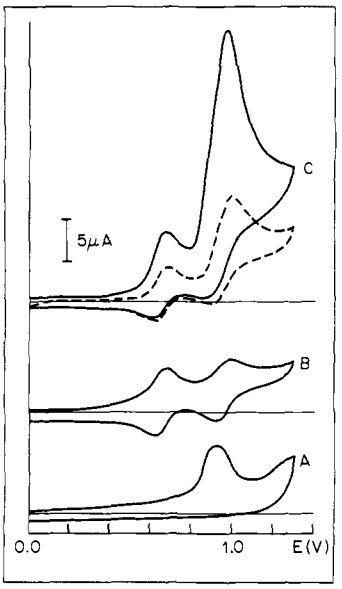


Figure 5. Voltammogram for IVa in the absence or presence of methylviologen: (A) IVa without MV^{2+}; (B) MV^{2+} on a bare electrode; (C) reduction of IVa in solution containing MV^{2+}. $\Gamma_i = 3.92 \times 10^{-8}$ mol cm^{-2}, $\nu = 0.05$ V s^{-1}, pH 7. $[MV^{2+}] = 8.4 \times 10^{-5}$ M. Dashed line is second cycle.

$Cr(ClO_4)_3$. Similar phenomena were observed with $FeCl_3$. For instance, soaking of electrode IVa with $\Gamma_i = 1 \times 10^{-8}$ mol cm^{-2} in a solution of 6.3 mM $FeCl_3$ increased the Q^*/Q to 7.8 when the cyclic voltammogram was performed using unbuffered 0.1 N KCl solution. In pH 7 buffer solution, on the other hand, $FeCl_3$ had almost no effect. No current enhancement was noted when electrodes IVa were soaked in $Ru(NH_3)_6Cl_3$, $Co(NH_3)_6Cl_3$, $CuCl_2$, $CdCl_2$, $ZnCl_2$, $CoCl_2$, or $HgCl_2$.

These qualitative observations suggest that the metal ions Fe^{3+} and Cr^{3+} can interact with carboxylate groups in the polymer layer to enhance the current. It is not clear how this happens, but it would not be surprising to have these ions act as Lewis acids to catalyze the reduction process. It seems less likely that we are observing catalysis of the reduction of Fe^{3+} and Cr^{3+} by the polymer, but even that cannot be ruled out.

B. The Release of Glutamic Acid Is Enhanced by Methylviologen. The viologens, *N,N*-dialkyl-4,4′-bipyridines, show two reversible redox couples with $E^{\circ\prime}$ values near −0.5 and −1.0 V. These compounds have been previously used to mediate slow electrochemical reduction reactions.[9] In those cases the viologen were rapidly reduced at the electrode and then, in turn, reduced a substrate in solution. It has also be shown that viologens attached as electrode surface modifiers are capable of mediating the reduction of substrates in solution.[10]

Using electrode IVa, we have shown that methylviologen (MV^{2+}) in the solution enhanced the release of Glu. The experiment was performed as before using the thin layer cell and HPLC analysis. Table IV shows that the amount of Glu released was increased eightfold in the presence of MV^{2+}. As expected from this preparative result, considerable current enhancements were also observed when cyclic voltammograms were run using IVa with MV^{2+} in the solution. As shown in Figure 5, 8.4×10^{-5}

(9) (a) Farrington, J. A. *Biochem. Biophys. Acta* **1973**, 314, 372. (b) Brugger, P. A.; Gratzel, M. *J. Am Chem. Soc.* **1980**, *102*, 2463, and references cited therein. (c) Ebbesen, T. W.; Levevy, G.; Patterson, L. K. *Nature (London)* **1982**, *298*, 545.

(10) Mortimer, R. J.; Anson, F. C. *J. Electroanal. Chem.* **1982**, *132*, 535. Martigny, P.; Anson, F. C. *Ibid.* **1982**, *139*, 385. Oyama, N.; Yamaguchi, S.; Nishiki, Y.; Tokuda, K.; Matsuda, H.; Anson, F. C. *Ibid.* **1982**, *139*, 371. Abruña, H. D.; Bard, A. J. *J. Am. Chem. Soc.* **1981**, *103*, 6898. Elliot, C. M.; Martin, W. S. *J. Electroanal. Chem.* **1982**, *137*, 377. William, K. W.; Murray, R. W. *Ibid.* **1982**, *133*, 211. Bookbinder, D. C.; Lewis, N. S.; Wrighton, M. S. *J. Am Chem. Soc.* **1981**, *103*, 7656. Bruce, J. A.; Wrighton, M. S. *Ibid.* **1982**, *104*, 74. Dominey, R. N.; Lewis, N. S.; Bruce, J. A.; Bookbinder, D. C.; Wrighton, M. S. *Ibid.* **1982**, *104*, 467.

Table VI. Electrode IVa in the Presence of MV^{2+} [a]

$\Gamma_i \times 10^8$ (mol cm^{-2})	$Q_{IVa} \times 10^9$ (F cm^{-2})	$Q_{IVa,MV} \times 10^9$ [c] (F cm^{-2})
1.0	2.32	8.16
3.9	2.44	13.12
4.7	3.8	19.12
9.3	4.36	22.36

[a] In buffer 7 solution, 0.1 M KCl, ν = 50 mV s^{-1}. [b] Q for IVa in the absence of MV^{2+}. [c] Q for IVa in the presence of 8.4×10^{-5} M MV^{2+}.

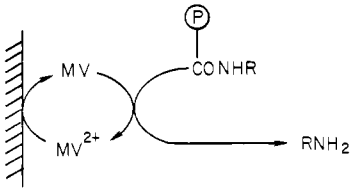
Table VII. Sweep Rate Dependence of i_p for IVa with MV^{2+} [a]

$\Gamma_i \times 10^8$ (mol cm^{-2})	ν (mV s^{-1})	i_{CAT} [b] (μA)
1	20	7.1
	50	13
	100	21
	200	36
3.9	10	5.3
	20	13
	50	26
	100	53
	200	66

[a] 8.4×10^{-5} M solution of MV^{2+} in buffer 7, 0.1 M KCl. [b] $i_{CAT} = i_p$ for IVa in the presence of MV^{2+}, minus i_p for MV^{2+} on bare carbon, where both i_p values were measured at ca. −0.9 V.

M MV^{2+} on a bare carbon electrode undergoes two reversible one-electron reductions forming MV^+ and MV. The $E^{o'}$ values in pH 7 buffer containing 0.1 N KCl were −0.65 and −0.96 V. These values were pH independent as expected. Note that the second peak potential corresponds closely to that for IVa or IVb reduction. When MV^{2+} was reduced on electrode IVa (Figure 5), the cathodic currents at −0.65 and −0.96 V were substantially larger than those for MV^{2+} on bare carbon or IVa alone. On the reverse half-cycle, the anodic peaks for MV → MV^+ and MV^+ → MV^{2+} were essentially unchanged from those observed when bare carbon was used. On successive cycles, smaller enhancements of the MV^{2+} peak currents were observed.

The extra cathodic current came from a mediated reduction process in which MV^+ and MV were formed at the carbon surface and then in turn reduced otherwise inert isonicotinate units in the outer sublayers of the film. More precisely, MV^{2+} diffused through the polymer layer and, depending on E, it was reduced to MV^+ or MV. The MV^+ or MV then diffused back toward the solution, but they were capable of reducing some isonicotinamide sites in the layer. This reaction regenerated MV^{2+}, which could be reduced again leading to higher i. MV is a stronger reducing agent than MV^+, and larger current enhancements are seen at the second peaks than at the first. At −0.96 V this can be crudely conceptualized as:



In Table VI are shown the results from a set of experiments in which Γ_i was varied, and at 50 mV s^{-1}, Q was measured in the presence and absence of MV^{2+}. In each case the Q from MV^{2+} (integral of both peaks on bare carbon) was 0.56×10^{-9} F cm^{-2}. Several sweeps were required to exhaust the current from the polymer. It can be seen that the larger Γ_i layers gave larger Q values. This accords with expectations based upon the model for MV^{2+}/MV catalysis of slow charge propagation.

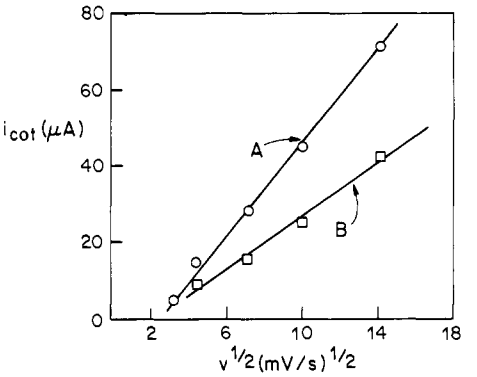


Figure 6. Variation of second peak catalytic current, i_{CAT}, with $\nu^{1/2}$ (see text for definition of i_{CAT}), pH 7. (A) $\Gamma_i = 3.96 \times 10^{-8}$ mol cm^{-2}; (B) $\Gamma_i = 1.02 \times 10^{-8}$ mol cm^{-2}.

In Table VII are data which describe the dependence of i_p at −0.95 V on scan rate for two electrodes IVa, one with $\Gamma_i = 1.0 \times 10^{-8}$ and the other 3.9×10^{-8} mol cm^{-2}, each in the presence of 8.4×10^{-5} M MV^{2+}. In this table are i_{CAT} values. These values are determined by subtracting the i_p (second cathodic peak) for MV^{2+} on bare carbon from the i_p for MV^{2+} on IVa. These i_{CAT} values may contain contributions from (1) mediated reduction of polymer, and (2) direct reduction of polymer. The relative contributions from these two factors cannot be determined, but based upon the small i_p seen in the absence of MV^{2+} it seems likely that (1) dominates. For example, at 50 mV s^{-1}, the i_p for IVa with $\Gamma_i = 3.9 \times 10^{-8}$ mol cm^{-2} used alone is only 4.1 μA. Using this electrode in the presence of MV^{2+}, i_{CAT} is 25.6 μA.

It is interesting to treat the data according to the mechanism as a mediated reduction of a semiinfinite layer of polymer. In this way, we ignore the small contribution from direct reduction process (2) and predict that because mediation is a diffusion process, i_p will increase with $\nu^{1/2}$.[11] Using the data from Table VII we have generated Figure 6, where there is a linear relationship between i_p^{CAT} and $\nu^{1/2}$.

Conclusion

The primary goal of this work has been achieved. An electrode has been designed and synthesized which can release specific molecules from its surface at a specific moment. The method has been shown to be applicable to the neurotransmitters Glu and GABA, but the approach should be useful for the electrically stimulated release of other amines. It was found that the chemical reactions leading to release were not as selective at pH 7 as desired. It was also found that propagation of the reduction process through the polymer films was not as fast as desired and this limited the amount of Glu or GABA which was released. This problem could be alleviated by the redox catalytic effect of methylviologen in the solution.

Future work will develop better examples of such devices which will promptly release larger amounts of material in a fashion where the amount can be coulometrically controlled. Constructed in the form of a microelectrode, such a device should have interesting neuroscience applications.

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Registry No. 1a, 65201-55-0; **1b**, 86272-16-4; **2a**, 86272-15-3; **2b**, 86272-17-5; **3a**, 86272-08-4; **3b**, 86272-12-0; **4a**, 86272-10-8; **4b**, 86272-14-2; GABA, 56-12-2; glutamic acid, 56-86-0; benzenesulfonic acid monohydrate, 26158-00-9; benzyl alcohol, 100-51-6; isonicotinic acid, 55-22-1; chloromethylated polystyrene, 9080-67-5; chloromethylstyrene, 30030-25-2.

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