



Scanning electrochemical microscopy. 35. Determination of diffusion coefficients and concentrations of $\text{Ru}(\text{NH}_3)_6^{3+}$ and methylene blue in polyacrylamide films by chronoamperometry at ultramicrodisk electrodes

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Abstract—The chronoamperometric response at an ultramicrodisk electrode (diameter = 25 μm) inserted into polyacrylamide (PAAM) gel films containing reducible electroactive species [$\text{Ru}(\text{NH}_3)_6^{3+}$ and methylene blue (MB)] was used to determine the diffusion coefficients (D) and concentrations (C) of these species using a previously proposed treatment of the ratio of transient and steady-state currents. The diffusion coefficients obtained in film were slightly smaller (75–95%) than the values for the same species in solution, demonstrating that the film environment is mainly an aqueous one contained within large polymer pores. The calculated concentrations were in good agreement with those used to cast the films. Complexes of MB with DNA were also examined in solution and in a PAAM film. The diffusion coefficients in both cases were significantly smaller ($\sim 23\%$) than those of MB alone. The results indicate that, with some limitations, the chronoamperometric response of films probed with an SECM tip can be used to determine both D and C with knowledge of only the ultramicrodisk radius. © 1997 Elsevier Science Ltd

Key words: Ultramicrodisk electrode, SECM, chronoamperometry, polyacrylamide film, diffusion coefficients.

INTRODUCTION

Electrochemical techniques that can be employed to characterize microscale systems have been the subject of recent interest. For example, scanning electrochemical microscopy (SECM) [1–3] can be used to image surfaces with high spatial resolution and to determine rate constants of surface reactions. Among microsystems of interest, polymer films on conducting or insulating substrates have been the subject of considerable activity. Numerous investigations have dealt with electrodes modified by layers of polymers, such as polyvinylferrocene and Nafion

[4, 5]. In such studies, one wants to know the concentration (C) and diffusion coefficient (D) of electroactive species in the layer, often without knowledge of film thickness or density. One approach to studying such films with the SECM involves moving an ultramicroelectrode (*ume*) tip into a film and studying the steady-state current as a function of tip position (depth of penetration). Examples of this approach are the examination of a 2000 Å thick Nafion film containing $\text{Os}(\text{bpy})_3^{3+}$ on an indium tin oxide (ITO) substrate [6] and a polyvinylferrocene film on ITO [7]. These studies allowed the determination of film thickness and the characterization of the electrochemical process, but it was not possible to determine C and D from only the steady-state current ($i_{T,\infty}$) when the tip is far from the substrate, e.g., for a disk-shaped

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electrode:

$$i_{T,\infty} = 4nFDCa \quad (1)$$

where n is the number of electrons involved in the redox reaction, F is the Faraday constant, and a is the electrode radius.

An approach to the determination of D and C is that previously described employing chronoamperometry at ultramicroelectrodes [8, 9]. Here, the transient current at a microdisk stepped to a potential where the electrode reaction occurs at a diffusion-controlled rate, $i(t)$, is normalized by the steady-state current, given in equation (1). Based on the model for the transient behavior of a microdisk electrode [10], the equation for the normalized transient current is

$$i(t)/i_{T,\infty} = (\pi^{1/2}/4)a(Dt)^{-1/2} + \pi/4. \quad (2)$$

Thus, plotting $i(t)/i_{T,\infty}$ vs $t^{-1/2}$ results in a straight line that allows determination of D from the slope without foreknowledge of C . Application of equation (1) then allows determination of C . Conditions for the application of this approach have been discussed in detail [9].

In this paper we demonstrate this approach with a Pt *ume* that penetrates polyacrylamide (PAAM) gel films containing reducible electroactive species [$\text{Ru}(\text{NH}_3)_6^{3+}$ and methylene blue (MB)]. We also studied the effect of association of the MB with DNA on the diffusion coefficient of MB in aqueous solution and in the films. To our knowledge the only previous studies utilizing this approach were the determination of the number of electrons involved in the half-reaction for borohydride oxidation [9, 11] and the diffusion coefficient of oxygen in Nafion [12, 13].

EXPERIMENTAL

Reagents and polyacrylamide films

Hexamineruthenium(III) chloride ($\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, Strem Chemicals), methylene blue (MB, Fluka), NaCl (Aldrich), acrylamide (AAM, electrophoresis grade, Aldrich), N,N' -methylenebisacrylamide (X-AAM, electrophoresis grade, Aldrich), ammonium peroxydisulfate (APS, Fisher), and deoxyribonucleic acid (DNA, calf thymus, double stranded, Sigma) were used as received. Phosphate buffer solutions (pH = 6.7) were prepared by dissolving $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (J. T. Baker) to be 2.5 mM in each. A MB–DNA complex solution was produced by adding 50 μl of a 5 mM MB buffered solution to 4.95 ml of a 0.46 mM DNA base pair buffered solution. All solutions were prepared with deionized water (Milli-Q, Millipore Corp.).

To prepare PAAM films containing $\text{Ru}(\text{NH}_3)_6^{3+}$, 4.75 g of AAM, 0.25–1.0 g of X-AAM, and 0.05 g of APS were dissolved in 100 ml of water. This aqueous solution was then made 5 mM in $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ and 0.1 M in NaCl. Glass cells were formed by two glass plates spaced with a 1 mm thick glass strip, and 3

faces were sealed with epoxy resin. These cells were filled with a small portion of the AAM solution and then placed in an 80°C oven for 1 h. Removal of one plate yielded a film about 1 mm thick. The PAAM films containing MB and MB–DNA were produced by immersing the PAAM films, made as above without a redox couple, in 50 μM MB and 50 μM MB–0.46 mM DNA aqueous buffered solutions overnight.

Electrodes and apparatus

The 25 μm diameter Pt *ume* was fabricated as described previously [1] and was polished with 0.05 μm alumina before each experiment. The glass insulating sheath was typically ground to a taper so RG (radius of glass/radius of Pt) was ≤ 2 . The electrode area of the Pt microdisk was determined by the reduction of 5 mM $\text{Fe}(\text{CN})_6^{2+}$ in a 0.1 M NaCl solution, where the steady-state limiting current [equation (1)] was 16.0 nA. With a D of 6.32×10^{-6} cm^2/s , the diameter of the electrode was 26.2 μm . A silver wire was used as a quasi-reference electrode (*Aggre*) and Pt foil was used as the counter electrode. All potentials reported were calibrated with respect to *sce*. The Pt microdisk electrode, biased at -0.4 V to reduce MB or $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, was moved toward the PAAM films, which were placed on the Pt counter electrode and contacted with the *Aggre*, in a cell contained in the SECM. Details of the construction and operation of the SECM are described elsewhere [2]. The cyclic voltammetric and chronoamperometric experiments were carried out with the tip 20 μm beneath the PAAM film/air interface. The tip position within the film was determined by noting the sharp current increase when the tip contacted the film/air interface and then using the calibrated z axis inchworm to move the tip into the film. Because the transient currents, in the nanoampere range, had to be measured at short times, it was necessary to signal average to obtain good signal-to-noise. Thus, for MB and MB–DNA reduction, a Norland 3001 digital oscilloscope with signal averaging capabilities was utilized. Repetitive potential steps of 2 s duration between 0.0 and -0.4 V for MB and -0.15 and -0.4 V for MB–DNA were employed with data collection triggered at the moment of each negative potential excursion and points taken every 2 ms. The resulting data was averaged for 100 cycles for MB and 400 cycles for MB–DNA reduction.

RESULTS AND DISCUSSION

$\text{Ru}(\text{NH}_3)_6^{3+}$ experiments. The SECM approach curve for the tip moved from the air toward and into the PAAM film containing nominally 5 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ on a Pt substrate is given in Fig. 1. During the forward approach, the current shows a sharp increase when the tip contacts the film/air interface ($z = 0$). The current rapidly decays to the

steady-state value, $i_{T,\infty}$, as the tip continues to penetrate the film. The value of $i_{T,\infty}$ was independent of the position of the tip inside the film, suggesting that the electroactive species was uniformly distributed within the PAAM. When the tip is withdrawn from the film, as shown in the lower curve of Fig. 1, the current stays near the level of $i_{T,\infty}$ for a short distance, and doesn't return to baseline until about $13\ \mu\text{m}$ past the $z = 0$ point, suggesting that the film adheres to the tip and is elastically displaced with it until the point where it suddenly breaks away and the current drops to zero.

The constancy of $i_{T,\infty}$ in the SECM approach curve also demonstrates that compression of the film as the tip enters and moves into the film does not cause an appreciable decrease in the electrode area, at least as long as the tip is far from the substrate. This is consistent with the highly aqueous nature of the gel film. We have found in later experiments that films with a higher concentration of polymer can show some blockage of the tip on compression of the film, especially when the tip is moved close to the substrate. In all studies in this paper, measurements were made with the tip far from the substrate in thick films.

A cyclic voltammogram with the tip $20\ \mu\text{m}$ into the film (PAAM/X-AAM 19/2) taken at a scan rate of $2\ \text{mV/s}$ and one obtained under the same conditions in an aqueous solution are shown in Fig. 2. While the $E_{1/2}$ for both curves is nearly the same, the $i_{T,\infty}$ for the tip in the film is significantly smaller, suggesting that the diffusion of $\text{Ru}(\text{NH}_3)_6^{3+}$ in the film or its concentration are smaller. Although the same concentration of $\text{Ru}(\text{NH}_3)_6^{3+}$ was used in film preparation ($5\ \text{mM}$), volume changes that occur during film preparation can affect the final concentration. Independent determination of D and C was

then carried out by potential step chronoamperometry. With the tip maintained $20\ \mu\text{m}$ into the film, the potential was stepped from 0 to $-0.4\ \text{V}$ vs *sce* and the current was monitored for times between 1 and $190\ \text{ms}$ ($0.1\ \text{ms}$ per point). The current normalized with respect to $i_{T,\infty}$ and plotted against $t^{-1/2}$ is shown in Fig. 3. A linear regression of the points over the interval 10 – $190\ \text{ms}$ was used to compute intercept and slope (Table 1). By applying equations (1) and (2) we calculated $D = 5.5 \times 10^{-6}\ \text{cm}^2/\text{s}$ and $C = 5.9\ \text{mM}$. Similar experiments were carried out with PAAM films with different degrees of cross-linking (PAAM/X-AAM 19/1 and 19/4) and for a solution of $5\ \text{mM}$ $\text{Ru}(\text{NH}_3)_6^{3+}$ in $0.1\ \text{M}$ NaCl; the results are shown in Table 1. The intercept of the $i(t)/i_{T,\infty}$ vs $t^{-1/2}$ lines for all cases ranged from 0.78 to 0.88 , close to the value $\pi/4$ (0.785) predicted from equation (2). The value of D for $\text{Ru}(\text{NH}_3)_6^{3+}$ in $0.1\ \text{M}$ NaCl was slightly larger than that found for $\text{Ru}(\text{NH}_3)_6^{3+}$ in pH 4 buffer, $6.0 \times 10^{-6}\ \text{cm}^2/\text{s}$ [14]. The diffusion coefficient for the films becomes smaller with an increased amount of cross-linking, although in all cases the observed values were on the order of those in aqueous solution, suggesting that the environment is one of water filled channels of a size much larger than $\text{Ru}(\text{NH}_3)_6^{3+}$ tied together by a PAAM network. This can be contrasted to films of polymers like Nafion, where decreases in D of one or more orders of magnitude are seen.

The observed concentration values in the film reflect the fact that the volume decreases upon film preparation. For example, for the 19/1 film about a 5% volume decrease was noted, while for the 19/2 and 19/4 films, the shrinkage was about 8 and 15% , respectively. Note also that the 19/4 film was less homogeneous than the others and looked translucent.

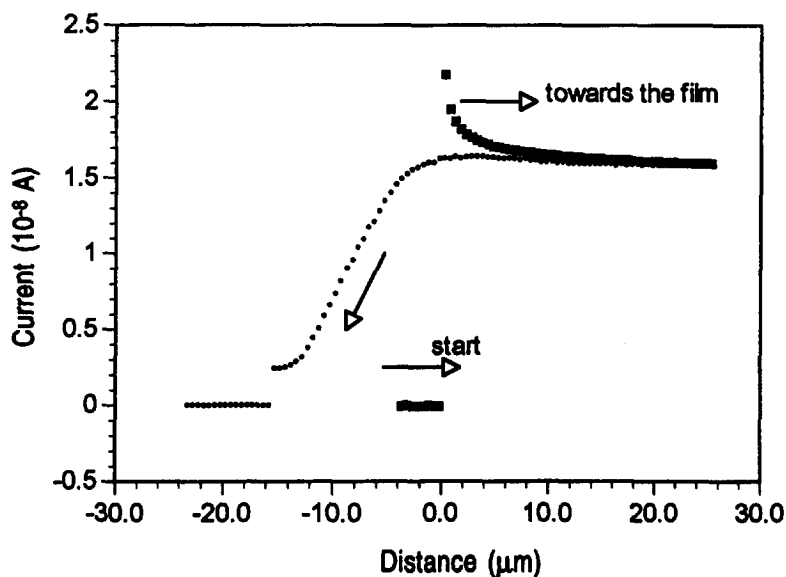


Fig. 1. Approach curve of a $25\ \mu\text{m}$ Pt microdisk electrode toward a PAAM film containing $5\ \text{mM}$ $\text{Ru}(\text{NH}_3)_6^{3+}$ and $0.1\ \text{M}$ NaCl. The tip was biased at $-0.4\ \text{V}$ vs *Ag/AgCl* and was moved at $1\ \mu\text{m/s}$.

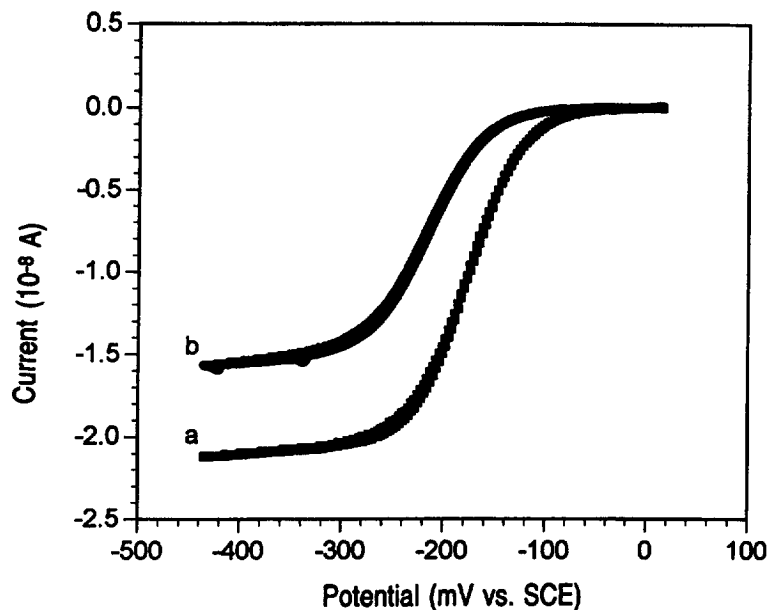


Fig. 2. Comparison of cyclic voltammograms scanned at 2 mV/s in (a) solution and (b) PAAM film. Both solution and film contained 5 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ and 0.1 M NaCl.

Methylene Blue experiments. Similar experiments were carried out for MB reduction in PAAM films. The electrochemical reduction of this species to the leuco-form in a $2e^-$ -reaction has been studied extensively [15, 16]. In this experiment a PAAM/X-AAM film 19/1 was loaded by placing it in a 50 μM

MB in 5 mM phosphate buffer (pH 6.7) solution overnight. Cyclic voltammograms for MB in solution and in the film at 10 mV/s for scans to negative potentials are shown in Fig. 4(a) and (b). Reoxidation peaks appear on anodic scans following the reduction, as seen in voltammograms at larger

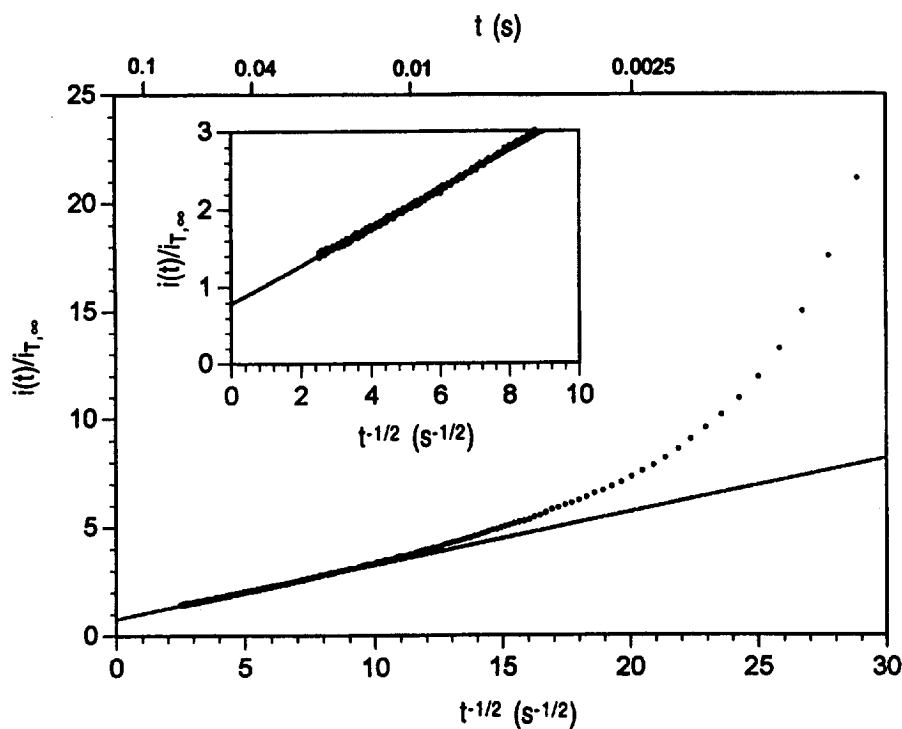


Fig. 3. Plot of the experimental $i(t)/i_{T,\infty}$ vs $t^{-1/2}$ for the reduction of 5 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in a PAAM film (PAAM/X-AAM = 19/2) with a 25 μm diameter Pt *ume*. The linear regression is also shown.

Table 1.
Experimental and calculated results for the reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ ^a

Medium	$i_{T,\infty}$ (10^{-8} A)	Slope	Intercept	D (10^{-6} cm ² /s)	C (mM)
Aqueous solution	2.12	0.205	0.881	8.02 (0.09)	5.23 (0.11)
PAAM/X-AAM = 19/1	1.60	0.238	0.780	5.85 (0.15)	5.40 (0.07)
PAAM/X-AAM = 19/2	1.63	0.246	0.784	5.48 (0.18)	5.93 (0.22)
PAAM/X-AAM = 19/4	1.65	0.251	0.799	5.28 (0.13)	6.22 (0.25)

^aSolution and PAAM films contained 0.1 M NaCl. D and C calculated from equation (2) using $i_{T,\infty}$, slope, and intercept found experimentally. $a = 13.1 \mu\text{m}$ and $n = 1$. Values in parentheses are standard deviations based on triplicate measurements.

electrodes; this has been attributed to oxidation of adsorbed multilayers of the leuco-form [15, 16]. As in the experiments above, film voltammetry was carried out with a $25 \mu\text{m}$ diameter tip placed $20 \mu\text{m}$ into the film. The $i_{T,\infty}$ for the tip in the PAAM film is about 6% smaller than that in solution, demonstrating only a small effect of film structure on the diffusion coefficient.

Methylene blue is known to interact strongly with double-stranded DNA through intercalation between the base pairs [17–19]. Such complexes are usually studied by spectroscopic methods because of the high extinction coefficient of MB. However, electrochemical methods can also be used to probe interactions of DNA with electroactive molecules through measurements of shifts in potential or decreases in diffusion coefficient with complexation [20, 21]. Thus it was of interest to study the effect of DNA on the diffusion of MB both in solution and in the film. In solution MB shows a

well-defined voltammetric wave at the tip (Fig. 4(a)). The limiting current, $i_{T,\infty}$, shows a large decrease on addition of DNA, consistent with past studies that showed large decreases in the diffusion coefficient of electroactive species when they associate with DNA. PAAM/X-AAM (19/1) films were soaked in a phosphate buffer (pH 6.7) containing $50 \mu\text{M}$ MB with or without 0.46 mM base pairs of calf thymus DNA for 24 h. The values of $i_{T,\infty}$ for MB reduction in the film in the absence of DNA is about 6% smaller than those for MB in solution. This decrease is smaller than that found with $\text{Ru}(\text{NH}_3)_6^{3+}$, suggesting that movement of the monocationic MB is less hindered than that of the tricationic Ru species. The decrease in $i_{T,\infty}$, comparing solution and film, is also larger for DNA-intercalated MB (27%) than for free MB (6%). This suggests that motion of the complex through the PAAM network may be more restricted by the presence of the larger DNA molecule, as

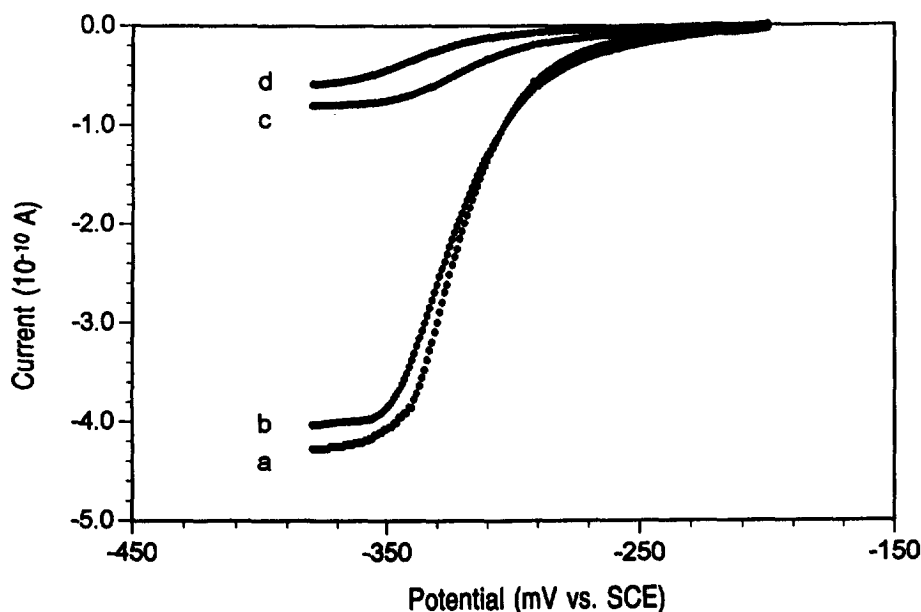


Fig. 4. Cyclic voltammograms of $50 \mu\text{M}$ MB and $50 \mu\text{M}$ MB– 0.46 mM base pairs DNA complexes in solution (pH 6.7 buffer) and in PAAM films (PAAM/X-AAM = 19/1) with a $25 \mu\text{m}$ diameter Pt *ume*. (a) MB in solution, (b) MB in film, (c) MB-DNA in solution, and (d) MB-DNA in film. Scan rate = 10 mV/s .

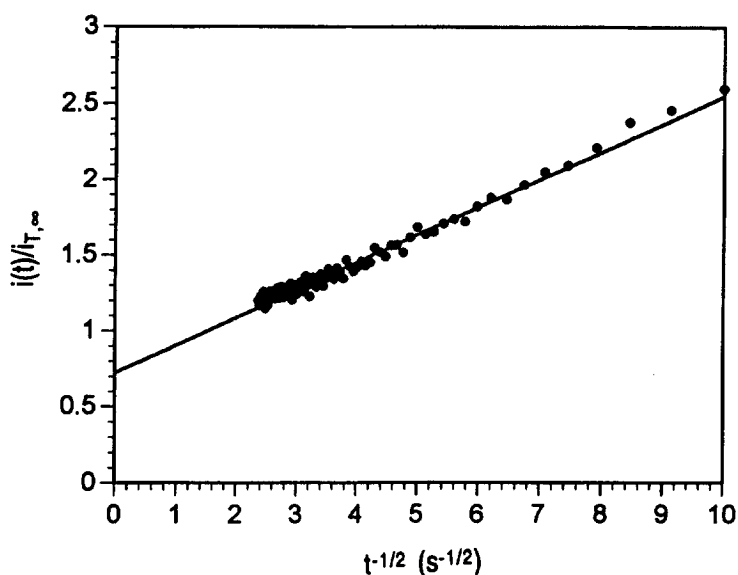


Fig. 5. Plot of the experimental $i(t)/i_{T,\infty}$ vs $t^{-1/2}$ for the reduction of 50 μM MB in a phosphate buffer solution (pH 6.7) with a 25 μm diameter Pt *ume*. The linear regression is also shown.

expected for a gel medium which is frequently employed for DNA separations by electrophoresis.

Chronoamperometric experiments were conducted in both solution and film with 2 s potential steps between -0.15 and -0.4 V with the current sampled every 2 ms. Because of the small currents observed at the concentrations of MB and MB-DNA employed, it was necessary to use signal averaging with a computer of average transients, averaging 100 and 400 separate potential step experiments, respectively. Typical results for $i(t)/i_{T,\infty}$ vs $t^{-1/2}$ for MB in solution are shown in Fig. 5. The diffusion coefficients obtained from plots like this are given in Table 2. The decrease in D for MB in the absence of DNA on incorporation in the film is smaller ($\sim 2\%$) than that suggested by the $i_{T,\infty}$ values, which are also affected by the decreased concentration of MB in the film compared to solution. The decrease in D for the DNA complex in the film is larger ($\sim 8\%$), but not as large as anticipated from the $i_{T,\infty}$ measurements because of a concentration decrease. These results are somewhat compromised by the known tendency of

MB to form dimeric species and bears further investigation.

CONCLUSIONS

With the use of *umes* and the SECM for tip positioning, it is possible to obtain reasonable estimates of the diffusion coefficients and concentrations of electroactive species within thin films without previous information about film thickness or other properties (knowing only the *ume* radius). Moreover, the positioning capabilities of the SECM should allow studies in which the magnitudes of D and C are probed as a function of film depth (z direction) or x, y position with respect to a defect or crack. This should allow one to probe whether loading of a film yields a thickness-dependent concentration or whether polymerization of a film on a substrate gives a variation in polymer properties with distance from substrate which is reflected in the D value. The resolution in such studies depends upon the size of the *ume* available. It is also critically

Table 2.
Experimental and calculated results for the reduction of MB and MB-DNA

Medium ^a	$i_{T,\infty}$ (10^{-10} A)	Slope	Intercept	D (10^{-6} cm ² /s)	C (μM)
MB in solution	4.28	0.183	0.721	10.1 (0.1)	41.9 (2.1)
MB in PAAM film	4.04	0.184	0.780	9.89 (0.14)	40.4 (1.3)
MB-DNA in solution	0.82	0.384	0.699	2.30 (0.07)	35.3 (1.8)
MB-DNA in PAAM film	0.60	0.398	0.654	2.12 (0.11)	28.0 (2.5)

^aSolutions and PAAM films contained 5 mM phosphate buffer. D and C calculated from equation (2) using $i_{T,\infty}$, slope, and intercept found experimentally. $a = 13.1 \mu\text{m}$ and $n = 2$. Values in parentheses are standard deviations based on triplicate measurements.

important in the application of this technique, *ie*, to use equation (2), that the *ume* has a known geometry that corresponds to the appropriate theoretical model. Information on the shape and validity of a tip can be obtained from SECM by the recording of the approach curves, i_T vs d (where d is the distance between the tip and substrate), and comparing these to theoretical curves valid for different geometries [22]. Small tip diameters are also needed in thin films, since the treatment based on transients requires that the tip be several tip diameters away from the substrate (either conductive or insulating) to avoid feedback effects. As shown above, however, with small tips, especially under conditions where diffusion coefficients are decreased well below solution values or accessible concentrations are small, the current levels will be small and signal averaging may be necessary. Moreover, small tips require very short times to allow measurements in the transient region. Thus, the following guidelines for making these measurements apply for measurements with a film of thickness δ :

To prevent SECM feedback effects: $\delta \gg 4-6a$.

For measurements in transient regime: $t \ll a^2/\pi D$.

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