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by

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**Microelectrochemistry and Measurement of the Diffusivity of
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ABSTRACT

Microelectrochemical methods are shown to be useful in determining the diffusivity of biological redox molecules. The electrochemistry of both ferri- and ferrocytochrome c has been examined at a microelectrode array using *bis*(4-pyridyl)ethylene as an electrocatalyst. Sigmoidal current-voltage scans are observed in the cyclic voltammometry typical of the cylindrical diffusion-limited response found for reversible redox couples at microelectrodes. The arrays used consist of eight Au electrodes of dimensions 2.7 μm wide, ~ 70 μm long, and separated from each other by 1.4 μm . Steady-state and transient microelectrochemical techniques have been used to measure the diffusivity of horse heart ferri- and ferrocytochrome c in solution. The transient technique involves electrochemical generation of the diffusing species at one microelectrode and collecting (detecting) it electrochemically at a second microelectrode, a fixed distance away. The time dependence of the collector current after the generation allows determination of the diffusivity. The interelectrode spacing is such that the peak collection current following a generation pulse occurs between ~ 20 and ~ 920 ms for collection 1.4 and 26 μm away from the generator. From limiting steady-state microelectrode currents and direct microelectrode transit time measurements, the diffusion coefficients have been determined to be $1.10 \pm 0.04 \times 10^{-6}$ cm^2/s and $1.00 \pm 0.04 \times 10^{-6}$ cm^2/s for horse heart ferrocytochrome c and ferricytochrome c, respectively.

INTRODUCTION

In this article we wish to demonstrate the applicability of microelectrochemical techniques to the measurement of the diffusivity of horse heart ferri- and ferrocytochrome c in aqueous solution. Determination of diffusion coefficients in solution is of importance to a number of fields. In biology, sedimentation measurements are used to determine molecular weights of proteins and the key measurements are critically dependent on the diffusion coefficient, D , of the protein.¹ Typically, D is measured by the boundary layer spreading of a ultracentrifuged sample.² The mobility of a molecule, u , in an electrophoretic experiment is also related to D through the Einstein relation, equation (1),³ where Z is the charge on

$$u = ZeD/kT \quad (1)$$

the ion, e is the unit of elementary charge, k is the Boltzmann constant and T is the temperature.

The mechanism by which cytochrome c mediates the charge transport between its physiological redox partners, cytochrome c oxidase and reductase is still not well defined. Homogeneous electron-transfer kinetics between outer-sphere redox components and cytochrome c have been studied extensively.⁴ However, cytochrome c is a solution protein where cytochrome c oxidase and reductase are found in the membrane fraction of isolates. Cytochrome c is believed to provide a charge transport mechanism in oxidative

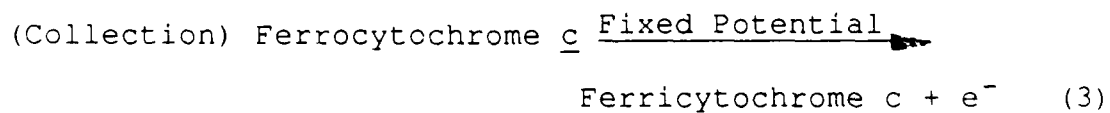
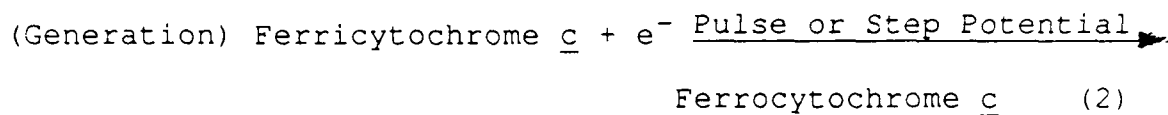
phosphorylation as depicted in Figure 1a. The electrochemistry of cytochrome c, both modified and native, has been studied at a variety of electrodes to investigate the heterogeneous electron-transfer chemistry.⁵ Varying degrees of reversibility are observed in electrochemical measurements. Modifications to amino acids in the region of the heme pocket have significant effects on the heterogeneous rate of electron transfer.⁶ This suggests that interaction between this area and the surface of the electrode must be favorable for fast kinetics. Consequently, irreversible binding at the surface of the electrode would be deleterious to the electrochemistry as at a Hg electrode.⁷ Although the diffusion coefficient of cytochrome c is generally accepted to be 1.1×10^{-6} cm²/s, the values in the literature range from 1.4×10^{-7} to 1.1×10^{-6} cm²/s depending on the technique and electrode surface.⁵

In 1984, our group introduced microelectrode arrays consisting of eight, individually addressable, closely-spaced microelectrodes.⁸ The small gaps allow the measurement of diffusion of species generated at one electrode and collected at a second electrode a distance, *d*, away on a convenient time scale. The microelectrode arrays have been shown to be useful to carry out experimentation analogous to rotating ring-disk electrodes.⁹ Recently, we extended a "time of flight" method used to study diffusion of carriers in a redox polymer¹⁰ to studies of physical diffusion of electrogenerated species.¹¹

Figure 1b and Scheme I summarize the essential features of our methodology. The key measurement is the determination

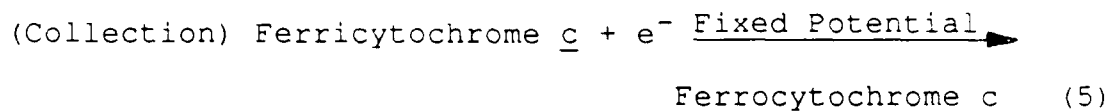
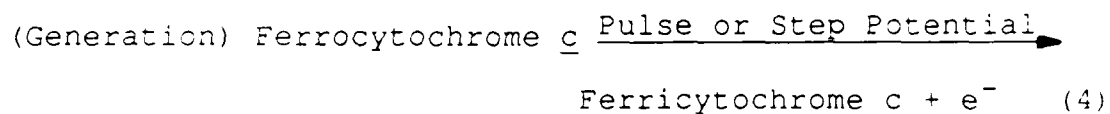
of the time dependence of the collector current resulting from oxidation or reduction of the electrochemically generated diffusing species. The generation can be accomplished either in a short pulse or in a potential step always holding the collector at a fixed potential where the electrochemical generation step is reversed. Thus, for studying diffusion of ferrocytochrome \underline{c} , the solution contains initially only ferricytochrome \underline{c} . The generation pulse or step results in production of ferrocytochrome \underline{c} , equation (2), and the

Ferrocytochrome c Diffusion



collection regenerates ferricytochrome \underline{c} , equation (3). For studying the diffusion of ferricytochrome \underline{c} , the solution initially contains only ferrocytochrome \underline{c} and the generation and collection processes are given by equations (4) and (5),

Ferricytochrome c Diffusion



respectively.

For the short pulse generation method a key measurement is the time to obtain the peak collector current, t_{mt} , (cf. Scheme I) given by equation (6) for the geometry of

$$t_{mt} = 0.22d^2/D \quad (6)$$

generator and collector used in this work. Thus, measurement of t_{mt} for known distance, d allows determination of D . In the stepped potential generation method the key measurement is the time to achieve a given fraction, say 1/3 or 2/3, of the ultimate steady-state collection current, Scheme I, for the microelectrode arrays used. Equations (7) and (8) give the time to achieve 1/3 and 2/3 of the ultimate steady-state

$$t_{1/3} = 0.37d^2/D \quad (6)$$

$$t_{2/3} = 1.34d^2/D \quad (7)$$

current, respectively. From measurements of t_{mt} , $t_{1/3}$, or $t_{2/3}$ values of D can be established accurately and relative values of D can be determined with smaller errors, since the constants of proportionality in equations (6)-(8) are empirically determined and have an estimated error of $\pm 10\%$. The values of D reported here reveal a small, but experimentally significant, difference in D for ferri- and ferrocytochrome c.

Our transient microelectrochemical methodology is analogous to the use of transients at rotating ring-disk electrodes with a few important exceptions. No rotational perturbations to the system are required. This fact is important because the microelectrochemical methodology can be applied to media such as solid polymer electrolytes¹² where rotating electrodes cannot be used. The microelectrode array can have many collector electrodes as opposed to only one ring electrode and the distance between the generator and collector electrodes can be varied. Collection efficiencies can exceed 90% and transit times (for 33% of steady-state collection current) can be less than 5 ms as opposed to rotating ring-disk electrodes where for a thin gap ($r_2/r_1 = 1.07$) at 1000 rpm only 2% of the steady-state value occurs in 30 ms.¹³

In this report the focus of the effort involves the applicability of the methodology represented by Scheme I and Figure 1 and the associated equations (6)-(8) to large biological molecules. In some cases simple linear sweep

voltammetry is adequate to measure D (as we will show for cytochrome c), but the use of the transient techniques allows determination of D when the concentration is not knowable or where the species of interest is not as durable.

Experimental Section

Microelectrode Arrays. Arrays of eight Au microelectrodes each ~ 70 μm long, 2.74 μm wide, and 0.1 μm thick and spaced 1.37 μm apart were used in this work. Fabrication of the microelectrode arrays on p-Si/SiO₂/Si₃N₄ substrates has been described previously.^{8,14} The device surface, except for the electrochemically active area was encapsulated using Hysol Corporation clear and white epoxies.

Prior to use, arrays, mounted on electrically accessible transistor headers, were sonicated for several minutes in acetone to remove residual photoresist. This was followed by a chemical etch in a fresh 3:1 solution of concentrated H₂SO₄ and 30% H₂O₂. The cycle of chemical etch/rinse was repeated for 10, 3, and 3 second etches.

Clean, dry arrays were tested for electrical isolation. When the intermicroelectrode resistance is greater than 10^8 Ω the microelectrodes are considered to be isolated from each other. Arrays were then immersed in an aqueous 0.1 M K₂HPO₄ solution for an electrochemical cleaning by potentiostatic cycling of each electrode between -1.5 and -2.0 V vs. saturated calomel electrode (SCE) at 100 mV/s for 5 seconds, to evolve H₂. A final characterization for electrode isolation and electrochemical activity of the array was carried out in a 5 mM solution of Ru(NH₃)₆Cl₃ in pH 7 phosphate buffered 0.1 M KCl. Individual and adjacent pairs of microelectrodes were cycled between 0.3 and -0.6 V vs. SCE. Such a test reveals, for an "active" microelectrode, a

sigmoidal current-voltage curve with a plateau current of 20-25 nA, and an increased plateau for isolated adjacent electrode pairs. The Au microelectrodes were then scanned from 0.0 to -2.0 V vs. SCE in a 0.1 M NaClO₄, pH 6.5 phosphate buffer saturated with bis-1,2-(4-pyridyl)ethylene to enhance surface activity toward cytochrome c⁵ as demonstrated by attainment of a stable voltammogram and electrochemically reversible surface. All measurements were made at 23 ± 1 °C.

Chemicals. Horse heart ferricytochrome c was obtained from Sigma and used as received. Cytochrome c concentrations were determined by reducing the sample with a minimum amount of sodium dithionite and measuring the absorbance at 570 and 520 nm.¹⁵ Ferrocycytochrome c was obtained by bulk electrolysis of ferricytochrome c solutions using a Au electrode (approximately 4 cm² surface) and the concentration was obtained as above. Bis-1,2-(4-pyridyl)ethylene was obtained from Aldrich and used as received. Electronic grade solvents were used for chemical etching. Omnisolv H₂O was used as solvent in all cases. All other chemicals were analytical grade and were used as received. All solutions were gently deoxygenated with welding grade Argon for at least 10 min prior to measurements.

Equipment. Cyclic voltammetry measurements and bulk electrolyses were carried out using a 100 nA scale modified Pine Instruments RDE-4 bipotentiostat and a Kipp and Zonen BD 91 X-Y-Y' recorder. In addition the potential step and potential pulse measurements used a Princeton Applied Research

175 Universal Programmer as two electrode setup for the generator electrode and a BAS Low Current module using a Nicolet 4904 Digital Collection Oscilloscope to record the signals at the collector electrode.

Microscopy. Microelectrode dimensions were determined using a Cambridge Mark 2A Stereoscan electron microscope and a Bausch & Lomb MicroZoom microscope.

Results and Discussion

Steady-State Voltammetry.

Figure 2 shows the cyclic voltammetry at a macroscopic (approximately 0.04 cm^2) Au electrode of 2.5 mM ferricytochrome c in a 0.1 M NaClO_4 solution buffered to pH 6.5 with phosphate and saturated with *bis*-1,2-(4-pyridyl)-ethylene. The anodic peak to cathodic peak separation of $\sim 60 \text{ mV}$ is consistent with a one-electron reversible process. The peak current corresponds to that expected for a species at 2.5 mM having a diffusion coefficient of $\sim 1.1 \times 10^{-6} \text{ cm}^2/\text{s}$. These results indicate that the system exhibits essentially electrochemically reversible kinetics, as reported previously.⁵

Figure 3 shows cyclic voltammograms for cytochrome c at a microelectrode array. Voltammogram (a) of Figure 3 shows the cyclic voltammetry at a microelectrode in an array consisting of eight separate, individually addressable, microelectrodes. The microelectrodes give a reversible steady-state voltammogram expected for a band microelectrode where the current is governed by cylindrical diffusion.¹⁶ The cylindrical diffusion profile at a band microelectrode gives nearly steady-state currents which can be directly compared to $\text{Ru}(\text{NH}_3)_6^{3+}$ or $\text{Fe}(\text{CN})_6^{4-}$ currents at the same electrode. The value of the nearly steady-state current is proportional to D and the concentration of the diffusing species and since D is known for $\text{Ru}(\text{NH}_3)_6^{3+}$ and $\text{Fe}(\text{CN})_6^{4-}$, comparison of the data allow determination of D for cytochrome c. The double-layer

charging currents are smaller relative to the faradaic currents due to enhanced diffusion to the electrode. This effect is most advantageous for cytochrome c since electrochemistry at macroscopic electrodes shows large capacitive currents limiting the resolution of many techniques.⁵

Figure 3 also shows the generation-collection voltammograms associated with the reduction of ferricytochrome c at one microelectrode and the reoxidation at either a single adjacent microelectrode ((b) and (e)) or symmetric adjacent microelectrodes ((c) and (d)). The collection efficiencies of 58% at a single adjacent and 72% at dual symmetric adjacent microelectrodes are similar to those observed for outer-sphere electrochemically reversible inorganic species such as $\text{Ru}(\text{NH}_3)_6^{2+/3+}$ and $\text{Fe}(\text{CN})_6^{4-/3-}$. The fact that the collection efficiencies do not change regardless of redox active species implies that geometry is not important to the mode of transport. Similar results have been noted on rotating ring-disk electrodes.¹³

Figure 4 shows the steady-state voltammogram of six of the eight microelectrodes at an array after the solution has been electrolyzed in a two compartment cell then transferred to a cell for microelectrode transit time measurements. The currents are consistent from electrode to electrode indicating that all have equivalent surface areas. Also shown are the generation-collection voltammograms for symmetric adjacent

(1.4 μm gap) ((b) and (d)) and symmetric electrodes spaced 6.8 μm away ((a) and (e)).

Since the steady-state voltammogram at a microelectrode is only dependent on the concentration and D of the species, using the limiting current to determine D is a valid method.¹⁷ In Table I is shown the limiting currents for a 2.5 mM ferricytochrome \underline{c} solution as compared to a 1 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ solution. The ratio of the limiting currents is 2.59 so that the diffusion coefficient (using the literature value of $7.1 \times 10^{-6} \text{ cm}^2/\text{s}$ for $\text{Ru}(\text{NH}_3)_6^{3+}$)⁹ for ferrocyclochrome \underline{c} is $1.10 \pm 0.05 \times 10^{-6} \text{ cm}^2/\text{s}$ and $0.99 \pm 0.05 \times 10^{-6} \text{ cm}^2/\text{s}$ for ferricytochrome \underline{c} .

Microelectrochemical Transient Measurements.

The microelectrochemical transit times, t_{mt} , of solutions of 2.5 mM ferricytochrome \underline{c} , ferrocyclochrome \underline{c} , and $\text{Fe}(\text{CN})_6^{3-}$ are shown in Figure 5. The pulse duration determines the total amount of generated species which is proportional to the current obtained upon collection. For $\text{Fe}(\text{CN})_6^{3-}$ $t_{\text{mt}} = 42 \text{ ms}$ as opposed to ferrocyclochrome \underline{c} , where $t_{\text{mt}} = 290 \text{ ms}$ and ferricytochrome \underline{c} , where $t_{\text{mt}} = 320 \text{ ms}$. $\text{Ru}(\text{NH}_3)_6^{2+}$ (not shown) traverses this distance with $t_{\text{mt}} = 41 \text{ ms}$.¹¹ The ratios of the transit times for $\text{Fe}(\text{CN})_6^{3-}$ and cytochrome \underline{c} (using $D = 7.6 \times 10^{-6} \text{ cm}^2/\text{s}$ for $\text{Fe}(\text{CN})_6^{3-}$)¹⁸ correspond to diffusion coefficients of $1.1 \times 10^{-6} \text{ cm}^2/\text{s}$ and $1.0 \times 10^{-6} \text{ cm}^2/\text{s}$ for ferrocyclochrome \underline{c} and ferricytochrome \underline{c} , respectively. These values correspond closely to values accepted in the literature.¹⁹

In vivo cytochrome c need not travel very far to accomplish the transport of charge. The slow self-exchange rate²⁰ and low concentration of cytochrome c rule out charge transport from the generator to the collector via a self-exchange process. However, to show that the mechanism of diffusion in our system is actual movement of redox active material the distance dependence of the microelectrochemical transit time is shown in Figure 6. The linearity of the pulse time as a function of $(d)^2$ implies that diffusion is occurring in a random walk process according to the Einstein-Smolukowski equation. Notice that ferrocyanochrome c transit times are faster for a given interelectrode spacing than the corresponding ferricytochrome c. The ratio of the two slopes is 1.1 implying the diffusion coefficient of the ferro- is faster than the ferricytochrome c, i.e. $1.1 \times 10^{-6} \text{ cm}^2/\text{s}$.

Microelectrochemical Potential Step Measurement.

The diffusion coefficient can also be measured by a step in the potential as opposed to a pulse. The time dependence of the collector current to a steady-state value is related to d and D using equation (7) and (8). Digital simulations as well as previous experimental results on $\text{Ru}(\text{NH}_3)_6^{2+}$ have confirmed the direct relationship between the diffusion coefficient and the time taken for the current to reach 1/3 and 2/3 its steady-state value.¹¹ Figure 7 shows the collector current at a microelectrode as a function of time when the generator electrode is held at either a reducing potential for ferricytochrome c or at an oxidizing potential for

ferrocytochrome c. For the data shown $t_{1/3}$ and $t_{2/3}$ for ferrocytochrome c and ferricytochrome c are 205, 945 ms and 230, 954 ms, respectively. For similar measurements on $\text{Fe}(\text{CN})_6^{3-}$ and $\text{Ru}(\text{NH}_3)_6^{2+}$, $t_{1/3}$ and $t_{2/3}$ are 34, 138 ms and 30, 120 ms, respectively. The distance dependence of $t_{1/3}$ and $t_{2/3}$ in the step experiment are shown in Figure 8 for the transit of ferricytochrome c. The ratio of the $t_{1/3}$ to $t_{2/3}$ slopes is similar to that of $\text{Ru}(\text{NH}_3)_6^{2+}$ and $\text{Fe}(\text{CN})_6^{3-}$ and is qualitatively predicted by previous simulations.¹¹ This is consistent with simple diffusion. The diffusion coefficient measured by this method as compared to a similar measurement made on $\text{Ru}(\text{NH}_3)_6^{2+}$ corresponds well to those determined by the pulse method. For ferricytochrome c D is $1.00 \times 10^{-6} \text{ cm}^2/\text{s}$ and for ferrocytochrome c D is $1.10 \times 10^{-6} \text{ cm}^2/\text{s}$ in good agreement with values of D from the pulse method.

Conclusions

There is a significant difference in the diffusion coefficients of ferri- and ferrocytochrome c, from all three types of microelectrochemical measurements reported here: steady-state, pulse, and potential step techniques all show a ~10% larger D for ferro- compared to ferricytochrome c. Our best measures of D are $1.10 \pm 0.04 \times 10^{-6} \text{ cm}^2/\text{s}$ and $1.00 \pm 0.04 \times 10^{-6} \text{ cm}^2/\text{s}$ for ferrocytochrome c and ferricytochrome c, respectively. Well documented differences occur in the diffusion coefficients of the two halves of some inorganic redox couples.²¹ Most of the differences are rationalized in terms of the size of the ion itself or the size of the ion and solvation cage (i.e. the solvent molecules which are associated with the ion on a significant time scale). However, if the charge on cytochrome c should change with the redox state of the heme, it is unlikely that the state of solvation should change appreciably. As an alternative explanation, the tertiary structure of cytochrome c may change depending on redox state. Long range structural changes may be responsible for differences in binding to substrates, for example differential binding to ferri- and ferrocytochrome c to cytochrome c oxidase.

This study profiles the diffusion of species over $1.4\ \mu\text{m}$ to $26\ \mu\text{m}$ and the characteristic collection concentrations as a function of time. The utility of the microelectrode transit time measurement as applied to biologically relevant molecules is demonstrated. The limiting current method of diffusion coefficient determination at microelectrodes is applied to ferri- and ferrocytochrome c. Our results show that the better faradaic to capacitive current ratio of microelectrodes can be exploited to characterize biomolecules.

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SCHEME AND FIGURE CAPTIONS

Scheme I. Schematic representation of the time dependence of generator potential and collector current during a microelectrode transit time experiment. (Upper curves) With reference to Figure 1b, the generator is pulsed to a relatively reducing potential. Arrival of the diffusing species at the collector is detected by anodic current. As indicated, t_{mt} is measured as the time from the midpoint of the generator pulse to the point of maximum collector current. (Lower curves) As above, but the generator is stepped to a constant potential and the resultant time dependence of the collector current monitored. As indicated $t_{1/3}$ or $t_{2/3}$ are measured from the origin of the generator step to the time to achieve 1/3 or 2/3 of the steady-state collector current, respectively.

Figure 1. (a) Illustration of the role of cytochrome c in oxidative phosphorylation. Cytochrome c is located between the inner and outer mitochondrial membranes shuttling electrons from cytochrome c reductase to cytochrome c oxidase. (b) Representation of the experimental configuration for microelectrochemical studies showing the reduction of the oxidized form (generation) and diffusion of the reduced form to the collector electrode where it is oxidized (collection).

Figure 2. Cyclic voltammetry of 2.5 mM ferricytochrome c in 0.1 M NaClO₄, pH 6.5 saturated with bis-1,2-(4-pyridyl)

ethylene at a macroscopic Au electrode of $\sim 0.04 \text{ cm}^2$ area at 10 mV/s.

Figure 3. (a) Cyclic voltammogram at a microelectrode of length $\sim 50 \mu\text{m}$, $1.37 \mu\text{m}$ in width and $0.1 \mu\text{m}$ height scanned from -0.2 V to $+0.25 \text{ V}$ vs. Ag at 50 mV/s, (b) cyclic voltammogram at a microelectrode scanned as above with a single adjacent (separated by $1.4 \mu\text{m}$) microelectrode held at a fixed potential of $+0.25 \text{ V}$ vs. Ag, (c) cyclic voltammogram at a microelectrode scanned as above with two adjacent (separated by $1.4 \mu\text{m}$) microelectrodes symmetrically disposed each held at $+0.25 \text{ V}$ vs. Ag, (d) current measured at each of the symmetrically disposed "collector" microelectrodes (held at a fixed potential) as a function of the potential of the intervening "generator" microelectrode shown in (c), (e) current measured at a single adjacent "collector" microelectrode as a function of the potential of the nearby "generator" microelectrode shown in (b).

Figure 4. (Left half) Cyclic voltammograms of microelectrodes #2, #4, #5, #6, #7, #8 in a 2.5 mM ferrocyanide solution obtained by bulk electrolysis of a ferricyanide solution in pH 6.5 phosphate buffer. (Right half) Generation-collection voltammograms of electrodes in the array at a scan rate of 10 mV/s, (a) generation current at microelectrode #6 with microelectrodes #5 and #7 collecting at -0.2 V vs SCE, (b) generation current at microelectrode #6

with microelectrode #4 and #8 collecting at -0.2 V vs SCE, (c) generation current at microelectrode #6 scanned from -0.2 V to $+0.2$ V vs SCE with no collector, (d) collector current at microelectrodes #4 and #8 held at -0.2 V vs. SCE with microelectrode #6 scanned as in (b), (e) collector current at microelectrodes #5 and #7 held at -0.2 V vs. SCE with microelectrode #6 scanned as in (a).

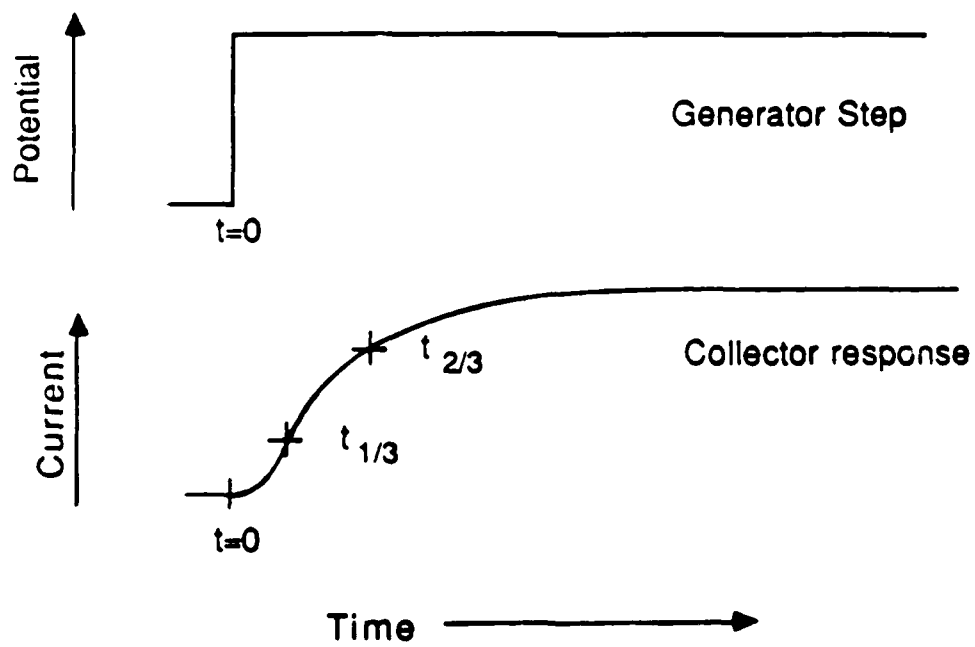
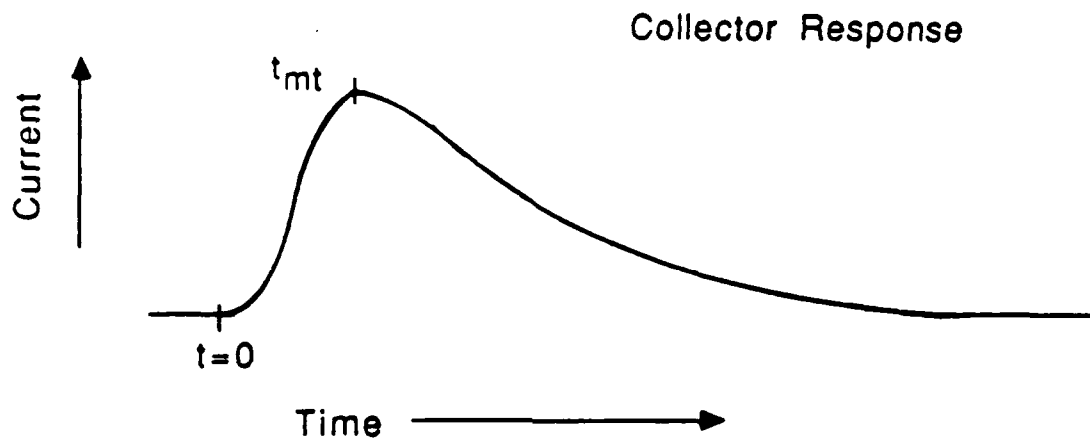
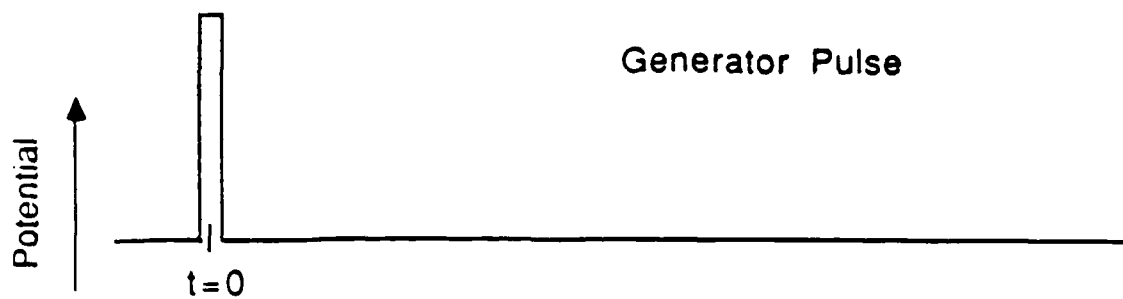
Figure 5. Microelectrode transit time experiments for ferricytochrome c, ferrocyanochrome c and $\text{Fe}(\text{CN})_6^{3-}$. (a) The time dependence of the collection current of microelectrode #4. with microelectrode #1 pulsed from -0.2 V to 0.2 V for 50 ms in 2.5 mM ferrocyanochrome c in 0.1 M NaClO_4 , pH 6.5 phosphate buffer generated by bulk electrolysis of the oxidized form of cytochrome c. (b) Time dependence of the collection current at microelectrode #5 initiated by a 0.5 ms pulse from 0.0 V to 0.4 V vs. SCE of 2.5 mM $\text{Fe}(\text{CN})_6^{3-}$ in 0.1 M NaClO_4 , pH 6.5 phosphate buffer at microelectrode #8. (c) Time dependence of the collection current at microelectrode #4 initiated by a 20 ms pulse from 0.2 V to -0.2 V vs. SCE in 2.5 mM ferricytochrome c in 0.1 M NaClO_4 , pH 6.5 phosphate buffer at microelectrode #1.

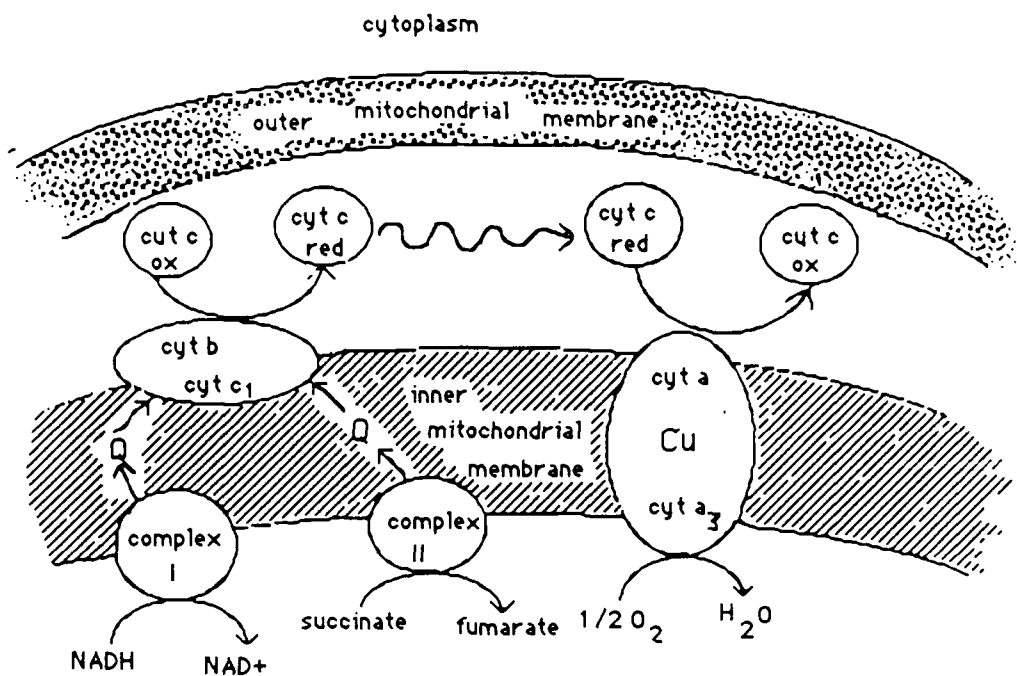
Figure 6. Distance dependence of the peak pulse time of Microelectrode transit time experiments of both ferri- and ferrocyanochrome c in 0.1 M NaClO_4 pH 6.5 buffered solution. Each point is an average of >8 individual measurements similar

to Figure 5 and are plotted vs. the square of the center to edge distance of the generator and collector microelectrodes.¹¹ The ratio of the slopes gives the ratio of the individual diffusion coefficients of 1.1.

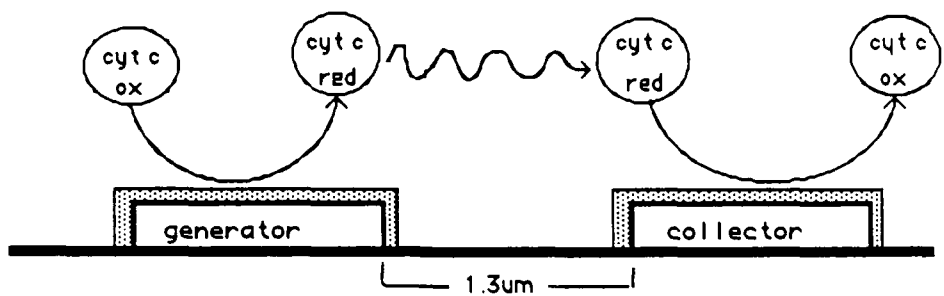
Figure 7. Step measurements of the transit time of cytochrome c. (a) Collector current at microelectrode #4 as a function of time. The generator, microelectrode #1, was stepped from +0.2 to -0.2 V vs. SCE in a 2.5 mM ferricytochrome c, 0.1 M NaClO₄, pH 6.5 phosphate buffer. (b) Collector current at microelectrode #6 as a function of time. The generator, microelectrode #3, was stepped from -0.2 to +0.2 V vs. SCE in a 2.5 mM ferrocyanochrome c, 0.1 M NaClO₄, pH 6.5 phosphate buffer.

Figure 8. The distance dependence of the $t_{1/3}$ and $t_{2/3}$ times for the diffusion of ferricytochrome c in 0.1 M NaClO₄, pH 6.5 phosphate buffer. Each point is an average of ≥ 5 individual measurements similar to Figure 7 and are plotted vs. the square of the center to edge distance of the generator and collector microelectrodes as in Figure 6.



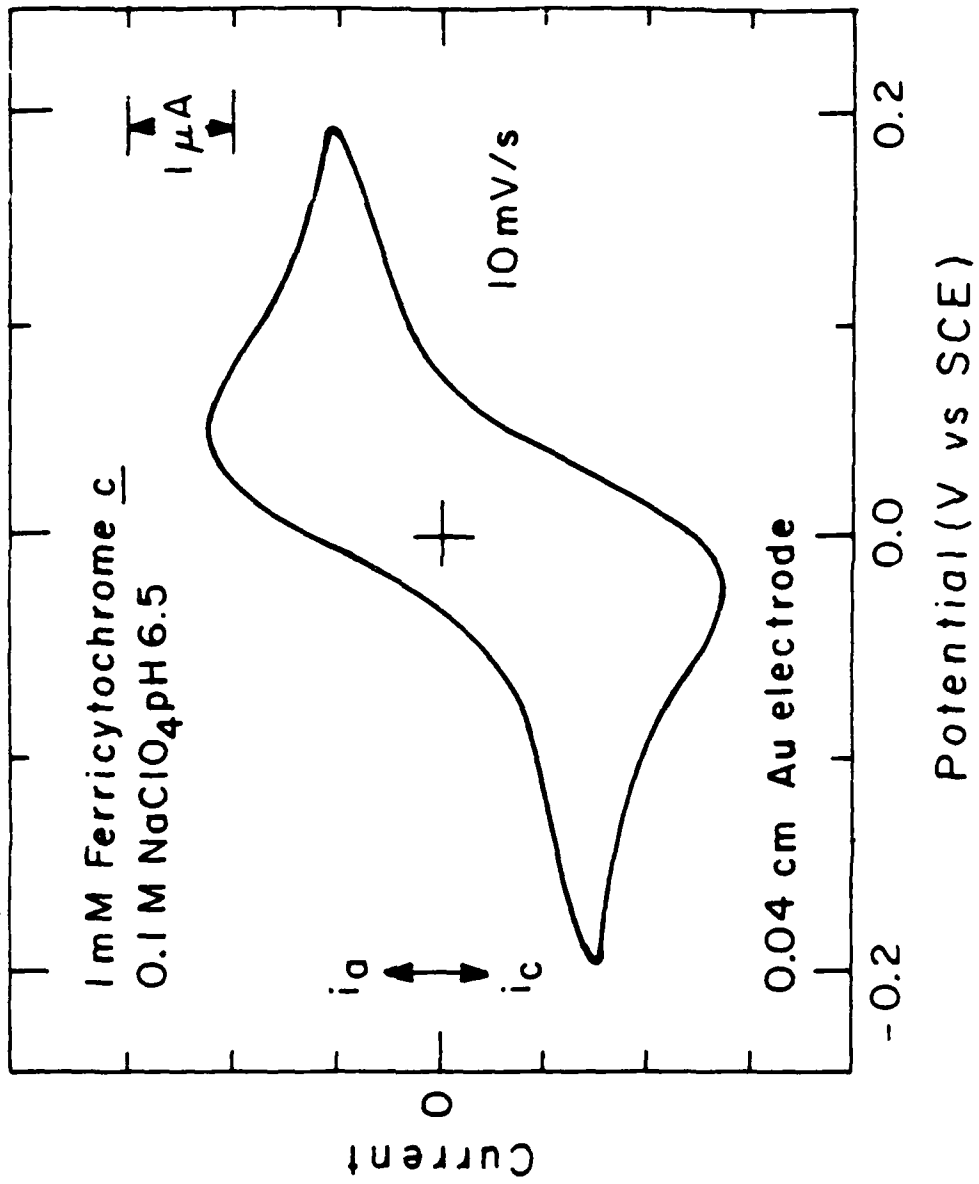


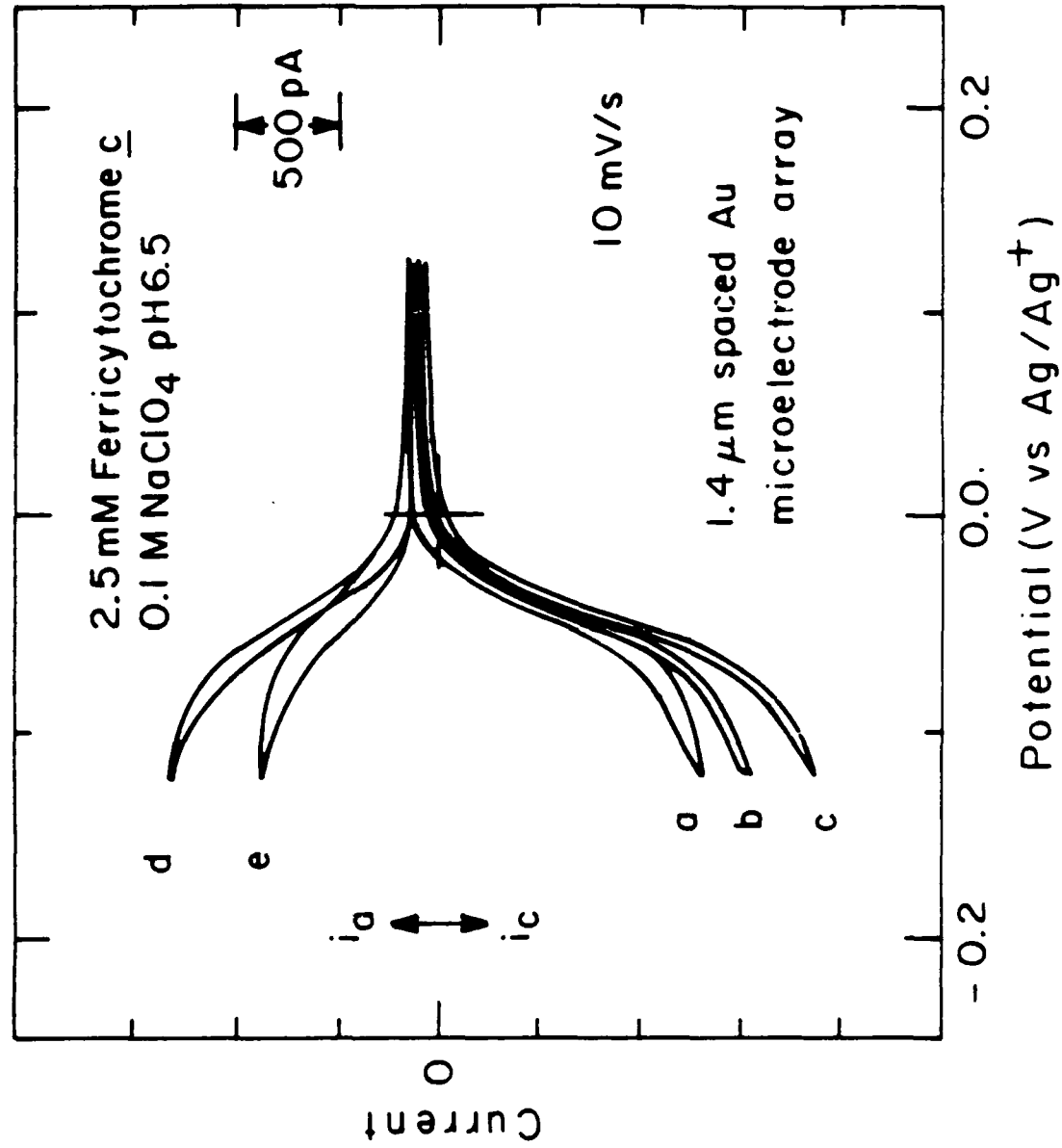
in vivo e-transport
by cytochrome c

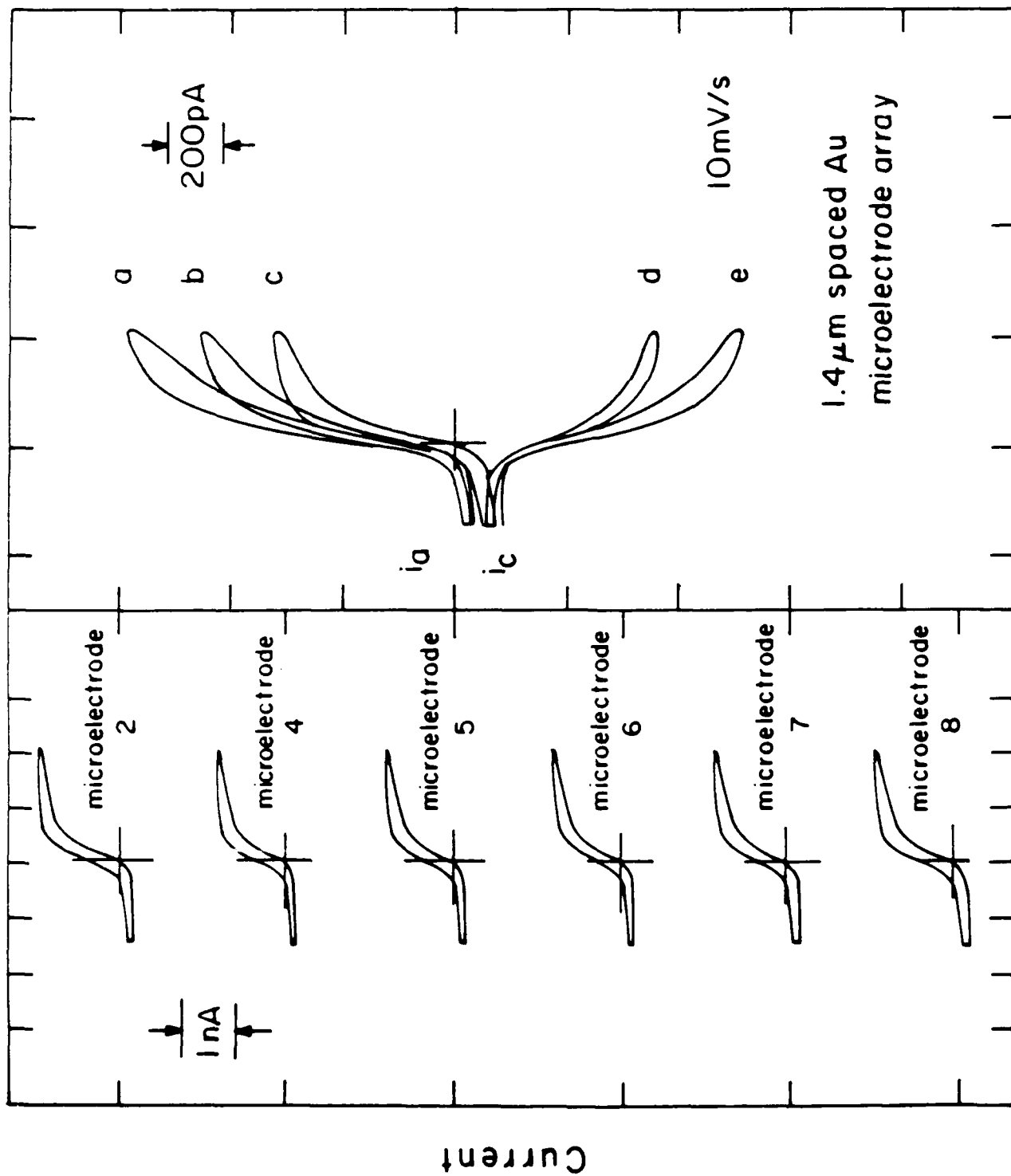


Generator, Collector = Au microelectrodes 80um x 2.6um
 = 1,2 bis(4-pyridyl) ethylene

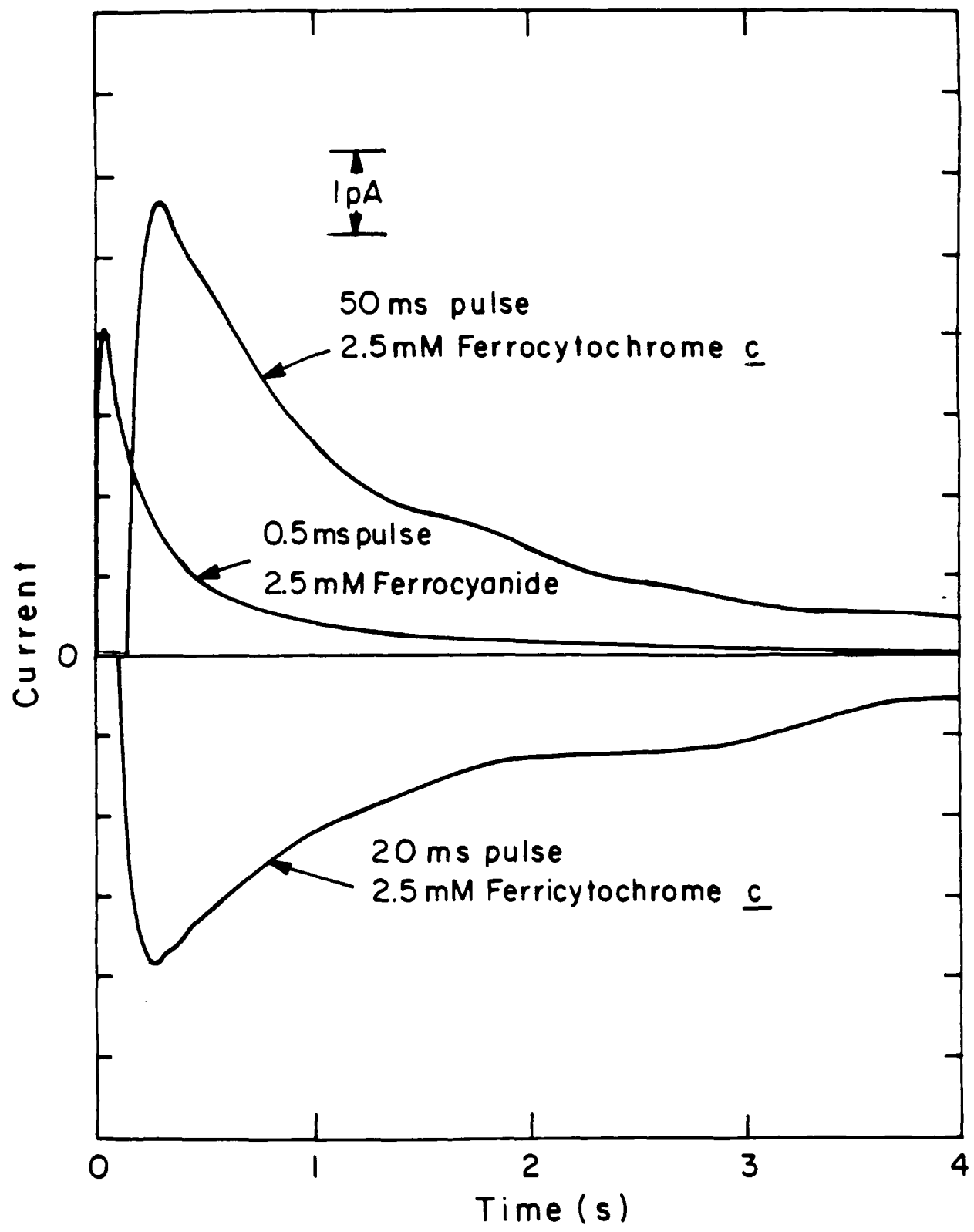
Experimental in vitro e-transport
by cytochrome c

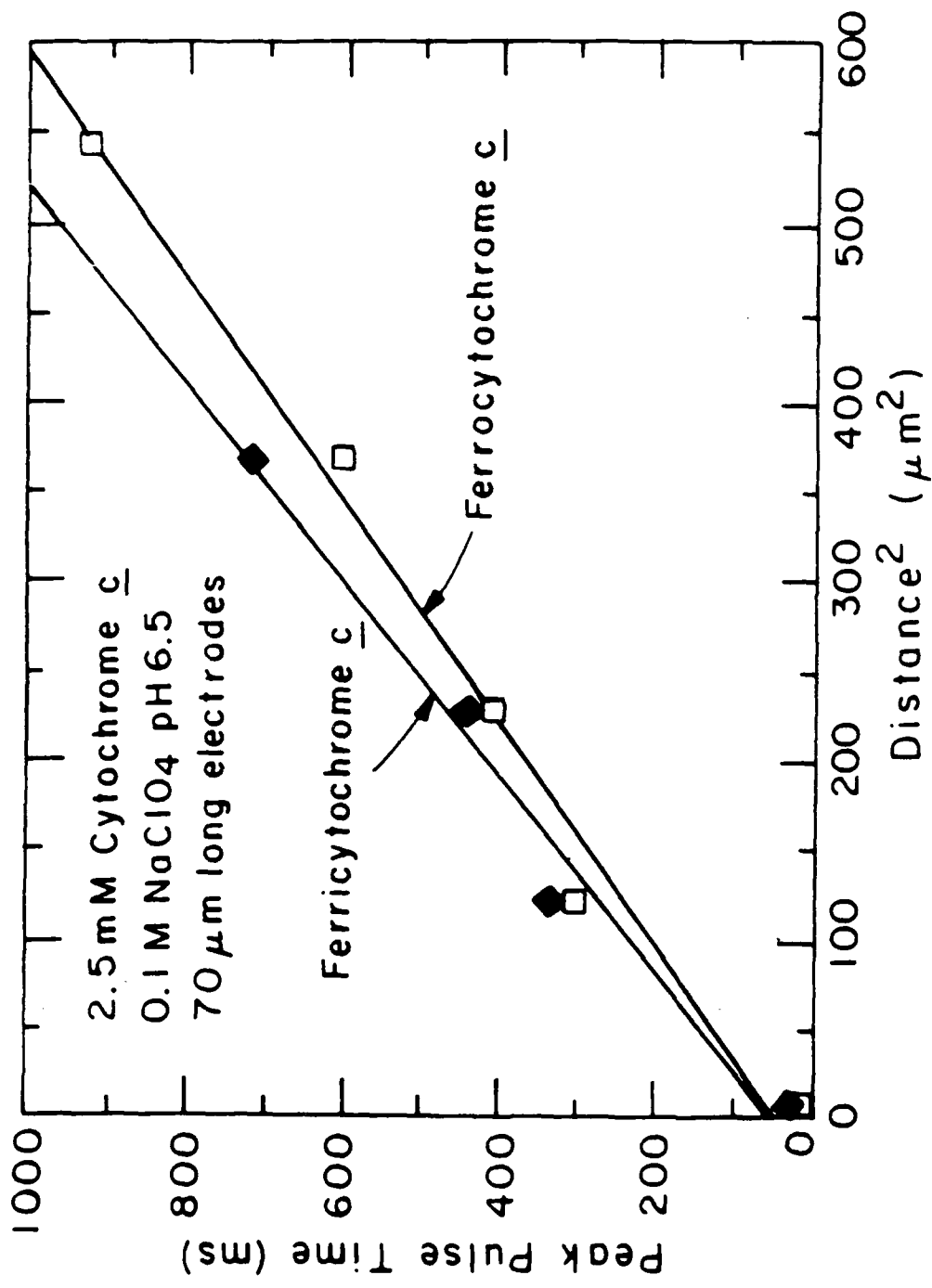


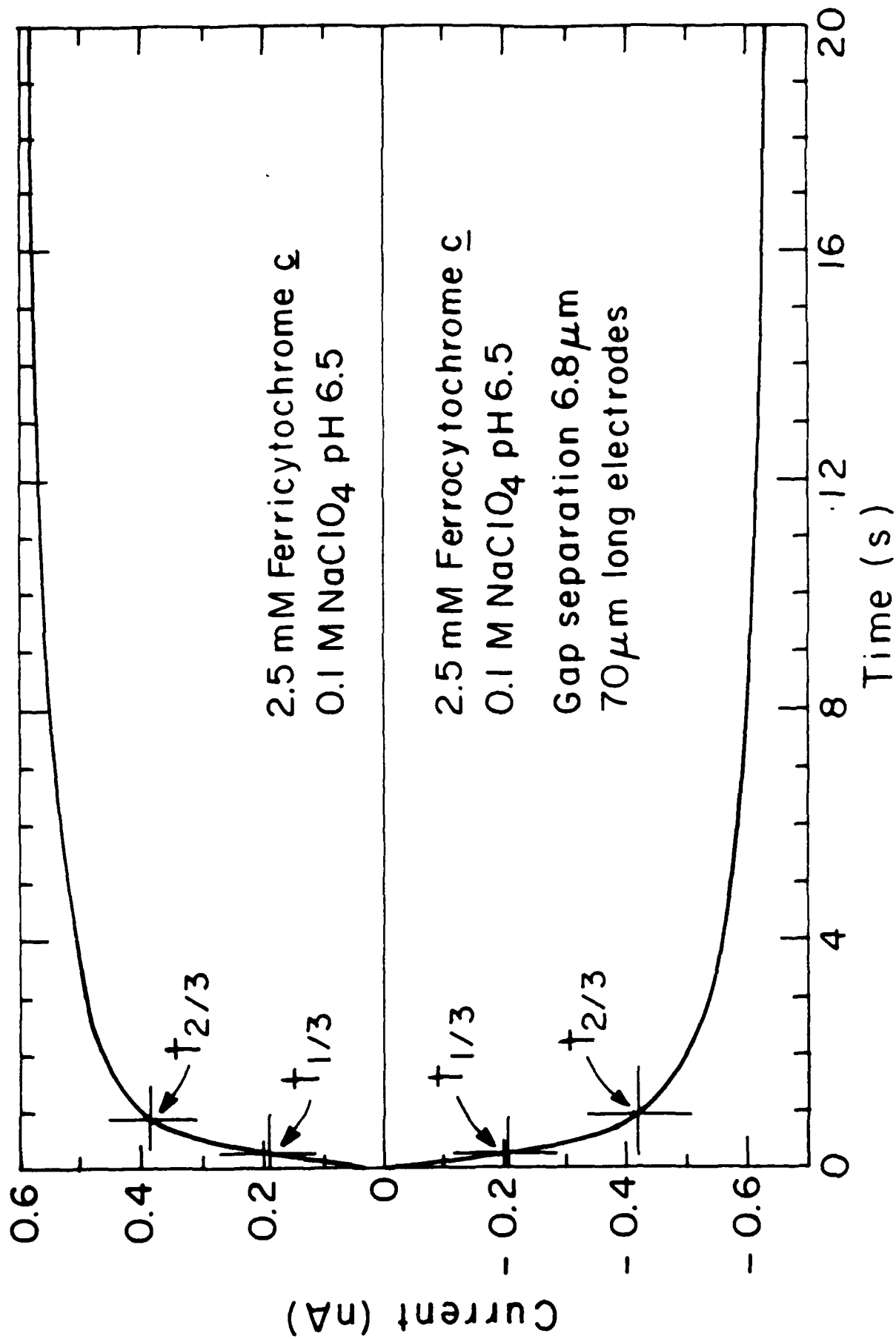


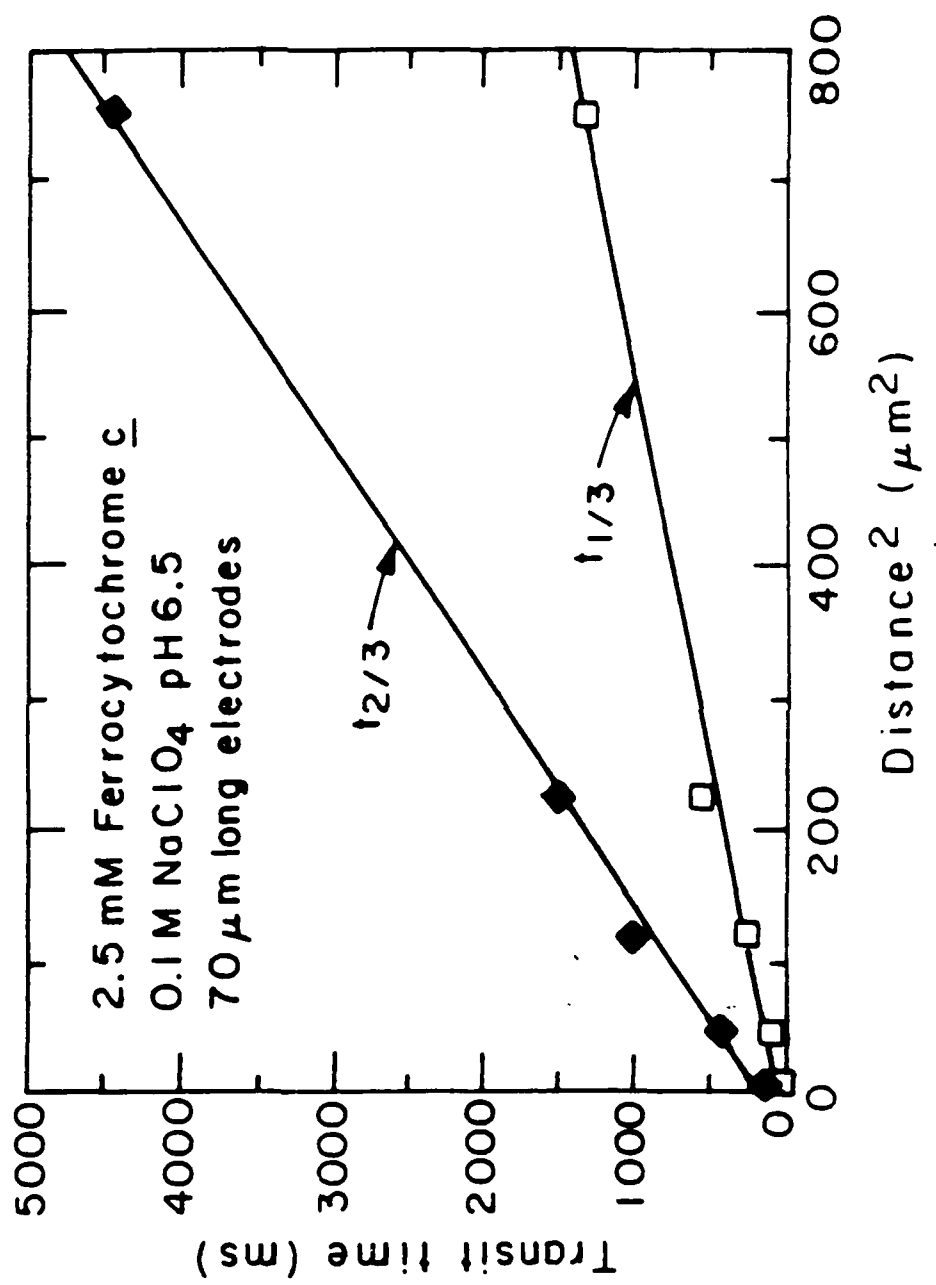


Potential (V vs Ag/AgCl)









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