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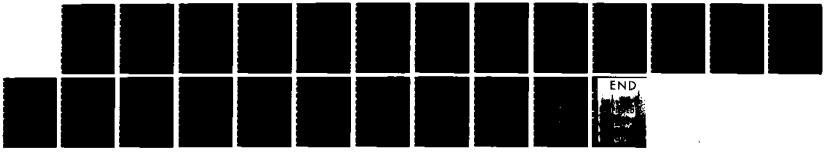

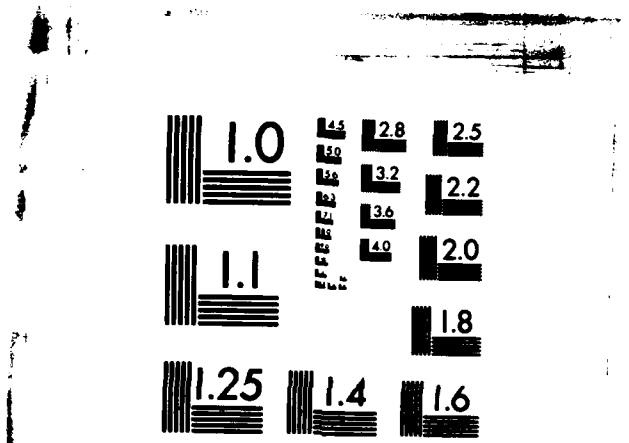

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Electrochemical Studies on Minoxidil and its Determination in Tablets by Differential-Pulse Polarography

By

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ELECTROCHEMICAL STUDIES ON MINOXIDIL AND ITS DETERMINATION IN TABLETS BY DIFFERENTIAL-PULSE POLAROGRAPHY

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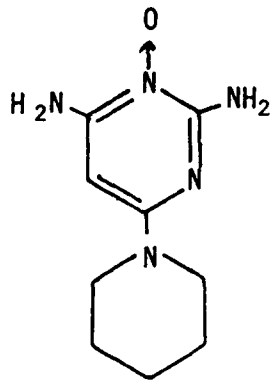
ABSTRACT

A simple differential-pulse polargraphic (d.p.p.) method has been developed for the analysis of minoxidil in pharmaceutical dosage forms. The extracting solvent was methanol and the supporting electrolyte was 1.0 N sulphuric acid. An excellent linear relationship was obtained between concentration and current peak height with a correlation coefficient of 0.9999. Good agreement was obtained between results with the d.p.p. method and those by the manufacturer's method of assay. There was no interference by the tablet excipients. In acid solution, a mechanism for reduction at -1.2 V is proposed which involves four electrons transferred as well as dehydration and deamination steps.

Keywords: Minoxidil determination;
differential-pulse polarography;
controlled potential coulometry;
cyclic voltammetry

Minoxidil (2,4-diamino-6-piperidino-pyimidine-3-oxide)(I) is a recently marketed, orally administered peripheral vasodialator that is useful in treating patients with refractory hypertension[1].



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The detection and determination of this substance continues to be of interest, particularly because of its associated numerous side effects[2]. As yet, no official method of analysis for minoxidil has been listed in the U.S.P.[3] and the few reported methods of assay involve radioimmunoassay[4], T.L.C.[5,6], or radiochromatogram scanning[7]. Investigation with a GLC procedure revealed that substantial amounts of some derivatives were lost on the column[4].

In addition to its basic properties, minoxidil has two structural features that could be used for analytical purposes (earlier reports[8-10] have shown that both the N-oxide bond and the carbon-nitrogen double bond are electroreducible). Accordingly, in this report, the authors are presenting a differential-pulse polarographic procedure for the determination of minoxidil in tablets that involves only a single

extraction prior to the electroreduction. The method is sensitive, accurate and easy to perform for routine analysis.

EXPERIMENTAL

Apparatus and Conditions for Polarographic Analysis

A Fisher, Model 320, pH meter fitted with a glass-calomel electrode system was employed to measure the pH values of the solutions.

A PAR, Model 174, polarographic analyser equipped with a drop timer (Model 172A) and a Houston Ominigraphic, Model 2000, recorder were used in the investigations. A three-electrode combination was employed which consisted of a saturated calomel-electrode, a dropping-mercury electrode and a platinum wire as the auxiliary electrode. A conventional H-type cell was maintained at 25 ± 1°C and all sweeps utilized a scan rate of 2 mVs^{-1} and a drop time of 2s.

In 1.0 N H_2SO_4 (pH ≈ 0.5) the instrumental parameters were: applied potential range -0.6 to -2.1 V; current 100 μA full scale; height of mercury column 75 cm; flow rate of mercury 1.176 mg s^{-1}; modulation amplitude, set at 50 mV; and low pass filter, set at a time constant of 1s. The instrument was operated in the differential-pulse mode.

Controlled-potential Coulometry

A PAR, Model 173, potentiostat-galvanostat, equipped with a PAR, Model 377A, three component coulometric cell system was connected to a Hi-Tek digital integrator and digital voltmeter.

Nineteen ml of 1.0 N sulphuric acid were placed in the coulometric cell on top of a 5 ml layer of triple distilled mercury and 1 ml of 10^{-2} M

solution of minoxidil in methanol was added. The system was purged for 10 min with purified nitrogen. The applied potential was set at -1.2 V with a current range of 10 μA full scale and the solution was electrolysed until the digital readout indicated a constant but small count. One hour was required to complete the electrolysis. The process was repeated with a blank consisting of 19 ml of 1.0 N sulphuric acid and 1 ml of methanol.

Cyclic Voltammetry

Cyclic Voltammetric experiments at a hanging mercury drop electrode were performed with a four-component system consisting of a PAR EG and G, Model 175, Universal Programmer, a PAR, Model 173, potentiostat-galvanostat, a Houston, Model 2000, Omnigraphic recorder and a PAR, Model 9323, hanging mercury drop electrode fitted with a polarographic cell. Two supporting electrolyte systems were employed. In 1.0 N sulphuric acid, the instrumental parameters were: potential range -0.8 V to -1.3 V; current range, 10 μA; scan rate varied from 10 mVs^{-1} to 200 mVs^{-1}. In a dimethyl-formamide/tetraethylammonium bromide system, the settings were: potential range, -1.2 to -2.2 V; current range, 10 μA; scan rates were the same as in the previous system.

Reagents

The following reagents were used, all of analytical-reagent grade: barbital, boric acid, citric acid, potassium hydrogen phosphate, dimethyl-formamide, anhydrous methanol, tetraethylammonium bromide, 0.2 N sodium hydroxide, 1.0 N sulphuric acid, and 1% tetraethylammonium bromide in DMF. Britton-Robinson buffers were prepared with distilled, deionized water at intervals of 0.5 pH unit over the pH range of 2.6-7.0.

Reference Standard

Minoxidil (100.4%) was obtained from Upjohn Company, Canada Ltd., and used without further purification.

pH Dependence Studies

These studies were carried out in Britton-Robinson buffer over the pH range 2.6 to 7.0.

Preparation of Calibration Graph

A stock solution of minoxidil (10^{-3} M) was prepared in anhydrous methanol. Five test solutions of varying concentrations, 1 to 5 x 10^{-5} M were prepared by appropriately diluting the stock solution with 1.0 N sulphuric acid, while in the total sample volume of exactly 20 ml, the amount of methanol was always maintained at 1 ml.

All samples were purged with oxygen-free nitrogen for 10 min prior to each run and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Samples of each of five concentrations were run five times and resulted in a correlation coefficient for the graph of 0.9999.

Diffusion Dependence Studies

These studies were carried out in the aforementioned sulphuric acid-methanol system on a 5 x 10^{-4} M solution of minoxidil. The applied potential was from -0.6 to -2.1 V and the height of the mercury column ranged from 60 to 80 cm. The weight of mercury was also obtained at each of five heights over that range.

Analysis of Pharmaceutical Dosage Forms

Two dosage forms, 2.5 and 10.0 mg tablets, were available from the manufacturer.

Twenty tablets were weighed, finely powdered and an amount of powder was taken which according to the label would result in approximately 10^{-3} M solution of minoxidil in 50 ml of solvent. The accurately weighed sample was stirred magnetically for 20 min in 20 ml of methanol. The mixture was quantitatively transferred into a 50 ml volumetric flask, diluted to volume with methanol and then filtered through a Whatman No. 1 paper discarding the first 5 ml of the filtrate. A 0.6 ml aliquot of the filtrate was transferred to the polarographic cell, 19 ml of 1.0 N sulphuric acid and 0.4 ml of methanol were added. As previously described, the solution was purged for 10 min with purified nitrogen prior to recording the polarogram.

The amount of minoxidil was computed from a calibration graph.

Content Uniformity Test

Ten tablets were randomly selected from the sample. Each tablet was placed in an individual 150 ml beaker, 20 ml of methanol were added and the system was allowed to stand for 5 min in order to promote disintegration of the tablets. The remaining larger lumps of tablet mass were crushed with a glass rod and the mixture stirred magnetically for 20 min. After transferring the mixture quantitatively to a 50 ml volumetric flask, the determination was continued as described in the previous section, except that 1 ml of the filtrate and 19 ml of 1.0 N H_2SO_4 were used. The amount of minoxidil in each tablet was computed by the direct comparison method,

using reference standard solutions of 0.2386×10^{-3} M and 0.9546×10^{-3} M for the 2.5 mg and 10 mg tablets, respectively.

Macroscale Electrochemical Synthesis of 2-Amino-6-piperidinopyrimidine (X) from Minoxidil

The procedure was similar to that for the controlled potential coulometry with the exception that the cell contained 150 mg of minoxidil in 25 ml of 20% v/v methanol in 1.0 N H_2SO_4. The applied potential was held at -1.2 V and the reduction time was 6 hr. Upon completion of the reduction, the product, together with the supporting electrolyte, was separated from the mercury, the pH adjusted to 7.0 with ammonium hydroxide and the resulting solution extracted with chloroform. The organic layer was separated, dried over magnesium sulphate and concentrated to 1 ml. The concentrated solution was applied to a 1 mm thin-layer silica gel plate and then developed with a methanol:ammonium hydroxide solvent system (100:1.5). The component with an R_F value of 0.60 was scaped off the plate, leached out with methanol and the methanolic solution was evaporated to dryness.

This isolation yielded (X), a yellow, crystalline powder which decomposed between 80-100°C. NMR (200 MHz, $CDCL_3$) δ; 1.6 (m,6H), 3.5 (m,4H), 5.9 (m,3H) and 7.9 (d,1H)(see discussion); M^+ m/e 178; IR (KBr disc): 930 cm^{-1}, 1060 cm^{-1}, 1090 cm^{-1}, 1400 cm^{-1}, 1680 cm^{-1}, and 3000 cm^{-1}.

RESULTS AND DISCUSSION

Minoxidil exhibits two d.c. and d.p. polarographic waves in 1.0 N H_2SO_4 and in Britton-Robinson buffer pH range 3.0 to 7.0. The first wave is intense and well resolved from the second wave which is partially

overlapped by the supporting electrolyte discharge current. Wave one had an $E_{\frac{1}{2}}$ value at -0.95 V, and both the $E_{\frac{1}{2}}$ and i_d are dependent on pH. The $E_{\frac{1}{2}}$ moves cathodically while the i_d decreases with increasing pH. The second wave has a diffusion current which is very much higher than that of the first and has an $E_{\frac{1}{2}}$ value at approximately -1.20 V (Fig. 1). Both the i_d and $E_{\frac{1}{2}}$ of this wave vary with pH in the same manner as those of wave one.

The first wave is attributed to the reduction of the fully protonat- N-oxide while the second probably results from the reduction of the 3, carbon-nitrogen double bond. The latter functional group is more difficu to reduce owing to the presence of the amino group at position 4 of the pyrimidine moiety[11].

The first step in the process then would involve protonation of the N-oxide to an N-hydroxy intermediate which undergoes a two electron reduction to 2,4-diamino-6-piperidinopyrimidine intermediate. At higher potentials, the 3,4 carbon-nitrogen double bond can be reduced by a two-electron process to an unstable intermediate, 3,4-dihydro-2,4-diamino-6-piperidinopyrimidine. This intermediate undergoes deamination to give the final product, 2-amino-6-piperidinopyrimidine which was isolated in this work. The coulometric analysis of minoxidil in 1.0 N H_2SO_4 indicates that four electrons per molecule were involved in the electroreduction process.

The graph of diffusion current versus the square root of corrected height of the mercury volumn is a straight line that does not pass through the origin.

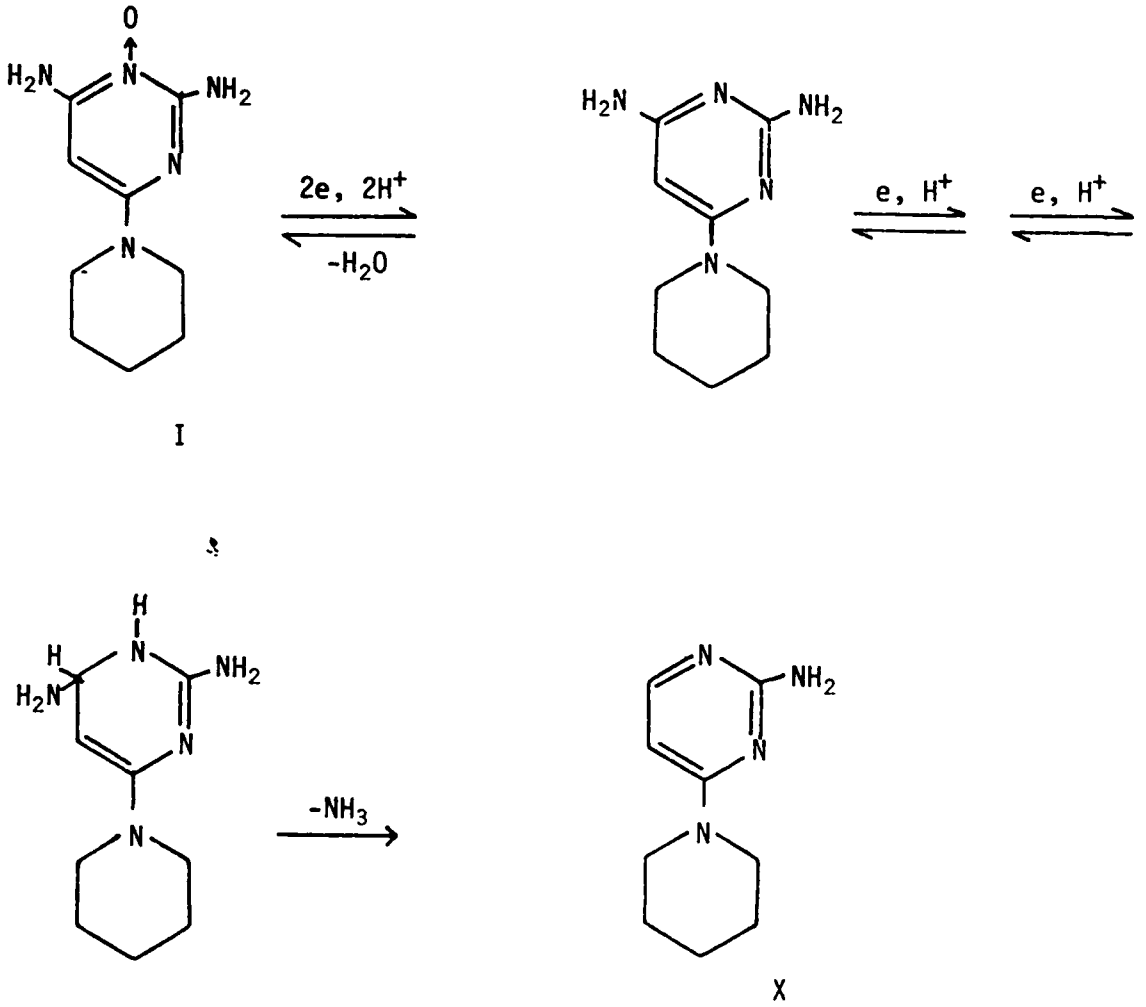
Figure 2 illustrates the cyclic voltammogram of minoxidil (5×10^{-4} M) in dimethylformamide/tetraethylammonium bromide. The E_{Pc} occurs at -2.04 V

while the E_{Pa} is at -1.96 V. At a scan rate of 20 mVs^{-1} and a τ value of 10 sec, the ratio of i_{Pa}/i_{Pc} [12] is 1.09. The cathodic peak current decreases with decreasing scan rate but the graph of $I_{Pc}/\bar{\upsilon}^{\frac{1}{2}}$ vs $\bar{\upsilon}$ is non linear. The electroreduction process of minoxidil in the aforementioned system probably involves a process that is more complicated than one of simple diffusion.

Strong multiple adsorption peaks, as illustrated in Figure 3, were observed in the cyclic voltammogram of minoxidil in 1.0 N H_2SO_4. The peak centre occurs at about -1.0 V, and the multiplicity of the peaks decreases with increasing scan rate.

The major product extracted from the macroscale experiment emits a pinkish fluorescence under short wave UV light. It also produces a negative result with the ferric chloride test indicating the absence of an N-oxide group. The strong IR N-oxide absorption peak between 1,250 and 1,300 nm is absent in the IR spectra of the product. The NMR (200 MHz) spectrum of the product exhibits four distinguishable peaks in $CDCl_3$. In $CDCl_3$-D_2O system, however, the peak at δ 5.9 is reduced to a doublet with an integration value corresponding to one proton. No other change in the spectrum was observed.

From all of the foregoing observations, the following pathway is proposed as minoxidil proceeds, by electrochemical reduction, to the final product, 2-amino-6-piperidinopyrimidine (Scheme I).


O
H_2N
N
NH_2
N
N
2e, $2H^+$
$-H_2O$
H_2N
N
NH_2
N
N
e, H^+
e, H^+
H
N
NH_2
H
H_2N
N
N
$-NH_3$
N
N
NH_2
N
N

I

X

Scheme I

Better resolution was provided by the differential-pulse wave at an $E_{\frac{1}{2}}$ value of -0.95 V, consequently, it was utilized for the analysis of the dosage forms. The peak height varied linearly with the concentration of the drug over the range 1×10^{-5} to 5×10^{-4} M. Table I provides the results of the assay for each of the minoxidil dosage forms. Values obtained by the manufacturer's quality control laboratory are presented for comparative purposes and excellent agreement is observed between the two results. Common excipients do not interfere with the electrochemical method. Table II lists the average of the results obtained for each dosage form when ten single tablets were analyzed.

The proposed method has the advantage of simplicity, high sensitivity and rapidity. It can distinguish between minoxidil and those degradation products that do not contain the N-oxide group and, consequently, the method can be applied in purity and stability studies on minoxidil.

Table I - ASSAY OF MINOXIDIL TABLETS BY DIFFERENTIAL-PULSE POLAROGRAPHY IN A 1.0 N H_2SO_4 - METHANOL SYSTEM

Tablet Lot No	Label Claim/mg	Recovery by Manufacturer/mg	% Recovery by Manufacturer	Recovery by * Differential-Pulse Polarography/mg	% Recovered by Differential Pulse Polarography
LOT H-651	2.5	2.49	99.6	2.46 ± 0.09	98.2
LOT H-710	10	9.69	96.9	9.66 ± 0.12	96.6

* Each value is the average of five determinations

Table II - AVERAGE VALUE FOR THE ANALYSIS OF TEN INDIVIDUAL MINOXIDIL TABLETS IN A 1.0 N H_2SO_4 - METHANOL SYSTEM

Tablet Lot No	Labeled Claim/mg	Recovery by d.p.p./mg	% Recovery by d.p.p.
LOT H-651	2.5	2.49 ± 0.1	99.9
LOT H-710	10	10.08 ± 0.7	100.8

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FIGURE LEGENDS

Fig. 1. Effect of pH on the d.p.polarographic waves of minoxidil (5×10^{-4} M) in; A, 1.0 N H_2SO_4; B, C, and D Britton-Robinson buffer pH's 3.0, 5.0 and 7.0, respectively.

Fig. 2. Cyclic Voltammogram of minoxidil (5×10^{-4} M) in DMF/TEAB.

Fig. 3. Effect of scan rate on the cyclic voltammogram of minoxidil (5×10^{-4} M) in 1.0 N H_2SO_4. Scan rates; A, 10 mVs^{-1}; B, 100 mVs^{-1}; C, 200 mVs^{-1}.

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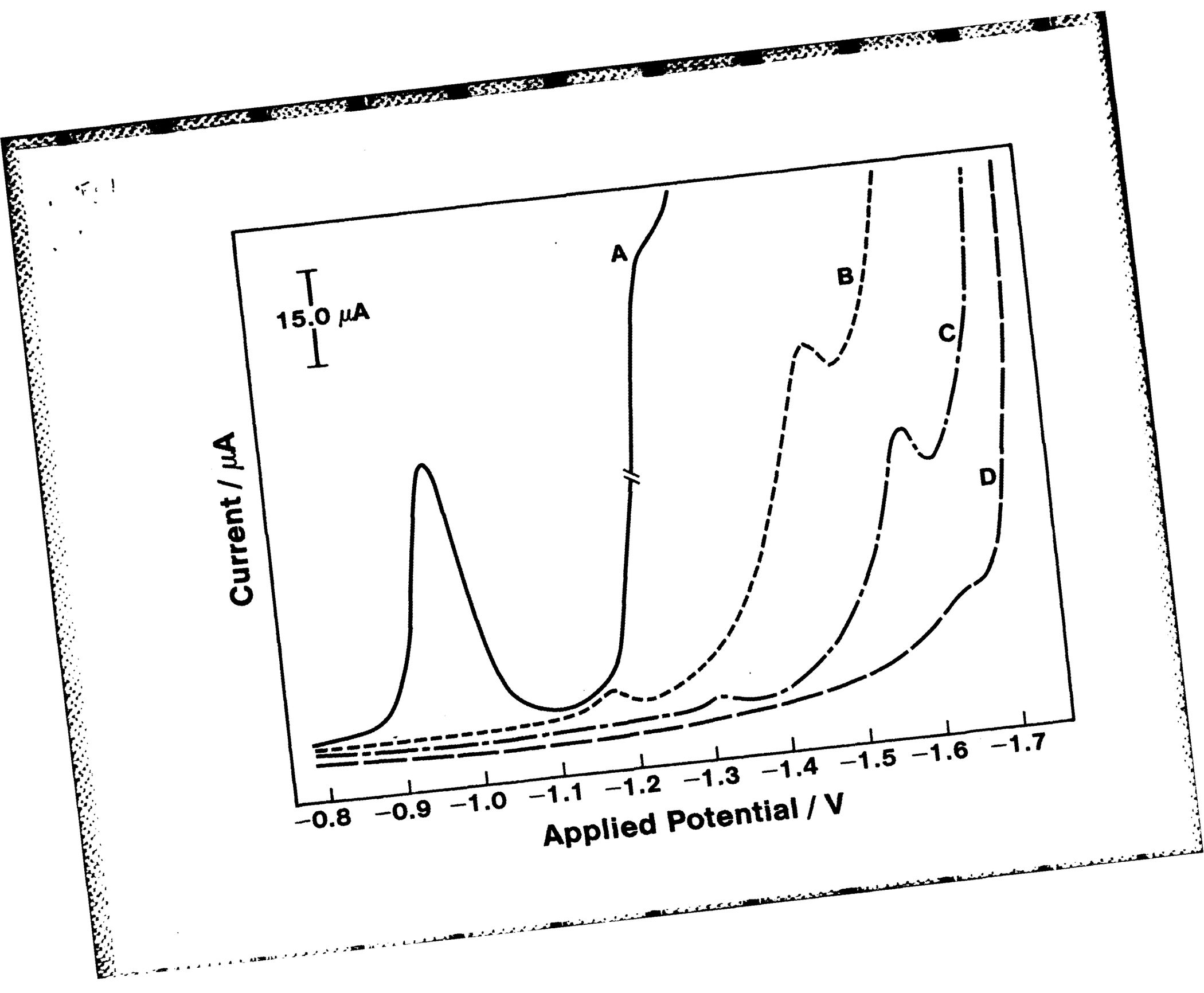

15.0 μA
A
B
C
D
Current / μA
−0.8 −0.9 −1.0 −1.1 −1.2 −1.3 −1.4 −1.5 −1.6 −1.7
Applied Potential / V

Fig 2

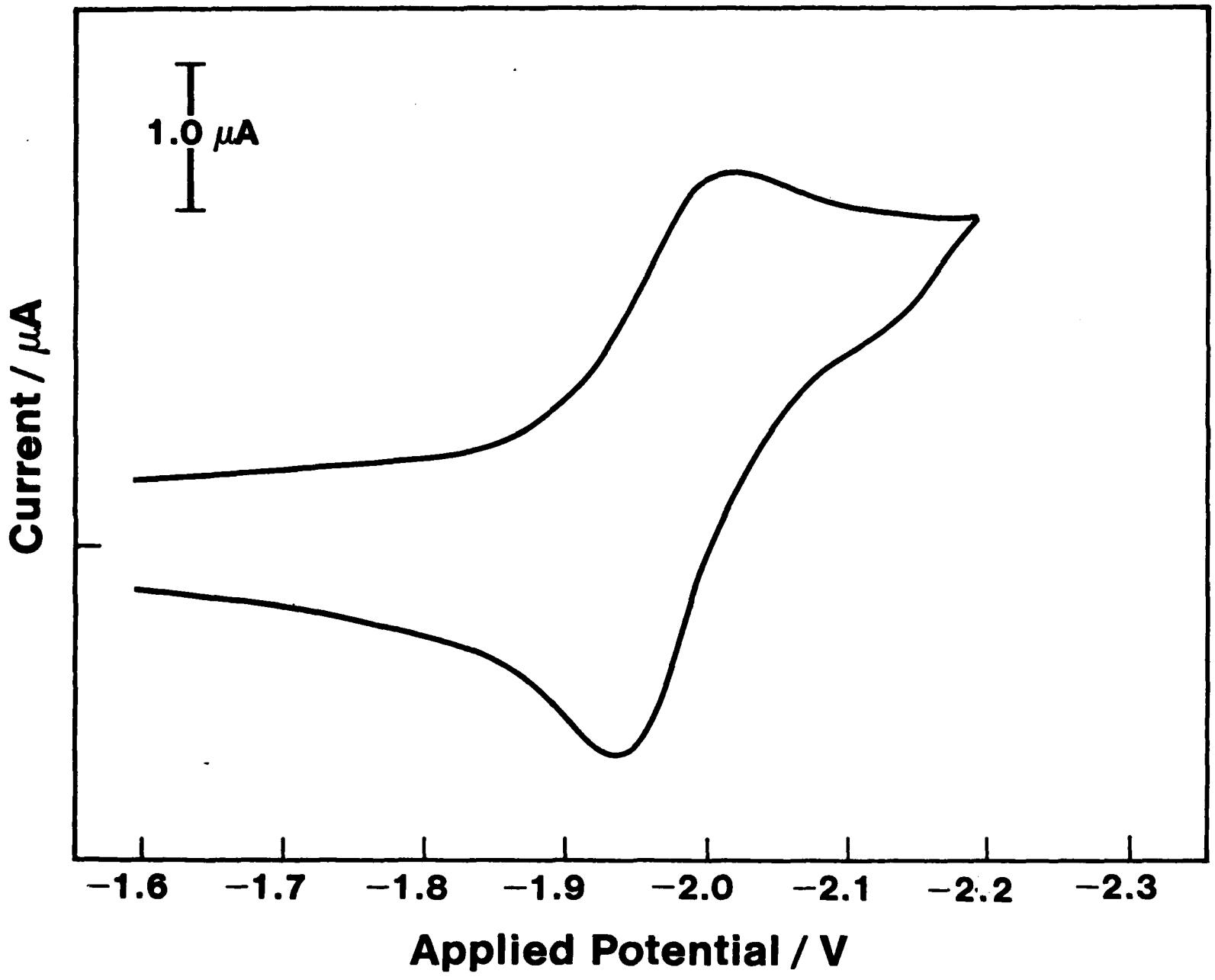
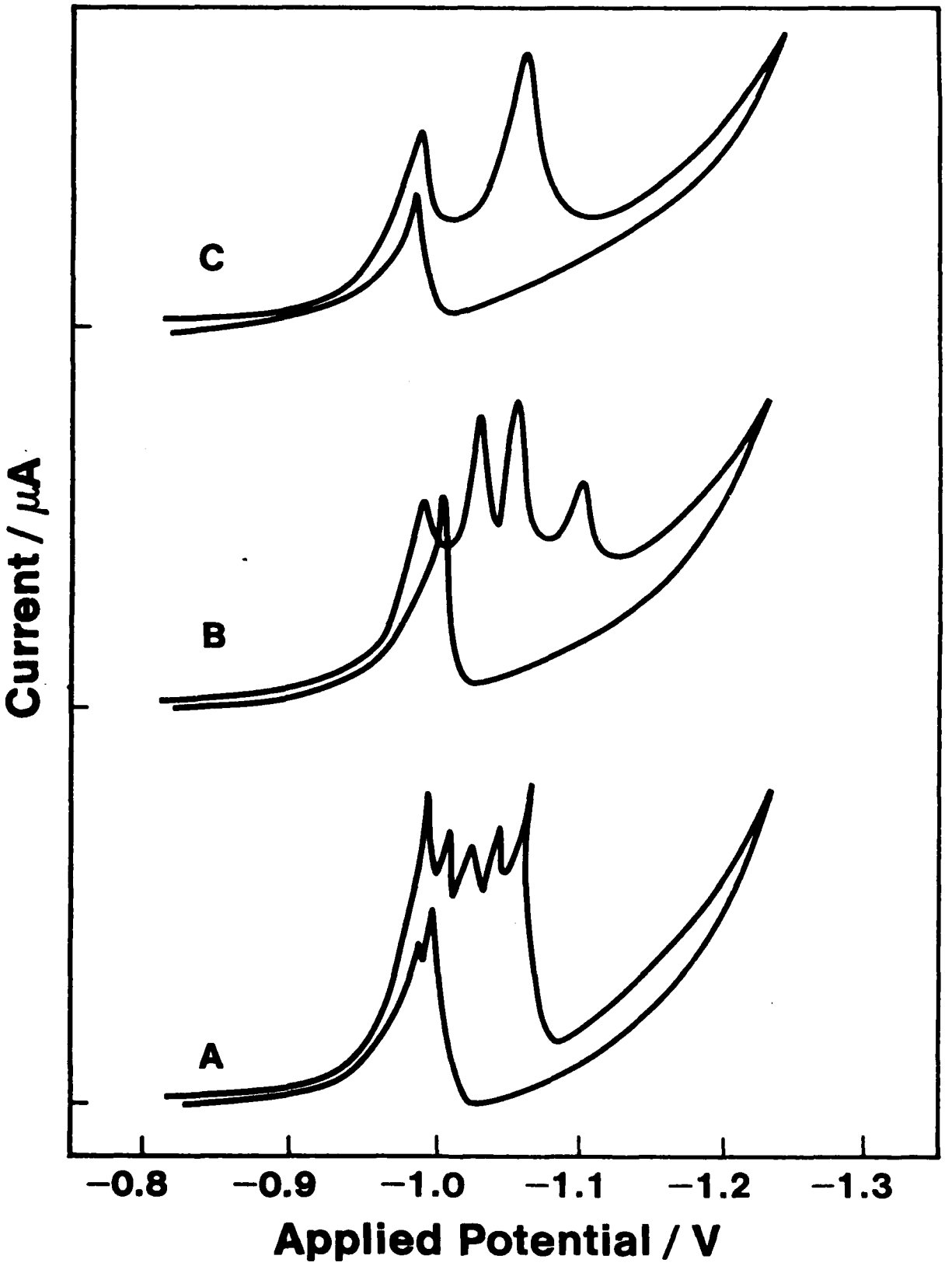


Fig 3



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