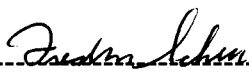
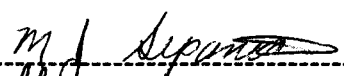
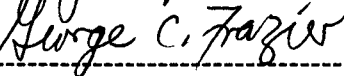


To the Graduate Council:

I am submitting herewith a dissertation written by Gregory J. Wagner entitled "A STUDY OF THE ELECTROCHEMICAL BEHAVIOR OF COVALENTLY MERCURATED URIDINE NUCLEOTIDES." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

  
-----  
J.Q. Chambers, Major Professor

We have read this dissertation  
and recommend its acceptance:

  
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Accepted for the Council:

  
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Associate Vice Chancellor and  
Dean of The Graduate School

**A STUDY OF THE ELECTROCHEMICAL BEHAVIOR OF  
COVALENTLY MERCURATED URIDINE NUCLEOTIDES**

**A Dissertation  
Presented for the  
Doctor of Philosophy  
Degree  
The University of Tennessee, Knoxville**

**Gregory J. Wagner  
December, 1996**

## DEDICATION

This thesis is dedicated to my parents

Mrs. Carolyn Sue Wagner

and

Mr. Thomas E. Wagner

## ACKNOWLEDGMENT

I would like to thank my wife, Myra, for all her help and encouragement throughout my graduate studies.

## ABSTRACT

The electrochemical behavior of the covalently mercurated uridine triphosphate (UTP-HgX), mercurated uridine diphosphate (UDP-HgX), and mercurated uridine monophosphate (UMP-HgX) have been characterized with a hanging mercury drop electrode in aqueous medium with linear and cyclic voltammetry. The voltammetry of these compounds was found to contain two components: diffusional and surface effects. The diffusional component, seen in the form of a large diffusional wave, resulted from the two-electron reduction of the diffusing mercurated nucleotides with the production of the parent compound and metallic mercury. The surface component resulted from the electroactive nucleotide surface layer which self assembles on mercury electrode at open circuit. The Langmuir adsorption isotherm was found to describe this adsorption event the majority of the time. Multiple one-electron surface waves were present during the surface voltammetry. A mechanistic interpretation was provided. The specific mechanism depended on the complexation state of the mercury substituent. Both intramolecular phosphate complexation and chloride complexation were observed. Unique to the mercurated UMP, intermolecular complexation through the nitrogen of the pyrimidine ring was observed to occur resulting in polymerization which dramatically effected the surface voltammetry. From the mechanistic interpretation of the surface voltammetry, Quick Basic programs were constructed to calculate theoretical voltammograms for each mechanism. Calculated and experimental were compared.

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## CHAPTER I

### Introduction

#### Electrochemistry of Organomercury compounds

Organomercury compounds, which were first reported in 1852 by Frankland (1), are among the oldest organometallics known. These compounds have the general formula,  $R-HgX$ , where  $R$  represents the organic component and  $X$  refers to a halide or another appropriate ligand usually with a formal charge of -1. Thus, organomercury compounds are often referred to as organomercuric salts. In water, organomercuric salts are ionized (equation 1.1) with the degree of ionization depending on the strength of the ligand  $X$ . For example, Waugh, Walton, and Laswick have shown that the order of ionization for methyl mercuric halides is  $Cl > Br > I$  (2). Other authors have also demonstrated this trend and reported semi-quantitative rate data (3).



Organomercury compounds are known to strongly adsorb on mercury surfaces. The electrochemical reduction of organomercury compounds on mercury has been the subject of numerous studies. Most of these studies were conducted with

classical voltammetric and polarographic techniques. One of the earliest studies was conducted on the electrochemical reduction of the phenylmercuric ion by Benesch and Benesch in 1951 (4). Hush and Oldham (3), have provided more extensive studies on various organomercuric halides as well as Laviron et al. (5,6). These studies, which were in general agreement, showed that in aqueous solutions the reduction of organomercuric compounds involves two steps. The first step consists of the reversible one-electron reduction of the organomercury compound yielding a strongly adsorbed radical (equation 1.2). The second step consists of the irreversible one-electron reduction of the radical yielding a carbanion (equation 1.3). The irreversibility of the second step is the result of rapid irreversible neutralization of the carbanion (equation 1.4).



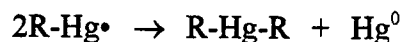
In Hush and Oldham's extensive polarographic study, these two one-electron steps were obtained with a variety of mercuric compounds and supporting electrolytes (3). Typical polarograms obtained contained two polarographic waves of equal height, hence, demonstrating the two one-electron steps. For the organomercuric halides (R-HgCl, R-HgBr, R-HgI), these authors demonstrated that the reduction characteristics (standard reduction potentials and diffusion coefficients)

were indistinguishable in 0.1 M tetraethylammonium iodide. In 0.1 M KCl, however, the results for both R-HgCl and R-HgBr were almost identical whereas those for R-HgI were quite distinct. These results demonstrated that the reduction characteristics of organomercury compounds are dependent on the identity of X. In tetraethylammonium iodide, in accord with the order of ionization previously established (Cl > Br > I), it was concluded that equations 1.5 and 1.6 have reached completion. However, in 0.1 M KCl, also in accord with the order of ionization previously established, equation 1.7 is either incomplete or lies wholly to the left.



Additional support for this conclusion was demonstrated by the progressive addition of iodide ion to a solution of R-HgCl in 0.1 M KCl. The results not only demonstrated a change in the  $E^0$  value, but a steady decrease in the diffusion coefficient, thus demonstrating the progressive replacement of R-HgCl as the diffusing species by the heavier R-HgI.

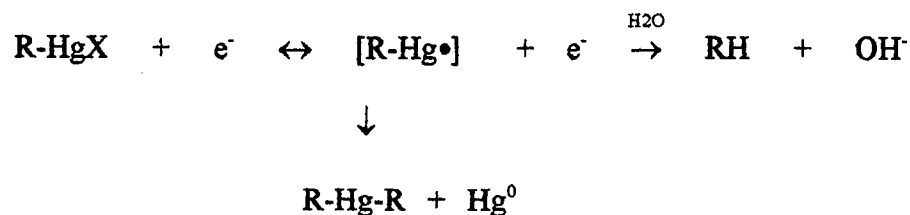
Although the polarographic results for the organomercury compounds featured two polarographic waves, studies concluded that the radical produced in the first step can also undergo disproportionation to form a non-reducible dimer (equation 1.8)(3,4,5,8). Evidence supporting this disproportionation was reported before these



1.8

polarographic studies were even conducted. For example, Kraus found in 1913 that methyl mercury radicals produced at a mercury cathode underwent dimerization to form dimethyl mercury (7). In Benesch and Benesch's first polarographic study of these compounds, dimerization with the production of diphenylmercury was also indicated (4). With the presence of the second polarographic wave, it can be concluded that the rate of disproportionation is slow compared to the rate of reduction of the radical in the second wave. In fact, several authors have studied the lifetime (stabilities) of the organomercury radicals. For example, Laviron (5), who applied cyclic voltammetry to 3-pyridylmercury chloride, reported  $10^{-2}$  to  $10^{-3}$  sec for the lifetime of the 3-pyridyl mercury radical. Reutov et al. provided a more extensive study and reported that the rate of disproportionation increased according to the following trend:  $\text{R} = \text{i-Pr} < \text{Et} < \text{n-Pr} < \text{n-C}_5\text{H}_{11} < \text{mesityl} < \text{2-naphtyl} < \text{Ph} < \text{CH}_2=\text{CH} < \text{PhCH}_2$  (8).

The electrochemical behavior of the organomercury compounds on mercury can now be summarized as shown in the scheme 1 below where the intermediate radical has two fates: disproportionation or reduction. It is interesting to note that, except for Laviron's work, there has not been extensive use of cyclic voltammetry in the study of these compounds. This is probably the result of the early period in which the majority of work on organomercury compounds was conducted.



**Scheme 1**

### Subject of Study

Mercury is recognized as a significant environmental pollutant with global emissions of mercury from both natural and anthropogenic sources on the order of thousands of tons per year. While the chemical fate of mercury in our environment is not well known, massive amounts of stored solid waste are being generated (greater than 109 Kg/yr in the USA alone) that contain mercury (9). It is also known that the residence time of mercury in these wastes is on the order of centuries. These wastes present a serious health hazard since organomercury compounds have been implicated as mutagenic and carcinogenic agents. For methyl mercury, the most toxic mercury compound known, teratogenic activity in man has been demonstrated (10). Thus, an understanding of the interaction of mercury with biologically important molecules is of great interest.

The interaction of mercury (II) with nucleotides and polynucleotides has been the topic of several investigations. One such interaction occurs through the formation of a coordination complex between the nucleotides and the mercuric ion. Although not fully understood, this binding appears to result from the interaction of

the mercuric ion with the amino groups and nitrogens of the purine and pyrimidine bases (11). Studies have documented the profound effect upon the composition and physical properties of nucleotides and polynucleotides after the formation of these coordination complexes (12).

Another interaction results in the formation of covalently mercurated nucleotides and polynucleotides. Covalent mercuration involves the formation of a covalent bond between the Hg atom and a carbon of the base, thus forming an organomercury compound. Dale et al. first demonstrated covalent mercuration of nucleotides by heating nucleotides at 37-50°C in buffered aqueous solutions (pH=5-8) containing the mercuric ion (13,14). They reported that cytidine (deoxycytidine) and uridine (deoxyuridine) nucleotides underwent covalent mercuration at the C-5 position of the base. Less than 5% of the thymine, adenine, and guanine containing nucleotides were mercurated under identical conditions. Polynucleotides, both single and double stranded, were mercurated under similar conditions. For example, 23s rRNA, bacteriophage R17 RNA, single stranded fd DNA, and native T<sub>7</sub> DNA were covalently mercurated. The percent total bases mercurated in these polynucleotides was shown to be less than 30% for the RNA polymers and less than 15% for the DNA polymers. The higher base mercuration for the RNA polymers was the result of the thymine bases in DNA being replaced by uridine in RNA.

Figure 1-1 shows the synthesis scheme for the uridine nucleotides where Y represents either the mono, di, or triphosphate arm.

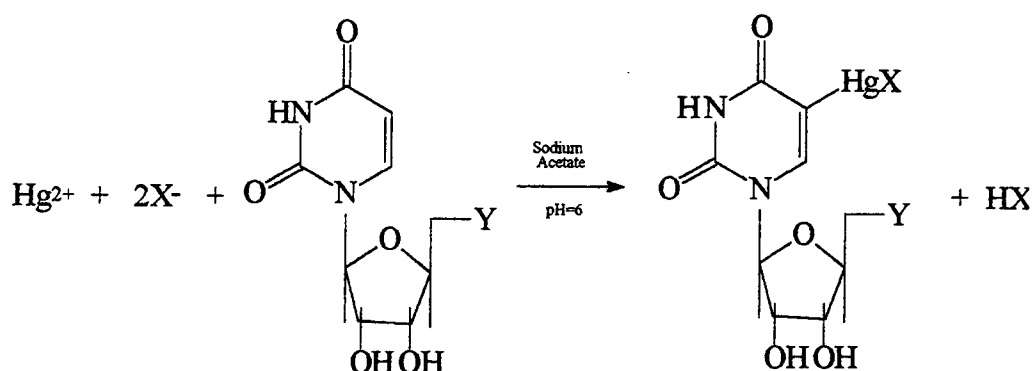


Figure 1-1: Preparation of the mercurated uridine nucleotides

Since their introduction, mercurated nucleotides, particularly the mercurated uridine nucleotides, have found many applications including the following: the purification of specific DNA sequences (15), preparation of halogenated derivatives (16), and as intermediates in the preparation of biotin-labelled nucleotides and polynucleotides (17). But no investigation into the electrochemical behavior of covalently mercurated nucleotides has been conducted to date. Hence, a study of the electrochemical behavior of covalently mercurated uridine nucleotides has been undertaken and presented in this thesis.

Since mercurated nucleotides are strongly adsorbed on mercury electrodes, the majority of the work will be conducted with a static mercury drop electrode. It will be shown that the mercurated nucleotides behave similarly to other organomercury compounds where a two-step reduction is operative during the electrochemical reduction. However, it will be demonstrated that the identity of ligand not only affects the reduction characteristics, but also plays a crucial role in the mechanism of the electrochemical reduction.

It will also be demonstrated that the voltammetry of the mercurated uridine nucleotides varies strongly with pH. Thus, a brief review of the effect of pH on protonation in the uridine nucleotides will be provided in the next section. In distinction from previous studies of organomercury compounds, the work presented here will draw on the theoretical understanding of surface voltammetry which has developed throughout the years. Hence, appropriately, a theoretical section on surface voltammetry has been provided to better present the material in this thesis.

#### Protonation/Deprotonation of the Mercurated Uridine Nucleotides

The uridine nucleotide illustrated in Figure 1-1 is one of the building blocks of ribonucleic acids (RNA). It consists of a base (uracil), a sugar (D-ribose), and a phosphate arm. Protonation and deprotonation of the three units of this nucleotide will be briefly reviewed in this section in order to present material in subsequent text in a more orderly fashion. It should be noted that this review is easily extended to other pyrimidine and purine nucleotides.

Figure 1-2 summarizes the effect of pH on the uridine nucleotides with the triphosphate species as an example. As shown, an active area of protonation and deprotonation occurs within the phosphate arm. For example, at pH values less than 2, the phosphate arm of the uridine triphosphate is either fully protonated or has a charge/phosphate ratio of 1/3 or 2/3. For the uridine diphosphate, the phosphate arm is either fully protonated in this region or has a charge/phosphate ratio 1/2. The uridine monophosphate is fully protonated in this region. The first of three main pKa values for the uridine nucleotides results from the removal of a proton from the above species. The result is a single negative charge per phosphate group and a charge/phosphate ratio of 1.0. The pKa value for this proton is in the vicinity of 1.6 for the three uridine nucleotides (mono, di, and triphosphate) (18). The second main pKa value for the uridine nucleotides results from the removal of the last proton on the phosphate arm. Figure 1-2 shows that this proton is located on the terminal phosphate group of the triphosphate species. The same is true for the diphosphate species. For the triphosphate species, the pKa value for this proton is 7.6 (19). The pKa value for the the diphosphate and monophosphate species is 7.2 and 6.6 (19).

Figure 1-2 shows that the third pKa for the uridine nucleotides results from protonation/deprotonation of the N-3 atom of the base (uracil). The pKa value for this proton has been demonstrated to vary only slightly between the triphosphate, diphosphate, and monophosphate. A value of 9.6 has been reported (18).

It should be noted that one additional deprotonation can occur with the uridine nucleotides. This occurs with the hydroxyls in the ribose component. As

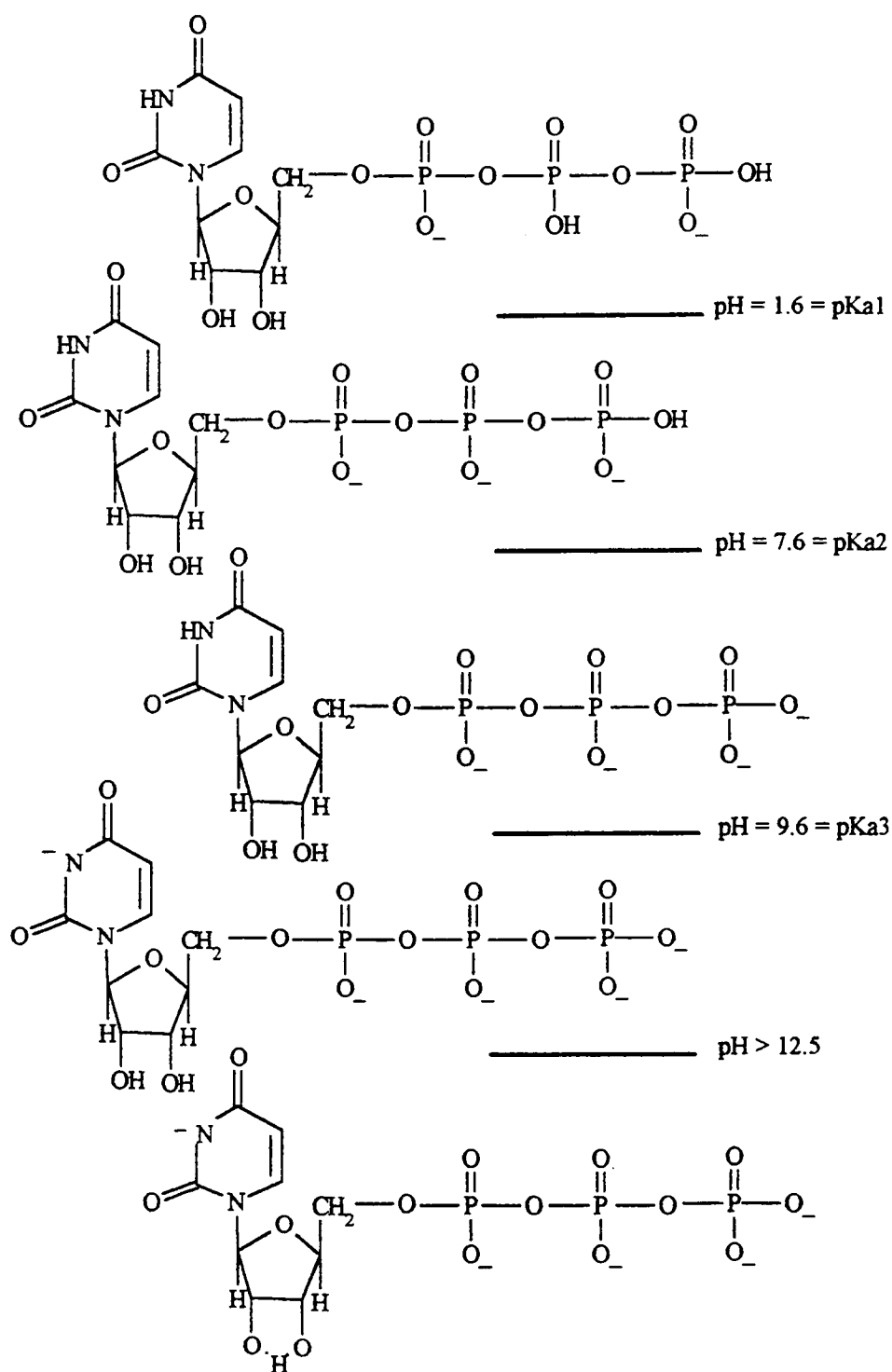


Figure 1-2: Protonation/deprotonation in the uridine triphosphate species

shown in Figure 1-2, removal of this proton occurs at pH values greater than 12.5 for the triphosphate species. This is also the case for the diphosphate and monophosphate species.

## CHAPTER II

### Voltammetry of Specifically Adsorbed Species

#### Linear Sweep Voltammetry

The current-potential relationship during the application of a potential sweep to an electrode with an surface confined species that can undergo electron transfer (equation 2.1) is given by equation 2.2, where  $n$  is the number of equivalent electrons per mole,  $F$  is Faraday's constant,  $A$  is the electrode area,  $v$  is the scan rate,  $\Gamma_T$  is the total surface concentration of the electroactive species,  $R$  is the gas constant,  $T$  is temperature, and  $\zeta$  is given by equation 2.3 where  $E$  is the applied potential and  $E_{pk}$  is the potential at maximum current (20). Equation 2.2 assumes fast electron transfer rates, a homogeneous electrode surface with no lateral interactions between adsorbed molecules, and no preceding or follow-up chemical reactions. It also assumes that the total surface coverage,  $\Gamma_T = \Gamma_{Ox} + \Gamma_R$ , is constant.



$$i = (n^2 F^2 v \Gamma_T / RT) [\zeta / (1 + \zeta)^2] \quad 2.2$$

$$\zeta = \exp[nF/RT(E - E_{pk})] \quad 2.3$$

The relationship between  $E_{pk}$  and the formal reduction potential ( $E^0$ ) is as follows. The Nernst equation describes the variation in oxidation state of a species with applied potential (equation 2.4). From equation 2.4, where  $C_{Ox}^{x=0}$  equals the concentration of the electroactive species in the oxidized form near the surface, and  $C_R^{x=0}$  equals the concentration of the electroactive species in the reduced form near the surface, one would expect  $E_{pk}$  to equal the formal reduction potential because at the point of maximum current the surface concentration of the reduced and oxidized species are equal. But as shown in equations 2.5 and 2.6, the activities of the oxidized ( $a_{Ox}^{ads}$ ) and the reduced species ( $a_R^{ads}$ ) are the product of the adsorption coefficients ( $b_{Ox}$  and  $b_R$ ) and the concentration of the species near the surface.

$$E = E^0 + (RT/nF) \ln (C_{Ox}^{x=0} / C_R^{x=0}) \quad 2.4$$

$$a_{Ox}^{ads} = b_{Ox} C_{Ox}^{x=0} \quad 2.5$$

$$a_R^{ads} = b_R C_R^{x=0} \quad 2.6$$

If equations 2.5 and 2.6 are substituted into equation 2.4, the Nernst equation becomes as that shown in equation 2.7. In addition, if it is assumed that the ratio of the activities is equivalent to the ratio of the surface concentrations, the Nernst equation takes the form of equation 2.8. Since the surface concentration of the oxidized and reduced form are equal at  $E_{pk}$ , equation 2.8 reduces to equation 2.9 at  $E_{pk}$ . Hence,  $E_{pk}$  is not equal to the formal reduction potential unless the adsorption coefficients for the oxidized and reduced species are equal.

$$E = E^0 + RT/nF \ln(b_R/b_{Ox}) + RT/nF \ln(a_{Ox}^{ads}/a_R^{ads}) \quad 2.7$$

$$E = E^0 + RT/nF \ln(b_R/b_{Ox}) + RT/nF \ln(\Gamma_{Ox}/\Gamma_R) \quad 2.8$$

$$E_{pk} = E^0 + RT/nF \ln(b_R/b_{Ox}) \quad 2.9$$

The current-potential relationship given in equation 2.2 can be expressed using the adimensional function  $\psi$  (equation 2.10) and plotted as a function of  $(E - E^0)$ . This is shown in Figure 2-1 with  $b_R/b_{Ox}$  values of 0.02, 1, and 50. Unlike diffusional behavior, these waves are symmetrical in shape and have peak widths at half the maximum current of 90.6 mV/n at 25°C, (In subsequent text in this chapter, all numerical values of diagnostic criteria will be given at 25°C). The results also illustrate that if R is more strongly adsorbed than Ox ( $b_R > b_{Ox}$ ), then there is a positive shift from  $E^0$ ; and when  $b_R < b_{Ox}$ , there is a negative shift from  $E^0$ , but there is no effect on the peak width or shape. In the former situation when  $E_{pk} > E^0$ , the voltammetric wave is termed an adsorption "pre-wave"; in the latter case, it is an adsorption "post-wave". From equation 2.10, it is also evident that  $i_{pk}$  varies linearly with the scan rate. This differs from diffusional behavior where the peak current is proportional to the square root of the scan rate.

$$\psi = i / (n^2 F^2 A v \Gamma_T / RT) = \zeta / (1 + \zeta)^2 \quad 2.10$$

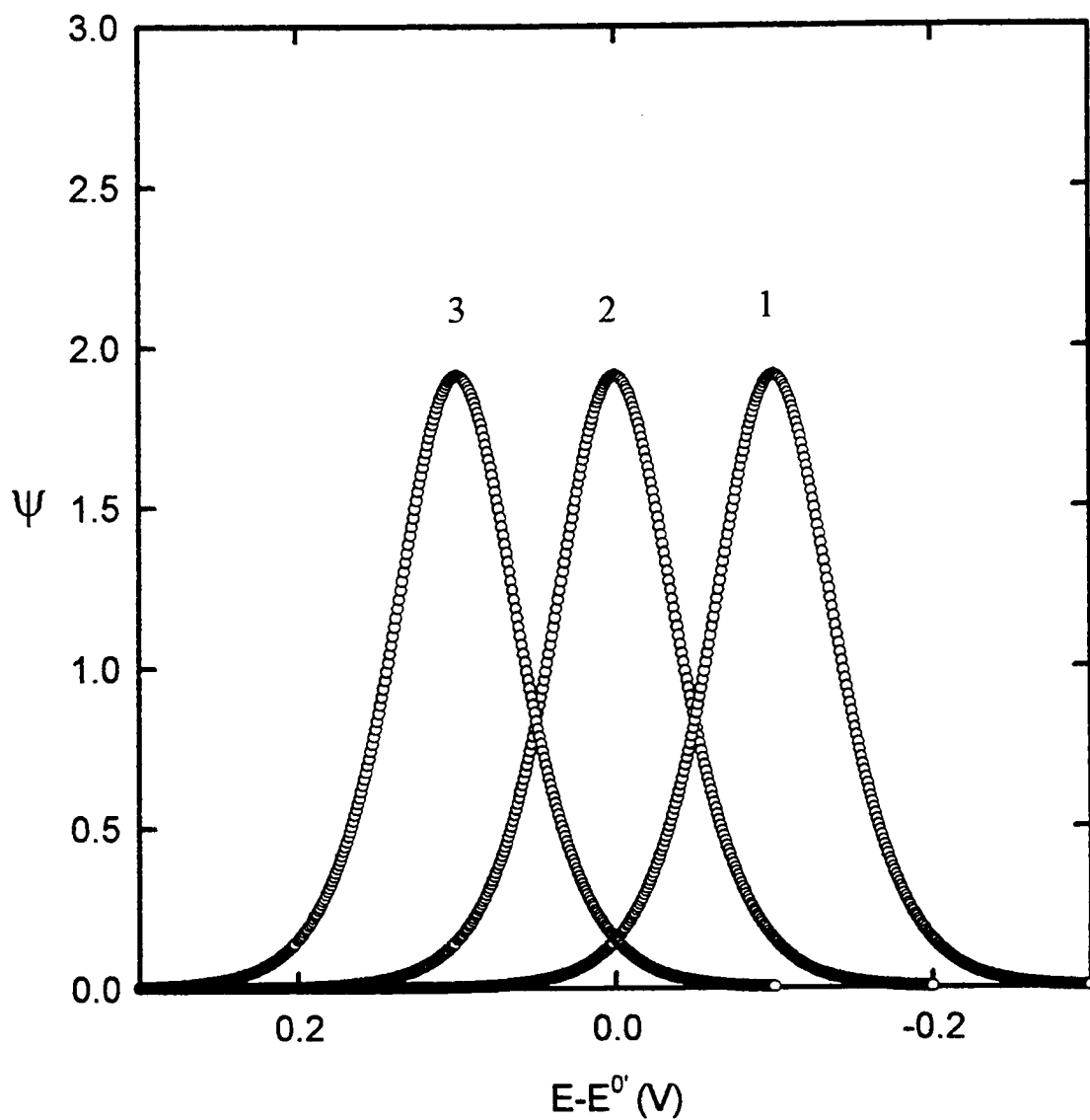


Figure 2-1: Ideal surface waves for a one-electron process at 25°C.

1:  $b_R/b_{Ox}=0.02$ ; 2:  $b_R/b_{Ox}=1$ ; 3:  $b_R/b_{Ox}=50$ .

The ideal behavior outlined above assumes fast electron transfer, no coupled chemical reactions, and no interactions between adsorbed molecules. If these assumptions do not hold, deviation from ideal behavior is observed. However, when the deviation is interpreted correctly, it can provide important information about the electrochemical process. For example, to take into account slow electron transfer rates, the general current potential characteristics using Butler-Volmer kinetics can be described by equation 2.11 where  $k_e$  is the rate constant for electron transfer,  $\Gamma_{ox}$  is the surface concentration of the electroactive species in the oxidized form,  $\Gamma_R$  is the surface concentration of the electroactive species in the reduced form, and  $\alpha$  is the transfer coefficient (21).

$$i = nFAk_e (\Gamma_{ox}\zeta^{-\alpha} - \Gamma_R\zeta^{1-\alpha}) \quad 2.11$$

Substitution into equation 2.10 provides a new equation for the function  $\psi$  which now includes a term for electron transfer rates (equation 2.12). Figure 2-2 demonstrates the effect of varying degrees of  $k_e$  expressed in terms of  $m$  where  $m = RTk_e/nFv$  (21). As shown, when  $m \rightarrow \infty$ , the symmetrical ideal wave is obtained. When  $m$  decreases, the shape of the wave changes and there is a shift towards more negative potentials for a cathodic wave or a shift towards more positive potentials for an anodic process. In addition, the peak width increases with decreasing  $m$ , thus, deviating from the ideal value of 90.6 mV/n.

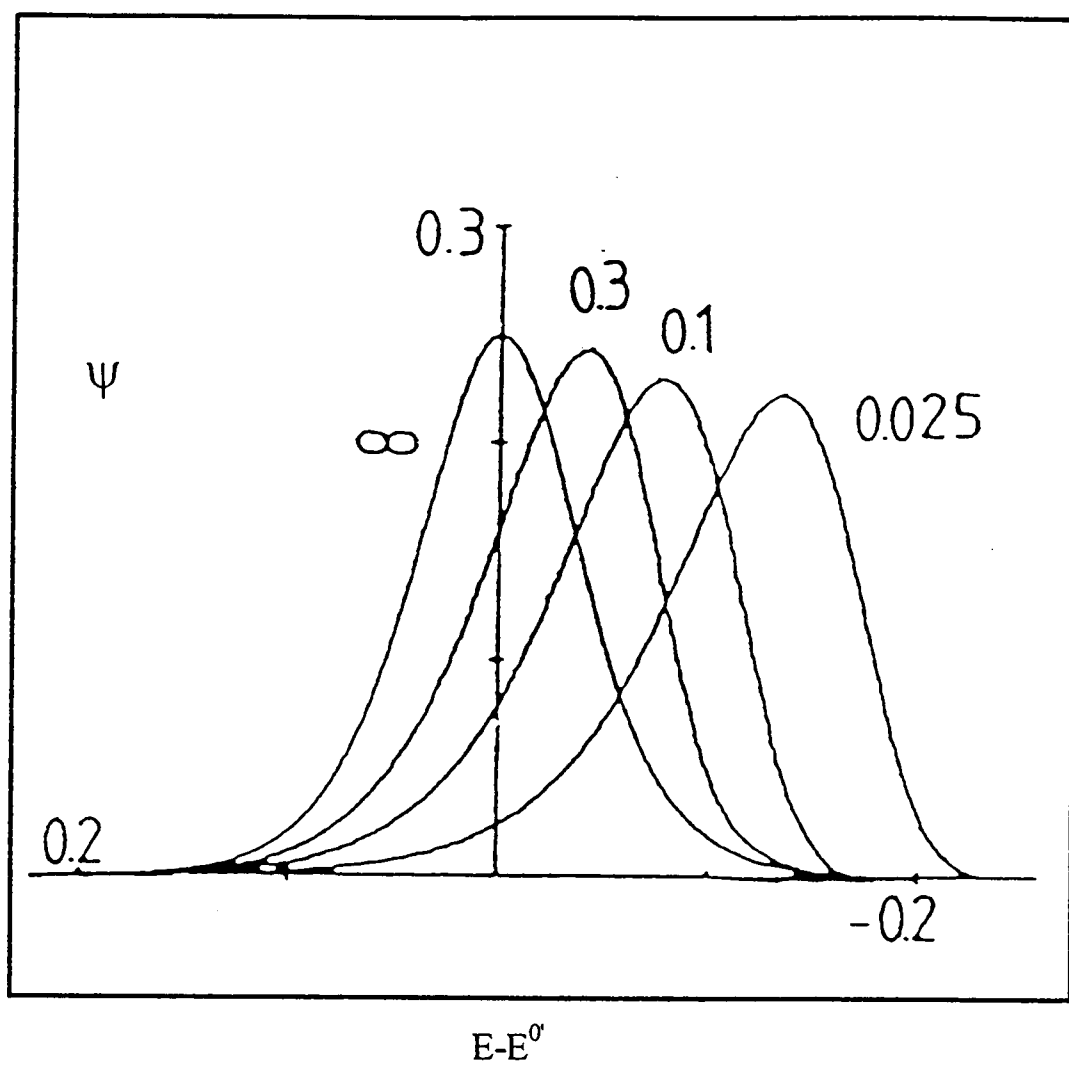


Figure 2-2: Effect of slow electron transfer on ideal surface waves.

Figure obtained from Laviron E., J. Electroanal. Chem. 100, 1979, 263.

$$\psi = [nFAk_e(\Gamma_{Ox}\zeta^{-\alpha} - \Gamma_R\zeta^{1-\alpha})] \div [nF^2Av\Gamma_T/RT] \quad 2.12$$

Deviation from ideal surface behavior can also be the result of follow-up chemical reactions. For example, Laviron (22) has provided an extensive theoretical study of adsorption peaks in linear sweep voltammetry for a reversible electrochemical reaction followed by an irreversible chemical reactions (EC process). Three EC processes considered by Laviron are illustrated in the scheme below.



Process I describes a dimerization process. Process II describes a second order coupling process and process III describes a first-order process. For all three cases, the reduction current from Laviron's theory can be described by equation 2.13.

$$I = (n^2F^2/RT) Av\Gamma_{Ox} \psi \quad 2.13$$

For the dimerization process (process 1),  $\psi$  is given by equation 2.14 where the value of  $b$  is inversely proportional to the rate constant  $k_c$  for the chemical step (equation 2.15) and  $\chi$  is equal to the ratio of the surface concentrations of the electroactive species in the reduced and oxidized form (equation 2.16) (23). From

these equations it is obvious that as the rate constant for the chemical step is increased,  $b$  decreases and the current increases. When  $b$  approaches infinity, the current approaches ideal behavior for a process consisting of only an electron transfer with no follow-up chemical step. This is illustrated in Figure 2-3 where  $\psi$  is plotted as a function of  $(E-E_{pk})$  with  $b$  values of  $\infty$ ,  $10^{-1}$ ,  $10^{-3}$ , and  $10^{-4}$ . Also evident in figure 2-3 is the change in peak width and peak potential with  $b$ . As  $b$  decreases (rate constant increases), the peak width decreases and compensates for the increase in current so that the total area under the waves is constant at any value of  $b$ . This demonstrates that the dimerization process has no effect of the amount of adsorbed species reduced. With a  $b$  value approaching infinity (no dimerization), the peak width at half the maximum current is 90.6 mV/n and with a  $b$  value approaching  $-\infty$ , the peak width is 47 mV/n. The peak potential shift towards more positive potentials with decreasing  $b$  can be expressed in terms of equation 2.17 where an additional term taking into account the chemical step has been added to equation 2.9. This presents yet another difficulty in reporting  $E^0$  values but can be diagnostic for the elucidation of the chemical step because the peak potential thus varies linearly with  $\log_{10}(v)$  with a slope of 29.6 mV.

$$\psi = b\chi[\ln(1+\chi) + b] \div [(1+\chi)\ln(1+\chi) - \chi + b(1+\chi)]^2 \quad 2.14$$

$$b = nF/RT (v/k_c\Gamma_{Ox}) \quad 2.15$$

$$\chi = 1/\zeta = \Gamma_R/\Gamma_{Ox} = 1/\exp[nF/RT (E-E_{pk})] \quad 2.16$$

$$E_{pk} = E^0 - RT/nF \ln(b_{Ox}/b_R) - RT/nF \ln(b) \quad 2.17$$

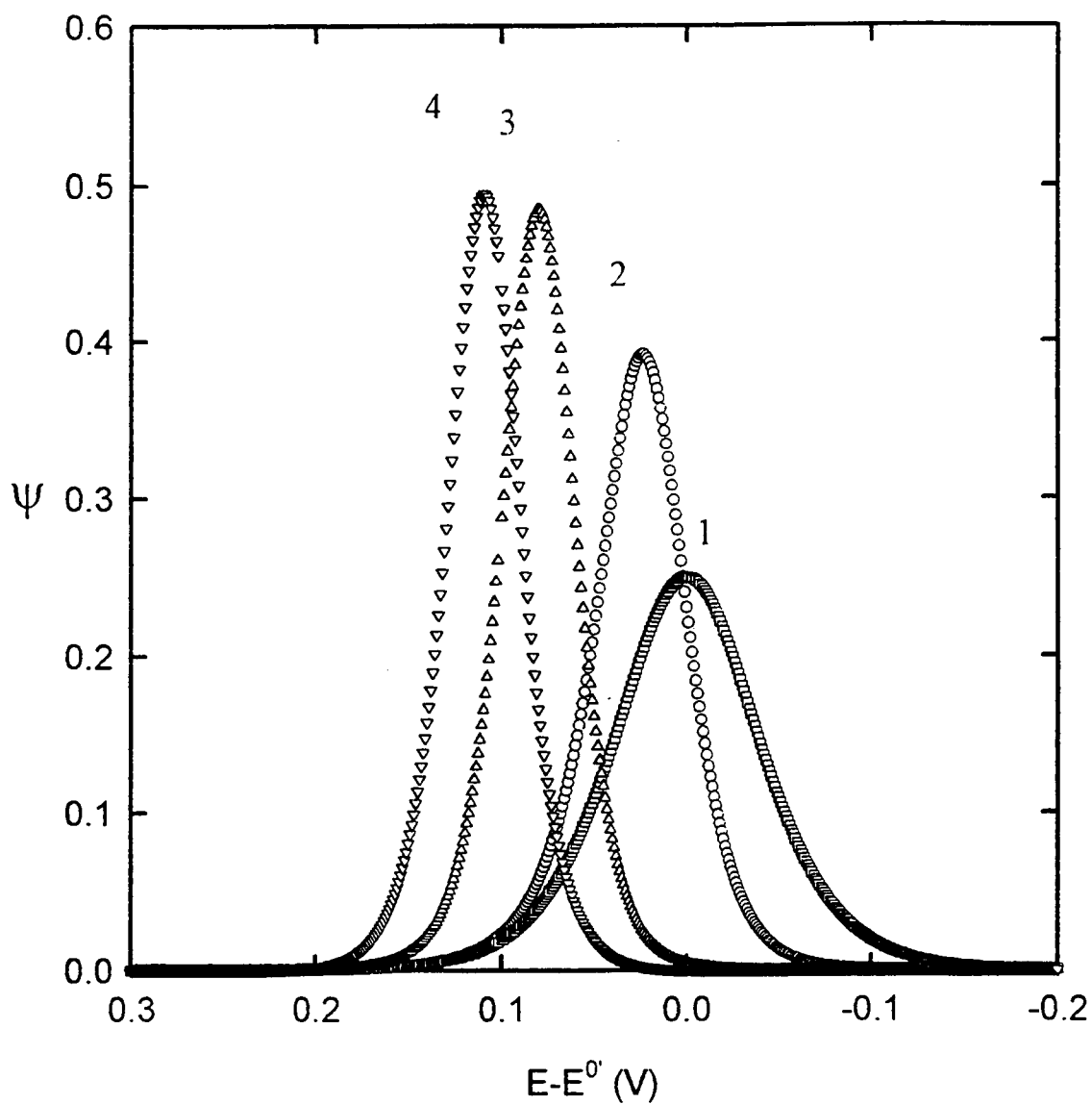


Figure 2-3: Theoretical surface waves with dimerization following electron transfer for a one-electron process at 25°C. 1:  $b=\infty$ ; 2:  $b=10^{-1}$ ; 3:  $b=10^{-3}$ ; 4:  $b=10^{-4}$ .

For the second chemical process,  $\psi$  is given by equation 2.18, where  $u$  equals  $1/b$  (equation 2.15) (24). The result, shown in Figure 2-4 with  $b$  values of  $\infty$ , 1,  $10^{-1}$ , and  $10^{-2}$ , is that as the rate constant of the chemical step increases,  $b$  and the current decrease, resulting in a decrease in the total area under the wave. The decrease in coulombic charge is a result of the chemical step removing the starting material Ox from the electrode surface. It is also interesting to note that the rate constant for this coupling process has no effect on the peak width. The peak width at half the maximum current remains constant at 90.6 mV/n. The variation of the peak potential with  $k_c$  for this EC process can also be described by equation 2.17 and varies linearly with  $\log_{10}(v)$  with a slope of 29.6 mV.

$$\psi = (1 + u)\chi \div [1 + (1 + 2u)\chi]^2 \quad 2.18$$

For the third chemical step (process III)  $\psi$  is given by equation 2.19 where  $b$  is now given by equation 2.20 (25). For this process, there is an increase in the peak current and a decrease in peak width with an increase in the rate of the chemical step. This behavior is illustrated in Figure 2-5 for  $b$  values of 0, 1, 10, and 100 where  $b$  now increases with increasing  $k_c$ . The behavior demonstrated in Figure 2-5 is similar to the behavior seen with process I, however, for this process the peak shape varies slightly, becoming more asymmetric with increasing  $b$ . For process 1, the peak shape remained symmetric with varying  $b$ . In addition, the peak width at half the maximum current varies from 90.6 mV/n for  $b=0$  (no chemical step) to 66

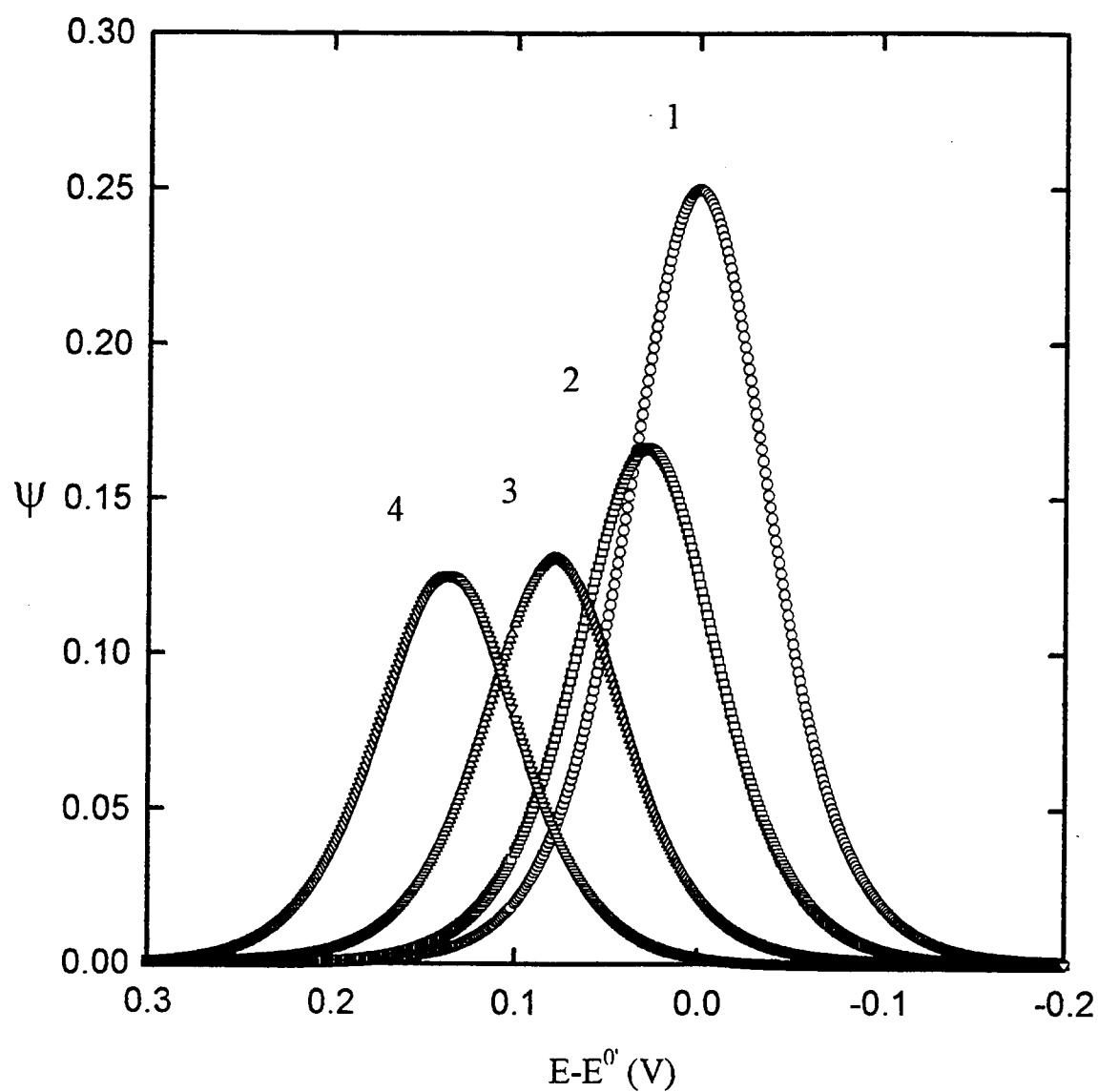


Figure 2-4: Theoretical surface waves with a coupling chemical reaction following electron transfer for a one-electron process at 25°C. 1:  $b = \infty$ ; 2:  $b = 1$ ; 3:  $b = 0.1$ ; 4:  $b = 0.01$ .

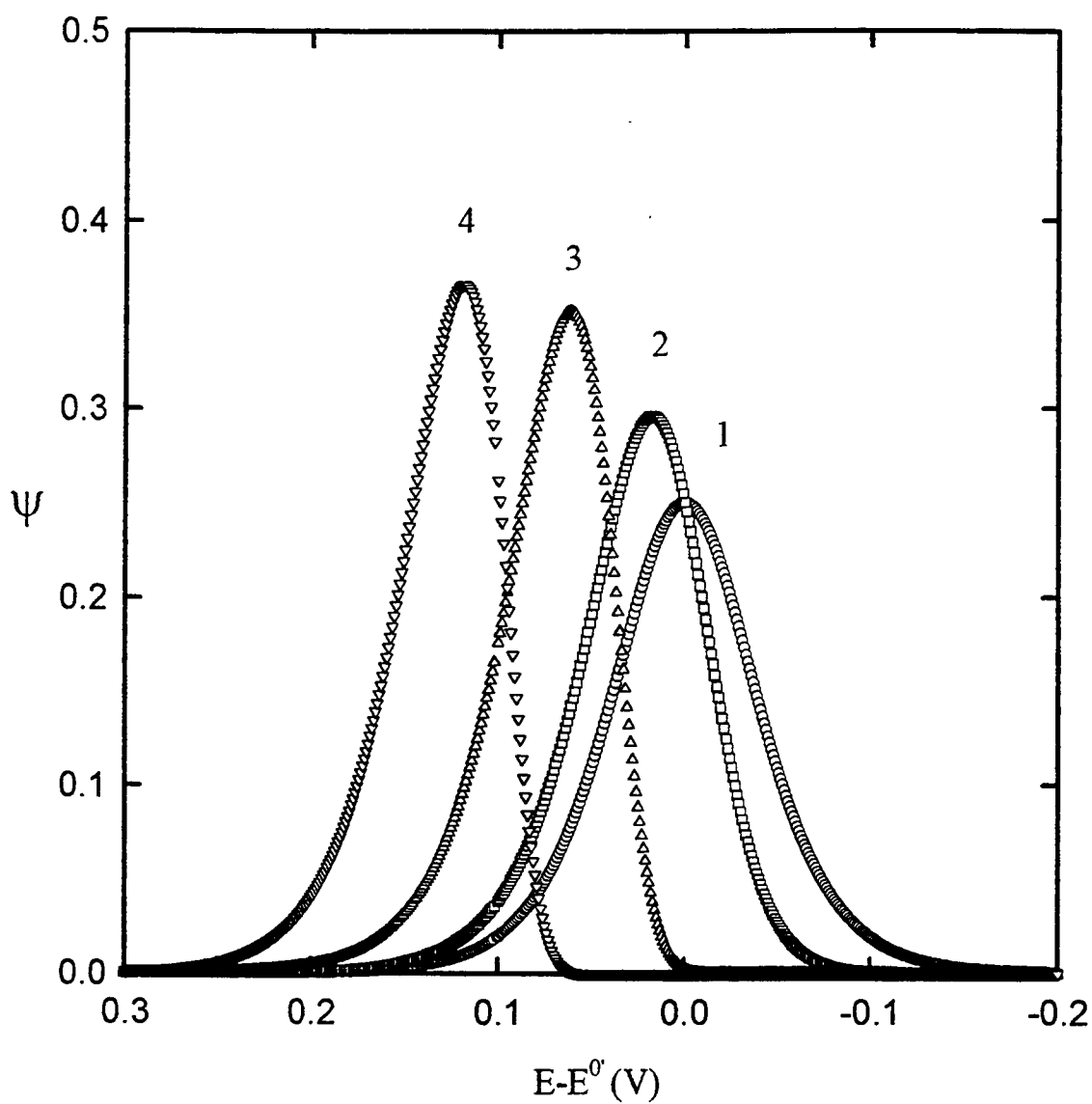


Figure 2-5: Theoretical surface waves with a first-order chemical reaction following electron transfer for a one-electron process at 25°C. 1:  $b=0$ ; 2:  $b=1$ ; 3:  $b=10$ ; 4:  $b=100$ .

mV/n with  $b$  approaching infinity. Figure 2-5 also illustrates that, like processes 1 and 2, the peak potential shifts towards more positive potentials with increasing  $k_c$ . The variation in peak potential for this process is described by equation 2-21. Like processes 1 and 2, the peak potential varies linearly with  $\log_{10}(v)$ , but now with a slope of  $59.2/n$  mV.

$$\psi = (1 + b)\chi \div (1 + \chi)^{2+b} \quad 2-19$$

$$b = RT/nF (k_c/v) \quad 2-20$$

$$E_{pk} = E^o - RT/nF \ln(b_{ox}/b_R) + RT/nF \ln(b) \quad 2-21$$

The general effect of interactions between adsorbed molecules will not be presented here, but a theoretical treatment taking into account interaction parameters can be found in the work presented by Brown and Anson (26). However, another process which could possibly be classified as "interactive" in nature and results in deviation from ideal surface behavior will be presented. This process results from a phase transition accompanying the electron transfer. The theory which best describes the behavior obtained with a phase transition is the nucleation-growth-collision (NGC) theory (27). This theory treats the growth of the electron transfer induced phase transitions at the electrode surface by assuming that the formation of nuclei is followed by growth around the nuclei until the growing areas collide. Typically this phenomenon is seen with metal electrodeposition, but the adsorption and desorption

of various organic substances have been found to conform to this phase transition/nucleation mechanism (28,29). Both two-dimensional and three-dimensional nucleation is possible but in the remaining text only two-dimensional will be discussed.

The effect of nucleation on voltammetric waves has been demonstrated by various authors (30,31). Typically, very narrow, sharp, tailless peaks are obtained. To the authors knowledge, a complete theoretical treatment of the voltammetric response obtained with nucleation has not been presented. Camacho and co-workers, however, have illustrated a theoretical treatment which is obeyed at low scan rates ( $<1000$  mV) (32). In their theory, the current-potential relationship with nucleation accompanying electron transfer is given by equation 2.22, where  $f$  equals  $RT/nF$ ,  $q$  is the overall charge exchanged, and  $t_n$  is given by equation 2.23, where  $k_n$  is the rate constant for nucleation and  $B$  is a constant. The constant  $B$  contains a term for the rate of an activation controlled electron transfer step related to  $k_e$  of equation 2.11.

$$i = (qt_n / f v) \zeta^4 e^{\pm (t_n \zeta / 5 v^2)} \quad 2.22$$

$$t_n = (2/3)\pi f^2 k_n B \quad 2.23$$

Figure 2-6 illustrates equation 2.22 plotted in the adimensional form ( $\psi = If/qt_n^{1/2}$ ) against  $(E-E^0)/f$  at  $t_n^{1/2} = 1000$  mV/s and  $v$  values of 1, 5, and 10 mV/s. These plots show a large increase in peak width at half the maximum current (40 mV - 100 mV) with scan rate. This is indicative of phase transition /nucleation

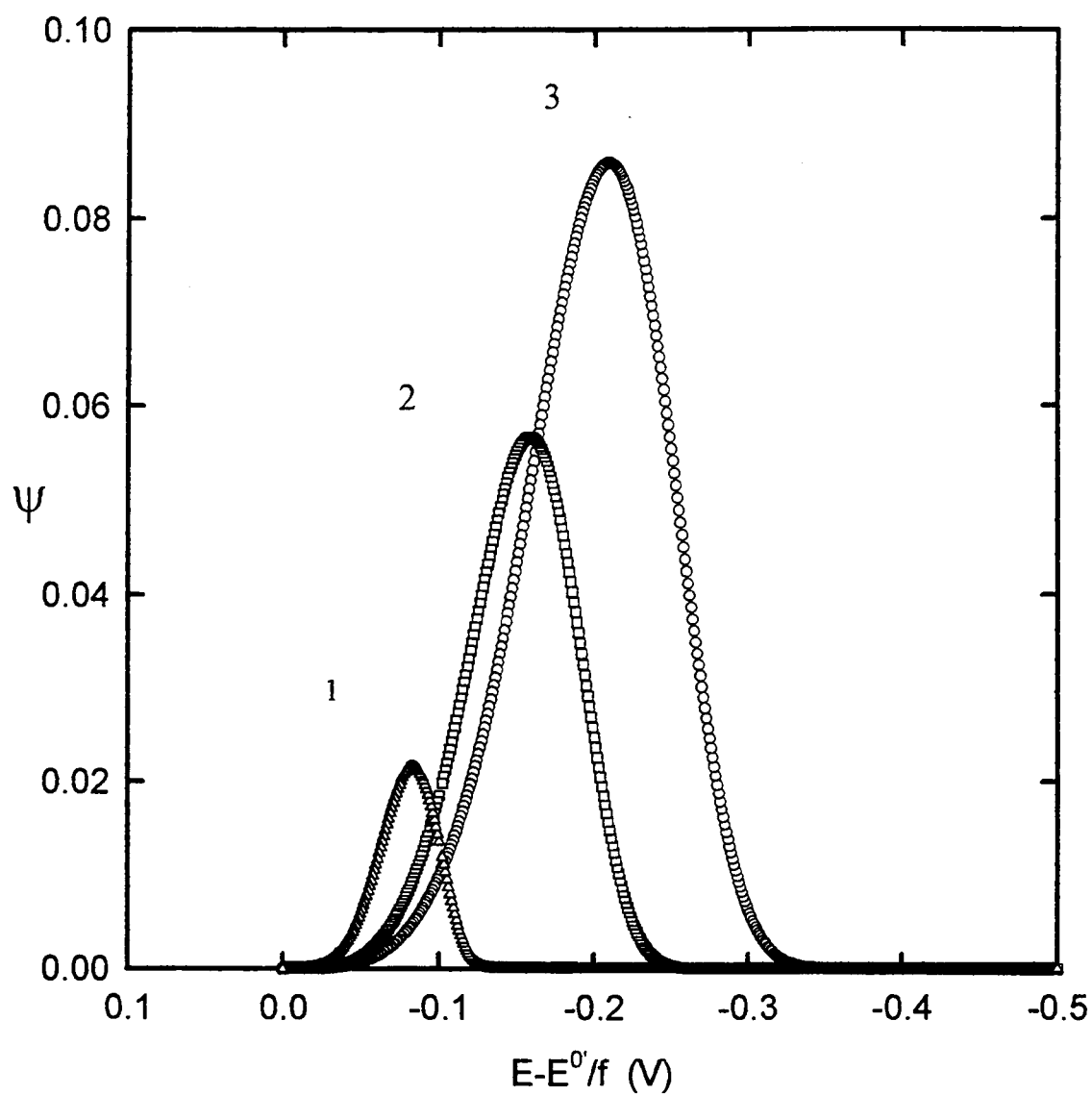


Figure 2-6: Theoretical surface waves with phase transition/nucleation accompanying electron transfer for a one-electron process at 25°C.

1:  $\nu=1$ ; 2:  $\nu=5$ ; 3:  $\nu=10$  mV/s.

accompanying electron transfer. In fact, according to Camacho et al., a log-log plot of the peak width vs. the scan rate must be linear with a slope of 0.4 (equation 2.24).

$$\Delta E_{pk}^{1/2} = 0.5263f(4/t_n)^{1/2}v^{2/5} \quad 2.24$$

### Cyclic Voltammetry

The theoretical treatments demonstrated here for ideal and non-ideal surface waves are readily extended to voltammetry in which the potential sweep is reversed after sweeping through the cathodic or anodic wave. The convention throughout this work will be that cathodic current is positive and anodic current is negative.

For the ideal behavior, equation 2.2 can again be used except that upon sweep reversal the current is give an opposite sign. Figure 2-7 illustrates the results using the adimensional function  $\psi$ . As shown, for ideal surface voltammetry, with all the assumptions listed previously, the cathodic and anodic surface waves are mirror images with the peak potentials ( $E_{pk}^{cath}$  and  $E_{pk}^{anod}$ ) being equal. Systems illustrating this voltammetric behavior are typically referred to as reversible.

By again using Butler-Volmer kinetics (equation 2.11) the effect of slow electron transfer on the voltammetric response can be demonstrated for cyclic voltammetry. Figure 2-8 illustrates the behavior at varying degrees of the rate constant, expressed in terms of  $m$ . As shown, when the rate constant for electron transfer decreases, the voltammetric waves become more drawn out and a peak

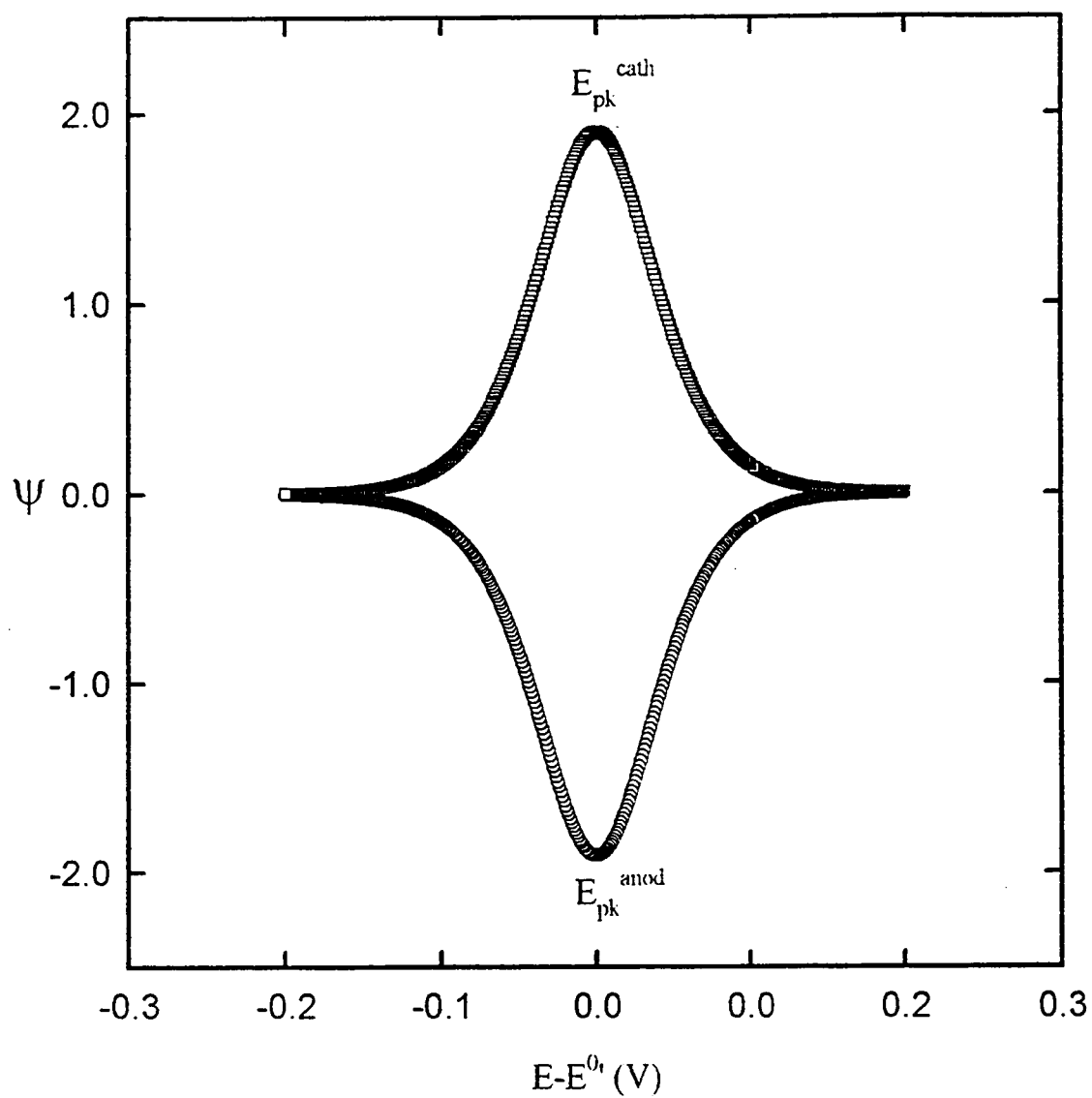


Figure 2-7: Ideal surface cyclic voltammogram for a one-electron process at 25°C and  $b_R/b_{OX}=1$ .

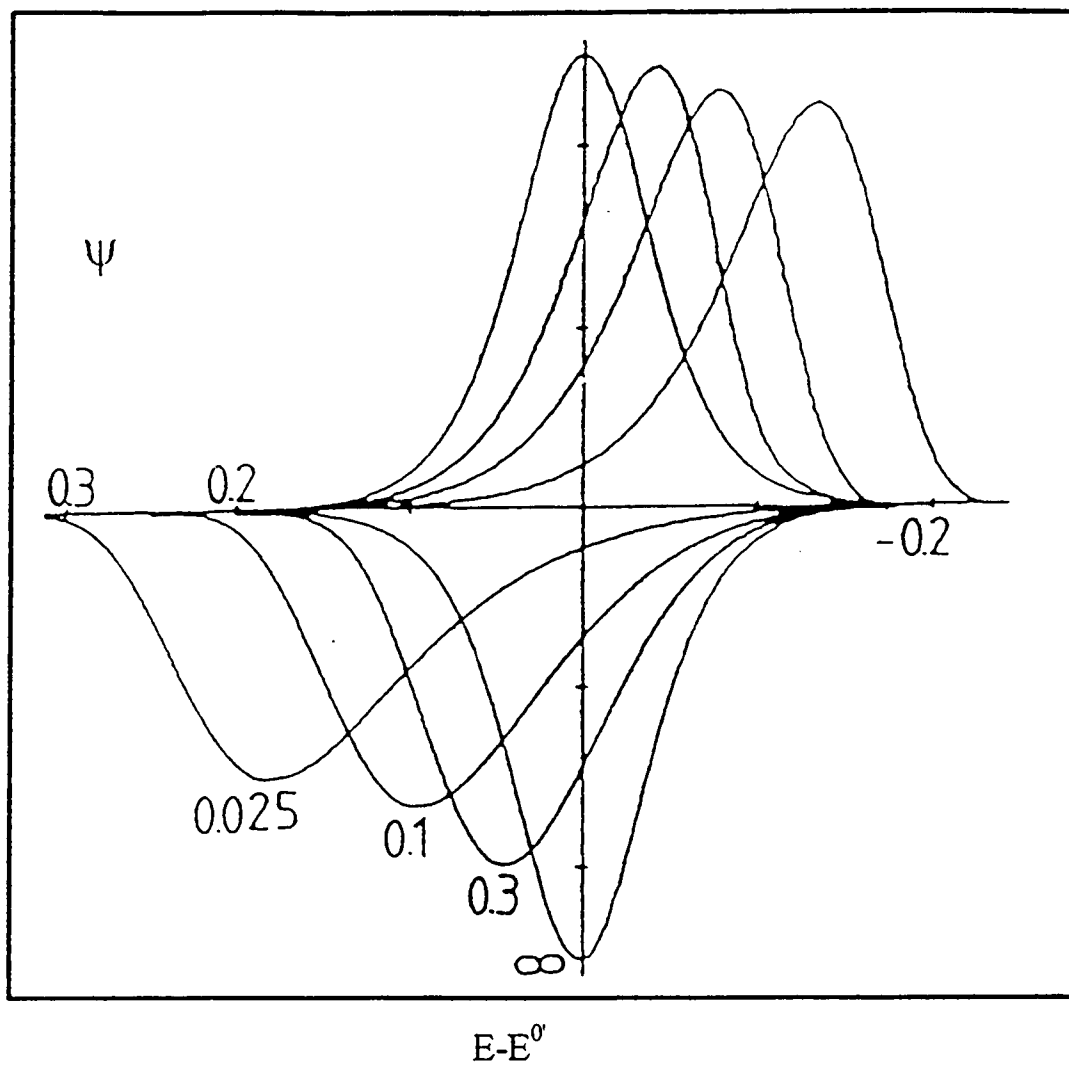
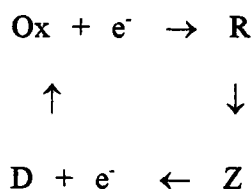


Figure 2-8: Effect of slow electron transfer during cyclic voltammetry. The rate constant is expressed in terms of  $m$  where  $m = RTk_0/nFv$ . Figure obtained from Laviron, E. J. *Electroanal. Chem.* **100**, 1979, 263.

separation between the cathodic and anodic waves develops ( $\Delta E_{pk}^{C,A}$ ). Cyclic voltammograms demonstrating this behavior are typically referred to as quasi-reversible.

Theoretical cyclic voltammograms for the three Laviron EC mechanisms, are not as easily demonstrated and depend on the particular system. For example, if Z is not oxidizable, then no anodic wave will be seen on the reverse sweep. Systems illustrating this behavior are called irreversible. For process III, one example will be illustrated which is seen throughout the literature. This example results from what is called a square scheme mechanism and is illustrated in the scheme below. In this mechanism, a fast follow-up reaction follows



the reduction of Ox to R, hence producing species Z. Species Z is then oxidized to a new species D during the anodic sweep which is then followed by another chemical reaction which produces the original compound Ox. For process III, where the follow-up reactions are first order, the theoretical cyclic voltammetric response can again be obtained by plotting an adimensional function of the current (equations 2.13 and 2.19). The response is illustrated in Figure 2-9. Typical of square scheme

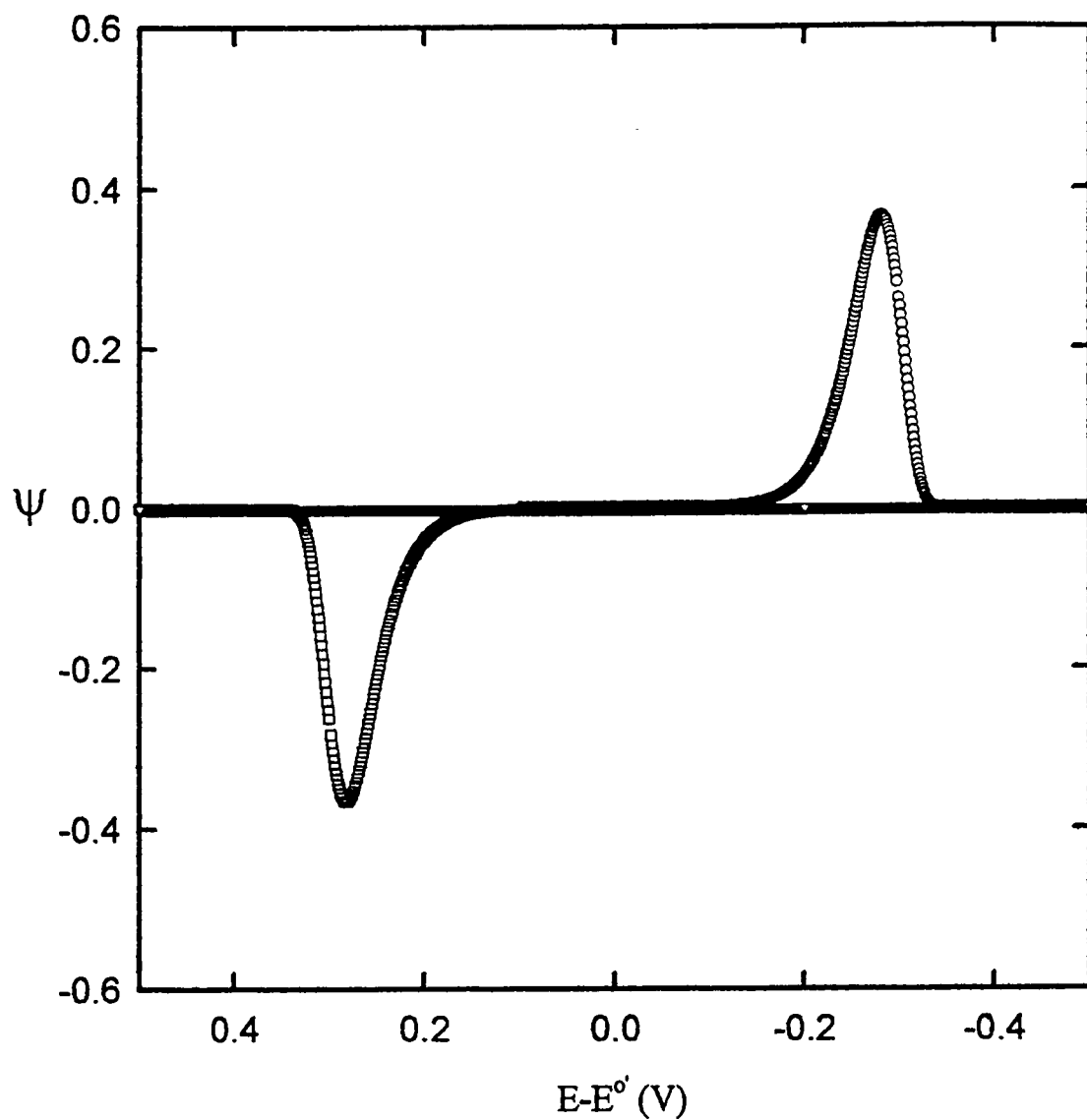


Figure 2-9: Theoretical cyclic voltammogram for a first-order square scheme mechanism.  $n=1$ ;  $b=100$ ;  $dE_{pk}^{c,A}=0.56 \text{ V}$ .

behavior, this voltammogram shows a large peak separation between the cathodic and anodic waves ( $\Delta E_{pk}^{C,A}$ ). In contrast to the results of slow electron transfer, however, the large peak separation does not accompany broadening of the voltammetric waves. In fact, for this process, there are two  $E^0$  values; one for the Ox/R couple and one for the D/Z couple.

The effect of phase transition/nucleation during cyclic voltammetry is also readily demonstrated. Figure 2-10 illustrates a cyclic voltammogram obtained by plotting the adimensional form of equation 2.22 at  $t_n^{1/2} = 1000$  mV/s and a scan rate of 5 mV/s. Similar to the square scheme behavior, this voltammogram also displays a peak separation between the cathodic and anodic wave. This is typical of nucleation and is often referred to as hysteresis. A difference from square scheme behavior, however, is that this process is characterized by one  $E^0$  value which lies between the cathodic and anodic waves.

It should be noted that the above theoretical treatments are not an exhaustive treatment of deviation from ideal surface behavior. They have been provided, however, because they will be used in the analysis of the voltammetry of the title compounds of this thesis.

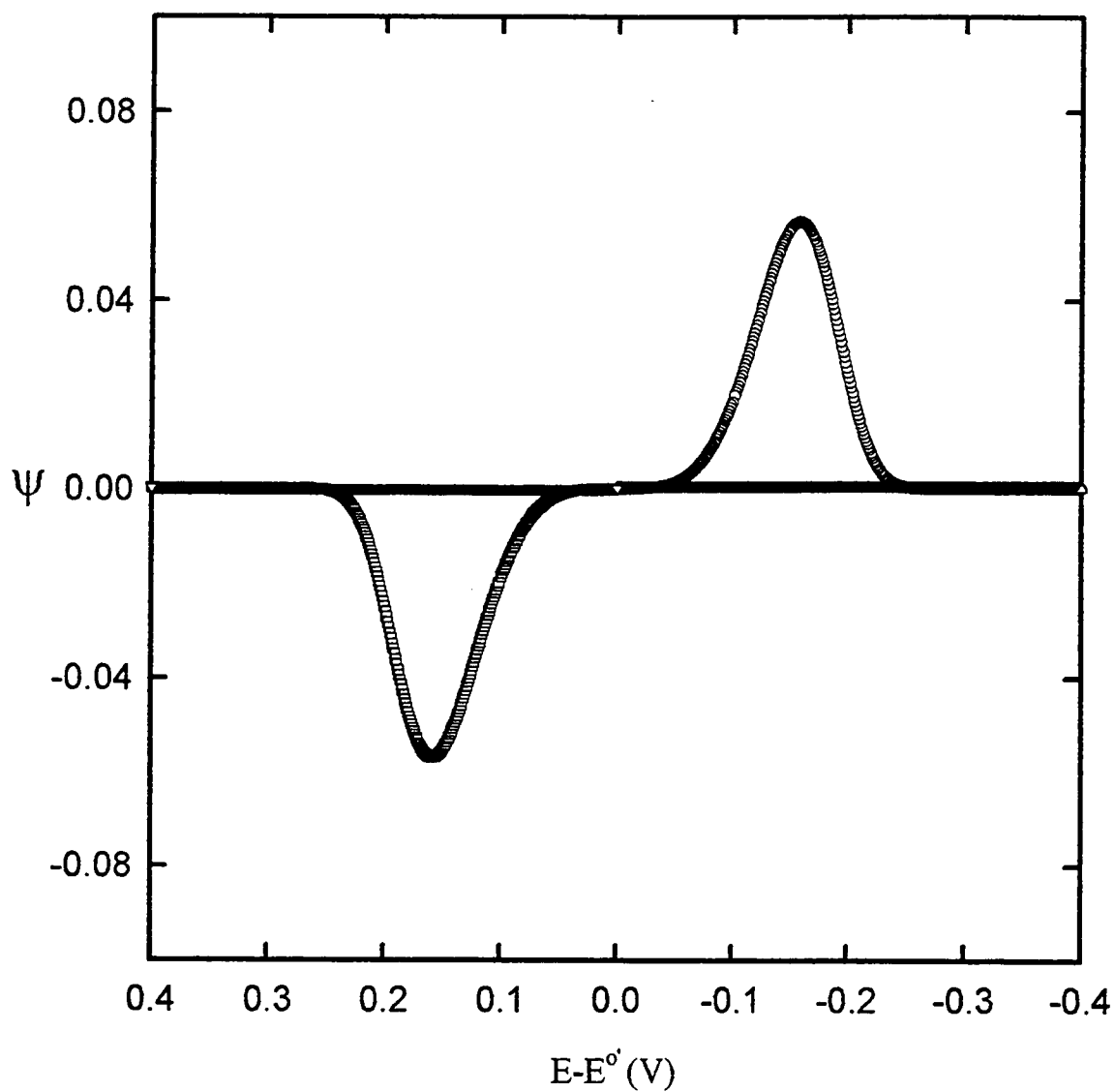


Figure 2-10: Theoretical cyclic voltammogram with phase transition nucleation accompanying electron transfer.  $n=1$ ;  $t_n^{1/2}=1$  V/s;  $v=5$  mV/s.

## CHAPTER III

### Experimental

#### Chemicals

Electrolyte solutions and buffer solution were prepared with reagent grade materials purchased from Sigma or Aldrich. All water was distilled and passed through a Millipore purification system. The uridine monophosphate (UMP), uridine diphosphate (UDP), and uridine triphosphate (UTP) were purchased from Sigma and the compressed nitrogen was obtained from National Welders Supply Co.

#### Preparation of the mercurated nucleotides

The 5-mercurated derivatives of UMP, UDP, and UTP were prepared by the method of Langer et al. (17). This method consisted of the addition of 368 mg of the monophosphate, 404 mg of the diphosphate, or 484 mg of the uridine triphosphate (1.00 mmole) to 100 mls of 0.1 M sodium acetate, pH=6.0. Then 1.59 g (5.00 mmoles) of mercuric acetate was added and the solution heated for 4 hrs. After cooling to room temperature, 392 mg (9.25 mmoles) of lithium chloride was added and the excess  $\text{HgCl}_2$  was extracted with five equal volumes of ethyl acetate. The progress of the extraction process was monitored by evaporating each ethyl acetate extraction, dissolving the resulting residue in 0.1 M KCl, and determining

the amount of  $\text{Hg}^{2+}$  present with linear sweep voltammetry. The voltammetry for  $\text{Hg}^{2+}$  determination was conducted with a standard three-electrode cell with a glassy carbon working electrode, a Pt counter and a SCE reference electrode. The glassy carbon electrode was scanned from 400 to -1000 mV vs. SCE; the amount of  $\text{Hg}^{2+}$  present was related to the coulombic charge under the voltammetric wave assigned to the oxidation of  $\text{Hg}^0$  which was deposited on the cathodic sweep. By the fifth extraction, no voltammetric wave assignable to the  $\text{Hg}^{2+}$  reduction or  $\text{Hg}^0$  oxidation was seen. The mercurated nucleotide in the aqueous layer was then precipitated with the addition of three volumes of cold ethanol and collected by centrifugation. The nucleotide was washed with ethanol, followed by acetone, and then dried under vacuum.

The down-field proton NMR spectra of the uridine monophosphate, diphosphate, triphosphate, and the mercurated derivatives in  $\text{D}_2\text{O}$  are illustrated in Figures 3-1, 3-2, and 3-3 (an equivalent amount of mercaptoethanol was added to the mercurated derivatives so that the species in solution were  $\text{UTP-HgSEtOH}$ ,  $\text{UDP-HgSEtOH}$ , and  $\text{UMP-HgSEtOH}$ ). The parent compounds show three diagnostic chemical shifts assigned to the H-5 and the H-6 of the pyrimidine ring and the  $\text{H-C}_1$  of the sugar. After mercuration, the H-6 doublet has collapsed to a singlet and the H-5 chemical shift has disappeared verifying that mercuration occurs quantitatively at the C-5 position of the pyrimidine ring in agreement with previous literature (13). Chemical shifts are given in Table 1. Elemental analysis (Atlantic Labs and Galbraith Labs) gave: %C, 16.94%; %H, 3.06%; %N, 4.35%; %Hg, 32.57%; in agreement with theoretical %C, 17.32%; %H, 2.60%; %N, 4.49%;

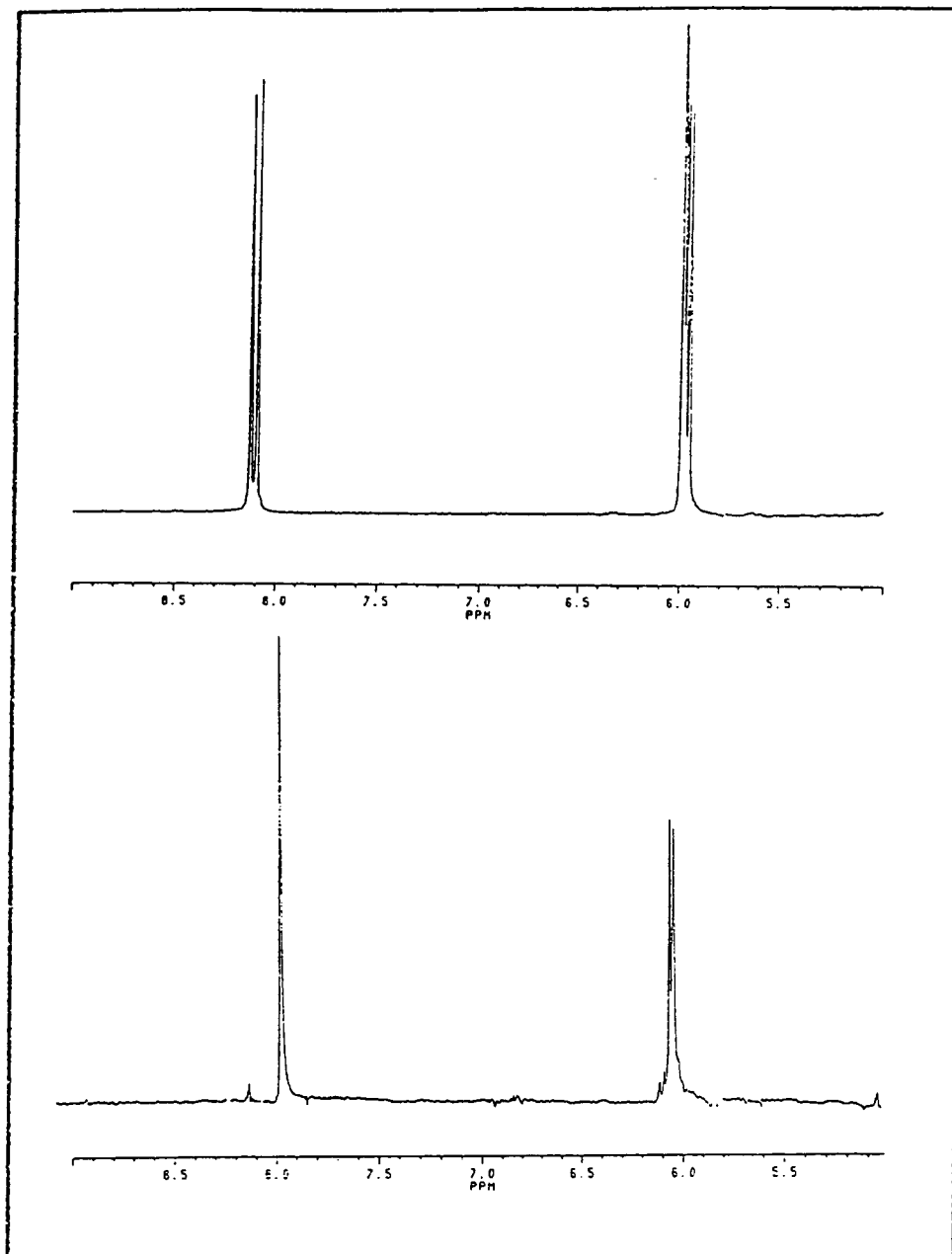


Figure 3-1: Down-field proton NMR spectra of the mercurated uridine monophosphate (top) and mercurated derivative (bottom).

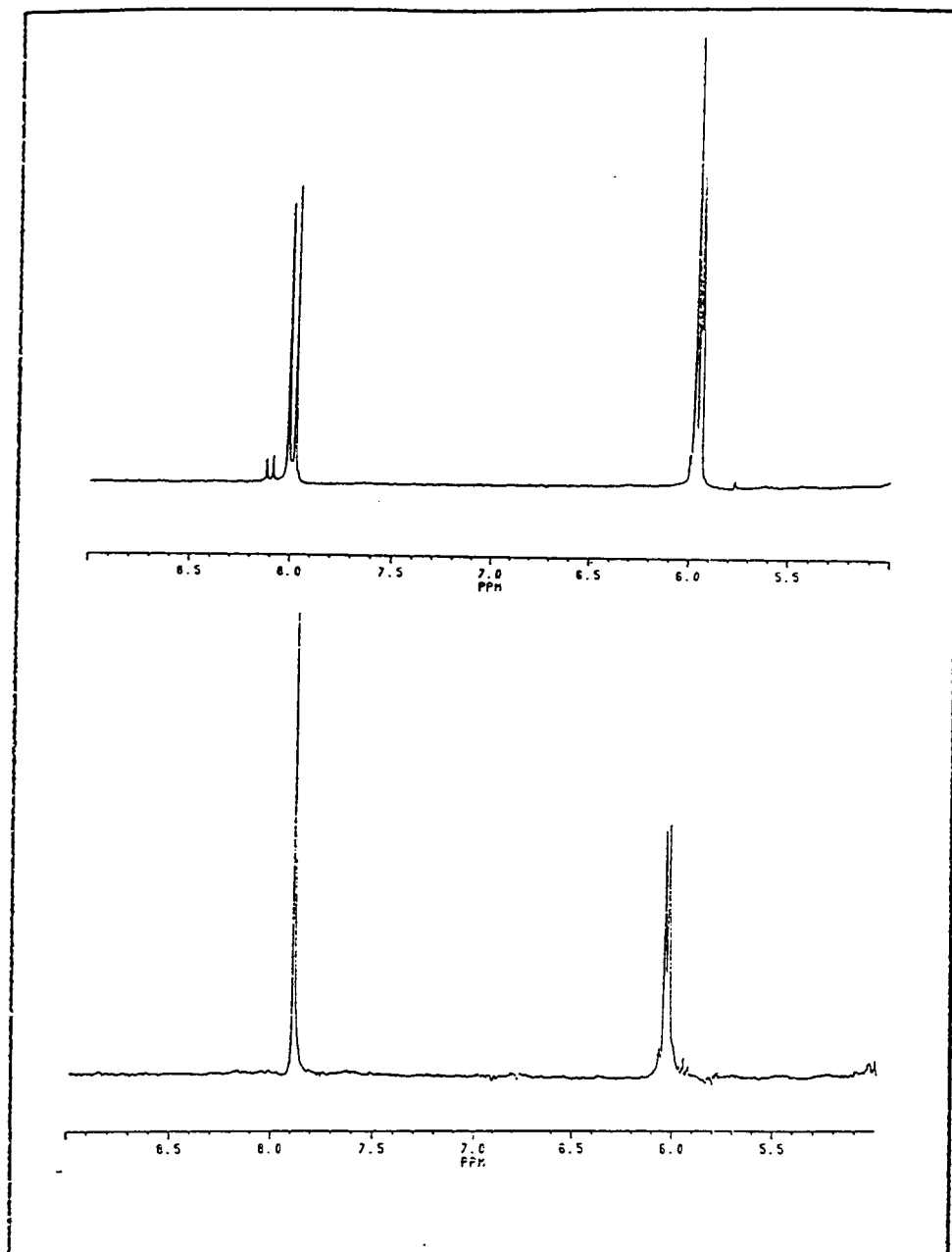


Figure 3-2: Down-field proton NMR spectra of the mercurated uridine diphosphate (top) and mercurated derivative (bottom).

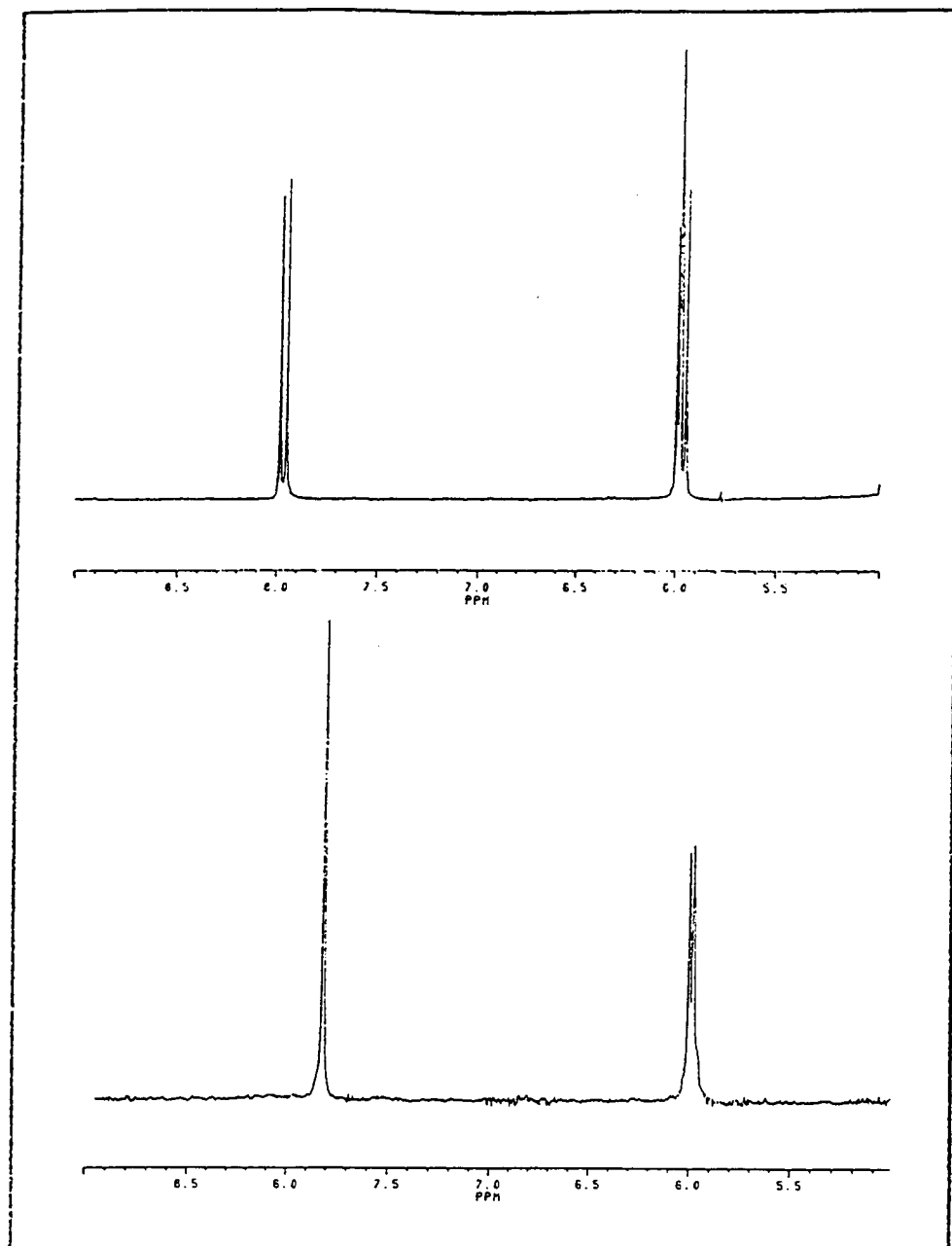


Figure 3-3: Down-field proton NMR spectra of the mercurated uridine triphosphate (top) and mercurated derivative (bottom).

Table 1: Down-field proton NMR shifts for the uridine nucleotides and mercurated derivatives. d = doublet; s = singlet; H-6 = Proton 6 of the uracil; H-5 = Proton 5 of the uracil; H-C1 = Proton on C1 of sugar.

| species | H-6    | H-5    | H-C1   |
|---------|--------|--------|--------|
| UTP     | d-7.95 | d-5.98 | d-5.94 |
| UDP     | d-8.00 | d-5.98 | d-5.95 |
| UMP     | d-8.10 | d-5.98 | d-5.94 |
| UTP-HgX | s-7.91 | -      | d-6.00 |
| UDP-HgX | s-7.89 | -      | d-6.00 |
| UMP-HgX | s-7.98 | -      | d-6.05 |

%Hg, 32.14% for the monophosphate ( $C_9H_{10}N_2O_9PLi_2HgCl \cdot 3H_2O$ ). Several experiments were also performed with a small quantity (5 mg) of the 5-mercurated uridine triphosphate that was purchased from Sigma. Voltammetric results with this material were identical to those obtained with the synthesized compound.

The prepared nucleotides were further evaluated by potentiometric (pH) titrations with standardized HCl solutions. In this manner, the effect of the mercury substituent on the acid base properties of the phosphate arm was examined. For a standard, the UMP purchased from Sigma was titrated with 0.104 M HCl. The titration consisted of first dissolving 0.316 grams of the UMP in 30 mls of  $H_2O$ . The initial pH of this solution was 8.2. An NaOH solution was then added to bring the pH up to 9.5. In this manner, the first break in the titration curve could be measured. Figure 3-4 illustrates this titration. Consistent with literature (18), this titration curve shows a  $pK_a$  in the vicinity of  $pH = 6.8$ . This  $pK_a$  corresponds to the

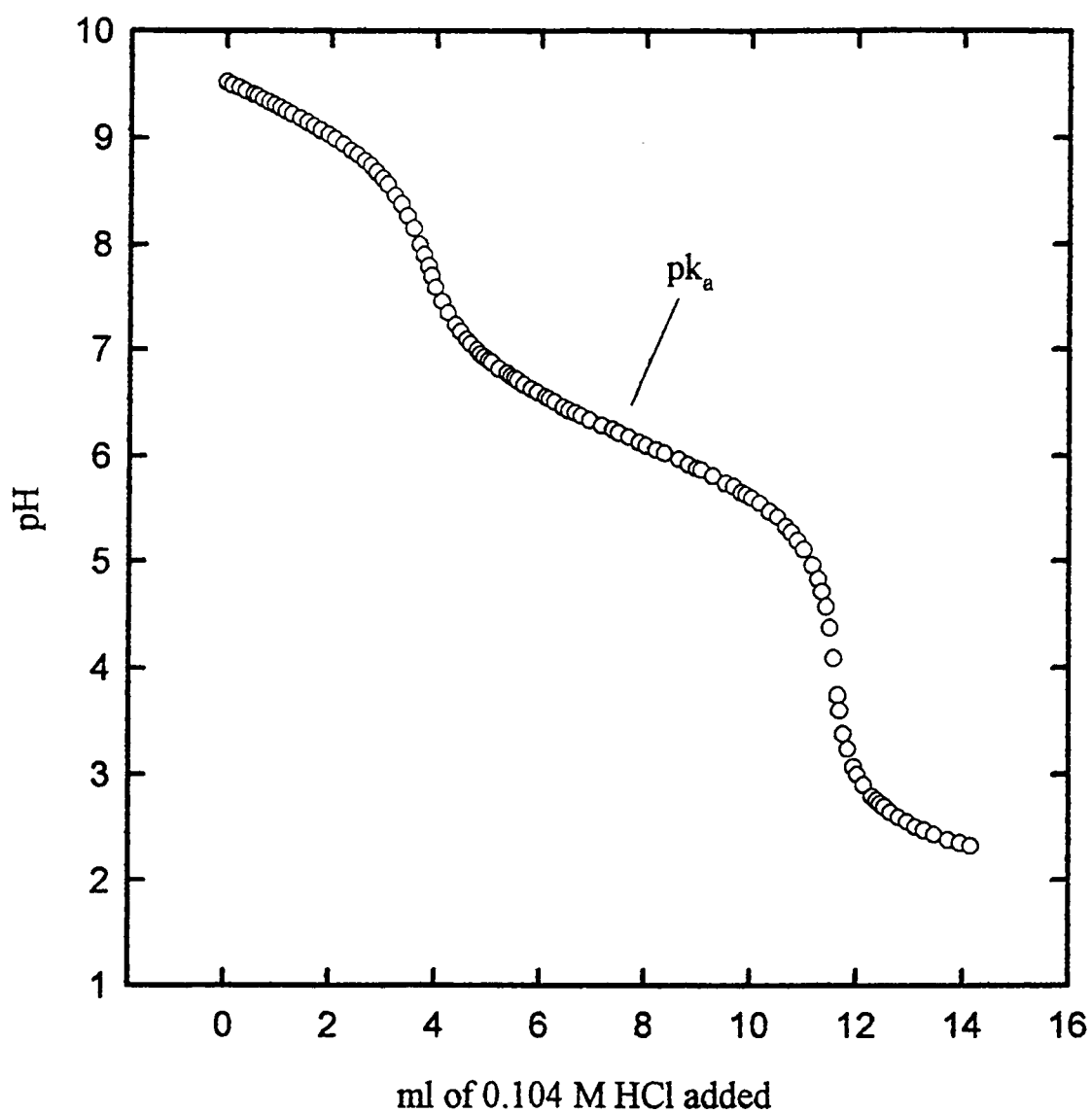


Figure 3-4: Titration of 0.316 grams of UMP with 0.104 M HCl.

addition of the first proton to the phosphate arm.

From the data in Figure 3-4 it was determined that  $8.01 \times 10^{-4}$  equivalent protons were required to titrate the  $8.59 \times 10^{-4}$  moles of UMP. Hence an error of 7.0 % which is probably the result of impurities. The prepared mercurated UTP was also titrated. This titration required  $6.98 \times 10^{-5}$  equivalent protons to titrate  $6.07 \times 10^{-5}$  moles of UTP-HgX; an error of 14.0 %. This higher error may again be the result of impurities introduced during the synthesis.

Figure 3-5 illustrates the titration curve obtained while titrating 0.0223 grams of the synthesized UMP-HgX with  $9.61 \times 10^{-3}$  M HCl. From the data it was determined that  $7.21 \times 10^{-5}$  equivalent protons were required to titrate the  $3.57 \times 10^{-5}$  moles of UMP-HgX. This large excess of HCl is a full equivalent in excess and was also obtained after extensive purification of the prepared UMP-HgX. An explanation of this result will be presented in chapter VII where it gives support for the proposed voltammetry scheme of the mercurated UMP.

### Instrumentation

Most voltammetric work was carried out using a model CS-2RA Cypress Systems potentiostat and a three-electrode cell with a static mercury drop electrode, a Pt counter, and a Corning SCE reference electrode. All potentials are given with respect to the saturated calomel electrode. The static mercury drop electrode consisted of a glass capillary/reservoir arrangement with a screw/piston to push the mercury through the capillary to form the drop (Figure 3-6). To ensure that the

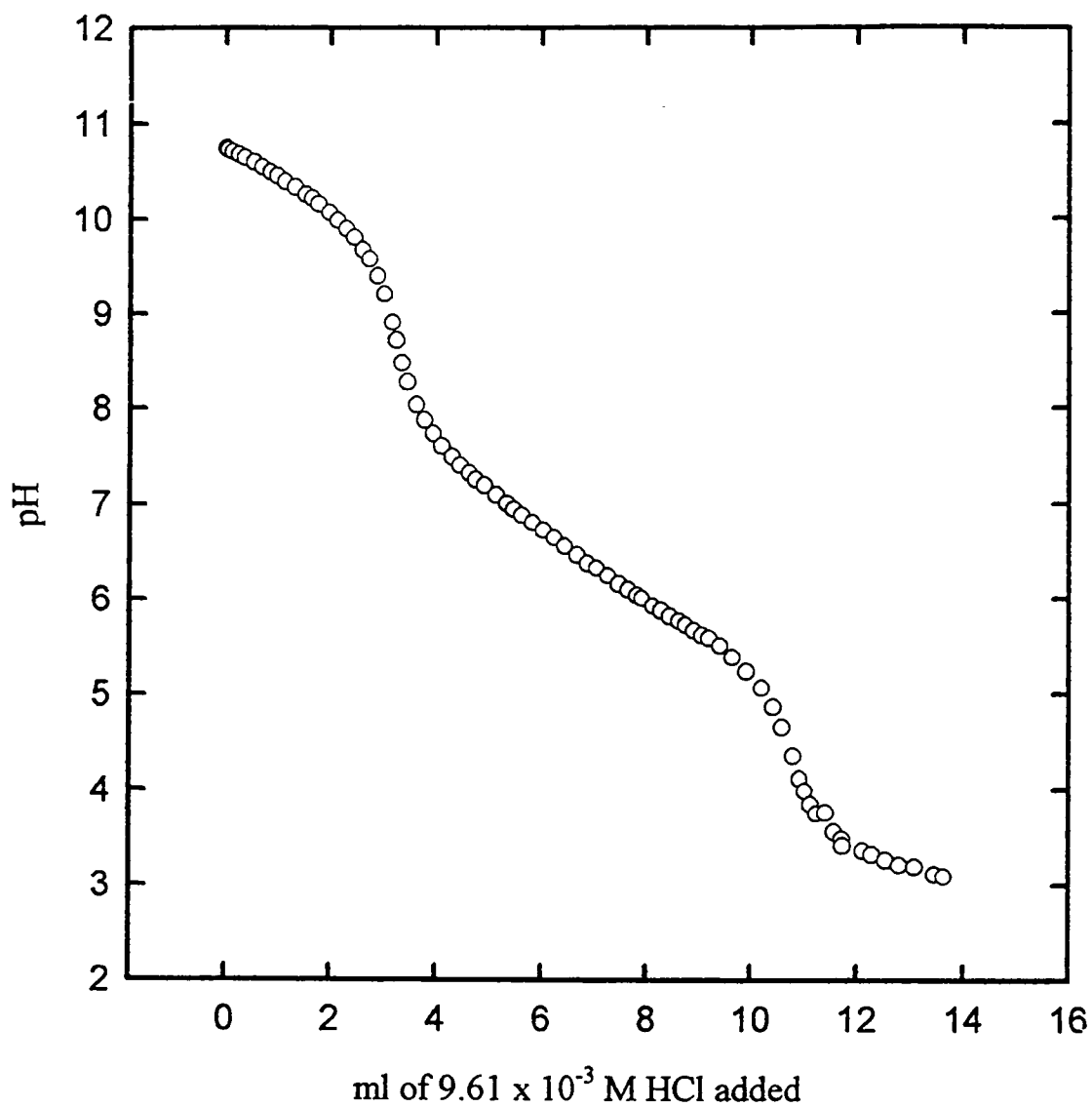


Figure 3-5: Titration of 0.0223 grams of UMP-HgX with  $9.61 \times 10^{-3}$  M HCl.

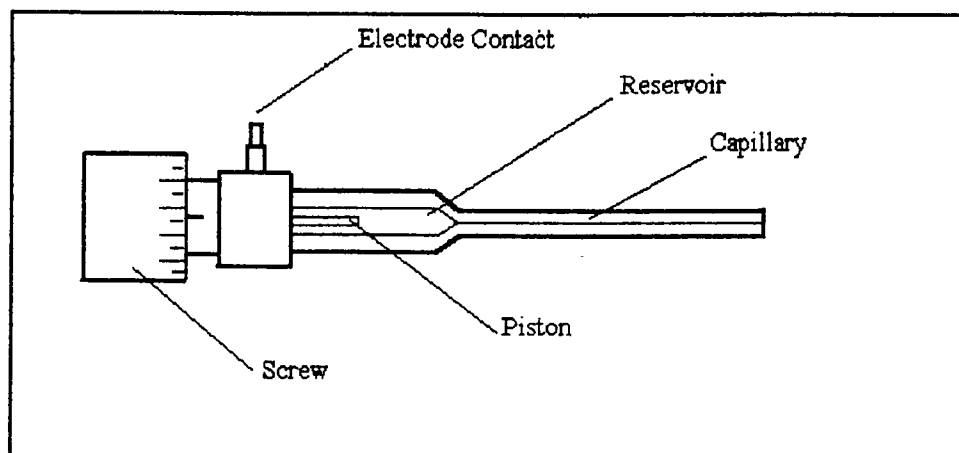


Figure 3-6: Static mercury drop working electrode.

electrode worked properly, a silicone film was deposited on the interior of the capillary (33). Silanization consisted of first thoroughly cleaning the interior of the capillary with 3 M sodium hydroxide, 3 M nitric acid, and distilled water. After thoroughly drying, a 5% solution of dichlorodimethylsilane in  $\text{CCl}_4$  (Aldrich) was pulled through the capillary, followed by air. The capillary was heated to  $50^\circ\text{C}$  overnight, cooled, and then used. The silicone coating was renewed when the hanging drop tended to fall during an experiment.

Polarography was conducted with both the CS-2RA Cypress System potentiostat and a model 174A PAR polarographic analyzer with a PAR drop timer. Typically, the Cypress potentiostat was utilized for the application of the potential sweep and current measurements. The polarographic analyzer was used for timing the drop knocker.

Proton NMR was conducted with a Bruker 250 MHz NMR and  $^{31}\text{P}$ -NMR was

conducted with a Bruker 400 MHz NMR. A Hewlett Packard 8452A diode array spectrophotometer was employed for UV/VIS measurements. The diode array was interfaced with a personal computer by a HP-IB board and Hewlett Packard 89532A UV-VIS software was used for data acquisition.

### Procedures

The potential excitation signal for linear voltammetry and cyclic voltammetry consisted of a staircase waveform with a scan resolution of 1 mV. Oxygen was removed prior to each experiment by purging with nitrogen and a nitrogen blanket was then maintained over the solutions throughout the experiment. The majority of experiments was conducted with nitrogen that had been passed through a MnO column to remove the small amount of oxygen present in the compressed nitrogen (34). Most experiments were conducted at ambient laboratory temperature,  $21 \pm 3^\circ\text{C}$ , unless stated otherwise. Voltammetric temperature studies were carried out with a non-isothermal cell where the main compartment, containing the working and counter electrodes, was thermostated using an ice bath or a heating element. The reference electrode was isolated from the rest of the cell by a salt bridge and was maintained at room temperature. This design was utilized to minimize changes in the liquid junction potential at the SCE/solution interface.

The area of the static mercury drop was determined by weighing multiple drops and controlled by the degree of rotation of the screw. Caliber lines made the amount of rotation and the electrode area reproducible. For example, rotating the

screw three units produced an electrode area of  $0.0197 \pm 0.0004 \text{ cm}^2$  and a rotation of 4 units produced an electrode area of  $0.0225 \pm 0.0004 \text{ cm}^2$ . The drop was dislodged before a new drop was formed by gently tapping the top of the electrode. Most mercury was reused after cleaning with 0.1 M HCl and filtering through gauze.

The concentration of the nucleotides in solution was initially determined by UV-VIS spectrometry. Except for a small shift in absorption maxima (5 nm), the spectra of the mercurated derivatives and the molar absorptivities were essentially identical to the parent compounds ( $\epsilon_{\text{UTP}} = 8890$ ;  $\epsilon_{\text{UDP}} = 8620$ ;  $\epsilon_{\text{UMP}} = 8560 \pm 50 \text{ L/mol cm}$ ). Thus, the amount of nucleotide in solution was readily determined by constructing a calibration curve of  $\lambda_{\text{max}}$  (absorbance) vs. concentration using the parent compounds as standards. This was conducted to minimize errors when weighing out the mercurated nucleotides because the nucleotides tended to adsorb water when being prepared. However, when the mercurated nucleotides were rinsed with acetone and air dried on the analytical balance, a reliable weight was obtained. Solutions prepared with this technique typically gave less than a 5% deviation in concentration from the spectroscopic value.

D<sub>2</sub>O was used as the solvent during proton NMR studies. Typically, broad chemical shifts were obtained for the mercurated derivatives. The addition of an equivalent amount of mercaptoethanol cleared up the signals by complexation with the mercury substituent. This technique was used extensively when characterizing new batches of prepared mercurated derivatives. However, no mercaptoethanol was added when studying the complexation state of the mercury substituent.

## CHAPTER IV

### Cyclic Voltammetry of the Mercurated Nucleotides Under Diffusional Conditions

#### Introduction

Cyclic voltammograms of the 5-mercurated derivatives of UTP, UDP, and UMP in aqueous buffer or KCl solutions showed complex behavior at a static mercury drop electrode. For example, Figures 4-1, 4-2, and 4-3 illustrate typical cyclic voltammograms of the mercurated derivatives in 0.1 M KCl at a bulk nucleotide concentration of 1 mM. As shown, these voltammograms contain both surface and diffusional components. For example, the UTP-HgX voltammogram (Figure 4-1) contains a cathodic surface wave at -0.59 V (A), an anodic surface wave at -0.14 V (B), a cathodic surface wave at -0.77 V, which was obtained during the anodic sweep (C), and a large diffusional wave at potentials more negative than -0.80 V (D). Similar results were obtained with the mercurated UDP (Figure 4-2). However, the UMP-HgX voltammogram (Figure 4-3), although still containing a diffusional component, deviated significantly from the mercurated UTP and UDP.

Although the bulk of this thesis will involve the study of the voltammetric surface behavior of the mercurated uridine nucleotides, it is appropriate to gain an understanding of the diffusional component seen with the three nucleotides in the Figures 4-1, 4-2, and 4-3 (wave D). Hence, in this chapter the diffusional wave obtained with the mercurated uridine nucleotides will be examined.

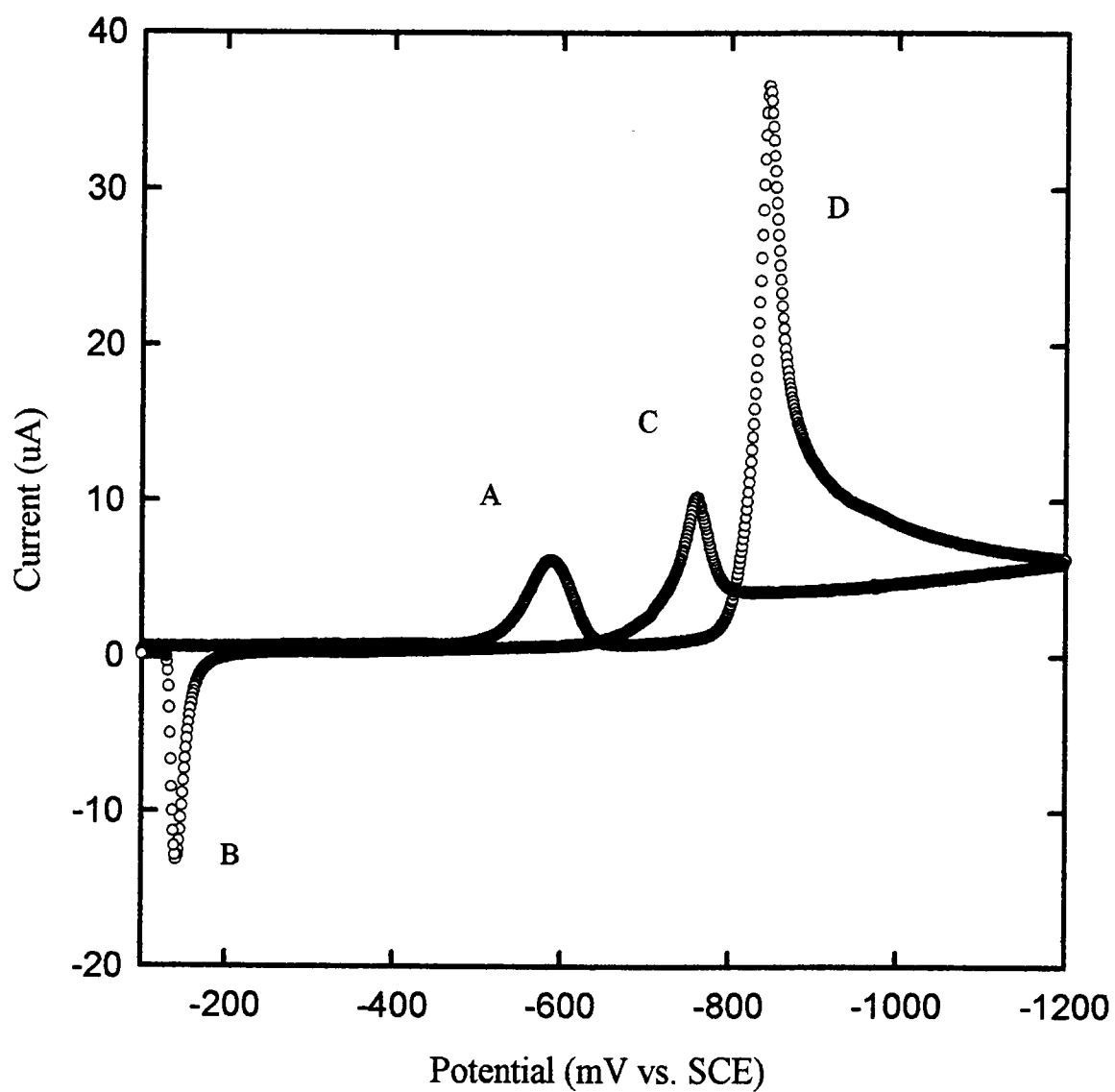


Figure 4-1: Cyclic voltammogram of 1 mM UTP-HgX in 0.1 M KCl with a 0.0225 cm<sup>2</sup> HMDE and a scan rate of 1 V/s.

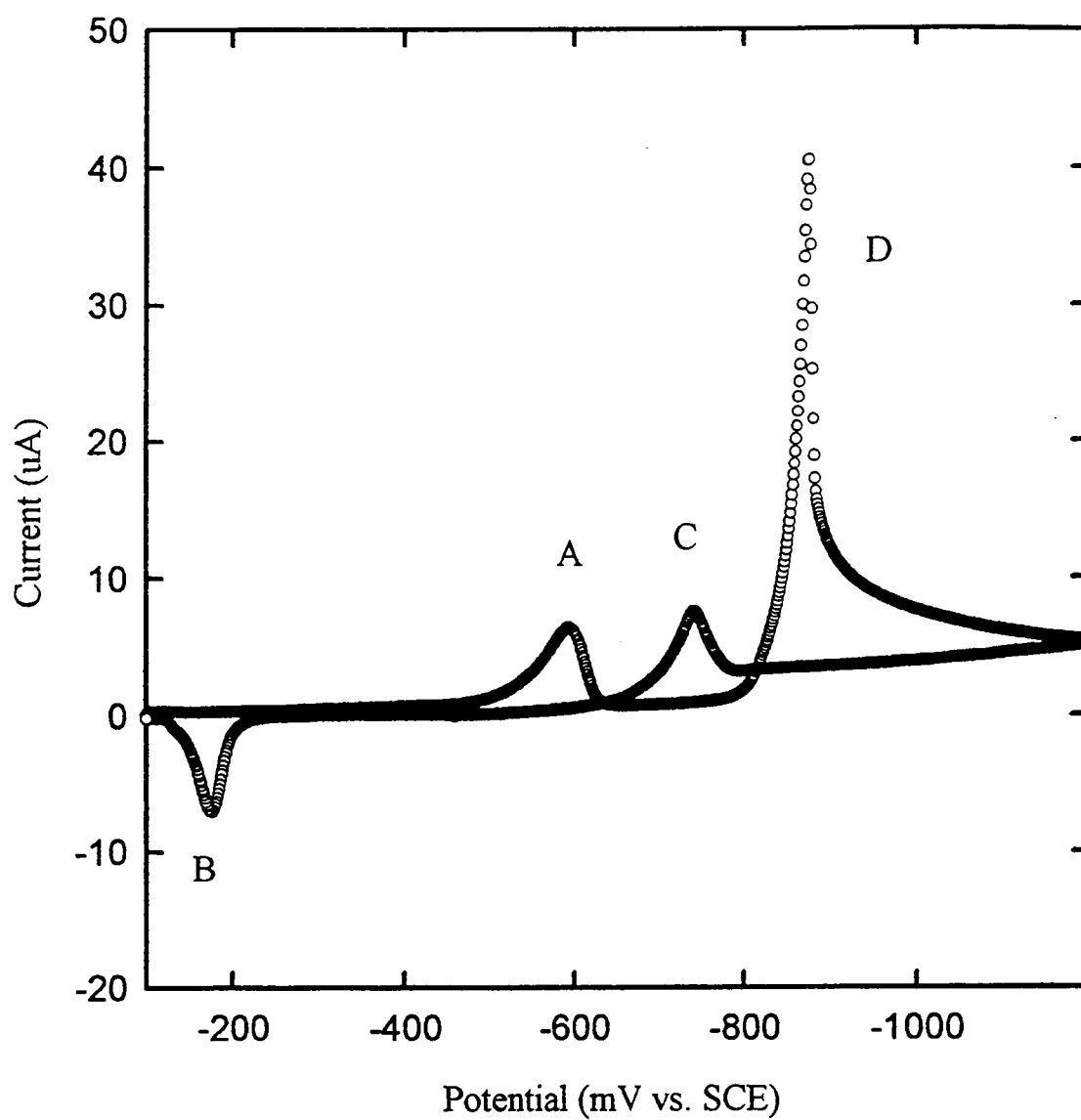


Figure 4-2: Cyclic Voltammogram of 1 mM UDP-HgX in 0.1 M KCl with a 0.0225 cm<sup>2</sup> HMDE and a scan rate of 1 V/s.

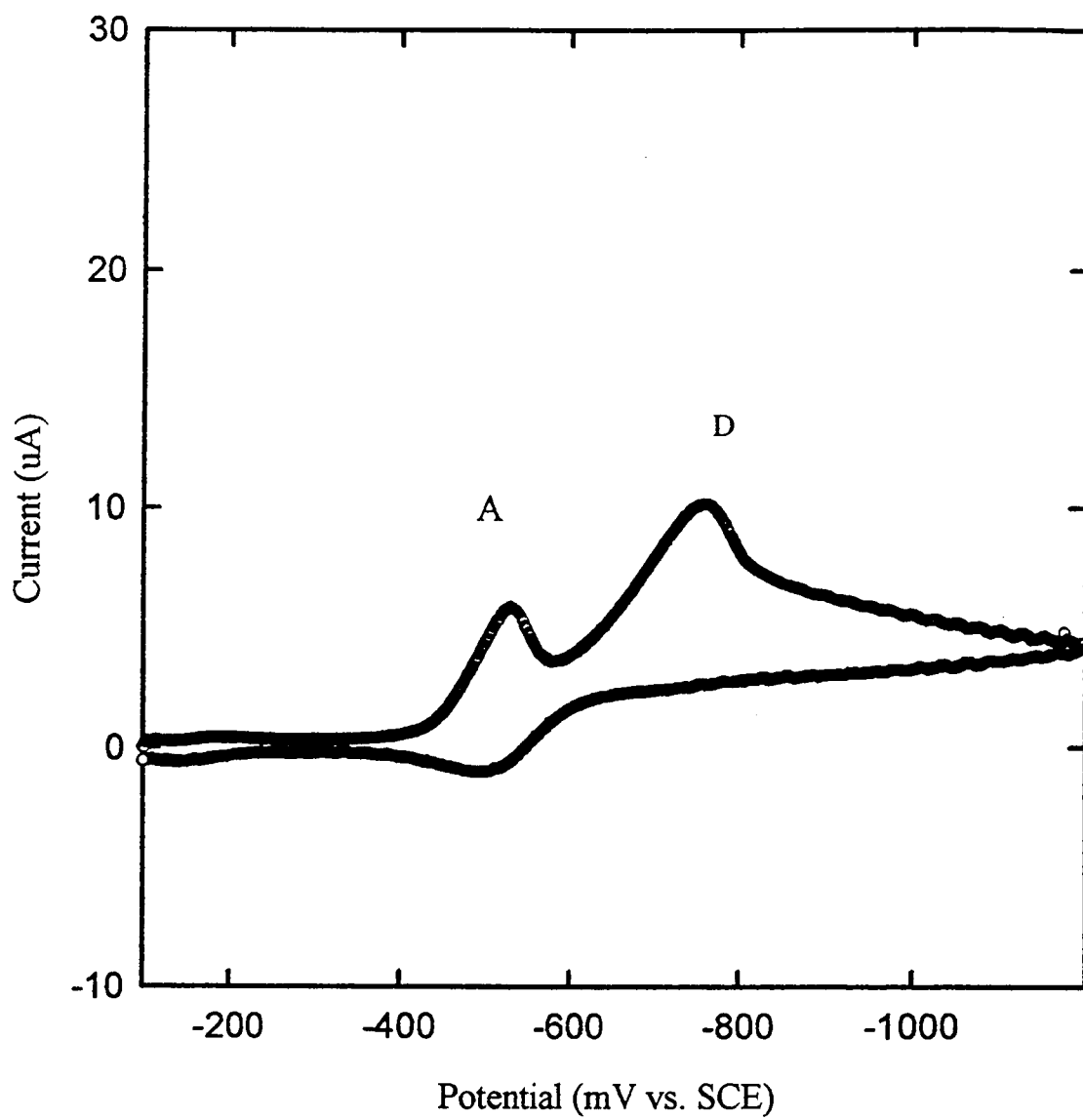


Figure 4-3: Cyclic voltammogram of 1 mM UMP-HgX in 0.1 M KCl with a 0.0225 cm<sup>2</sup> HMDE and a scan rate of 1 V/s.

## Experimental

From Figures 4-1, 4-2, and 4-3 it is evident that if a mercury electrode (immersed in a mercurated uridine nucleotide solution) is held at a potential less than -0.8 V vs. SCE, the mercurated uridine nucleotides will diffuse to the electrode and undergo an irreversible reduction. To gain an understanding of this electron transfer, the product, n-value, and diffusion coefficients were examined.

To determine the product and the n-value, a coulometric study was undertaken. The analysis was conducted in 0.1 M KCl on  $10^{-3}$  M UTP-HgX, UDP-HgX, and UMP-HgX with a  $10\text{ cm}^2$  mercury pool working electrode held at a potential of -1.0 V. For product determination, the nucleotides were precipitated by the addition of cold ethanol midway through electrolysis and when electrolysis was complete. The precipitated samples were then subjected to proton NMR analysis. The n-value was determined by plotting the coulombs passed during the electrolysis vs. the corrected polarogram plateau current (background subtracted). Extrapolating to  $Q_{\text{total}}$  provided the n-value.

The diffusion coefficients of the mercurated nucleotides were determined by several techniques. First, the peak currents at different scan rates were evaluated with a Randles Sevcik type equation. The scan rate data were generated from  $10^{-3}$  M UMP-HgX, UDP-HgX, and UTP-HgX in 0.1 M KCl with a  $0.0225\text{ cm}^2$  HMDE and scan rates ranging from 0.5 to 7.5 V/s. The Randles Sevcik equation, equation 4.1, describes the peak current as a function of scan rate. From this equation it is evident that a plot of the peak current vs. the square root of the scan rate should be linear

with a slope of  $269n^{3/2}AD^{1/2}C$ . Hence, the diffusion coefficients can be determined from the slope. However, the Randles Sevcik equation applies to a reversible n-electron process and clearly is not applicable to the irreversible diffusional waves seen in Figures 4-1, 4-2, and 4-3. But a modification of the Randles Sevcik equation is possible if the electron transfers are fast and the kinetic scheme is known. To take into account the irreversible behavior seen in Figures 4-1, 4-2, and 4-3, the Randles Sevcik equation can be written as shown in equation 4.2 (35,36). This equation holds if the first electron transfer is followed by a fast irreversible chemical reaction and the number of electrons per Ox is n. This mechanism most closely conforms to the reduction of these RHgX compounds. Hence, a plot of the peak current vs. the square root of the scan rate will again be linear but with a slope of  $298nAD^{1/2}C$  (the n-value is taken to be two for these reductions).

$$i_{pk} = 269n^{3/2}AD^{1/2}Cv^{1/2} \quad 4.1$$

$$i_{pk} = 298nAD^{1/2}Cv^{1/2} \quad 4.2$$

Secondly, the diffusion coefficients were determined from the cyclic voltammograms in Figures 4-1, 4-2, and 4-3 using the Cottrell equation. The Cottrell equation describes the current response during the application of a potential step from a region where electron transfer does not occur to a region where electron transfer proceeds readily (equation 4.3). Although the data in Figures 4-1, 4-2, and 4-3 were obtained with cyclic voltammetry, a modified Cottrell equation can be used for the

analysis (equation 4.4) (37). The data of interest in these figures lie past  $i_{pk}$  of the diffusional wave ( $1/2i_{pk}$  to  $-1.2$  V). Here equation 4.4 is applicable.

$$i = nFAD^{1/2}C/\pi^{1/2}t^{1/2} \quad 4.3$$

$$i = nFAD^{1/2}C/\pi^{1/2}(t-t_0)^{1/2} \quad 4.4$$

From equation 4.4, it is evident that a plot of  $1/i$  vs.  $(t-t_0)^{1/2}$  will be linear with a slope of  $\pi^{1/2}/nFAD^{1/2}C$ . Hence, the diffusion coefficients can be determined from the slopes. The values of  $t$  and  $t_0$  were determined as follows. By dividing the potential by the scan rate the value of  $t$  was determined for each current value of interest in Figures 4-1, 4-2, and 4-3. The value  $t_0$  was more difficult to determine. Since a plot of  $1/i$  vs.  $(t-t_0)$  theoretically should be linear, the  $t_0$  values were obtained by constructing these plots with increasing  $t_0$  values until a maximum linear coefficient was obtained. After the optimum  $t_0$  values were obtained, the diffusion coefficients were determined from the slope of the  $1/i$  vs.  $(t-t_0)$  plots.

The diffusion coefficients were finally determined with chronocoulometry. This procedure consisted of stepping the potential of a  $0.0225 \text{ cm}^2$  mercury electrode (immersed in a  $1 \times 10^{-3} \text{ M}$  UZP-HgX,  $0.1 \text{ M}$  KCl solution) from  $-0.1 \text{ V}$  to  $-1.0 \text{ V}$  and recording the current transient for  $0.5 \text{ s}$  after the potential step. Longer measurement times were also carried out but it was determined that spherical diffusion obscured the data at times greater than  $0.5 \text{ s}$ . Equation 4.5 (derived from the Cottrell equation) describes the coulombic charge as a function of  $t$ . From this equation, it is

seen that a plot of  $Q$  vs.  $t^{1/2}$  should be linear with the slope of  $2nFAD^{1/2}C/\pi^{1/2}$ .

Hence, the diffusion coefficients were evaluated from the slopes of the  $Q$  vs  $t^{1/2}$  plots (Anson plots).

$$Q = (2nFAD^{1/2}Ct^{1/2})/\pi^{1/2} \quad 4.5$$

### Results and Discussion

*Product identification and n-value.* Table 2 gives the down-field chemical shifts from the samples obtained midway through bulk electrolysis and when electrolysis was complete (the chemical shifts previously assigned to the parent compounds and the mercurated derivatives are given in Table 1, pg 39). The results illustrate that midway through electrolysis both the parent compound and mercurated derivative are present. For example, five chemical shifts were observed midway through electrolysis of the mercurated UMP. Three of these are consistent with the parent compound UMP (d-8.12, d-5.97, d-5.94) and the other two are consistent with the chemical shifts previously assigned to the mercurated UMP (s-7.99, d-6.05). With complete electrolysis, only the chemical shifts assignable to the parent compounds were present. Hence, the electrochemical reduction at  $E > -1.0$  V, appears to produce the parent compound with protonation at the H-5 position of the base.

Additional evidence for protonation at the H-5 position was seen during electrolysis of the mercurated UMP in  $D_2O$ . After electrolysis, the downfield NMR spectrum still consisted of a singlet and a doublet, but the NMR shifts were

inconsistent with those of the UMP (d-8.10, d-5.98, d-5.94). The values obtained were s-8.08 and d-5.95. These values are consistent with dueteration at the H-5 position.

Figure 4-4 shows the variation of the polarogram plateau current during the electrolysis of the mercurated UTP as a function of charge passed during the electrolysis. Extrapolation to zero current provided a  $Q_{\text{total}}$  value of 3.15 C. With the initial moles of the mercurated UTP equalling  $1.4 \times 10^{-5}$ , a n-value of 2.3 was calculated. Likewise, the n-values for the mercurated UDP and UMP were determined to be 2.1 and 1.9.

Table 2: Down-field proton NMR shifts obtained midway through electrolysis (1/2E) and when electrolysis was complete (F.E.)

| Species | Degree | # of Shifts | Chemical Shifts                        |
|---------|--------|-------------|----------------------------------------|
| UMP-HgX | 1/2E   | 5           | s-7.99, d-6.05, d-8.10, d-5.96, d-5.92 |
| UMP-HgX | F.E.   | 3           | d-8.12, d-5.97, d-5.94                 |
| UDP-HgX | 1/2E   | 5           | s-7.89, d-6.02, d-8.00, d-5.98, d-5.96 |
| UDP-HgX | F.E.   | 3           | d-8.00, d-5.97, d-5.94                 |
| UTP-HgX | 1/2E   | 5           | s-7.90, d-6.00, d-7.94, d-5.97, d-5.95 |
| UTP-HgX | F.E.   | 3           | d-7.94, d-5.98, d-5.94                 |

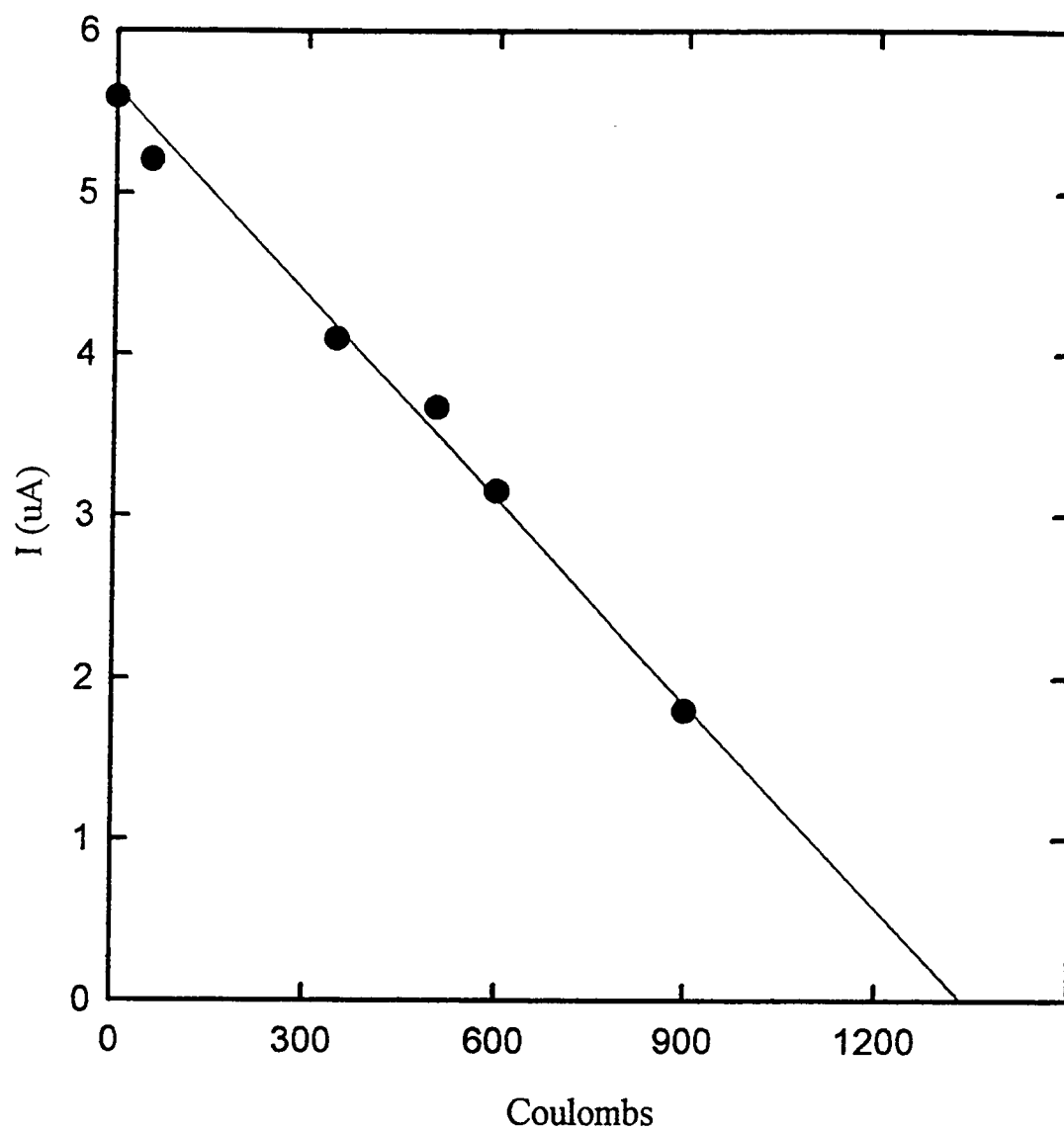


Figure 4-4: Polarogram plateau current vs. the coulombs passed during bulk electrolysis of  $1.4 \times 10^{-3}$  M UMP-HgX.

With the production of the parent compound and a n-value of 2, equation 4.6 can be written for the electrochemical reduction of the three nucleotides at the mercury surface. The assignment of metallic mercury as a product of the reduction is consistent with the lack of evidence of either  $\text{Hg}^+$  or  $\text{Hg}^{2+}$  being produced during cyclic voltammetry. In addition, the above results were obtained with a glassy carbon electrode (2-electron reduction to produce the parent compound). In fact, with a wider anodic sweep range, voltammetry at a glassy carbon working electrode provided unambiguous proof that metallic mercury was the product of the reduction because a wave assignable to the oxidation of  $\text{Hg}^0$  was observed during the anodic sweep. For example, Figure 4-5 illustrates a typical voltammogram of the mercurated UMP in 0.1 M KCl at the glassy carbon electrode. This voltammogram clearly shows a characteristic stripping wave for the oxidation of metallic mercury (wave B). Identical results were obtained for the mercurated UDP and UTP.



Z = mono, di, or triphosphate

*Determination of the diffusion coefficients with the Randles-Sevcik equation.*

Table 3 lists the peak currents as a function of scan rate for the mercurated nucleotides. With these data, plots of  $i_{\text{pk}}$  vs  $v^{1/2}$  were generated and found to be quite linear with a slopes of  $1.95 \times 10^{-5}$ ,  $9.97 \times 10^{-6}$ , and  $9.16 \times 10^{-6} \text{ cm}^3 \text{ mol L s}^{-1/2}$  and

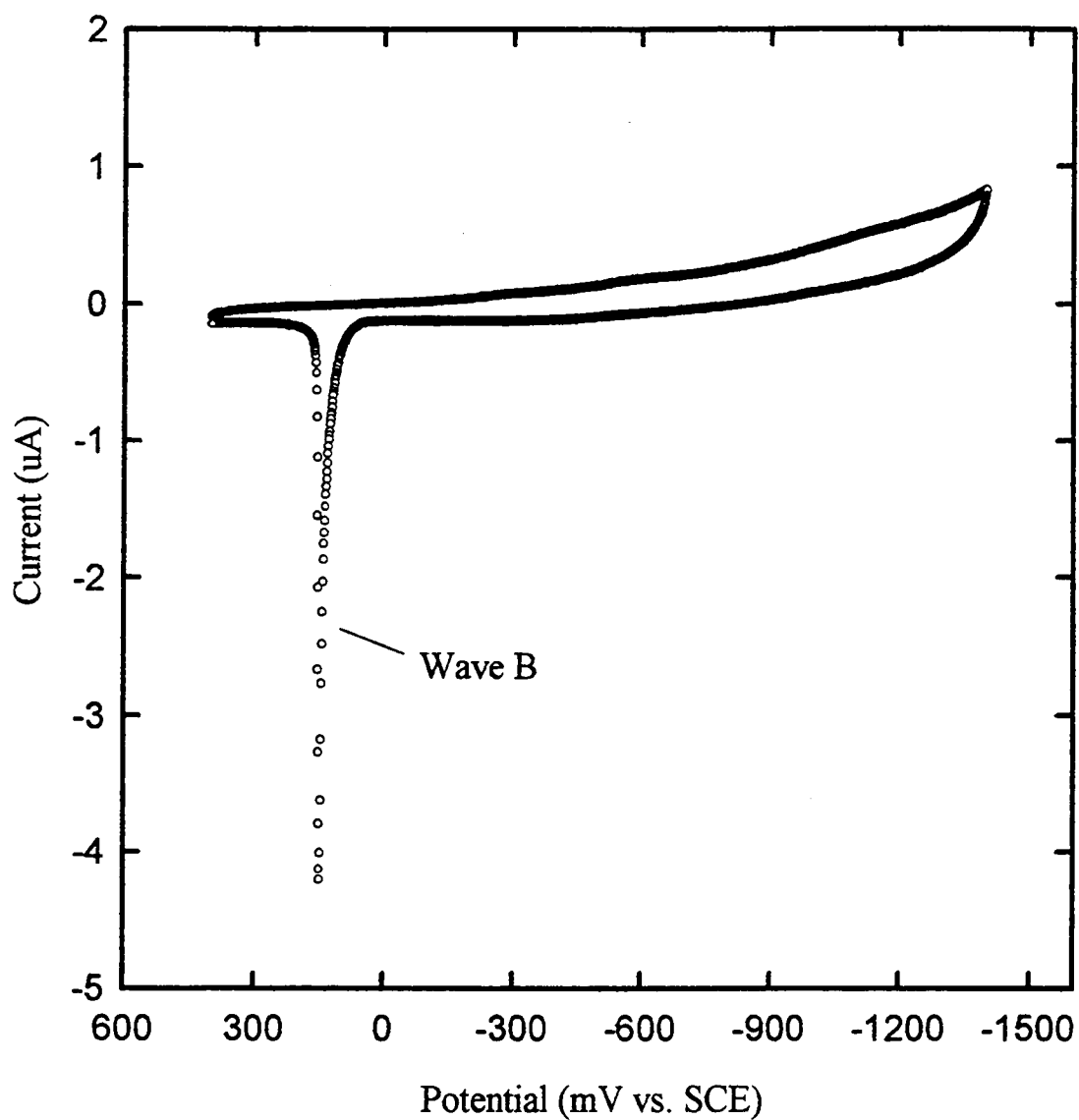


Figure 4-5: Cyclic voltammogram of  $2 \times 10^{-4}$  M UMP-HgX in 0.1 M NaCl with a  $0.1 \text{ cm}^2$  GCE and a scan rate of 50 mV/s.

Table 3: Peak currents of the diffusional waves of the mercurated UTP, UDP, and UMP at various scan rates and  $i/v^{1/2}$  values. Electrode area = 0.0225 cm<sup>2</sup>;  $C_{\text{UMP-HgX}} = 9.9 \times 10^{-4}$  M;  $C_{\text{UDP-HgX}} = 9.14 \times 10^{-4}$  M;  $C_{\text{UTP-HgX}} = 9.6 \times 10^{-4}$  M.

| UTP-HgX |             |        | UDP-HgX |             |        | UMP-HgX |             |        |
|---------|-------------|--------|---------|-------------|--------|---------|-------------|--------|
| v (V/s) | $i/v^{1/2}$ | i (uA) | v (V/s) | $i/v^{1/2}$ | i (uA) | v (V/s) | $i/v^{1/2}$ | i (uA) |
| 0.5     | 14.4        | 10.2   | 0.5     | 28.4        | 20.1   | 0.5     | 9.9         | 7      |
| 1.0     | 16.7        | 16.7   | 1.0     | 22.9        | 22.9   | 1.0     | 10.0        | 10     |
| 2.5     | 16.7        | 26.4   | 2.5     | 18.1        | 28.7   | 2.5     | 9.2         | 14.5   |
| 5.0     | 17.9        | 40.0   | 5.0     | 15.7        | 35.0   | 5.0     | 9.4         | 21     |
| 7.5     | 18.3        | 50.2   | 7.5     | 14.8        | 40.4   | 7.5     | 9.4         | 25.8   |

linear regression coefficients of 0.9987, 0.9996, and 0.9984. From the slopes, diffusion coefficients of  $2.3 \times 10^{-6}$ ,  $8.2 \times 10^{-7}$ , and  $4.8 \times 10^{-7}$  cm<sup>2</sup>/s were determined for the mercurated UTP, UDP, and UMP. These values appears to be quite low even for large nucleotide molecules. Hence, the Randles Sevcik type equation used to determine the diffusion coefficients (equation 4.2) was arranged as shown in equation 4.7. This expression states that the peak current divided by the square root of the

$$i_{\text{pk}}/v^{1/2} = 298n D^{1/2}CA = C \quad 4.7$$

scan rate should equal a constant which is the product of the n-value, the diffusion coefficient, the bulk nucleotide concentration, and the electrode area. For pure diffusional behavior, the value of C should be constant with v. The values of C for

the mercurated UTP and UDP were not constant and varied from 14.4 to 28.4. The values are provided in Table 3. Since it is improbable that the diffusion coefficient or the n-value will change within this scan rate range, these data suggest a possible surface component (a surface wave within the large diffusional wave) with the mercurated UTP and UDP. The values for the mercurated UMP were more constant ( $9.6 \pm 0.04$ ), however, the diffusion coefficient determined for the mercurated UMP is also unusually low. It should be noted that the  $i_{pk}/v^{1/2}$  values for the mercurated UDP vary in a manner inconsistent with that expected from a hidden surface component. An explanation of this observation has not been obtained.

*Semi-Integral of the diffusional wave of the mercurated UTP-HgX.* Further support for a surface component within the diffusional waves of the mercurated nucleotides was evident in the semi-integral curve of the diffusional waves. The transformation of the voltammetric i-E curves into a form resembling steady state voltammetry is frequently accomplished using semi-integral techniques. These steady-state forms are typically more convenient for data processing and can provide additional information such as the absence or presence of surface components. A review of this material can be found in Bard and Faulkner (38).

Equation 4.8 gives the equation utilized to obtain the semi-integral data in this section and Figure 4-6 illustrates a diffusional wave and the corresponding semi-integral obtained from Bard and Faulkner's review. As shown, the semi-integral curve reaches a plateau in the region of diffusion. The current at the plateau is given

by equation 4.9. Figure 4-7 illustrates the cyclic voltammogram of the mercurated UTP and the semi-integral curve for the diffusional wave. This semi-integral curve differs from the theoretical curve in Figure 4-6. Particularly, there is a peak before reaching the plateau. This is indicative of a surface component within the diffusional wave (39).

$$I(t) = 1/\pi^{1/2} \int_0^t [i(u)/(t-u)^{1/2}] du \quad 4.8$$

$$I_L = nFAD^{1/2}C_{\text{Bulk}} \quad 4.9$$

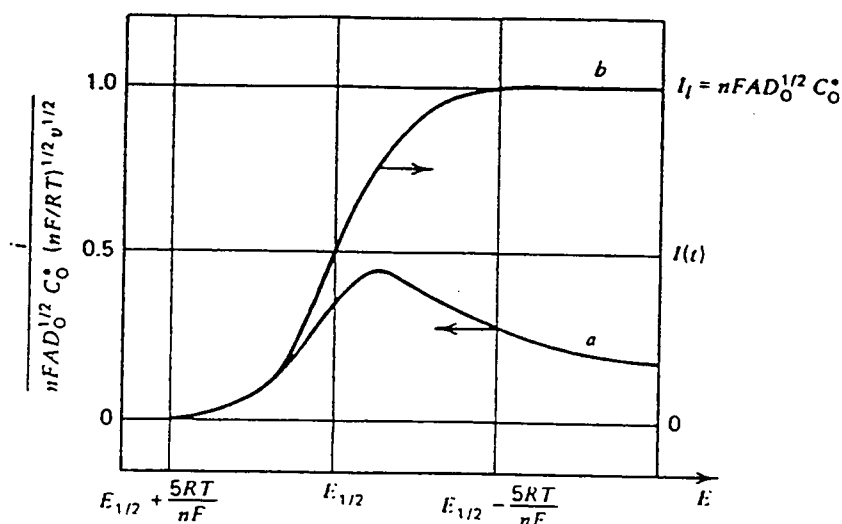


Figure 4-6: Diffusional wave (a) and semi-integral curve (b) obtained from Bard and Faulkner

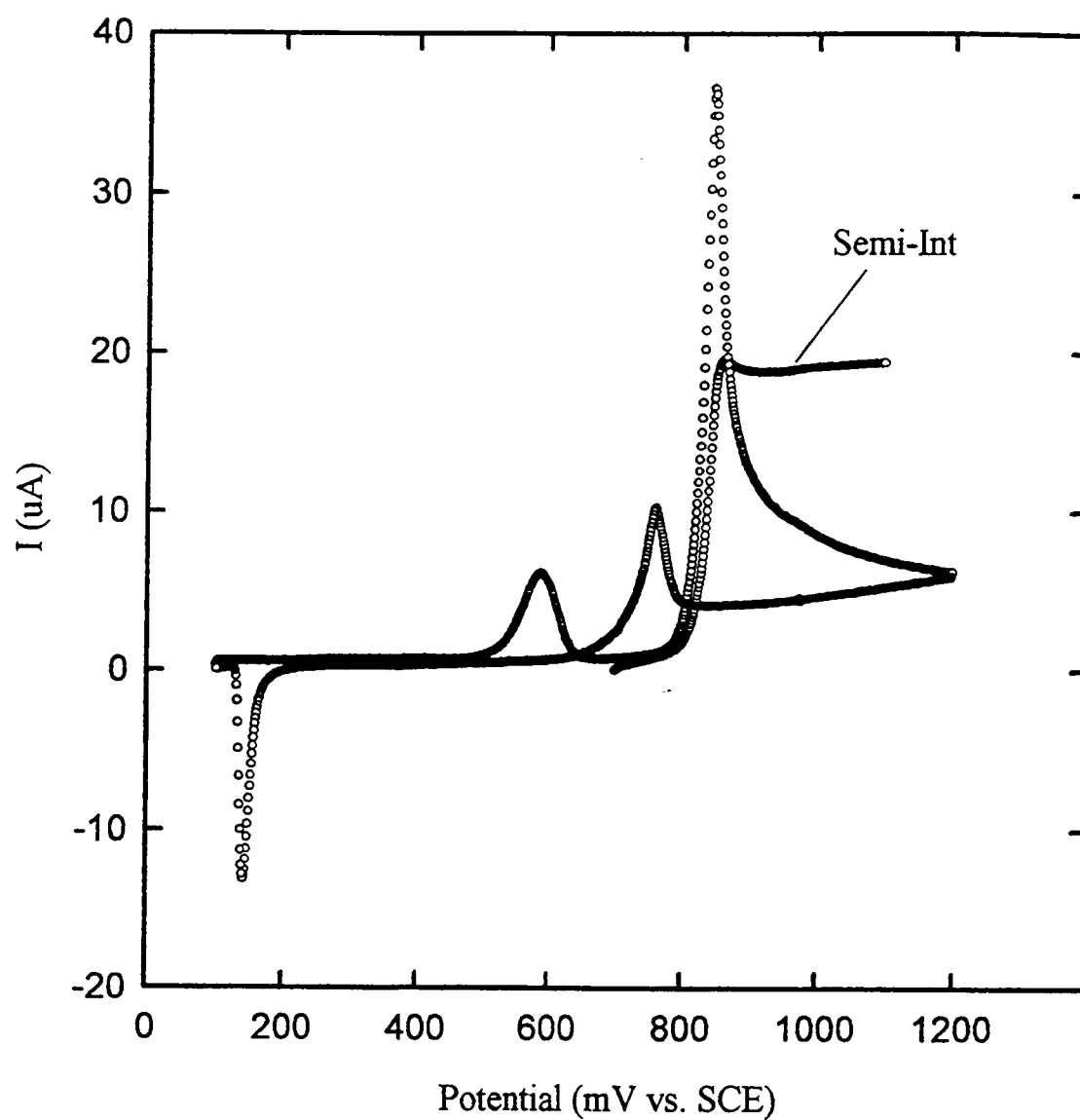


Figure 4-7: Cyclic voltammogram of the mercurated UTP obtained from Figure 4-1 and the semi-integral of the diffusional wave.

*Determination of the diffusion coefficients with the Cottrell equation.* The  $1/i$  vs.  $(t-t_0)^{1/2}$  plots for the mercurated UTP, UDP, and UMP are illustrated in Figure 4-8. Table 4 gives the determined  $t_0$  values, the slopes, and the linear coefficients. With slopes of  $2.07 \times 10^5$ ,  $2.45 \times 10^5$ , and  $2.67 \times 10^5$   $s^{1/2}/C$ , the diffusion coefficients were determined to be  $3.8 \times 10^{-6}$ ,  $2.8 \times 10^{-6}$ , and  $2.3 \times 10^{-6}$   $cm^2/s$  for the mercurated UTP, UDP, and UMP. These values which are more reasonable than those obtained with the modified Randles Sevcik equation, are probably the result of the elimination of the surface component seen with the Randles Sevcik data. This is a result of the fact that the above data were measured past the large current transient seen on the onset of the diffusional wave where a surface component may reside.

Table 4: Determined  $t_0$  values, slopes, and linear coefficients for  $1/i$  vs.  $(t-t_0)$  plots for the mercurated UTP, UDP, and UMP.

| species | $t_0(s)$ | slope ( $s^{1/2}/C$ ) | $R^2$  |
|---------|----------|-----------------------|--------|
| UTP-HgX | 0.87     | 207000                | 0.9989 |
| UDP-HgX | 0.88     | 245000                | 0.9997 |
| UMP-HgX | 0.70     | 267000                | 0.9878 |

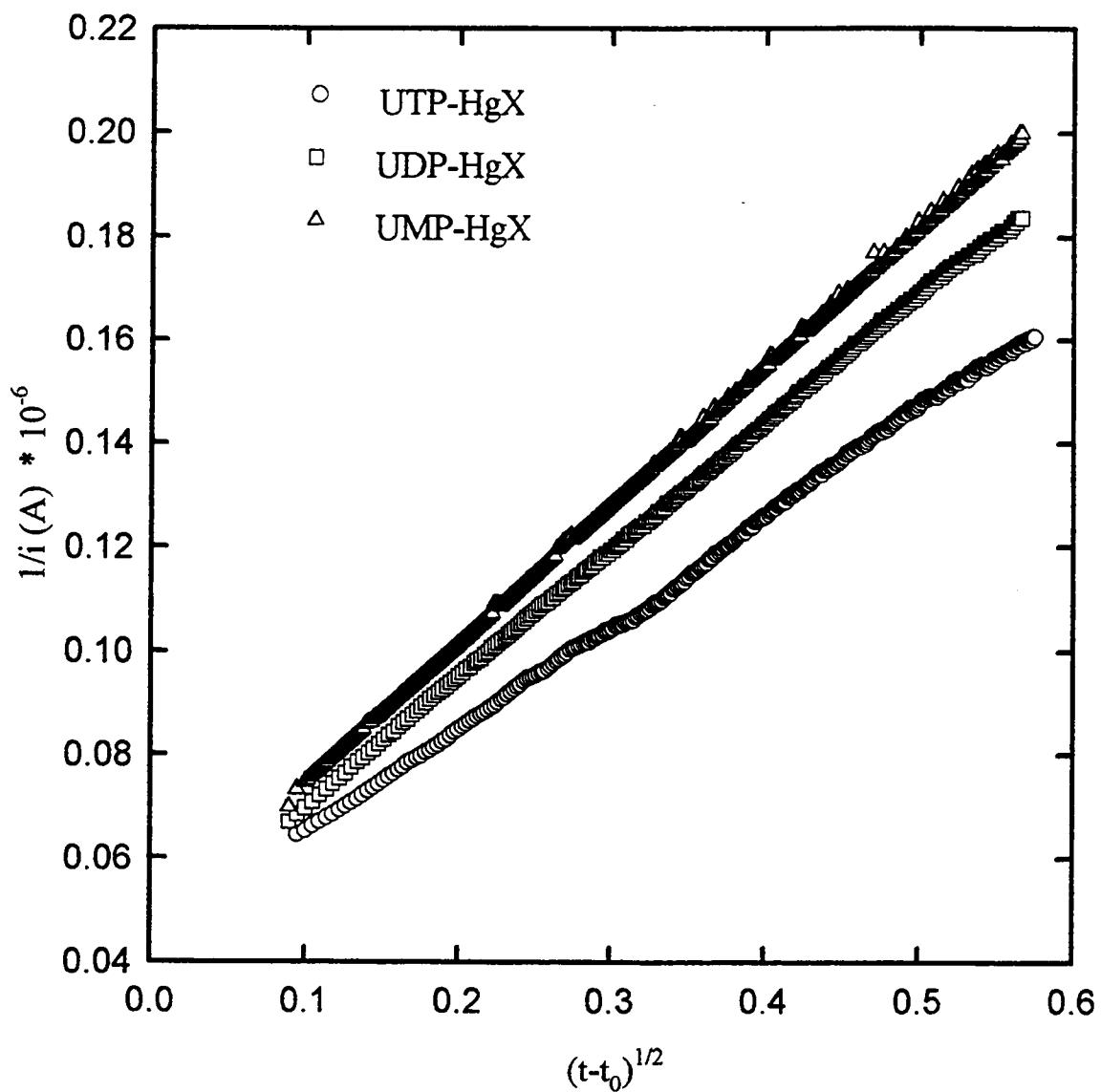


Figure 4-8:  $1/i$  vs.  $(t-t_0)^{1/2}$  plots for the mercurated nucleotides. Data obtained from the plateau region in figures 4-1, 4-2, and 4-3.

*Determination of the diffusion coefficients by chronocoulometry.* Figure 4-9

illustrate the  $Q$  vs  $t^{1/2}$  plots for the mercurated UTP, UDP, and UMP. These plots are indeed linear. The average slopes were determined to be  $1.2 \times 10^{-5}$ ,  $8.8 \times 10^{-6}$ , and  $9.0 \times 10^{-6} \text{ C/s}^{1/2}$ . Using a  $n$ -value of 2, an electrode area of  $0.0199 \text{ cm}^2$ , and the nucleotide concentrations given in Figure 4-9, the diffusion coefficients for the mercurated nucleotides were determined to be  $4.4 \times 10^{-6}$ ,  $3.2 \times 10^{-6}$ , and  $2.9 \times 10^{-6} \text{ cm}^2/\text{s}$  with this technique.

Table 5 lists the determined diffusion coefficients using the three techniques. The latter two are in good agreement. Since it was determined that the results obtained from the first technique were probably affected by a surface component, only the latter two were considered in the reported averages which are also listed in Table 5. It is interesting to note that the trend of increasing diffusion coefficient within increasing size of the molecule is the opposite one would expect. An explanation of this trend will be provided in a later section.

Table 5: Determined diffusion coefficients ( $\text{cm}^2/\text{s}$ ) for the mercurated uridine nucleotides in 0.1 M KCl.

| Technique           | UTP-HgX              | UDP-HgX              | UMP-HgX              |
|---------------------|----------------------|----------------------|----------------------|
| 1.Randles Sevcik    | $1.2 \times 10^{-6}$ | $3.3 \times 10^{-7}$ | $2.4 \times 10^{-7}$ |
| 2.Cottrell Equation | $3.8 \times 10^{-6}$ | $2.8 \times 10^{-6}$ | $2.3 \times 10^{-6}$ |
| 3.Chronocoulometry  | $4.4 \times 10^{-6}$ | $3.2 \times 10^{-6}$ | $2.9 \times 10^{-6}$ |
| Average of 2 & 3    | $4.1 \times 10^{-6}$ | $3.0 \times 10^{-6}$ | $2.6 \times 10^{-6}$ |

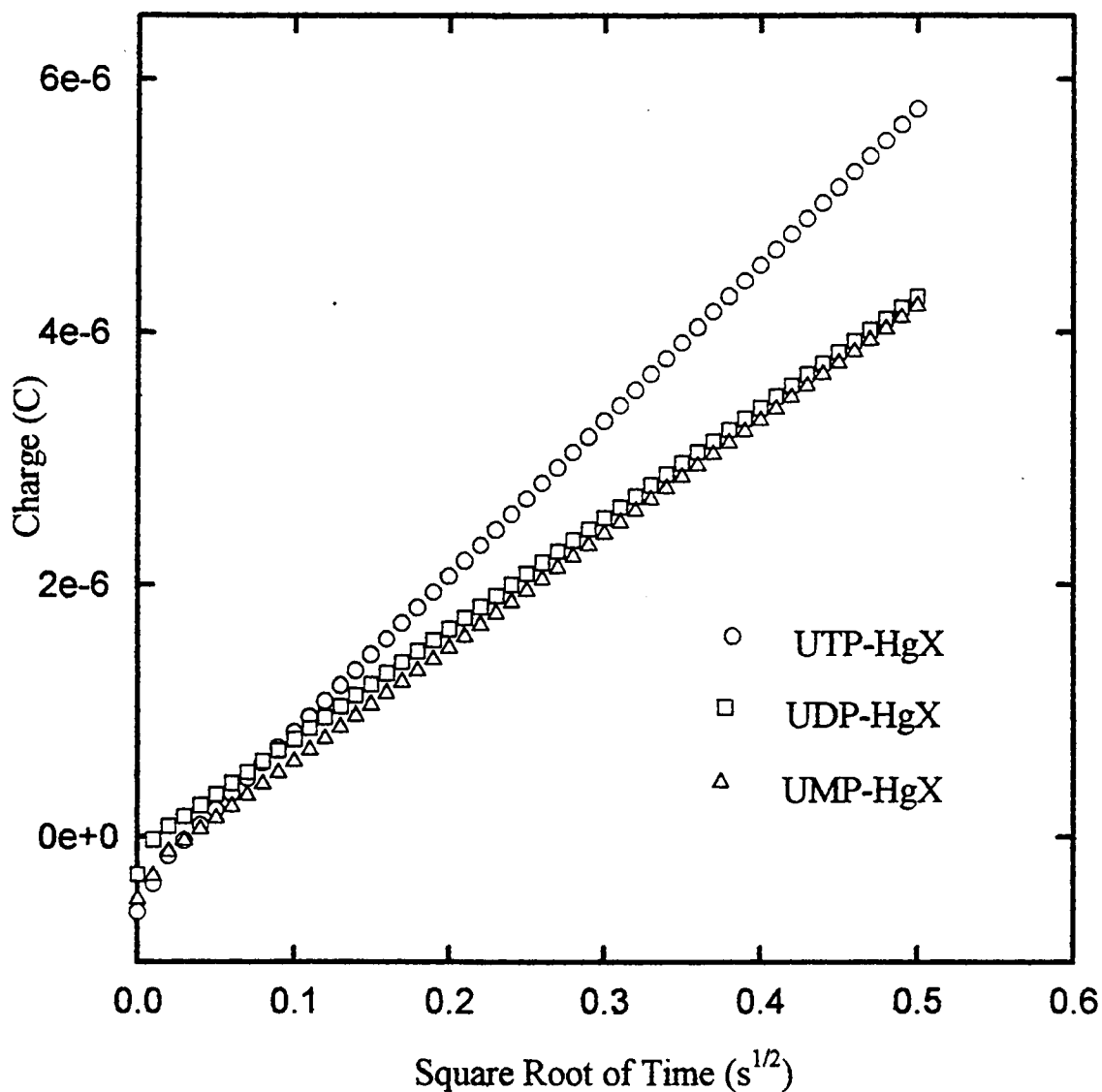


Figure 4-9: Anson plot from chronocoulometry of  $1.1 \times 10^{-3}$  M UMP-HgX,  $1.01 \times 10^{-3}$  M UDP-HgX, and  $1.2 \times 10^{-3}$  M UTP-HgX in 0.1 M KCl with a  $0.0199 \text{ cm}^2$  HMDE.  $E_{\text{initial}} = -0.1 \text{ V}$ ;  $E_{\text{step}} = -1.0 \text{ V}$ .

## Conclusion

In this chapter it was shown that the mercurated uridine nucleotides, in 0.1 M KCl, diffuse to the mercury electrode at a rate of  $3.9 \times 10^{-6}$ ,  $3.9 \times 10^{-6}$ , and  $2.5 \times 10^{-6}$  cm<sup>2</sup>/s and are reduced via a two-electron process if the electrode potential is held less than -1.0 V vs. SCE. This two-electron process results in the production of the parent compound and metallic mercury. These results were obtained in other supporting electrolytes such as NaCl and phosphate buffers (the diffusion coefficients will, however, vary slightly with supporting electrolyte). In addition, it was evident from the cyclic voltammograms in Figures 4-1, 4-2, and 4-3 that the mercurated UMP voltammogram differs from that of the mercurated UTP and UDP. An explanation of this and the other waves present in Figures 4-1, 4-2, and 4-3 will be provided in latter sections.

## CHAPTER V

### Linear Sweep Voltammetry of the Mercurated Uridine Nucleotides Under Conditions Where Diffusion is Minimal

#### Introduction

In the previous chapter it was shown that the mercurated uridine nucleotides diffuse to mercury electrodes and are reduced via a two-electron process if the potential of the electrode is held in the vicinity of -1.0 V or less. It was also evident from Figures 4-1, 4-2, and 4-3 that the voltammograms contained surface components (surface waves). These waves result from the reduction or oxidation of surface confined species that adsorb on the mercury electrode via diffusion at open circuit or are the result of surface confined species which are the product of a previous electron transfer.

The remainder of this thesis will involve the study of the surface behavior obtained with the mercurated nucleotides. To eliminate the diffusional wave obtained with the nucleotides, this study will be conducted with a bulk nucleotide concentration less than 1 mM and faster scan rates than those utilized in the work described in chapter IV. To present the material in an organized manner, this chapter will focus on the surface behavior of the mercurated uridine nucleotides obtained with a single sweep (cathodic). In the following chapter (chapter VI) the effect of sweep reversal (cyclic voltammetry) will be examined and then in chapter VII the effect of pH on the voltammetric behavior will be examined.

## Experimental

The linear sweep voltammetry of the mercurated uridine nucleotides was conducted with scan rates greater than 1 V/s and a supporting electrolyte of 0.1 M KCl. A ten second waiting time at open circuit was utilized with these conditions.

The adsorption equilibrium of the mercurated nucleotides on Hg was studied by measuring the total coulombs under the reduction wave A (Figure 4-1) while varying the bulk concentration of the nucleotide (In the discussion below, wave A is designated as R1). The typical waiting time at open circuit during the adsorption study was 2 minutes to establish equilibrium (longer waiting times did not effect the charge under the R1 wave) and a scan rate of 5 V/s or greater was used to minimize the contribution from diffusion. The longer waiting time during this study was required for solutions containing bulk nucleotide concentrations less than  $7.0 \times 10^{-5}$  M.

The Langmuir isotherm which describes adsorption equilibria with no lateral interactions between the adsorbants was utilized to analyze the adsorption data. The Langmuir isotherm is described by equation 5.1, where  $\Gamma_i$  is the limiting surface coverage of species i,  $\Gamma_s$  is the surface coverage at saturation,  $C_{bulk}$  is the concentration of species i in the bulk, and  $\beta_i$  is an energy parameter. From this equation, it is apparent that a plot of  $C_{bulk}/\Gamma_i$  vs  $C_{bulk}$  will be linear with a slope of  $1/\Gamma_s$  and an intercept of  $1/(\Gamma_s\beta_i)$  (Langmuir plot).

$$\Gamma_i/(\Gamma_s - \Gamma_i) = \beta_i C_{bulk} \quad 5.1$$

Chronoamperometry curves were obtained by applying a potential of -600 mV for 50 ms followed by a jump to a potential in the vicinity of -1.0 V and recording the current transient. The nucleotide solutions consisted of  $1 \times 10^{-4}$  M UZP-HgX in 0.1 M KCl.

## Results and Discussion

*Linear sweep voltammetry of the mercurated UTP and UDP.* Figures 5-1 and 5-2 illustrate typical linear sweep voltammograms of the mercurated UTP and UDP. These voltammograms, which are almost identical, contain two reduction waves labeled R1 and R2. The R1 wave in these figures corresponds to wave A in Figures 4-1, 4-2, and 4-3 of chapter IV. The R2 wave corresponds to the surface component of wave D. Figures 5-3 and 5-4 show that the peak heights for waves R1 and R2 approach linearly with scan rate over a range of 0.1-50 V/s. This fact and the lack of a diffusional tail on both waves gives solid evidence of true surface behavior.

The two surface waves, which were seen throughout a large scan rate range, demonstrate that, like other organomercury compounds, the mercurated nucleotides undergo a two-step reduction at mercury surfaces (3,4,5,6). Tables 6 and 7 summarize the experimental data for the mercurated UTP and UDP within the scan rate range of 1 to 50 V/s where  $E_{pk}$  is the potential at maximum current,  $Q$  is the coulombic charge, and  $\Delta E_{1/2}$  is the peak width at half the maximum current for waves R1 and R2.

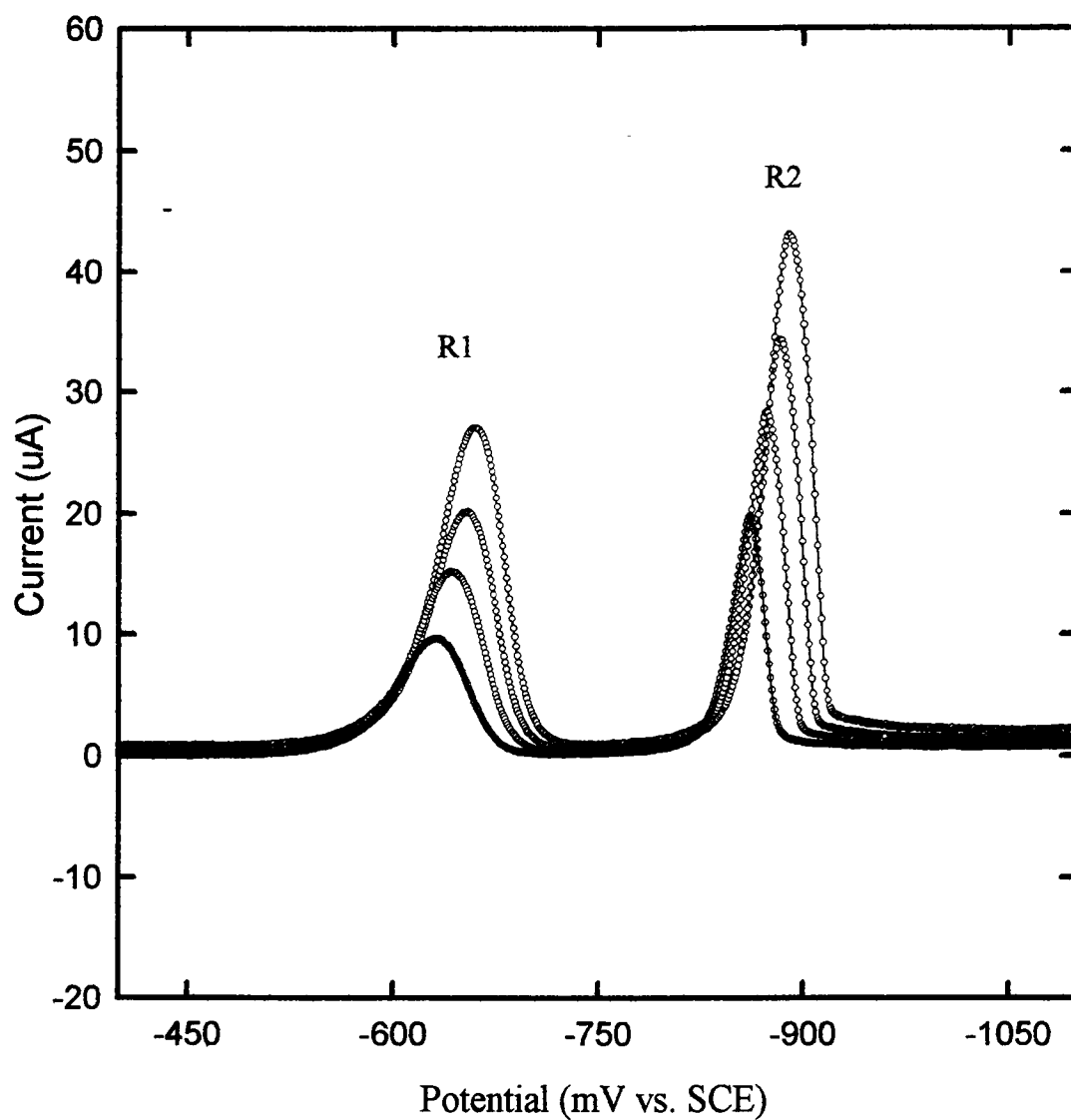


Figure 5-1: Linear sweep voltammograms of  $7.0 \times 10^{-5}$  M UTP-HgX in 0.1 M KCl at a  $0.0225 \text{ cm}^2$  HMDE; scan rates: 2, 3, 4, and 5 V/s.

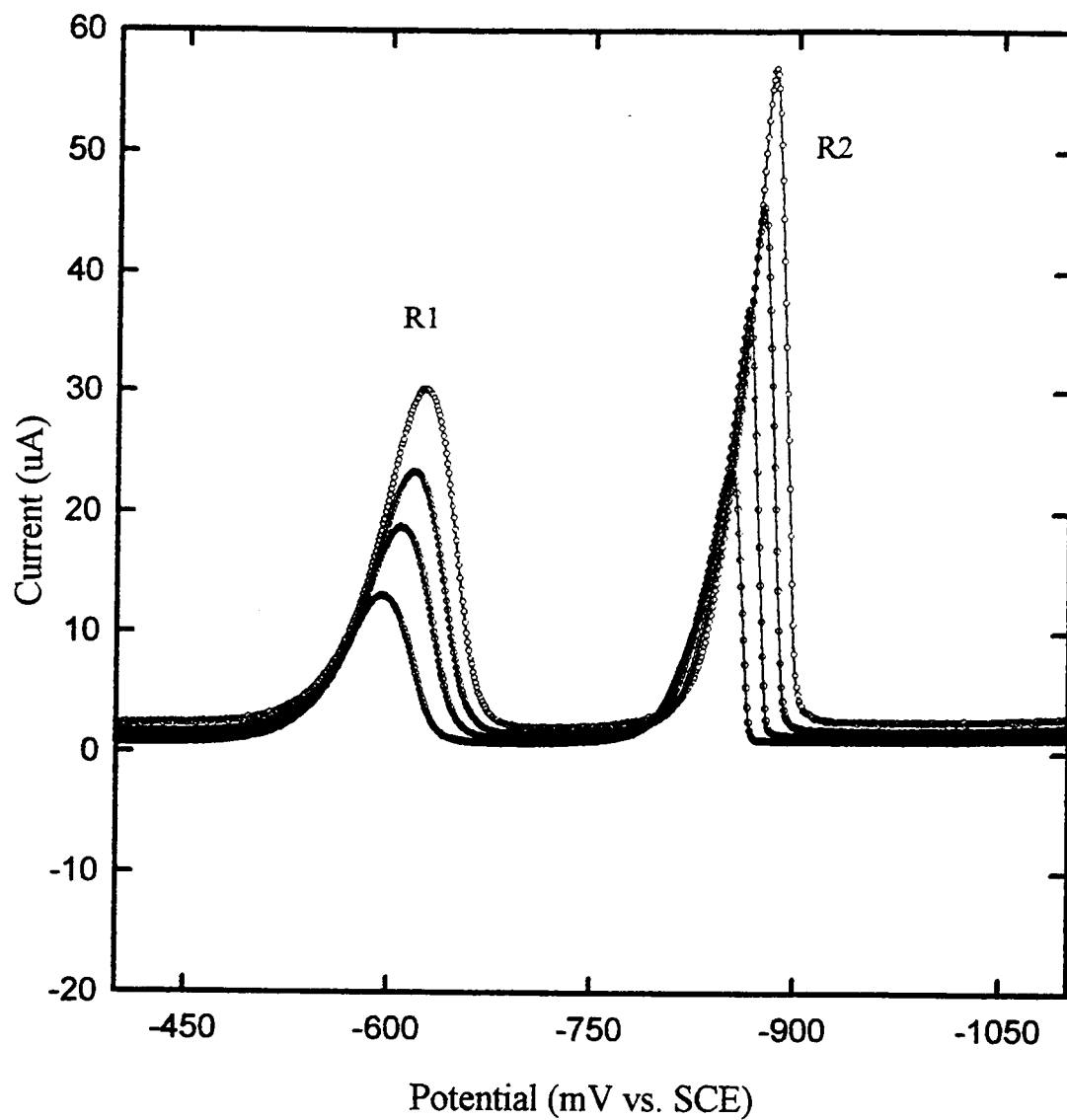


Figure 5-2: Linear sweep voltammograms of  $7 \times 10^{-5}$  M UDP-HgX in 0.1 M KCl at a  $0.0225 \text{ cm}^2$  HMDE; scan rate: 2, 3, 4, and 5 V/s.

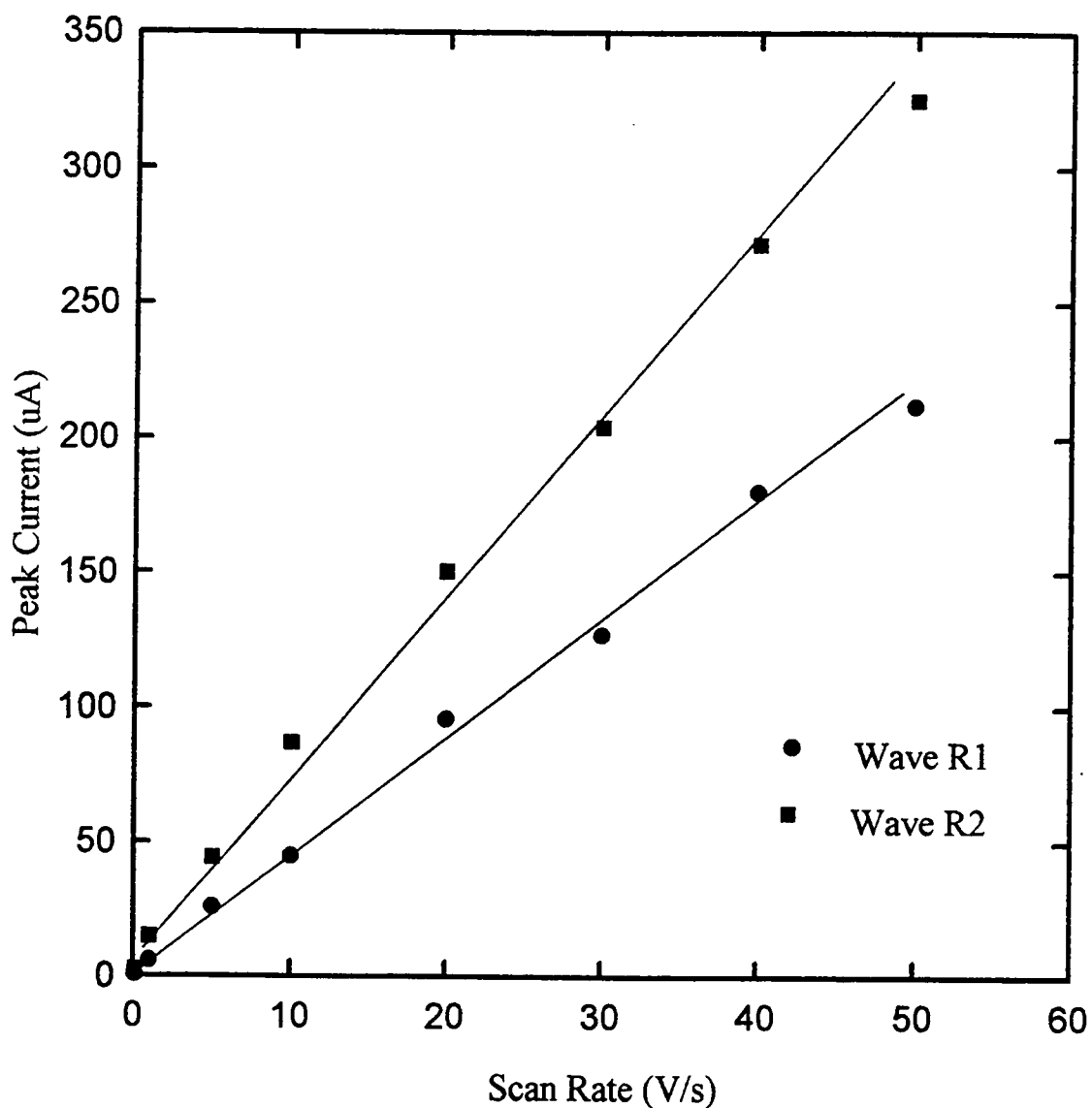


Figure 5-3. Variation of the peak currents for the R1 and R2 waves of UTP-HgX with scan rate.  $C_{\text{bulk}} = 7 \times 10^{-5} \text{ M}$ ; HMDE area:  $0.0225 \text{ cm}^2$ ; Electrolyte:  $0.1 \text{ M KCl}$ .

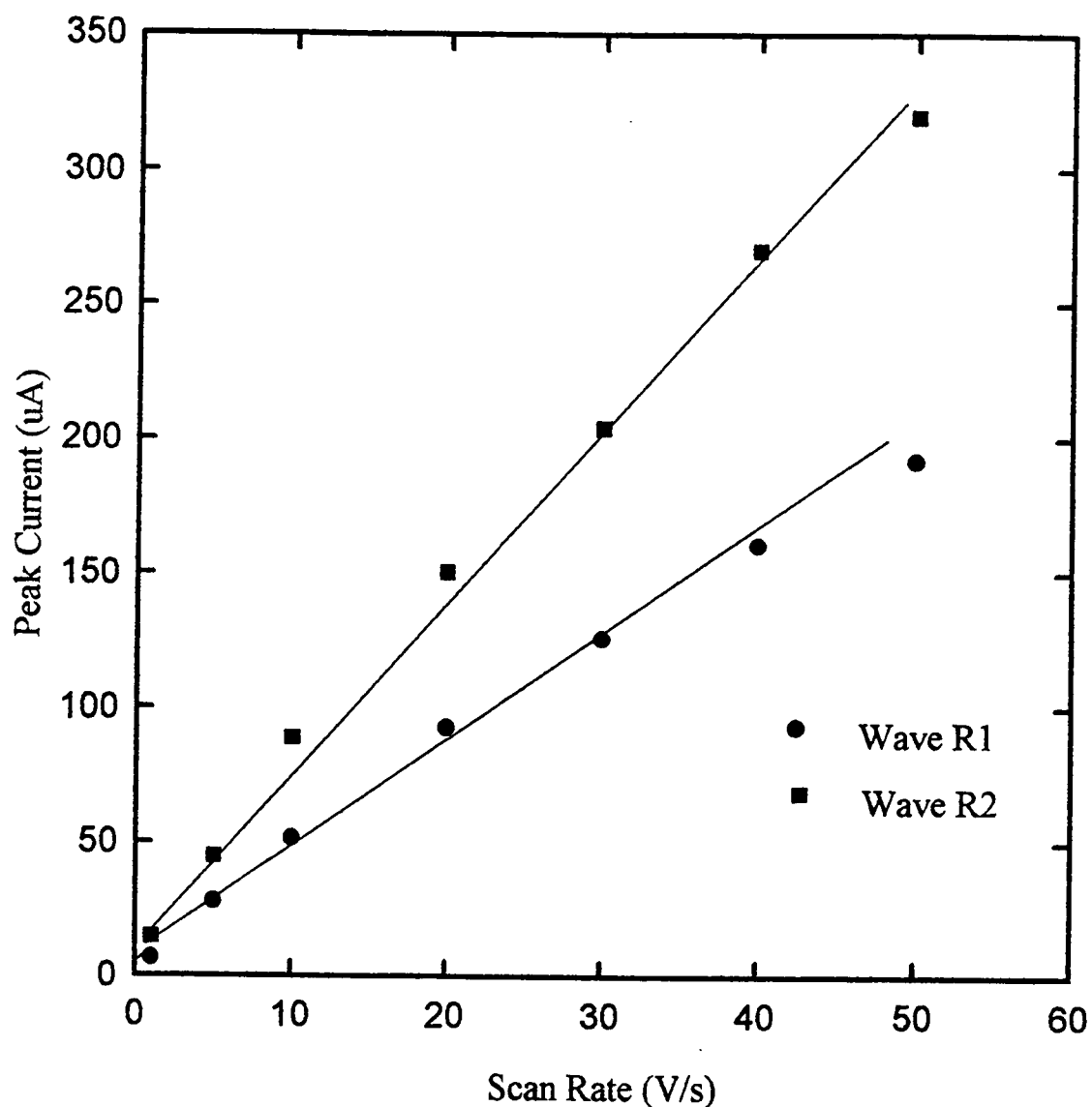


Figure 5-4. Variation of the peak currents for the R1 and R2 waves of UDP-HgX with scan rate.  $C_{\text{Bulk}} = 7 \times 10^{-5} \text{ M}$ ; HMDE area:  $0.0225 \text{ cm}^2$ ; electrolyte: 0.1 M KCl.

Table 6: Voltammetric data from linear voltammograms of  $7.0 \times 10^{-5}$  M UTP-HgX at a  $0.0225 \text{ cm}^2$  HMDE in 0.1M KCl.

| v<br>V/s | $E_{pk}(R1)$<br>mV | $E_{pk}(R2)$<br>mV | Q(R1)<br>uC | Q(R2)<br>uC | $\Delta E_{1/2}R1$<br>mV | $\Delta E_{1/2}R2$<br>mV |
|----------|--------------------|--------------------|-------------|-------------|--------------------------|--------------------------|
| 1        | -630               | -870               | 0.34        | 0.35        | 62                       | 30                       |
| 5        | -680               | -920               | 0.34        | 0.34        | 62                       | 34                       |
| 10       | -700               | -930               | 0.32        | 0.34        | 63                       | 45                       |
| 20       | -730               | -970               | 0.33        | 0.34        | 70                       | 65                       |
| 30       | -750               | -980               | 0.34        | 0.34        | 68                       | 79                       |
| 40       | -770               | -1000              | 0.34        | 0.36        | 70                       | 83                       |
| 50       | -780               | -1010              | 0.33        | 0.35        | 78                       | 86                       |

Table 7: Voltammetric data from linear voltammograms of  $7.0 \times 10^{-5}$  M UDP-HgX at a  $0.0225 \text{ cm}^2$  HMDE in 0.1 M KCl.

| v<br>V/s | $E_{pk}(R1)$<br>mV | $E_{pk}(R2)$<br>mV | Q(R1)<br>uC | Q(R2)<br>uC | $\Delta E_{1/2}R1$<br>mV | $\Delta E_{1/2}R2$<br>mV |
|----------|--------------------|--------------------|-------------|-------------|--------------------------|--------------------------|
| 1        | -610               | -860               | 0.39        | 0.40        | 62                       | 32                       |
| 5        | -670               | -910               | 0.40        | 0.40        | 63                       | 31                       |
| 10       | -690               | -930               | 0.39        | 0.40        | 64                       | 40                       |
| 20       | -720               | -960               | 0.40        | 0.40        | 64                       | 66                       |
| 30       | -740               | -970               | 0.39        | 0.39        | 66                       | 79                       |
| 40       | -750               | -990               | 0.40        | 0.41        | 69                       | 80                       |
| 50       | -760               | -1000              | 0.40        | 0.40        | 76                       | 84                       |

It was shown previously that the diffusional waves consisted of a two-electron process with the formation of the parent compound and  $\text{Hg}^0$ . This strongly suggests that waves R1 and R2 are due to one-electron processes and that wave R1 produces an intermediate species which is reduced in the second wave to the parent compound. Further evidence that wave R1 is due to a one-electron processes is demonstrated in Tables 6 and 7 where the average peak width at half the maximum current was found to be 67 mV. Theoretically, the peak width for an ideal surface wave is known to be  $90.6 \text{ mV}/n$ , hence, for a two electron process, a peak width in the vicinity of 45 mV would be obtained. The 67 mV peak width, which deviates from the ideal 90.6 mV, will be discussed shortly. In addition, the symmetrical nature of the R1 wave, shown in Figures 5-1 and 5-2, rules out the possibility that this wave arises from a process with slow electron transfer.

Although the peak width for wave R2 varies from 30 to 86 mV within the scan rate of study, this wave also appears to be due to a one-electron process because the coulombic charge under wave R2 is equivalent to that under wave R1. In addition, the wave shape of R2 is also inconsistent with slow electron transfer. Hence, the voltammetric surface behavior obtained with the mercurated UTP and UDP can be summarized as shown below in equation 5.2.



*Adsorption equilibria of the mercurated UTP and UDP.* Since the waves described above are surface in nature, it is appropriate to evaluate the adsorption equilibria at the mercury surface. The adsorption isotherms for the mercurated UTP and UDP are shown in Figure 5-5. These results indicate the formation of a saturated layer at bulk concentrations greater than  $3.0 \times 10^{-5}$  M.

The formation and study of condensed monolayers of purines and pyrimidines on mercury surfaces has been extensively studied by various authors (40, 41, 42, 43). Table 8 lists molecular areas determined by various techniques and authors for the uracil molecule adsorbed on mercury. These molecular areas cluster around 0.4-0.5 nm<sup>2</sup>/molecule and appear to correspond to a molecular orientation in which the plane of the ring is parallel to the mercury surface. With the formation of a saturated layer, it appears that the mercurated UTP and UDP also form a condensed monolayer at the mercury surface.

Surface areas calculated using the molecular modeling program PCmodel® were determined for the mercurated UTP and UDP in a variety of flat orientations. Typical values ranged between 0.76 and 0.84 nm<sup>2</sup>/molecule. From Figure 5-5,  $\Gamma_{\max}$  was determined to be 1.52 and 1.76 ( $\pm 0.04$ )  $\times 10^{-10}$  moles/cm<sup>2</sup> which correlates to surface areas of 1.10 and 0.94 nm<sup>2</sup> for the mercurated UTP and UDP. Since hydration and closest approach have not been taken into account in the above molecular modeling calculation, these results strongly suggest that the mercurated uridine nucleotides do behave like other purines and pyrimidines and form a self assembled monolayer on mercury. Additional support for this conclusion is also

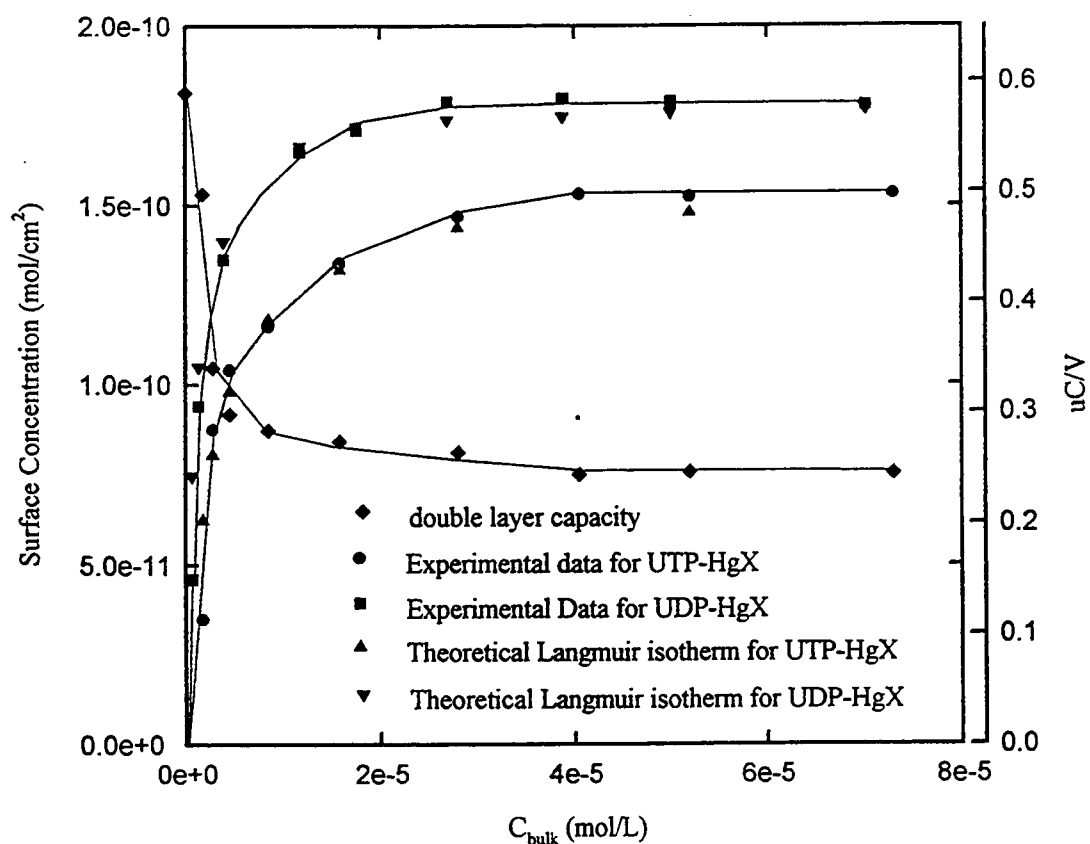


Figure 5-5: Experimental and theoretical adsorption isotherms for UTP-HgX and UDP-HgX on a HMDE. The change in double layer capacity is also illustrated for the UTP-HgX with the additional Y-axis

Table 8: Molecular surface areas (nm<sup>2</sup>/molecule) determined for the uracil molecule adsorbed on mercury.

| Technique                                    | Area       | Reference |
|----------------------------------------------|------------|-----------|
| Interfacial tension measurements             | 0.39, 0.40 | 40,41     |
| Molecular modelling with van der waals radii | 0.34       | 41        |
| Determined from crystal structure            | 0.36       | 43        |
| molecular modelling using a rectangular box  | 0.51       | 42        |

illustrated in Figure 5-5 with the double layer capacity (for the mercurated UTP) plotted as a function of the bulk nucleotide concentration. The double layer capacity was determined from the non-Faradaic current,  $i = C_{dl}v$ . As evident, in the plateau region of the isotherms, the double layer capacity is constant ( $2.5 \times 10^{-5}$  C/V). However, when the bulk concentration of the nucleotide decreases to zero, the double layer capacity decreases to  $5.8 \times 10^{-5}$  C/V. This illustrates a change in the surface structure. The decreased  $C_{dl}$  for the saturated surface is due to the lower dielectric constant of the organic layer.

Langmuir plots, constructed from the data in Figure 5-5, showed strong linear correlation for both the triphosphate and diphosphate with linear regression coefficients of 0.9996 and 0.9998 (Figure 5-6). In addition, using the experimentally determined energy parameter  $\beta_i$  and surface coverage  $\Gamma_s$ , the theoretical Langmuir isotherms for the two nucleotides were calculated and compared to the experimental data. The results, also illustrated in Figure 5-5, show a strong correlation between

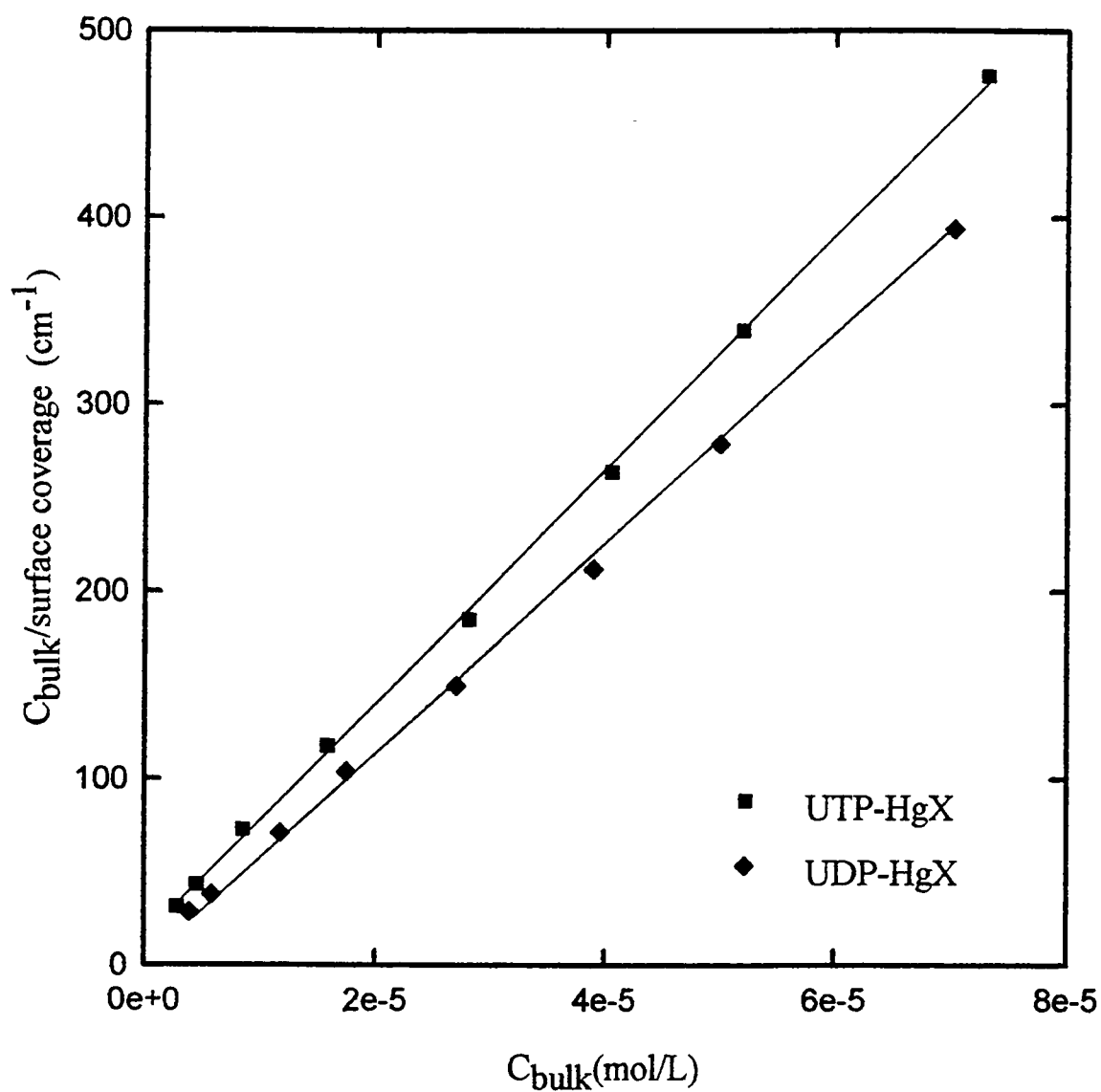


Figure 5-6: Langmuir plots for the mercurated UTP and UDP.

experimental and theoretical values. These results illustrate that the adsorption equilibria for the mercurated uridine triphosphate and diphosphate can be described by the Langmuir isotherm.

*Wave shape analysis of the R1 and R2 waves obtained with the mercurated UTP*

*and UDP.* It was shown above that the R1 wave has an average peak width at half the maximum current of 67 mV and the second wave, R2, has a peak width which varies from 30 to 80 mV within the scan rate range of study. The deviation from ideal surface behavior for the first wave, R1, does not appear to be the result of slow electron transfer because slow electron transfer would predict peak widths greater than 90.6 mV for a one-electron process. In addition, phase transistion/nucleation would predict a peak width which varies greatly with scan rate (32). Hence, the non-ideal surface behavior for wave R1 can be limited to chemical reactions following or proceeding electron transfer or interactions between absorbed molecules. However, the adsorption data demonstrated that the adsorption equilibria can be described by the Langmuir isotherm with minimal lateral interaction. This indicates that the non-ideal behavior seen with wave R1 is not the result of interactions between adsorbed molecules, but the result of a chemical reaction preceeding or following electron transfer.

A peak width of 67 mV for the R1 wave is consistent with Laviron's theory for a one-electron transfer followed up by a fast first order chemical reaction where the theoretical value is  $66 \text{ mV}/n$  (25). With the coulombic charge under wave R2

equaling that under R1 over a large scan rate range, it can be concluded that the product of this reduction is stable and surface confined. This is indicative of organomercury radicals which are produced via one-electron reduction of organomercury compounds. Hence, wave R1 can be assigned to the one-electron reduction of the adsorbed mercurated uridine triphosphate or diphosphate with the production of a radical (equation 5.3). The fast first-order chemical reaction, causing wave R1 to deviate from ideal behavior can now be assigned to the decomplexation of the radical as shown in equation 5.4. Additional support for the fast first-order reaction following electron transfer will be shown in chapter VIII where calculated voltammograms incorporating Laviron's b parameter show excellent agreement with experimental voltammograms.



Unlike wave R1, interactions between adsorbed molecules cannot be eliminated as a contribution to the non-ideal behavior seen with wave R2 because the radical is unstable in the bulk (3,4,5). This precludes obtaining an adsorption isotherm since it is impossible to reduce R-HgX in the bulk of the solution by one electron. Thus, an isotherm can not be determined for the radical and the non-ideal behavior could be a result of interactions between adsorbed molecules, phase transition/nucleation, or a chemical reaction. Although the peak width for R2 varies

strongly with scan rate, there must be a chemical reaction occurring after electron transfer. Evidence supporting this conclusion can be found in the bulk electrolysis data where protonation was observed. Thus, in accord with previously studied organomercury compounds, the R2 wave can be assigned to the one-electron reduction of the surface confined radical to the parent carbanion (equation 5.5) which reacts with H<sub>2</sub>O in a follow-up neutralization chemical reaction (equation 5.6).



The non-theoretical peak width, which varies with scan rate, appears to be the result of phase transition/nucleation accompanying the electron transfer. For example, Figures 5-7 and 5-8 illustrate the log-log plots of the peak width vs. the scan rate for the R2 waves of UTP-HgX and UDP-HgX ( $v < 5$  V/s). Although inconclusive, these plots approach linearity with slopes of 0.30 and 0.46. Camacho's theory predicts a slope of 0.40 (32). In addition, it was shown previously that the peak current of the R2 waves approaches linearity with scan rate (Figures 5-3 and 5-4). However, Camacho's theory predicts that the log of the peak current should vary linearly with the log of the scan rate with a slope of 0.60. A closer evaluation of Figures 5-3 and 5-4 does illustrate some non-linear behavior. In fact, the log-log plots for the R2 waves illustrate linearity with linear regression coefficients of 0.9972 and 0.9981. These plots are illustrated in Figure 5-9. The slopes were determined to

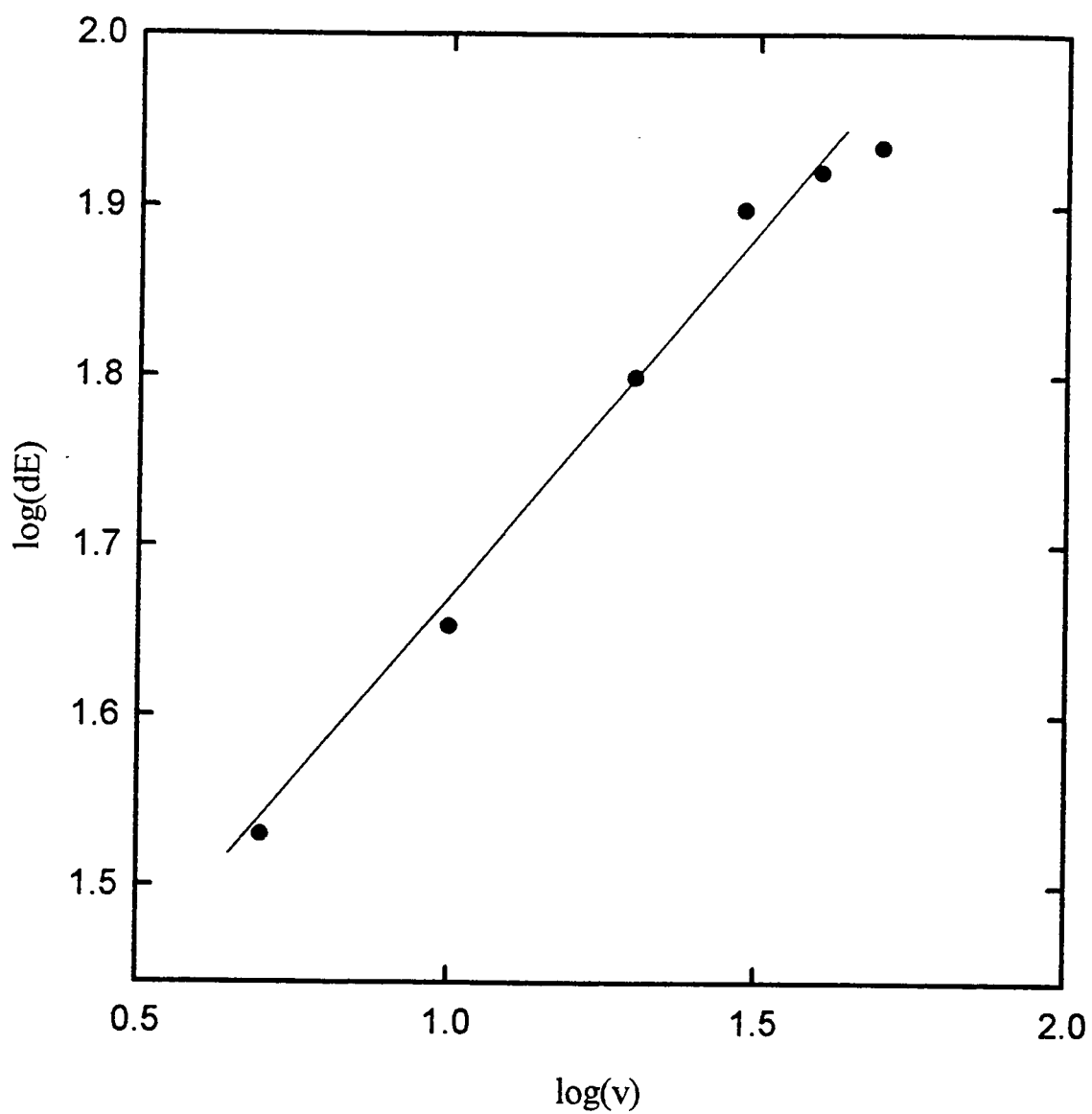


Figure 5-7: Log-log plot of the peak width vs. scan rate for the R2 wave of UTP-HgX.  $C_{\text{bulk}} = 7.0 \times 10^{-5}$  M UTP-HgX;  $0.0225 \text{ cm}^2$  HMDE; 0.1 M KCl.

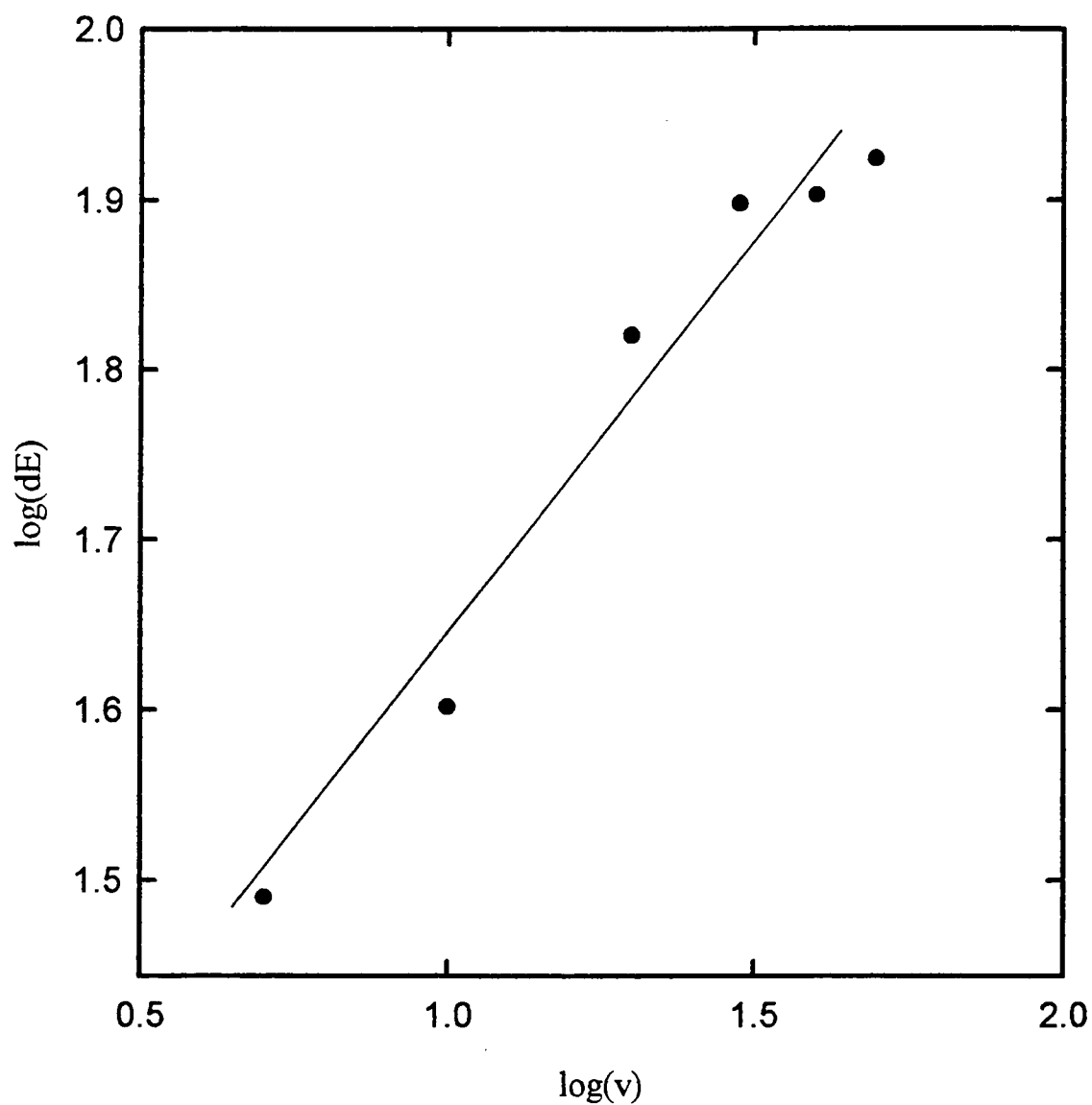


Figure 5-8: Log-log plot of the peak width vs. scan rate for the R2 wave of UDP-HgX.  $C_{\text{bulk}} = 7.0 \times 10^{-5}$  M UDP-HgX;  $0.0225 \text{ cm}^2$  HMDE; 0.1 M KCl.

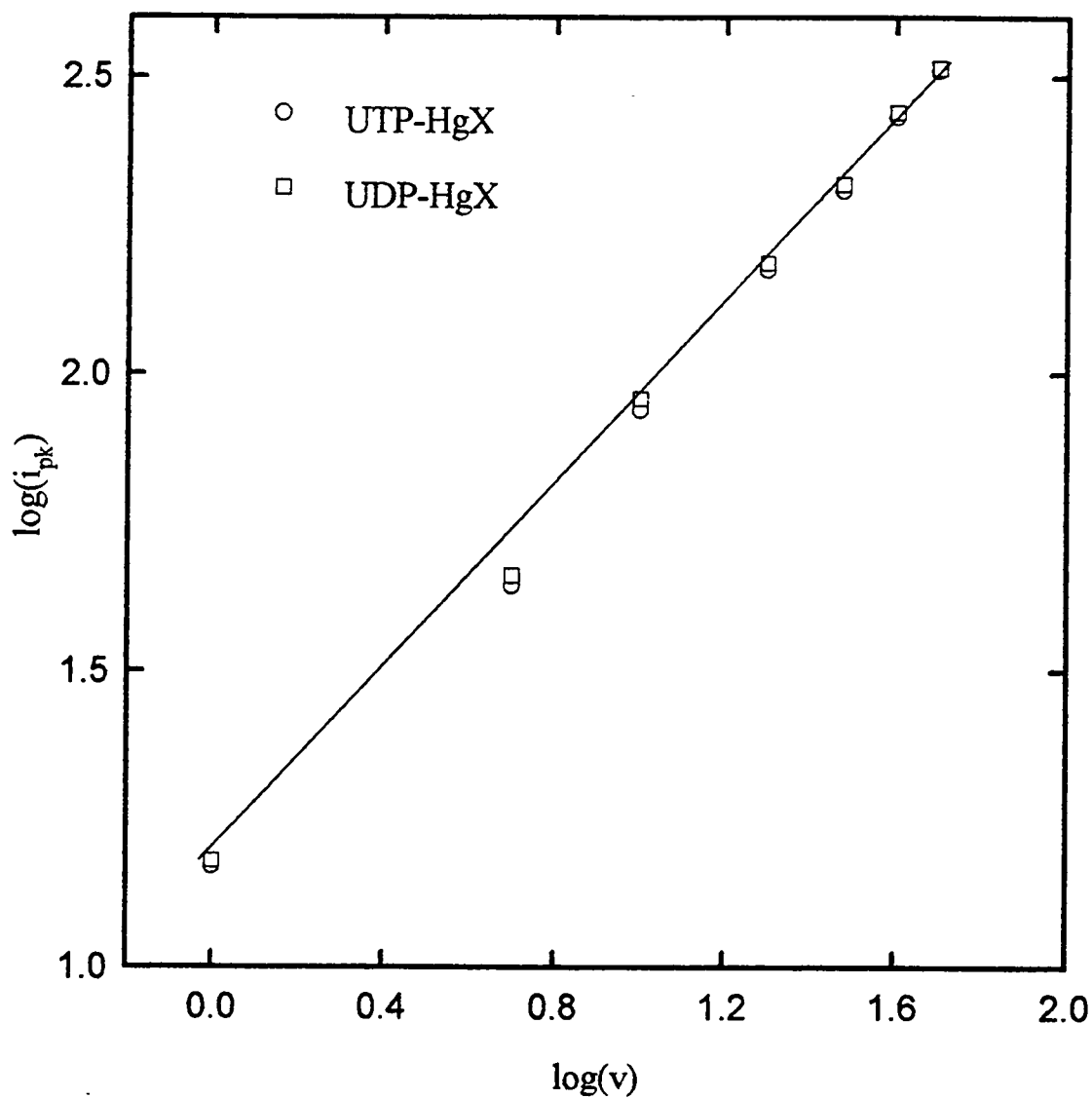


Figure 5-9: Log-Log plot of the peak current vs. the scan rate for the R2 waves of UTP-HgX and UDP-HgX. Data from Figures 5-1 and 5-2.

be 0.78 and 0.80. These values are again inconclusive, however, the chronoamperometry data given in the next section gives solid evidence of nucleation.

*Chronoamperometry varification of the phase transition/nucleation obtained with the R2 wave of the mercurated UTP and UDP.* The effect of nucleation on the chronoamperometry transient is especially diagnostic. Instead of a Cottrellian decay with  $t^{-1/2}$ , a maximum is seen during the decay. This maximum results from growth around the nucleation sites where in the early stages of growth there is an upward swing in current. At the point where the nuclei have begun to overlap the maximum is obtained. This subject has been treated thoroughly by Harrison and Thirsk in their 1971 review (44).

Two simple limiting cases are usually used to interpret the current transients; instantaneous and progressive nucleation. During instantaneous nucleation there are  $N_T$  nucleation sites initially after the potential step. Then nuclei growth follows until the growing areas collide. Progressive nucleation results when the number of nucleation sites increases with time and the original number of sites, initially after the potential step, is less than  $N_T$ . Two expressions have been commonly used to describe the response for instantaneous and progressive nucleation. These are given below in equations 5.7 and 5.8 where  $i$  is the current,  $i_m$  is the current at the maximum,  $t$  is time, and  $t_m$  is the time at the maximum. These equations will be used in the analysis of the mercurated UTP and UDP current transients.

$$i/i_m = t/t_m \exp [ -1/2 ( t^2 - t_m^2 / t_m^2 ) ] \quad \text{instantaneous} \quad 5.7$$

$$i/i_m = (t/t_m)^2 \exp [ -2/3 ( t^3 - t_m^3 / t_m^3 ) ] \quad \text{progressive} \quad 5.8$$

Figure 5-10 shows I-t curves for the mercurated UTP obtained by stepping the potential of the mercury drop electrode from -0.60 V to -0.85, -0.86, -0.87, -0.88, and -0.89 V. As shown these curves contain the nucleation indicative maximum in the transients. In Table 9 the current and time values ( $i_m$  and  $t_m$ ) are given. Figure 5-11 shows an experimental I-t curve after subtraction of the charging current, a curve produced with equation 5.6, and a curve produced with equation 5.7. The results show a very consistent fit between the experimental and progressive curve, hence giving almost conclusive evidence of nucleation via the progressive theory. The mercurated UDP also gave similar results with the progressive curve simulating the experimental curve almost perfectly (Figure 5-12). Thus, it can be concluded that the non-ideal behavior seen with the R2 waves of the mercurated UTP and UDP is the result of phase transition/nucleation. The origin of the nucleation could be the result of the metallic mercury joining with the HMDE or the desorption of the organic component.

*Linear sweep voltammetry of the mercurated UMP.* Figure 5-13 illustrates typical voltammograms of the UMP-HgX carried out under the same conditions as that of UTP-HgX and UDP-HgX. These voltammograms also show two reduction processes labelled R1 and R2. Table 10 summarizes the experimental data.

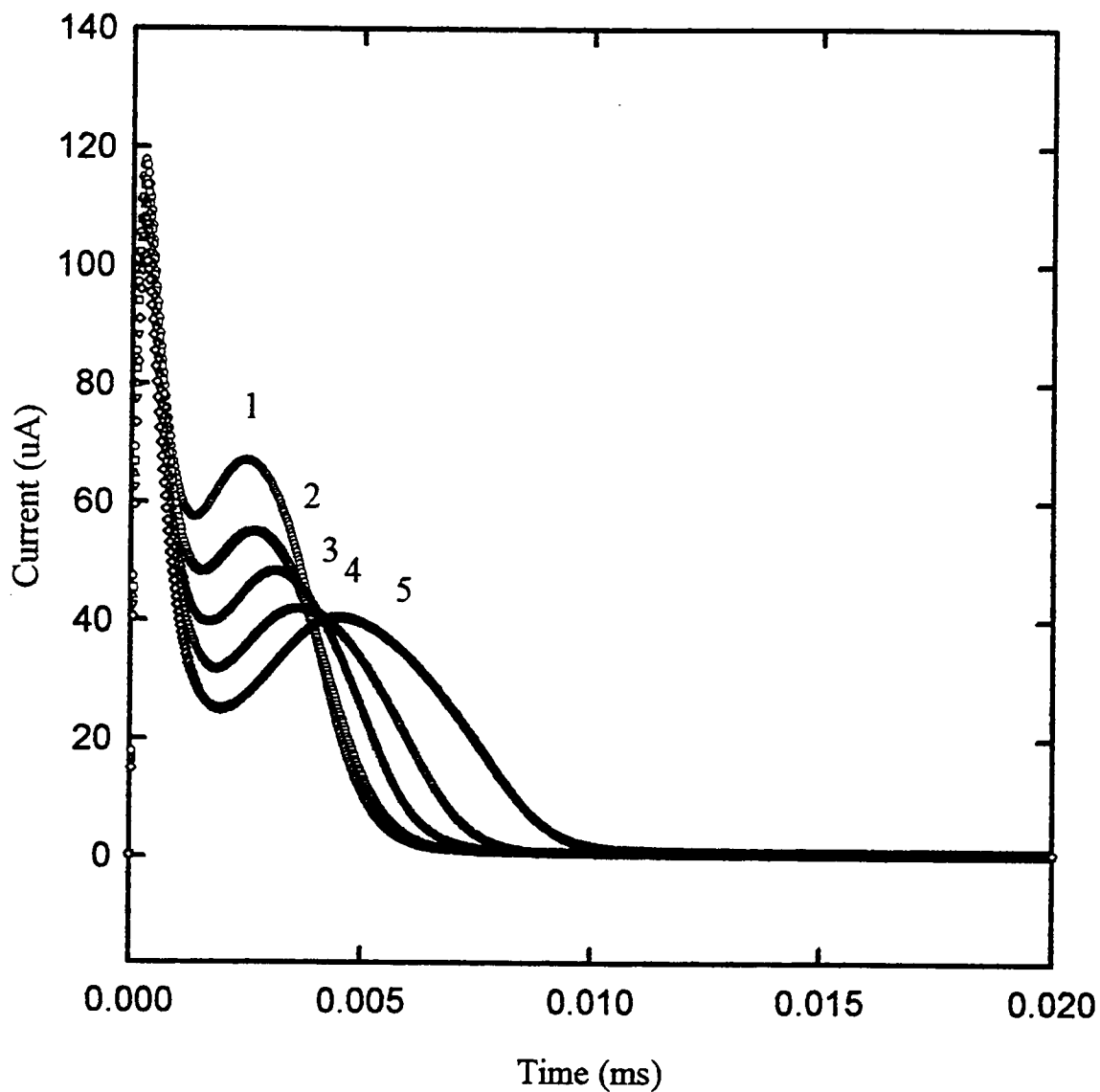


Figure 5-10: Experimental current-time curves for the mercurated UTP in 0.1 M KCl. The potential step consisted of a step from -0.6 V to 1: -0.89 V; 2: -0.88 V; 3: -0.87 V; 4: -0.86 V; 5: -0.85 V. HMDE area = 0.0225 cm<sup>2</sup>.

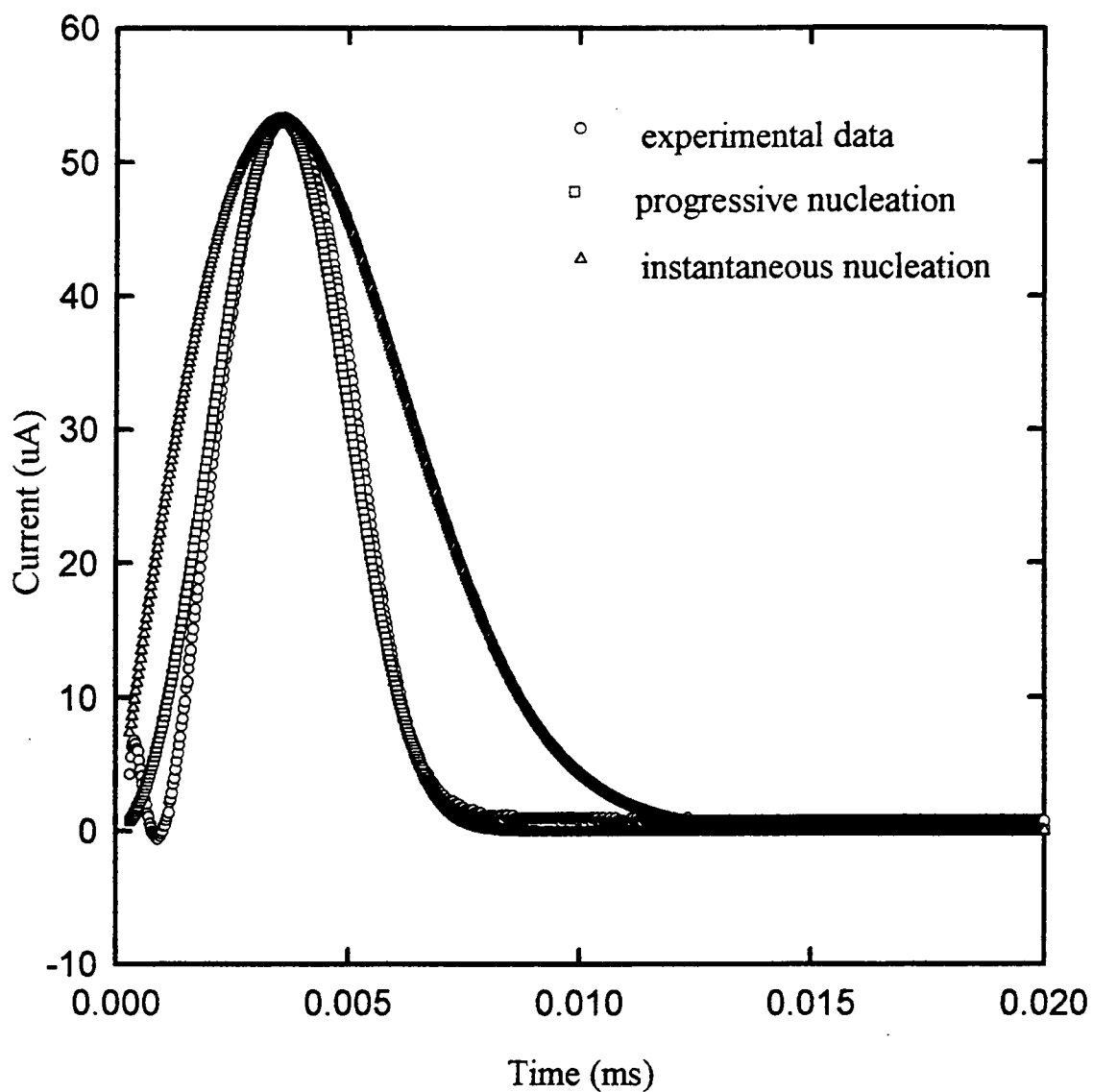


Figure 5-11: Comparison of the experimental current-time transient obtained from Figure 5-10 (curve 1) and theoretical current transients for both progressive and instantaneous nucleation.

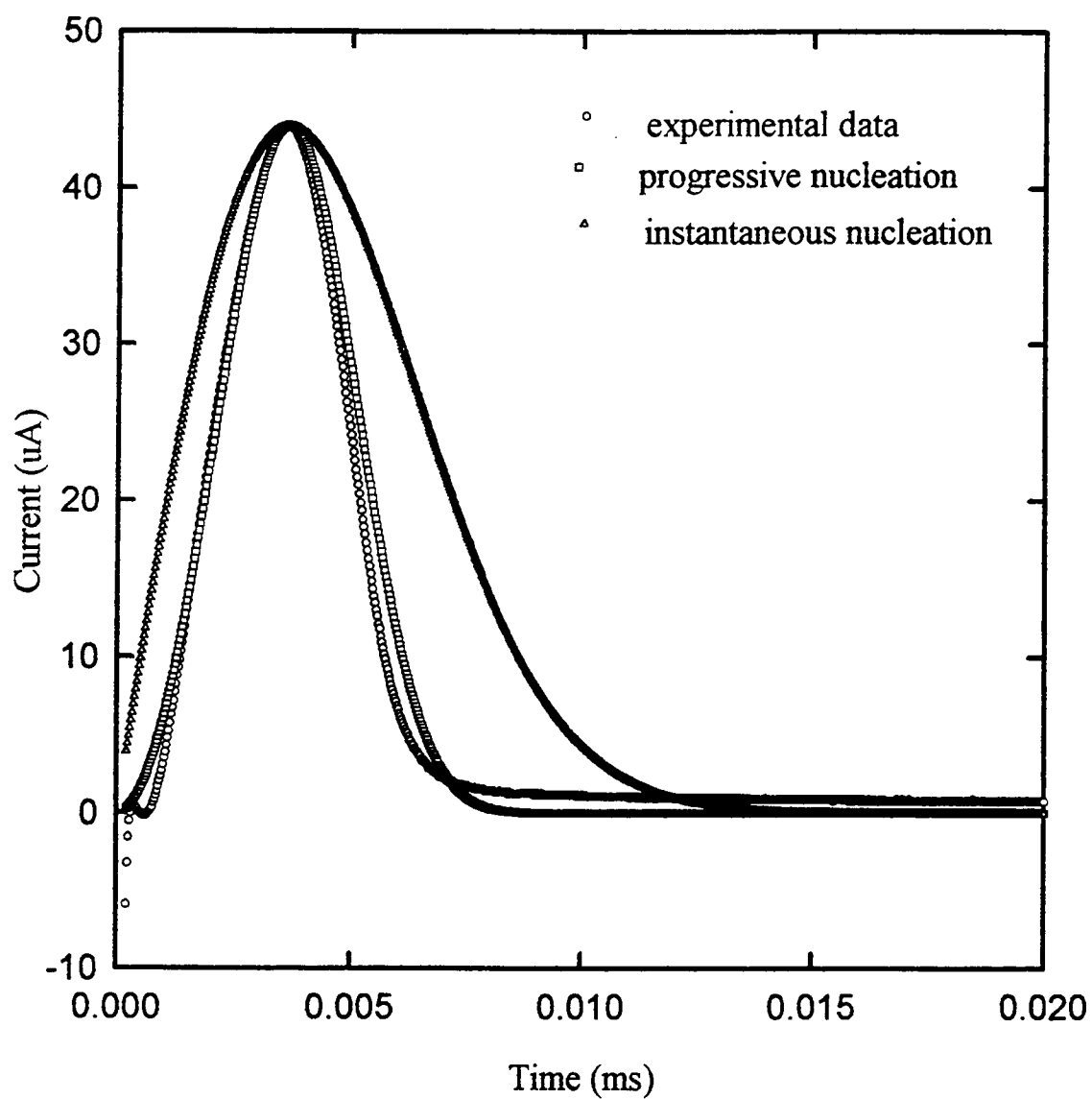


Figure 5-12: Comparison of the experimental current-time transient obtained with the mercurated UDP and the theoretical current transients for both progressive and instantaneous nucleation.

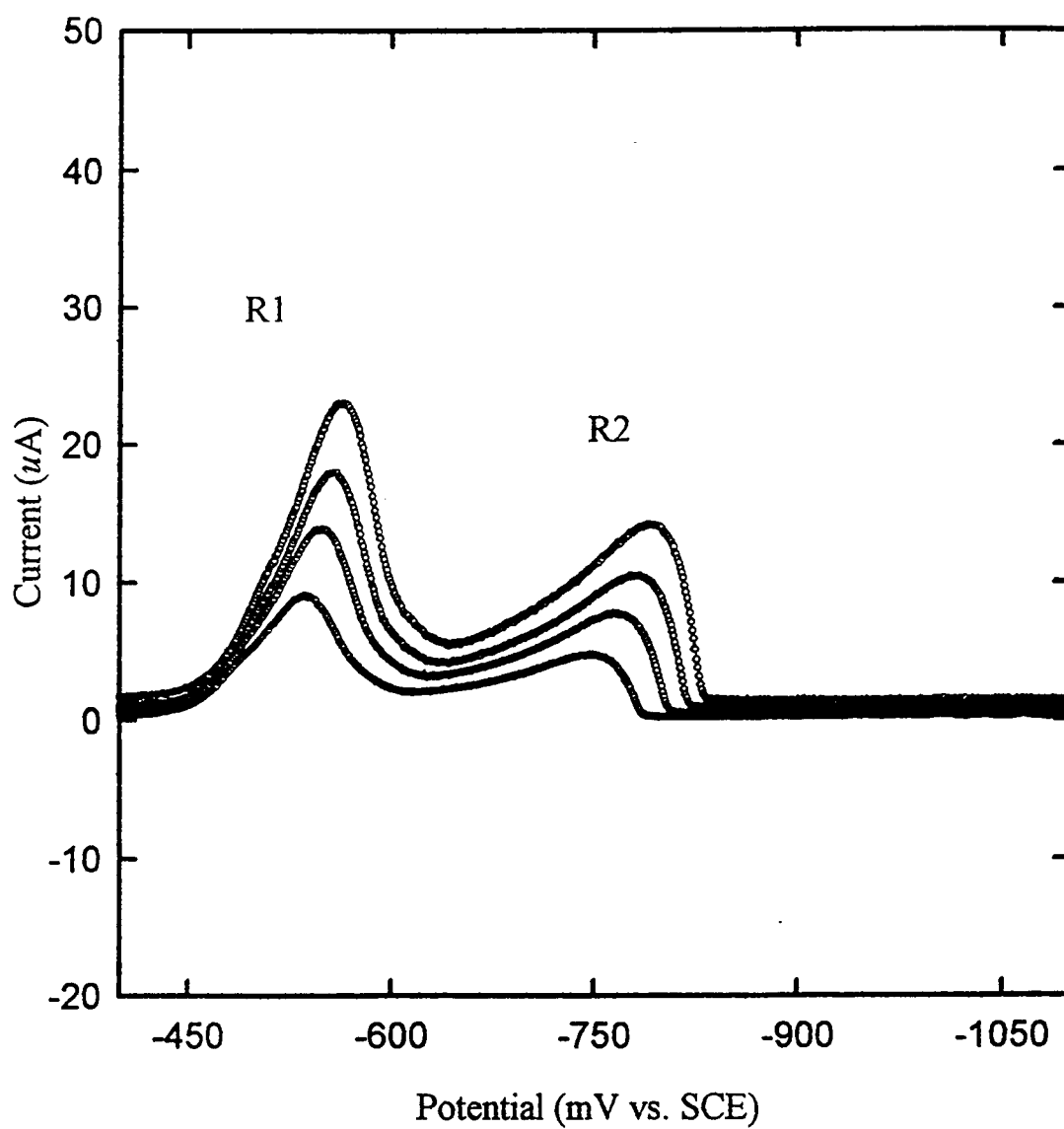


Figure 5-13: Linear sweep voltammograms of  $7 \times 10^{-5}$  M UMP-HgX in 0.1 M KCl at a  $0.0225 \text{ cm}^2$  HMDE; scan rates: 2, 3, 4, and 5 V/s.

Table 9: Current and time values at the maximum of the chronoamperometry transients of Figure 5-10.

| Potential Step (mV) | $i_m$ (uA) | $t_m$ (ms) |
|---------------------|------------|------------|
| -0.60 to -0.85      | 40.4       | 4.58       |
| -0.60 to -0.86      | 42.4       | 3.70       |
| -0.60 to -0.87      | 47.9       | 3.13       |
| -0.60 to -0.88      | 55.2       | 2.70       |
| -0.60 to -0.89      | 67.3       | 2.50       |

Table 10: Voltammetric data from linear voltammograms of  $7.0 \times 10^{-5}$  M UMP-HgX in 0.1 M KCl with a  $0.0225 \text{ cm}^2$  HMDE.

| $v$<br>V/s | $E_{pk}(R1)$<br>mV | $E_{pk}(R2)$<br>mV | $Q(R1)$<br>uC | $Q(R2)$<br>uC | $\Delta E_{1/2}R1$<br>mV | $\Delta E_{1/2}R2$<br>mV |
|------------|--------------------|--------------------|---------------|---------------|--------------------------|--------------------------|
| 1          | -565               | -740               | 0.42          | 0.27          | 84                       | -                        |
| 5          | -600               | -800               | 0.40          | 0.29          | 90                       | -                        |
| 10         | -630               | -854               | 0.45          | 0.32          | 85                       | 110                      |
| 20         | -660               | -880               | 0.40          | 0.32          | 90                       | 119                      |
| 30         | -685               | -915               | 0.45          | 0.35          | 92                       | 122                      |
| 40         | -700               | -944               | 0.43          | 0.37          | 95                       | 123                      |
| 50         | -720               | -962               | 0.44          | 0.39          | 115                      | 138                      |

As with the mercurated triphosphate and diphosphate, the wave shape and coulombic charge of wave R1 for the monophosphate are independent of scan rate and the peak height approaches linearly with scan rate over a range of 0.10 to 50 V/s (Figure 5-14). In contrast to the triphosphate and diphosphate voltammetry, however, the peak height for R2 does not vary linearly with scan rate and the coulombic charge under R2 is dependent on scan rate. In fact, at lower scan rates than that shown in Figure 5-13, R2 is not present, and only at scan rates greater than 50 V/s do the coulombic charges under R1 and R2 become equal.

*Effect of temperature on the coulombic charges under the R1 and R2 waves of the mercurated UMP.* Temperature had a strong influence on the coulombic charge under R2 of the mercurated UMP. For example, Table 11 illustrates the effect of temperature on the ratio of the coulombs under wave R2 to that under wave R1. The coulombic charge under wave R1 was essentially constant throughout this temperature range; thus, it is obvious that the coulombic charge under wave R2 increases significantly with decreasing temperature.

This behavior can be the result of either another follow-up chemical reaction (EC) where the radical, generated after electron transfer (R1), undergoes a chemical reaction producing a species that is not reducible under these voltammetric conditions or diffusion of the radical monophosphate from the mercury surface. With no significant diffusion of the radical triphosphate and diphosphate from the mercury surface under the same conditions, it is not likely that diffusion of the radical monophosphate occurs significantly during the time frame of the experiment. The

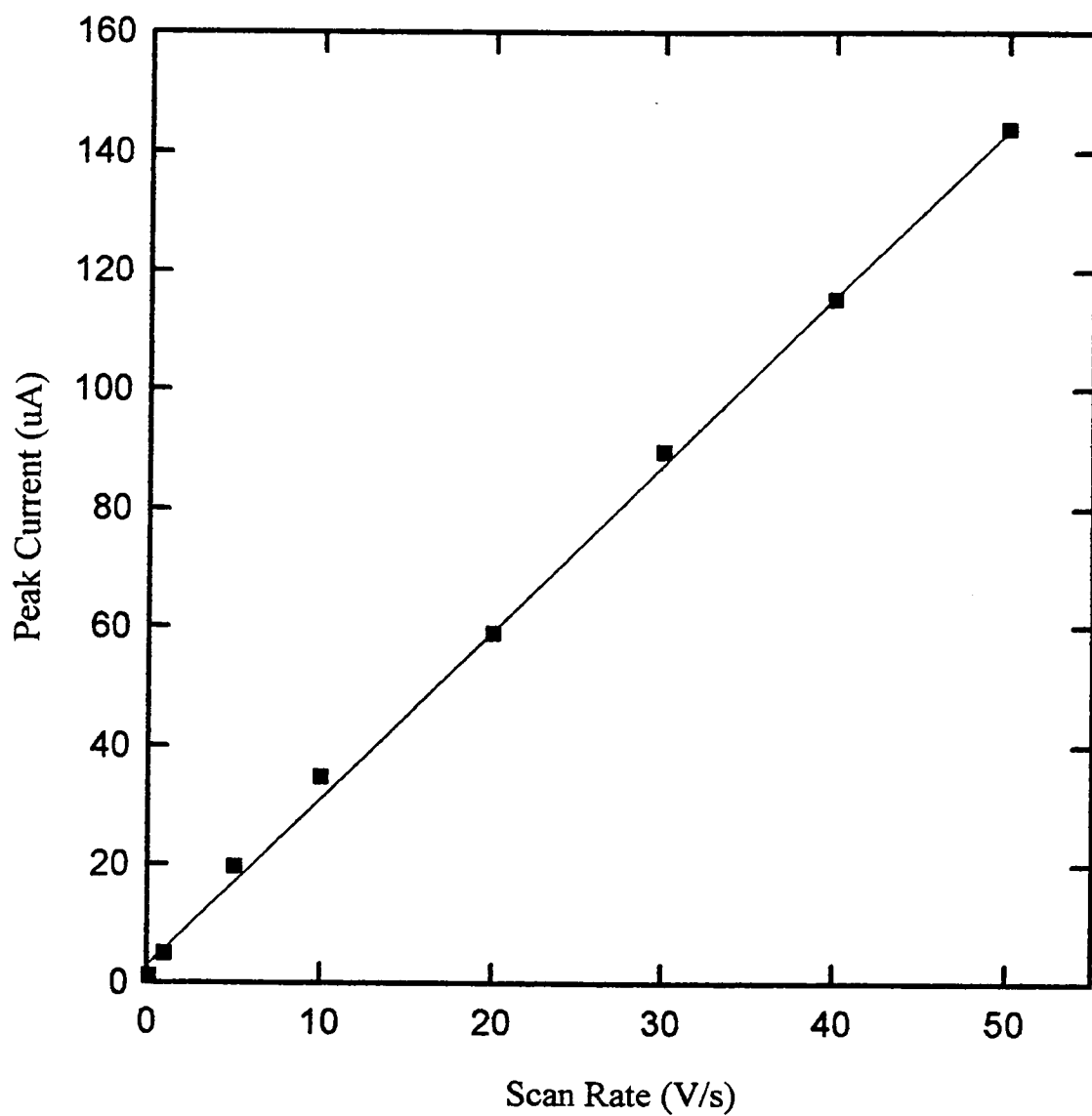
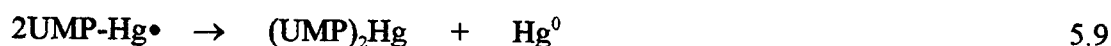


Figure 5-14: Variation of the peak current for the R1 wave of UMP-HgX with scan rate.  $C_{\text{bulk}} = 7.0 \times 10^{-5} \text{ M}$ ; HMDE area =  $0.0225 \text{ cm}^2$ ;  $0.1 \text{ M KCl}$

Table 11: Variation in coulombic charge ratios with temperature.  $C_{\text{bulk}} = 8.0 \times 10^{-5}$  M; Electrode area =  $0.0225 \text{ cm}^2$ ; 0.1 M KCl;  $V = 1 \text{ V/s}$ .

| °C              | 35   | 30   | 25   | 20   | 15   | 10   | 5    |
|-----------------|------|------|------|------|------|------|------|
| $Q_{R2}/Q_{R1}$ | 0.24 | 0.42 | 0.49 | 0.63 | 0.77 | 0.88 | 0.92 |

most likely reason for the scan rate dependence on the coulombic charge under R2 is a follow-up chemical reaction. This is supported by the electrochemical literature on organomercury compounds in which the radical (R-Hg•) intermediate either is reduced with the cleavage of the C-Hg bond in the second wave or undergoes disproportionation to produce a R-Hg-R species. Thus, the mercurated UMP after electron transfer, appears to undergo disproportionation (dimerization), which is sluggish on the time scale of the experiment in addition to possible decomplexation. The proposed reaction is given by equation 5.9.



*Adsorption equilibria of the mercurated UMP.* The adsorption isotherm for the mercurated UMP is shown in Figure 5-15.  $\Gamma_{\text{max}}$  was determined to be  $1.87 \times 10^{-10} \text{ mol/cm}^2$  which correlates to a surface area of  $0.89 \text{ nm}^2$  per molecule. Although the Langmuir plot for the above data demonstrated good correlation ( $R^2 = .9960$ ), the theoretical Langmuir isotherm, generated from the experimentally determined energy parameter and saturated surface coverage, indicated that the experimental isotherm

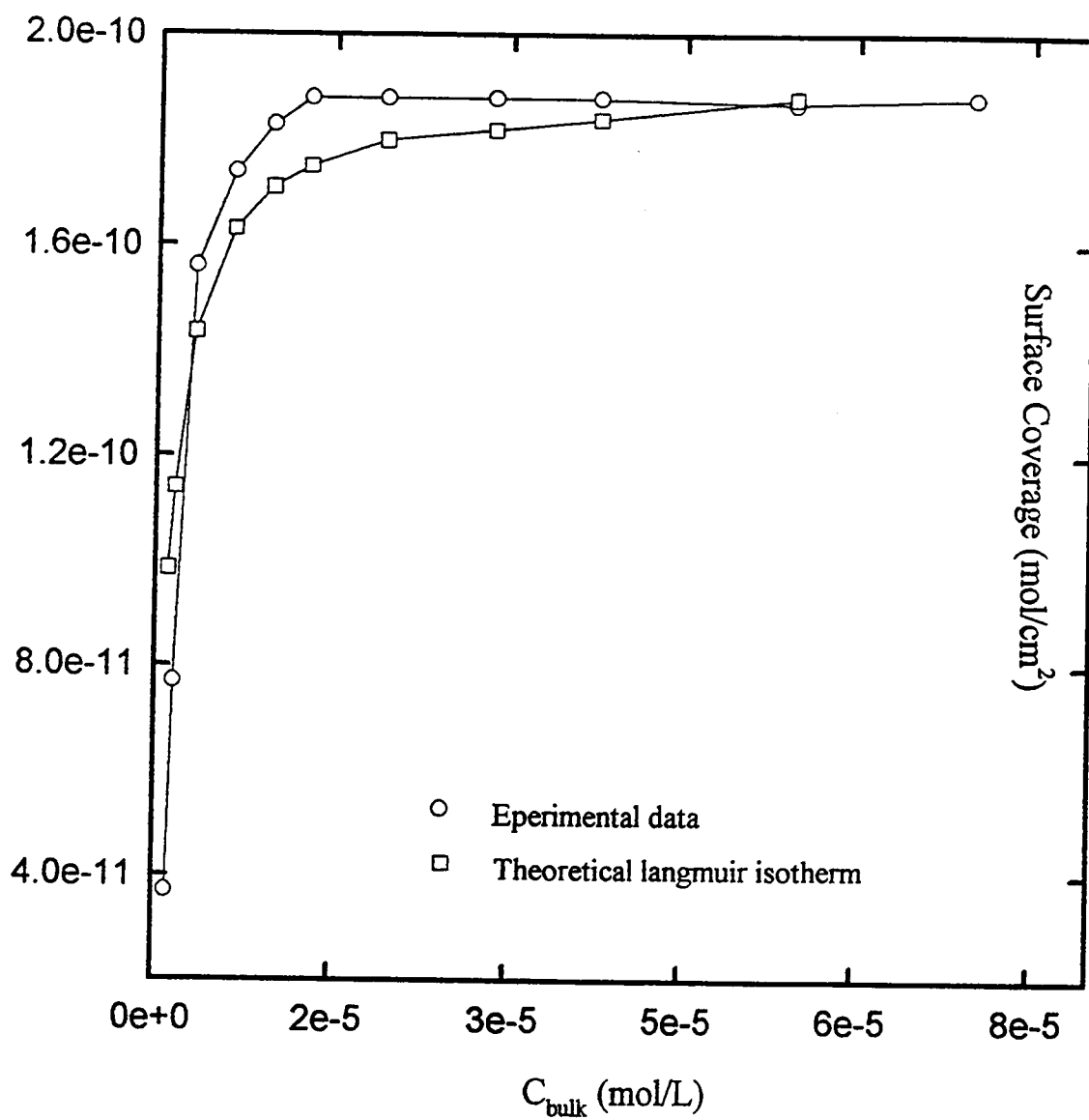


Figure 5-15: Experimental and theoretical isotherms for UMP-HgX on a HMDE.

contained a small positive deviation from true Langmuir behavior (Figure 5-15).

This suggests that there is some attractive interaction between the adsorbed monophosphate molecules. This result may give some insight on why dimerization is seen for only the mercurated UMP species. The only difference between the three uridine species is the length of the phosphate arm. The longer phosphate arm must block an adjacent molecule from reaching a distance which supports dimer formation. UMP-HgX, with its shorter phosphate arm allows this closer approach, apparently with some attractive interaction.

Hence, for the mercurated UMP, the R1 wave again corresponds to the one-electron reduction of the mercurated UMP to the surface confined radical where fast decomplexation of the radical probably follows electron transfer. The second wave, R2, corresponds to the one-electron reduction of the radical to the carbanion where neutralization of the carbanion occurs. Differing from the mercurated UTP and UDP, however, another chemical step, disproportionation, competes with R2 (equation 5.9) and changes the voltammetry significantly. In addition, it appears that the phase transition/nucleation seen with the R2 wave of the mercurated UTP and UDP does not occur with the mercurated UMP under the conditions of this study. Not only is this evident from the voltammetry data, but this is also evident from chronoamperometry conducted as described previously. For example, Figure 5-16 illustrates a typical I-t curve for the mercurated UMP where the potential was first held in the region of R1 for 50 ms and then stepped past R2. As shown there is no evidence of nucleation in this I-t curve.

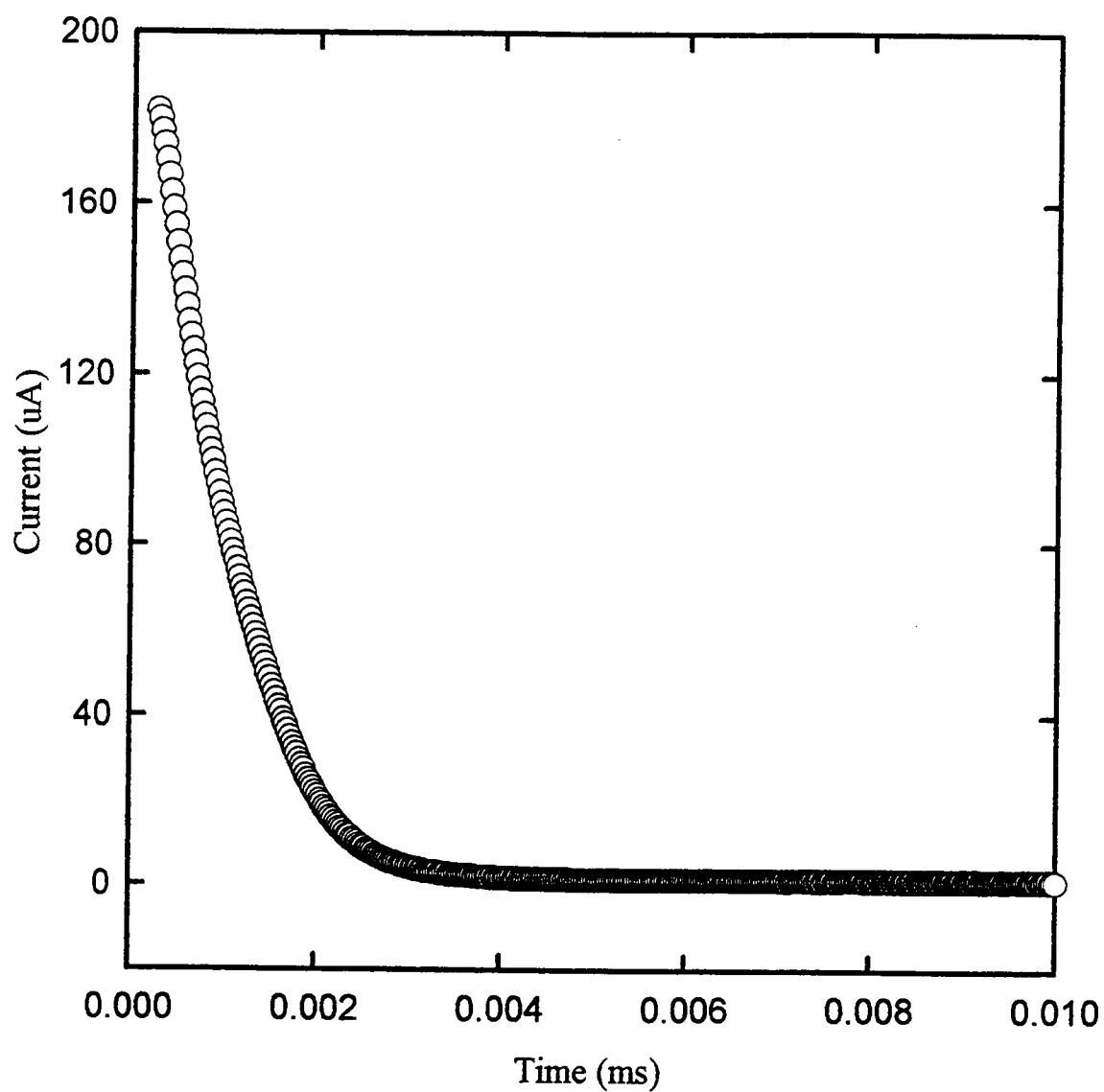


Figure 5-16: Experimental current-time curve for the mercurated UMP in 0.1 M KCl. The potential step consisted of a step from -0.58 V to -0.79 V.

## CHAPTER VI

### Cyclic Voltammetry of the Mercurated Nucleotides and the Determination of the Complexation State of the Mercurated Nucleotides in 0.1 M KCl

#### Introduction

In the previous chapter it was shown that the mercurated uridine nucleotides, in agreement with previously studied organomercury compounds, adsorb and form a saturated layer at the mercury electrode. Under conditions where diffusion was minimal, the application of a cathodic sweep produced two reduction waves, R1 and R2. Wave R1 resulted from the one-electron reduction of the mercurated uridine mono-, di-, or triphosphate to a surface confined radical. The second wave, R2 was the result of the one-electron reduction of the radical to the parent compounds.

It was also shown that the non-ideal behavior seen with the R1 waves of the mercurated UTP and UDP was the result of a follow-up chemical reaction, "decomplexation". Although the evidence wasn't as compelling, it was also concluded that decomplexation probably occurs with the R1 wave of the mercurated UMP. In this chapter, cyclic voltammetry conducted to gain a greater understanding of the decomplexation will be described. This will be carried out by reversing the potential sweep after the R1 wave for all three nucleotides. In addition, both phosphorous NMR and spectroscopy in the ultraviolet/visible range were used to study the complexation of the mercurated nucleotides.

## Experimental

The voltammetry in this section was conducted as previously described with a bulk nucleotide concentration in the vicinity of  $7.0 \times 10^{-5}$  M, scan rates greater than 1 V/s, and a supporting electrolyte of 0.1 M KCl. A ten second waiting time at open circuit was again utilized to allow for the complete formation of the self assembled surface layer. The potential at sweep reversal depended on the nucleotide and scan rate and generally was initiated when the faradaic current dropped below 1 percent of the R1 maximum current.

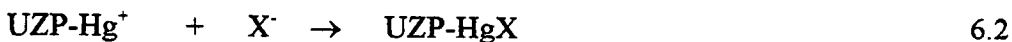
Phosphorous NMR was conducted with  $D_2O$  as the solvent. Typically, 10 mg of the nucleotide of interest was dissolved in 0.5 ml of  $D_2O$  for analysis. The cell consisted of a 1 cm<sup>3</sup> quartz tube. Reported chemical shifts are given with respect to an external standard of 0.1 M phosphoric acid which was assigned a value of 0.0 ppm. To take into account drift, the standard was analyzed before each new sample.

The ultraviolet/visible (UV/VIS) spectra of the mercurated nucleotides was conducted with  $7.0 \times 10^{-5}$  M UZP-HgX in KCl. (0.5 to 3.0 M). An appropriate KCl blank was subtracted from each spectrum before analysis. Reported absorbances are given at  $\lambda_{max}$  (260 nm).

## Results and Discussion

*Cyclic voltammetry of the mercurated UTP and UDP.* Figures 6-1 and 6-2 illustrate the effect of reversing the potential sweep after the first wave R1 for the mercurated uridine triphosphate and diphosphate. The results show that an anodic

wave (Ox1) appears around -100 mV which is not present if the cathodic sweep is swept through wave R2. Thus, the Ox1 wave appears to be coupled to the more positive of the cathodic waves, R1. This Ox1 wave corresponds to wave B of Figures 4.1 and 4.2. Tables 12 and 13 summarize the voltammetric data as a function of the sweep rate for the mercurated UTP and UDP. From these tables, it is seen that the peak separation between R1 and Ox1 increases with scan rate. More importantly, the data in Tables 12 and 13 show that the coulombic charges under the R1 and Ox1 waves were equivalent. This strongly suggests that the new wave, Ox1, is also a one-electron process. In addition, since the Ox1 wave is coupled to the R1 wave, Ox1 can now be assigned to the one-electron oxidation of the radical to regenerate UTP-HgX or UDP-HgX via equation 6.1 and 6.2 where equation 6.2 describes a follow-up complexation step which must be present if the nucleotide is truly regenerated. Multiple scans showed this to be true by the reappearance of the R1 wave on cathodic sweeps.



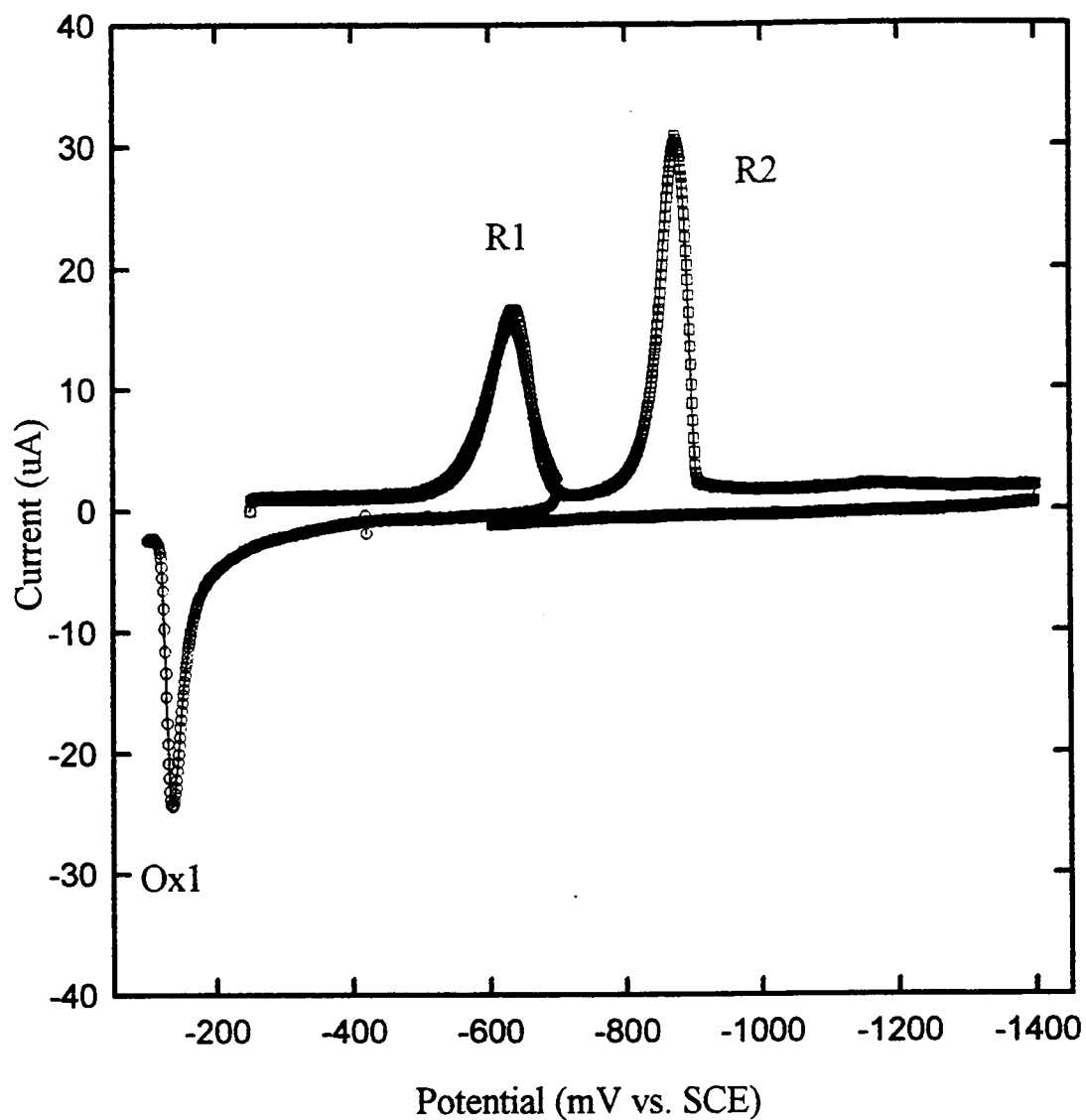


Figure 6-1. Voltammograms of  $7 \times 10^{-5}$  M UTP-HgX in 0.1 M KCl with a  $0.0225 \text{ cm}^2$  HMDE and a scan rate of 5 V/s.

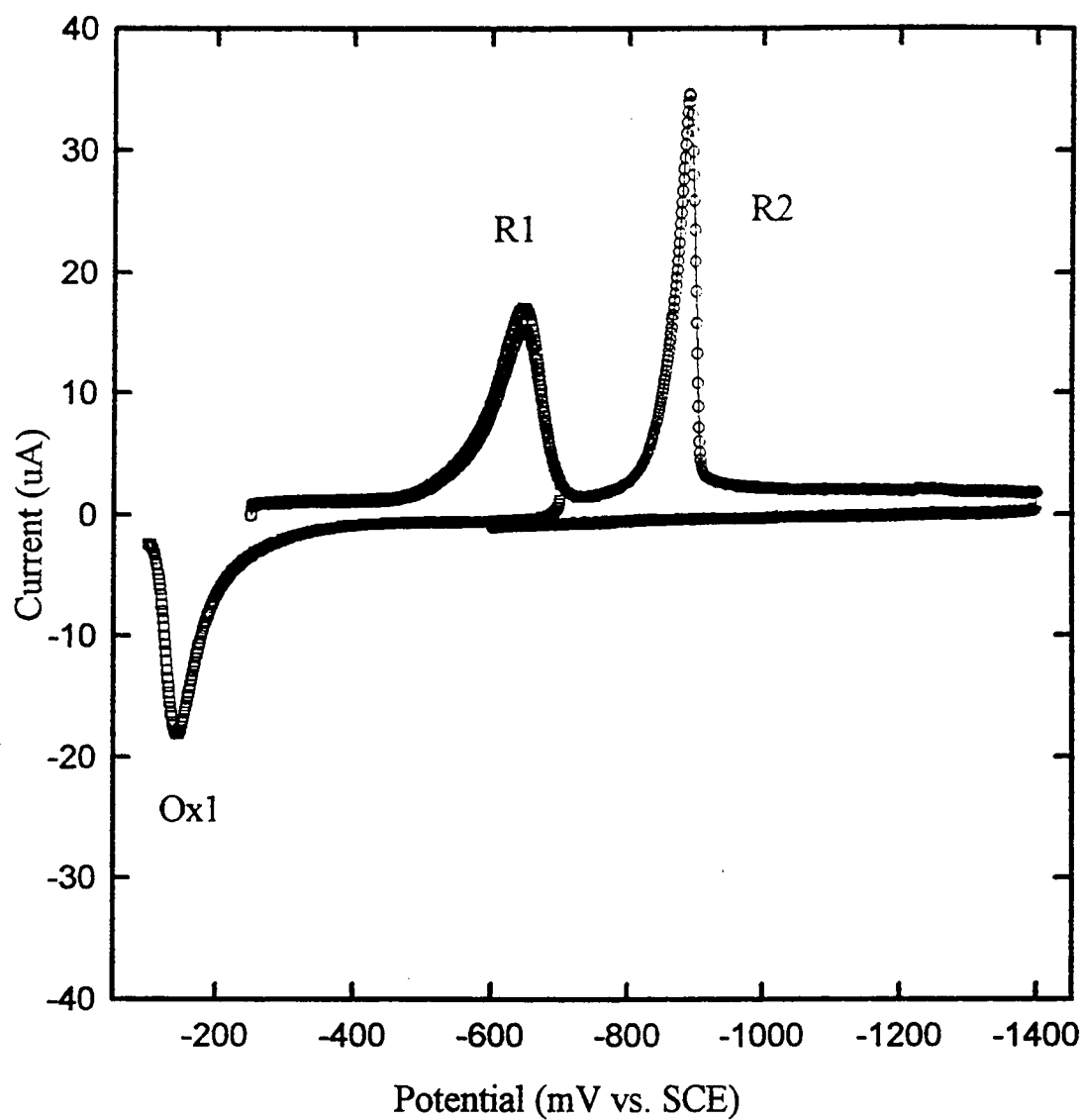


Figure 6-2: Voltammograms of  $7 \times 10^{-5}$  M UDP-HgX in 0.1 M KCl at a  $0.0225 \text{ cm}^2$  HMDE and a scan rate of 5 V/s.

Table 12: Voltammetric data from cyclic voltammograms of  $7 \times 10^{-5}$  M UTP-HgX in 0.1 M KCl with a  $0.0225 \text{ cm}^2$  HMDE.

| scan rate<br>V/s | $E_{pk}(R1)$<br>mV | $E_{pk}(Ox1)$<br>mV | $\Delta E_{pk}^{R1/Ox1}$<br>mV | $Q_{Ox1}/Q_{R1}$ |
|------------------|--------------------|---------------------|--------------------------------|------------------|
| 1                | -630               | -210                | 420                            | 1.00             |
| 5                | -680               | -170                | 500                            | 1.00             |
| 10               | -700               | -140                | 550                            | 0.98             |
| 20               | -730               | -120                | 595                            | 1.00             |
| 30               | -750               | -110                | 635                            | 1.00             |
| 40               | -770               | -100                | 660                            | 1.02             |
| 50               | -780               | -080                | 690                            | 1.00             |

Table 13: Voltammetric data from cyclic voltammograms of  $7 \times 10^{-5}$  M UDP-HgX in 0.1 M KCl with a  $0.0255 \text{ cm}^2$  HMDE.

| scan rate<br>V/s | $E_{pk}(R1)$<br>mV | $E_{pk}(Ox1)$<br>mV | $\Delta E_{pk}^{R1/Ox1}$<br>mV | $Q_{Ox1}/Q_{R1}$ |
|------------------|--------------------|---------------------|--------------------------------|------------------|
| 1                | -610               | -180                | 435                            | 0.89             |
| 5                | -670               | -160                | 525                            | 0.97             |
| 10               | -690               | -130                | 570                            | 1.00             |
| 20               | -720               | -110                | 620                            | 1.00             |
| 30               | -740               | -090                | 660                            | 0.98             |
| 40               | -750               | -080                | 680                            | 1.00             |
| 50               | -760               | -060                | 710                            | 1.00             |

*Determination of ligand X for the mercurated UTP and UDP.* So far, it was determined that the waves R1 and Ox1 consist of an electron transfer step followed by complexation or decomplexation of ligand X, but the identity of the ligand has not been assigned. It was initially assumed that the identity of the ligand was chloride because chloride was present in solution a factor of up to  $10^4$  times the concentration of the nucleotide. However, some of the voltammetric behavior did not correlate with this conclusion. For example, there was not a significant variation in voltammetry with supporting electrolyte concentration ( $\text{Cl}^-$ ). Thus, an electrolyte study was undertaken using the mercurated-uridine triphosphate in an effort to gain insight on this question. Cyclic voltammograms were carried out in 0.1 M of each of the electrolytes listed in Table 14. The results, illustrate that there is only a small variation with peak potential with the electrolyte anion and all show a large potential difference between R1 and Ox1. In addition, the voltammetric peak shapes did not change with the electrolyte anion. It is also interesting to note that even in noncomplexating electrolytes such as perchlorate or fluoride, the voltammetry does not change significantly. However, there does appear to be a variation with the electrolyte cation. This may be the result of change in the double layer structure or the formation of ion-pairs with the cation of the supporting electrolyte. This effect was not studied further.

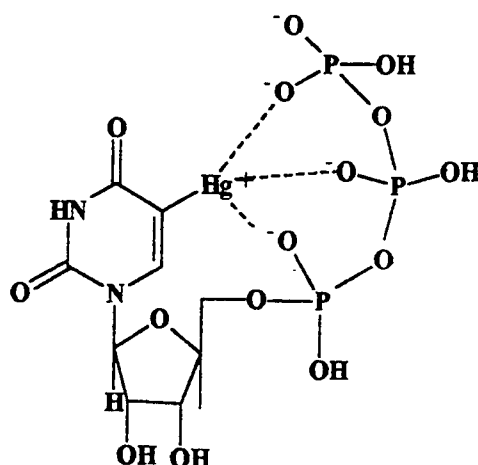
An explanation for the lack of substantial variation in voltammetry with the electrolyte anion, is that equation 6.2 is misleading and that the complexation of the mercury substituent occurs intramolecularly or intermolecularly via the phosphate arm

Table 14: Voltammetric data from cyclic voltammograms of  $7 \times 10^{-5}$  M UTP-HgX with a  $0.0225 \text{ cm}^2$  HMDE in 0.1 M electrolyte solution listed below.

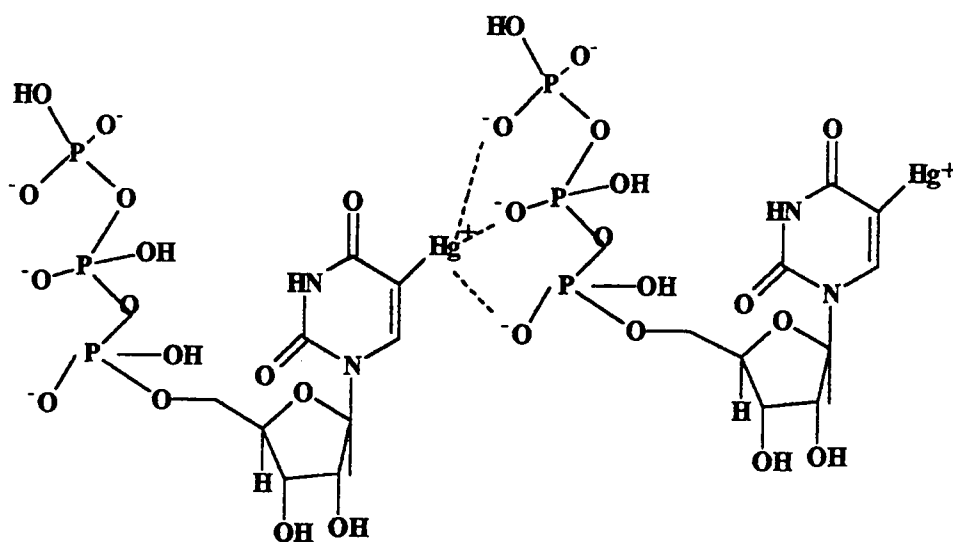
| Electrolyte       | $E_{pK} \text{ (R1)}$ | $E_{pK} \text{ (Ox1)}$ | $\Delta E_{pK}$ |
|-------------------|-----------------------|------------------------|-----------------|
| NaCl              | -720                  | -090                   | 640             |
| NaBr              | -710                  | -120                   | 690             |
| NaF               | -720                  | -100                   | 660             |
| LiCl              | -670                  | -080                   | 590             |
| KCl               | -680                  | -170                   | 530             |
| KBr               | -640                  | -160                   | 520             |
| KClO <sub>4</sub> | -660                  | -080                   | 580             |
| KNO <sub>3</sub>  | -660                  | -100                   | 560             |

of the nucleotide. This is illustrated in Figure 6-3 where instead of the formation of mercury chloride or bromide complexes, the phosphate arm is the ligand. Further support for this role of the triphosphate or diphosphate group will be given in Chapter 7 when the role of pH is considered.

*<sup>31</sup>P NMR study of the phosphate complexation seen with the mercurated UTP and UDP* Additional support for phosphate complexation comes from the <sup>31</sup>P NMR spectra obtained on the UTP, UDP and the mercurated derivatives. For example, Figures 6-4 and 6-5 illustrate the <sup>31</sup>P NMR spectra of the uridine triphosphate and diphosphate. The uridine triphosphate gave a very clean spectrum with the expected triplet (-22.3 ppm) and two doublets (-11.0 ppm and -9.1 ppm) with integrals of



Intramolecular Phosphate Complexation



Intermolecular Phosphate Complexation

Figure 6.3: Illustration of possible intermolecular and intramolecular phosphate complexation with the mercurated UTP

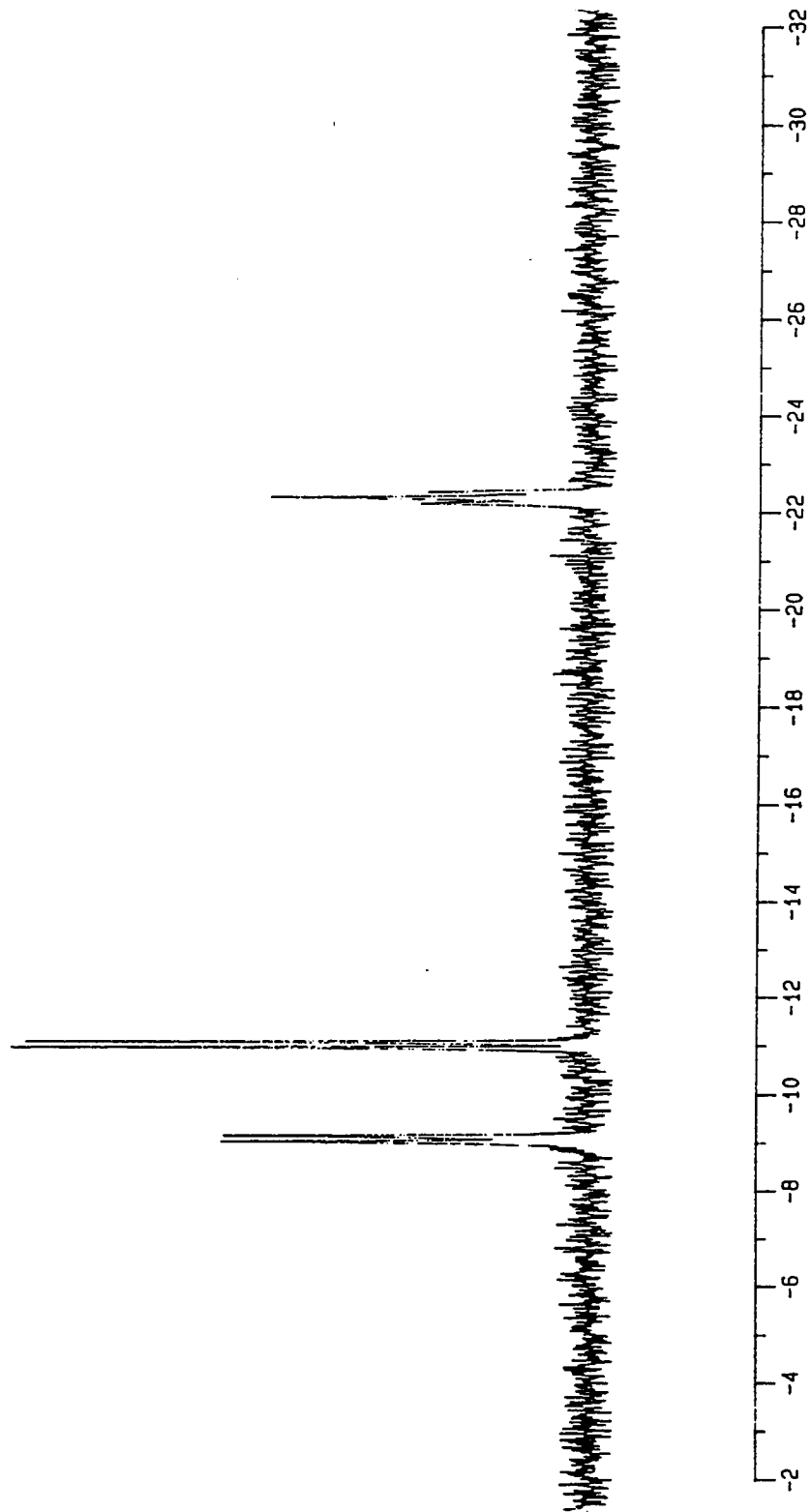


Figure 6-4: Phosphorus Magnetic Resonance Spectrum of the Uridine Triphosphate in  $D_2O$ .

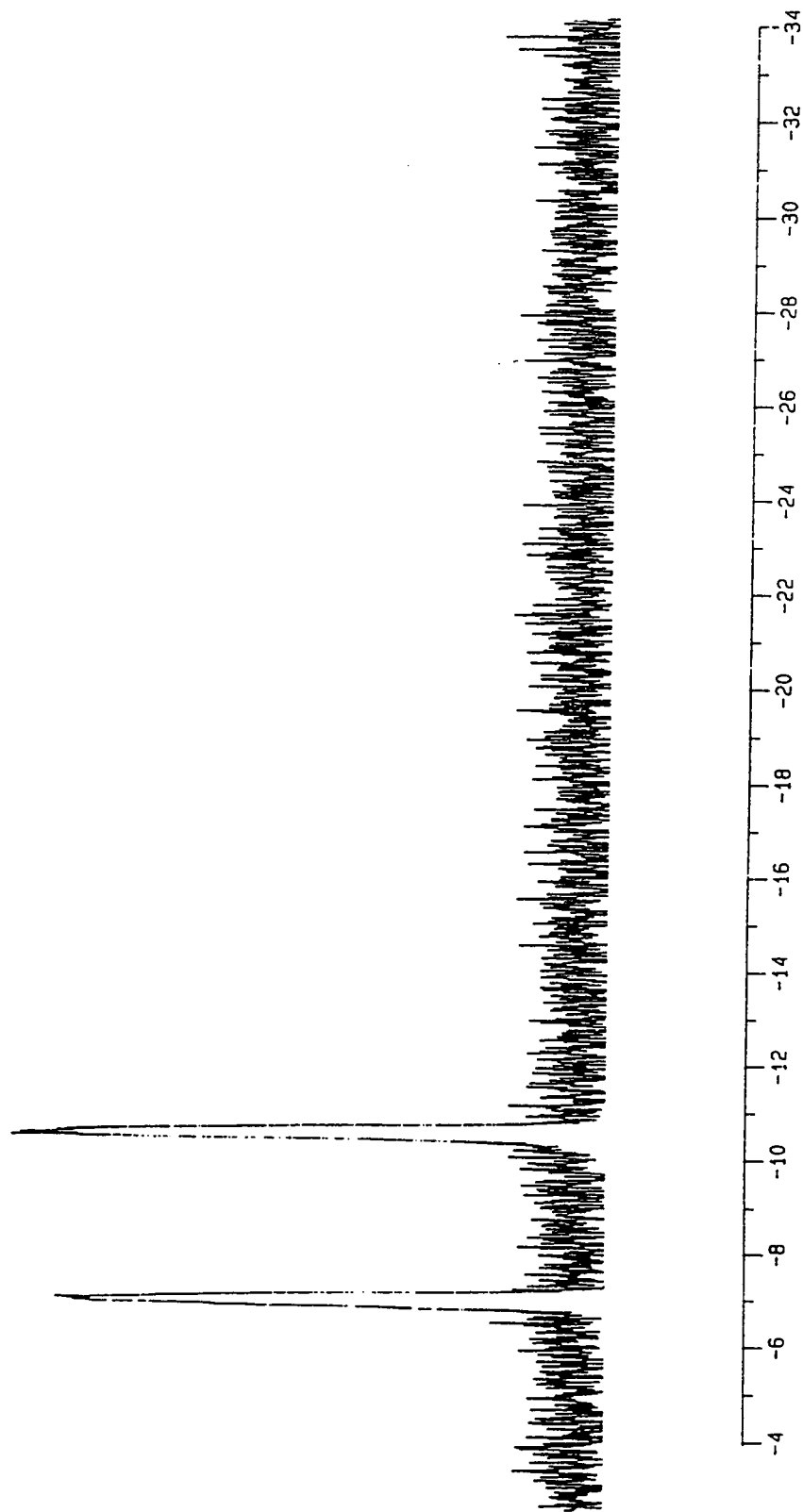


Figure 6-5: Phosphorus Magnetic Resonance Spectrum of the Uridine Diphosphate in  $D_2O$ .

1:1:1. Although at lower S/N ratio than the UTP spectrum, the spectrum of the uridine diphosphate shows the expected two chemical shifts (-7.0 ppm and -10.6 ppm) with integrals of 1:1. Figures 6-6 and 6-7 illustrate the  $^{31}\text{P}$  NMR spectra of the mercurated derivatives. It is evident from these spectra that the phosphate arm of the mercurated species sees a different environment. For example, for the mercurated UTP the integrals still remain 1:1:1, but the resonances are more complicated and shifted more down field (-20.5, -10.5, -5.0 ppm). Similar results were obtained with the mercurated UDP where chemical shifts of -9.6 and -5.2 ppm were obtained. It is also interesting to note that the phosphorus on the end of the phosphate arm, for both the mercurated UTP and UDP, gave the greatest change in chemical shift (assignments based on literature values for UTP and UDP). For example, the chemical shift for the third phosphorus on UTP changed from -9.1 ppm to -5.0 ppm, a difference of 4.1 ppm, upon mercuration. The other two chemical shifts for UTP shifted 1.0 and 1.8 ppm upon mercuration. Hence, not only does the phosphate appear to be interacting with the mercury substituent, but it appears that the end phosphate group is interacting the most. This would be explained by inter or intramolecular phosphate complexation.

*Differentiating between intra and intermolecular complexation.* Dale et al. (13) in their initial synthesis of mercurated nucleotides, were not able to detect a NMR resonance spectrum. They attributed this result to intermolecular interactions generating polymeric charge complexes such as  $[\text{PO}_4^--\text{R}-\text{Hg}^+-\text{PO}_4^--\text{R}-\text{Hg}^+]_n$  or

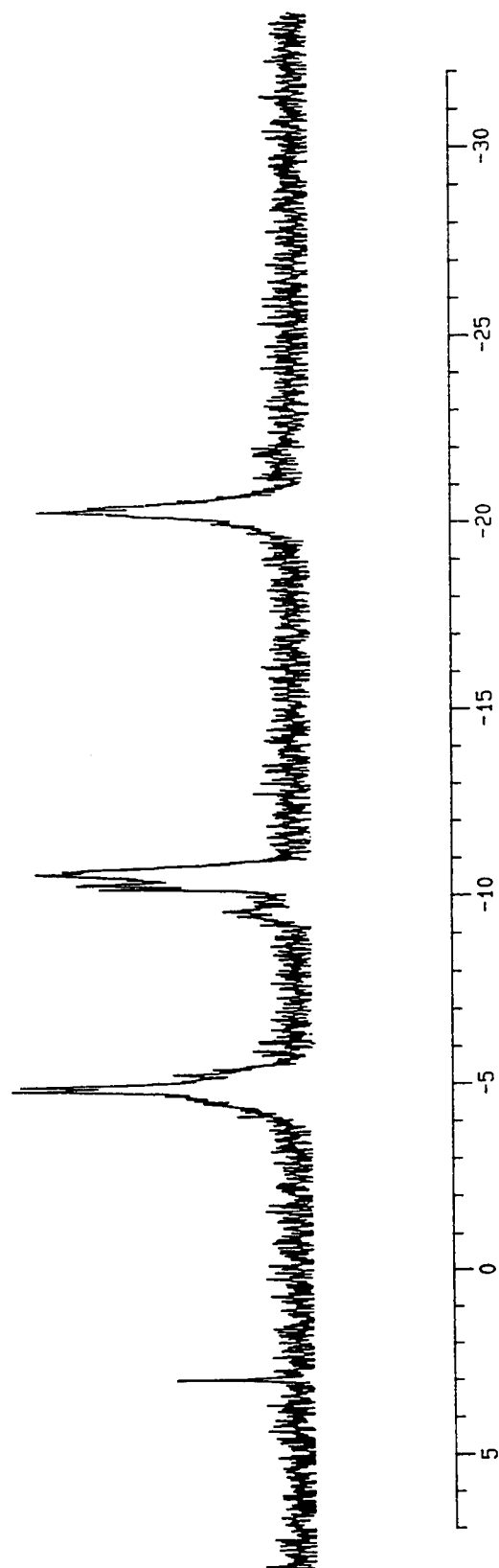


Figure 6-6: Phosphorus Magnetic Resonance Spectrum of the Mercurated Uridine Triphosphate in  $D_2O$ .

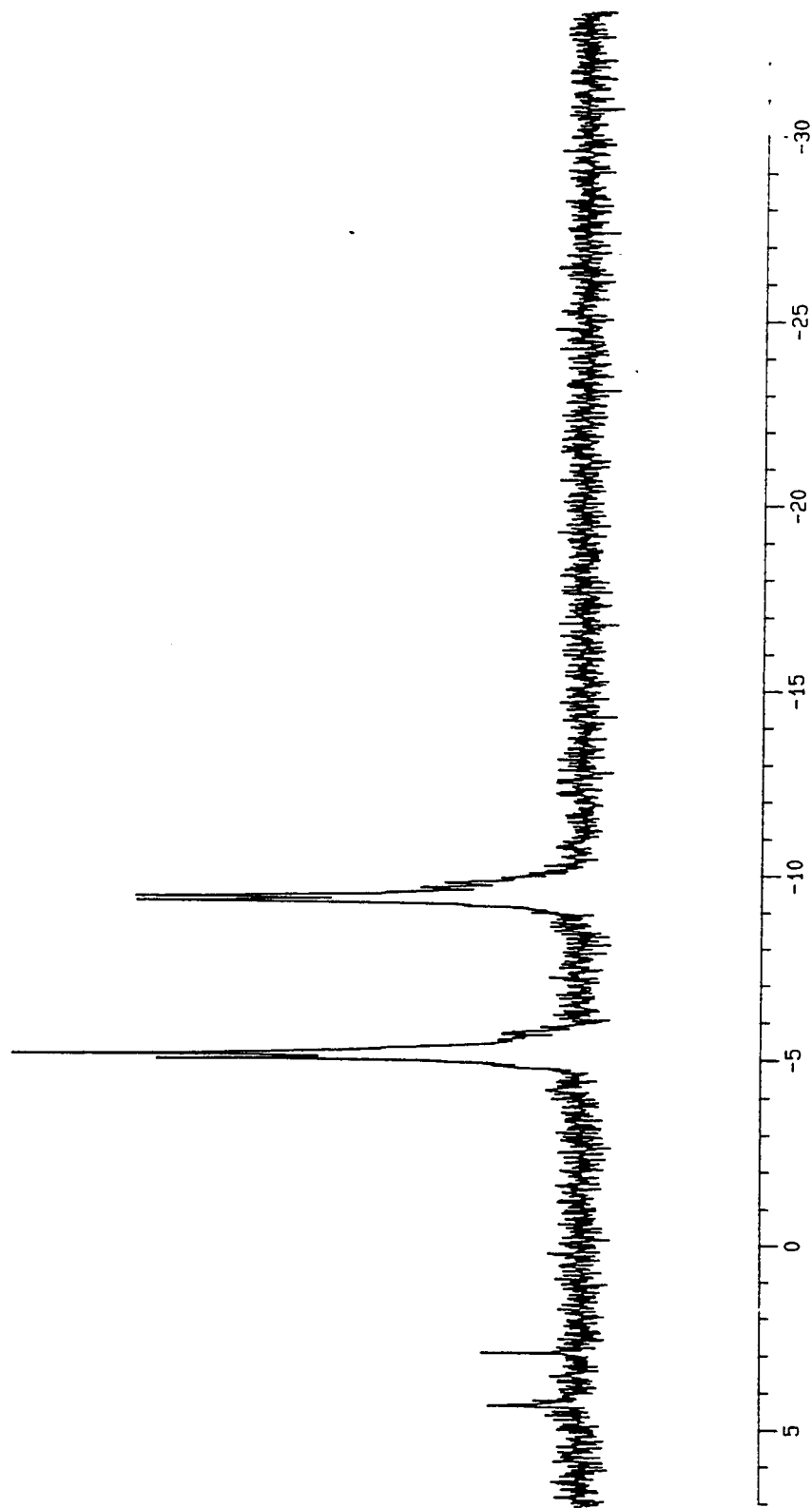


Figure 6-7: Phosphorus Magnetic Resonance Spectrum of the Mercurated Uridine Diphosphate in  $D_2O$ .

$[R-Hg--N-Ar-Hg--N-Ar]_n$  which abolish the monomer resonance. This conclusion was reached because the addition of mercaptoethanol appeared to disrupt the polymeric complex by forming the more stable mercurithioethanol adduct. Under the conditions of this study, however, polymeric charge complexes should not be formed because of the high chloride content. Chloride is known to disrupt these complexes (13). In addition, the low nucleotide concentration is unfavorable to complex formation. To determine if intermolecular complexation occurs under these conditions, the UV absorption of the mercurated triphosphate was monitored during the addition of chloride. It has been shown previously that the absorbance increases 5-15% when these complexes are disrupted (13). Table 15 shows that there is no significant increase in absorbance at  $\lambda_{max}$  with a bulk nucleotide concentration of  $7 \times 10^{-5}$  M. In fact, the entire spectrum was indistinguishable. Thus, at least in the bulk, intermolecular complexation does not appear to be occurring. However, when adsorbed on the electrode surface, the nucleotide molecules are closer together and it is possible that intermolecular complexation may be occurring. However, strong evidence for intramolecular complexation was seen earlier while studying the adsorption equilibrium. From the study it was determined that the adsorption equilibria of the mercurated UTP and UDP can be described by the Langmuir isotherm with no interactions between adsorbed molecules. Intermolecular complexation would cause the adsorption equilibrium to deviate from Langmuir behavior.

Table 15: Absorbance values at  $\lambda_{\text{max}}$  (260 nm) of  $7 \times 10^{-5}$  M UTP-HgX as a function of the chloride concentration.

| [Cl <sup>-</sup> ] | 0.05  | 0.10  | 0.20  | 0.30  |
|--------------------|-------|-------|-------|-------|
| Absorbance         | 0.976 | 0.978 | 0.977 | 0.977 |

*Cyclic voltammetry of the mercurated UMP.* Figure 6-8 illustrates the effect of reversing the potential sweep after the first wave, R1, for the mercurated UMP under the same conditions utilized for mercurated UTP and UDP ( $C_{\text{bulk}} = 7 \times 10^{-5}$  M,  $v = 5$  V/s). This voltammogram is drastically different from the mercurated UTP and UDP with no evidence of Ox1, but rather there appears to be two drawn out oxidation processes at more negative potentials. However, Figure 6-9 illustrates the effect of increasing the scan rate from 5 to 50 V/s. At this scan rate, the more positive of the two anodic waves (second anodic wave) has shifted towards more positive potentials and the first anodic wave does not appear to be present. The second anodic wave has been labeled Ox1 (in accord with previous notation) and the first anodic wave has been labeled Ox2.

Table 16 summarizes the scan rate dependence seen with the voltammetric waves. As shown in Table 16, below 5 V/s no anodic waves are seen. In the vicinity of 5 V/s, the two anodic waves Ox1 and Ox2 are present, and above 5 V/s only the Ox1 wave is present. Also evident from the table is the strong scan rate dependence on the coulombic charge under Ox1. As the scan rate increases, the

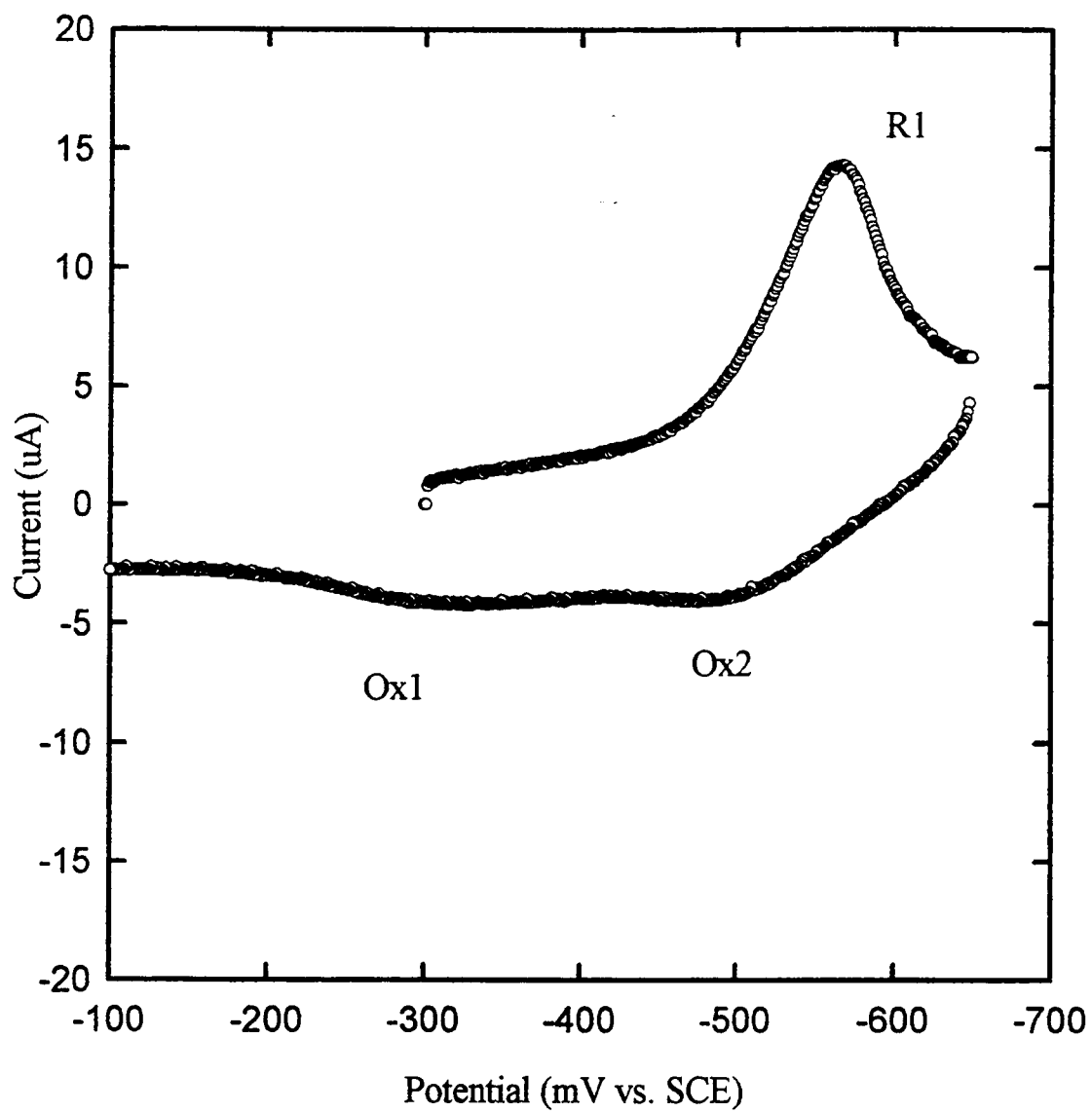


Figure 6-8: Cyclic voltammogram of  $7 \times 10^{-5}$  M UMP-HgX in 0.1 M KCl with a  $0.0225 \text{ cm}^2$  HMDE and a scan rate of 5 V/s.

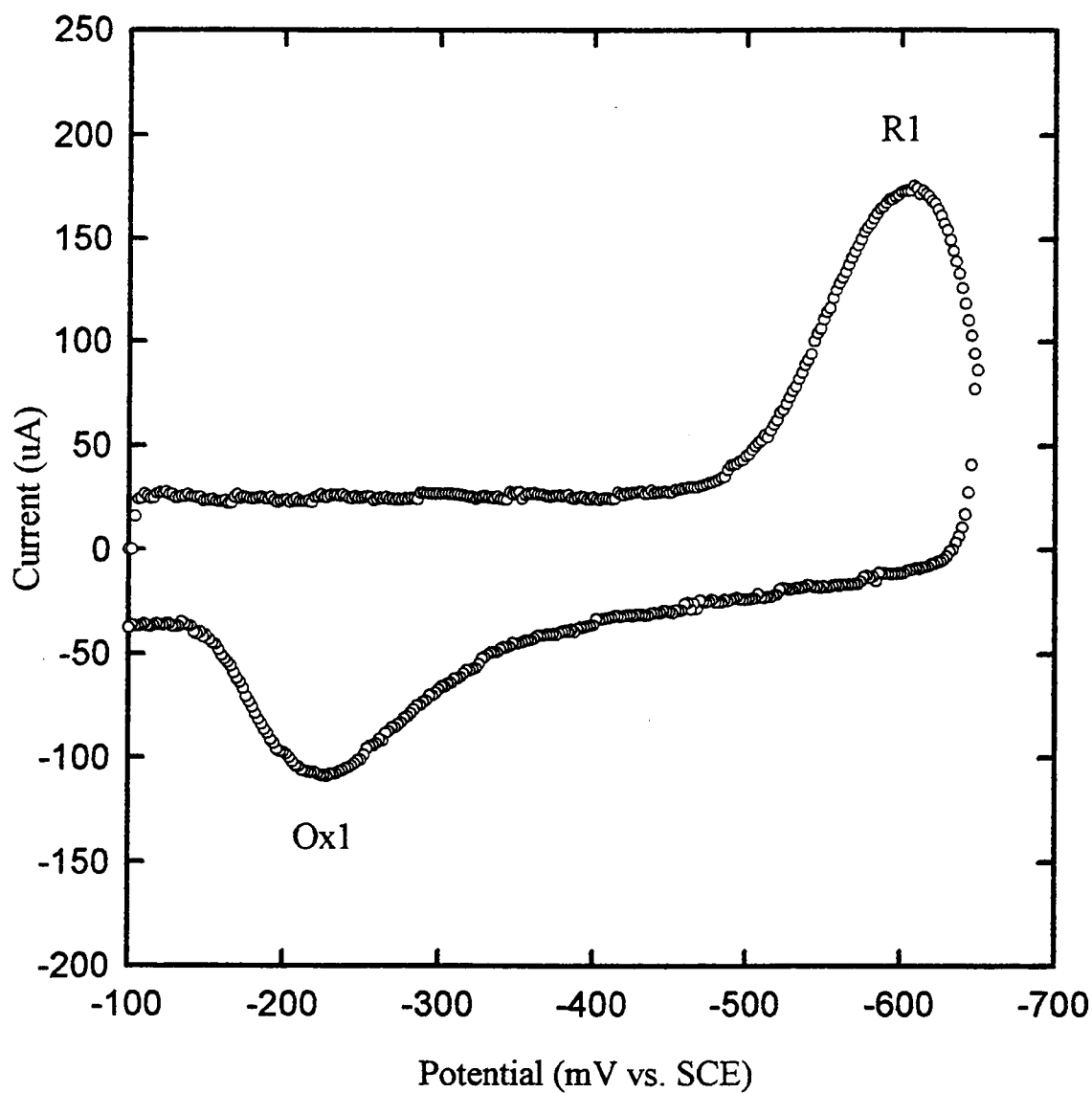


Figure 6-9: Cyclic Voltammogram of  $7 \times 10^{-5}$  M UMP-HgX in 0.1 M KCl with a  $0.0225 \text{ cm}^2$  HMDE and a scan rate of 50 V/s.

coulombic charge under Ox1 increases, but does not reach a value consistent with the charge under R1. An explanation of this behavior will be provided shortly.

*Determination of the complexation state of the mercurated UMP.* It was initially assumed that the ligand X was the phosphate arm. However,  $^{31}\text{P}$ -NMR showed only a small change in the chemical shift for the phosphorous between the UMP and the mercurated derivative. For example, Figure 6-10 and 6-11 show the phosphorous NMR of the UMP and it's mercurated derivative. The UMP gives the expected singlet at 5.0 ppm. The mercurated derivative also gives a singlet but shifted slightly to 5.2 ppm and broader. From the  $^{31}\text{P}$ -NMR results obtained with UTP and UDP, one would expect a greater variation between the two spectra, hence, an electrolyte

Table 16: Voltammetric data from cyclic voltammograms of  $7 \times 10^{-5}$  M UMP-HgX in 0.1 M KCl with a  $0.0225 \text{ cm}^2$  HMDE.

| Scan Rate<br>V/s | $E_{pk}(R1)$<br>mV | $E_{pk}(Ox1)$<br>mV | $E_{pk}(Ox2)$<br>mV | $Q_{(Ox1)}/Q_{(R1)}$ |
|------------------|--------------------|---------------------|---------------------|----------------------|
| 1                | -565               | -                   | -                   | -                    |
| 5                | -600               | -320                | -484                | -                    |
| 10               | -630               | -300                | -                   | 0.18                 |
| 20               | -660               | -280                | -                   | 0.28                 |
| 30               | -685               | -260                | -                   | 0.43                 |
| 40               | -700               | -240                | -                   | 0.45                 |
| 50               | -710               | -220                | -                   | 0.55                 |

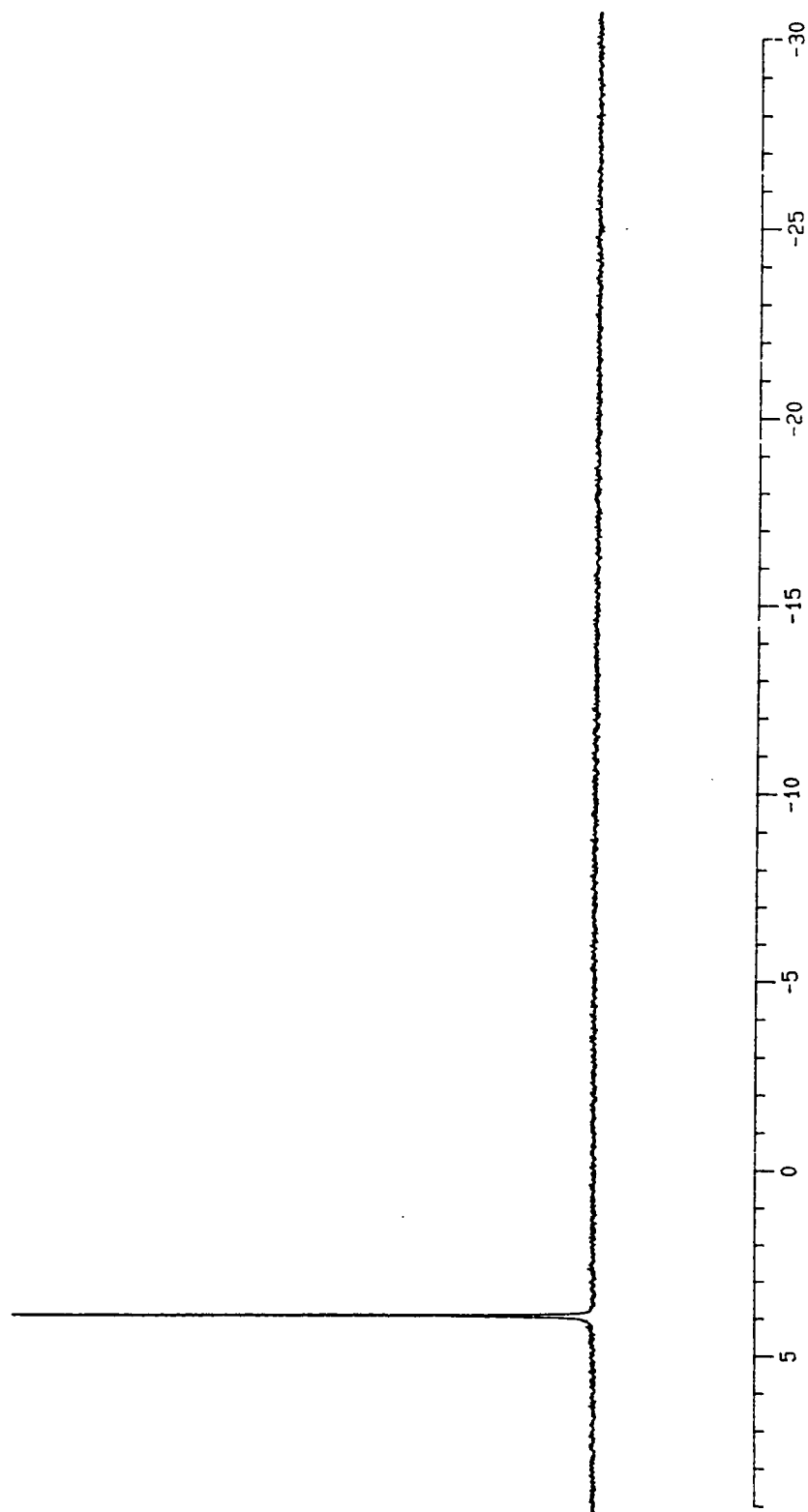


Figure 6-10: Phosphorus Magnetic Resonance Spectrum of the Uridine Monophosphate in  $D_2O$ .

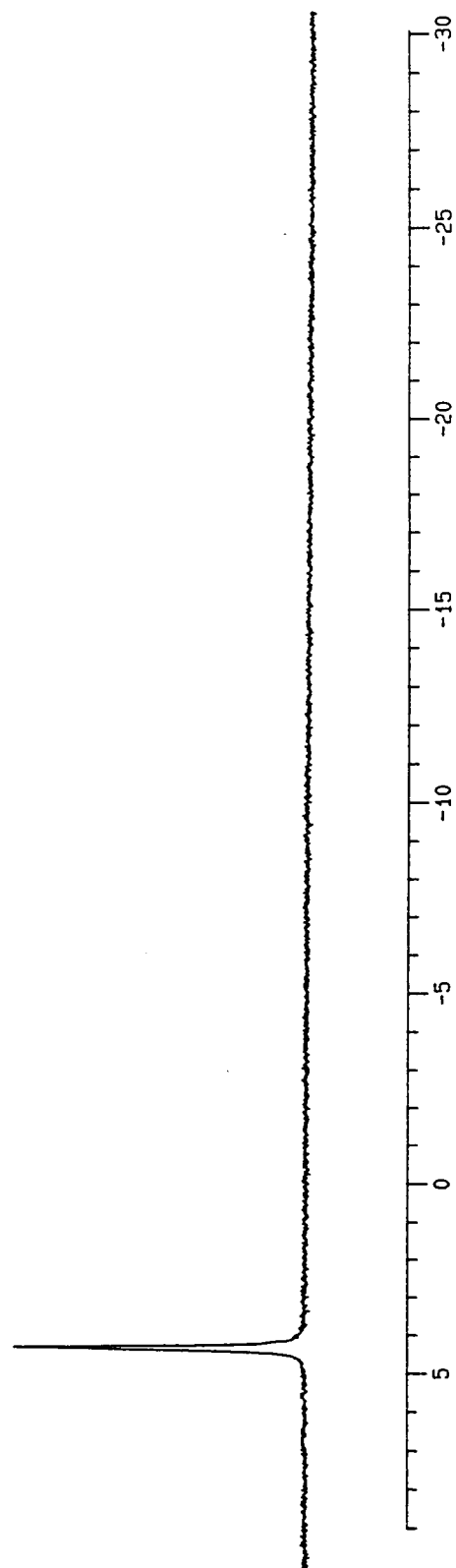
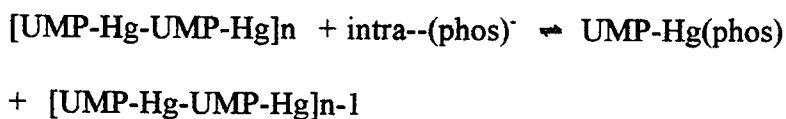


Figure 6-11: Phosphorus Magnetic Resonance Spectrum of the Mercurated Uridine Monophosphate in  $D_2O$ .

study was undertaken. It was found the voltammetry did not change significantly with chloride concentration or supporting electrolyte. This suggests that the ligand is not chloride.

One explanation of these results is that the mercury substituent is complexed intermolecularly via an adjacent UMP-HgX molecule. To determine if intermolecular complexation is occurring between the UMP-HgX species, the absorption spectra were obtained as a function of the chloride concentration. The results, illustrated in Table 17, demonstrate that the absorbance of a  $7 \times 10^{-5}$  M solution of UMP-HgX increases slightly with chloride concentration. This suggest that intermolecular complexation such as  $[\text{PO}_4^- \text{-R-Hg}^+ \text{-PO}_4 \text{-R-Hg}^+]_n$  or  $[\text{R-Hg--N-Ar-Hg--N-Ar}]_n$  may be occurring between the adsorbed UMP-HgX species. Although the adsorption equilibria did show a positive deviation from true Langmuir behavior, the deviation did not appear to be strong enough to contribute all complexation to a polymer type complex. Hence, the reaction in equation 5.1 appears to be occurring on the electrode surface. It should be noted that the error associated with the absorbance values in Table 17 was found to be  $\pm 0.02$ , hence, the data are inconclusive. However, stronger evidence of the above conclusion will be provided in the next chapter.



6.3

Table 17: Absorbance values at  $\lambda_{\text{max}}$  (260 nm) of  $7 \times 10^{-5}$  M UMP-HgX as a function of the chloride concentration.

|                           |      |      |      |      |
|---------------------------|------|------|------|------|
| KCl (mol/L)               | 0.05 | 0.10 | 0.20 | 0.30 |
| $A(\lambda_{\text{max}})$ | 1.14 | 1.15 | 1.16 | 1.18 |

*Identity of the R1, Ox1, and Ox2 waves of the mercurated UMP.* In chapter V it was shown that the R1 wave of the mercurated UMP deviated from the theoretical peak width of 66 mV for a first-order chemical reaction following electron transfer. Typical values varied from 80 to 115 mV. The results above suggest that this deviation is the result of the R1 wave consisting of the reduction of two species; the intramolecularly and intermolecularly complexed mercurated UMP. In fact, a close analysis of the R1 wave in Figure 5-12 shows a shoulder on the R1 wave, suggesting that the R1 wave may actually consist of two waves superimposed. Additional proof that the R1 wave consists of two superimposed waves will be demonstrated in the next chapter.

The identity of the Ox1 and Ox2 waves can now be assigned. The Ox1 wave of the mercurated UMP is consistent with the Ox1 wave of the mercurated UTP and UDP. Hence, the Ox1 wave of the mercurated UMP can be assigned to the oxidation of the surface confined radical where fast intramolecular complexation occurs. The Ox2 wave, which was not present during the voltammetry of the mercurated UTP and UDP, must result from the oxidation of the surface confined

radical with fast intermolecular complexation following electron transfer. The absence of these two anodic waves at slow scan rate could be the result of desorption of the UMP-Hg• radical from the electrode surface or formation of an electroinactive UMP-Hg-UMP species. The formation of the dimer species was suggested to occur with the mercurated UMP in the previous chapter. Thus, dimerization is a likely cause of the above observation. In addition, the above results suggest that dimerization is proceeded by intermolecular complexation which probably allows the proper distance or orientation for dimer formation.

## CHAPTER VII

### Effect of pH on the Voltammetric Behavior of Mercurated Uridine Nucleotides

#### Introduction

So far, the results and discussion pertain to the surface voltammetric behavior of the mercurated UTP, UDP, and UMP in unbuffered 0.1 M KCl. When the voltammetry of the nucleotides was examined in low pH solutions containing the chloride ion, however, a major change in the voltammetric behavior was observed. In this chapter, the effect of pH on the voltammetric behavior of the mercurated nucleotides will be examined. Potassium phosphate buffers were selected for this study because of the large pH window these buffers encompass. However, the results presented here were also obtained in other buffers such as sodium acetate and (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] ) (HEPES).

#### Experimental

In this chapter the effect of pH on the voltammetric behavior of the mercurated uridine nucleotides will be examined using potassium phosphate buffers. These buffer solutions were prepared with the appropriate potassium phosphate mixtures (mono-, di-, and tribasic) calculated from the Henderson-Hasselbalch equation. The pH of the prepared solutions were checked with a standard laboratory

pH meter. The measured pH values were found to be in excellent agreement with the calculated values ( $\pm 0.05$  pH units).

The most representative way to show the dependence of pH on the voltammetric behavior of a system is to construct a Pourbaix diagram which consists of a  $E^0$  vs. pH plot. These diagrams identify the species present in each pH/potential region and provide information on the involvement of protons during the electron transfer. However, it was demonstrated in chapter II, that to determine the  $E^0$  value of a surface confined species undergoing electron transfer, the adsorption coefficient of the species in both the oxidized and reduced form must be known. The species studied in this work undergo electron transfer to produce adsorbed radicals which are not present in the bulk. In addition, the peak potentials will be functions of the rate constant of the rapid coupled chemical reactions. Hence, the adsorption coefficient of the radical species can not be determined and  $E^0$  cannot be reported accurately. However, a similar plot can be constructed which can give the similar information as a Pourbaix diagram. This plot consists of a peak potential vs. pH diagram and will be referred to as a kinetic peak potential diagram. Since the peak potentials may contain kinetic terms which vary with pH, however, it is difficult to deduce the number of protons involved in the electrode reactions from the diagrams.

## Results and Discussion

### *Voltammetric behavior of the mercurated UTP in potassium phosphate*

*buffers.* Figure 7-1 shows the kinetic peak potential diagram of the mercurated UTP in 0.1 M potassium phosphate buffers. This diagram clearly indicates that there are two reduction processes which occur within the pH range of study (R1 and R2). Figure 7-2 illustrates a typical voltammogram obtained during this study (pH=7.5). This voltammogram is consistent with the voltammetric behavior obtained in 0.1 M KCl where the first reduction wave, R1, was assigned to the one-electron reduction of the nucleotide to the surface confined radical and the second wave, R2, was assigned to the one electron reduction of the radical to the parent compound UTP. Also illustrated in Figure 7-2 is the effect of reversing the potential sweep after the first reduction process, R1. The result, consistent with the voltammetry in 0.1 M KCl, shows that a wave, Ox1, appears on the anodic sweep. This typical behavior was seen throughout the pH range of study (Figure 7-1). In fact, the only significant difference between the voltammetry in KCl and the voltammetry in the buffer solutions was the position of the peak potentials. Thus, the intramolecularly complexed UTP-HgX species has been assigned to region A of the kinetic peak potential diagram. Likewise, the surface confined radical has been assigned to region B and UTP has been assigned to region C.

Like a typical Pourbaix diagram, the kinetic peak potential diagram illustrated in Figure 7-1 may give insight on the involvement of protons during electron

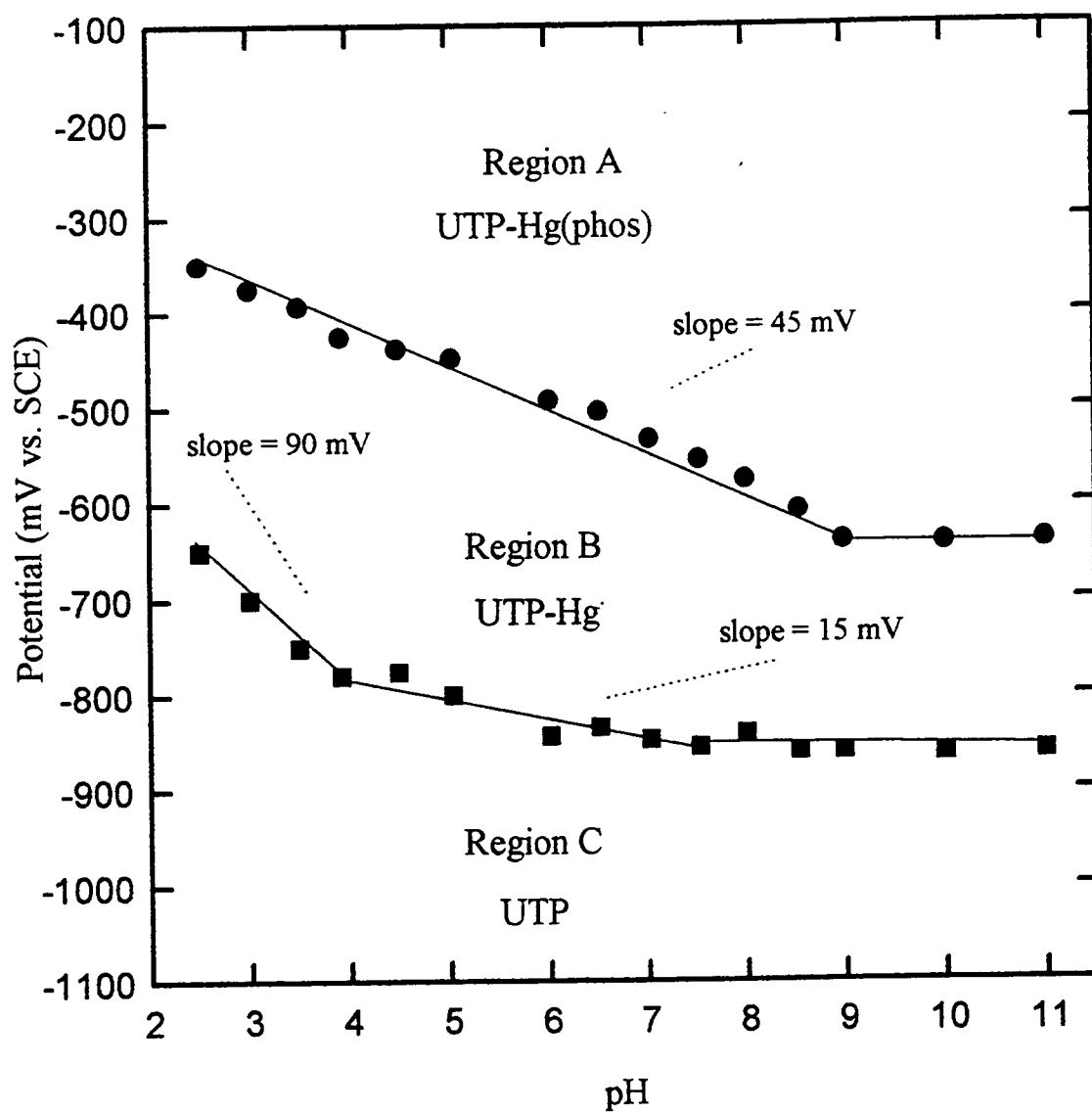


Figure 7-1: Kinetic peak-potential diagram for the UTP-HgX in 0.1 M potassium phosphate buffers.  $C^{\text{bulk}} = 0.07 \text{ mM}$ ;  $0.0225 \text{ cm}^2 \text{ HMDE}$ ;  $10 \text{ V/s}$

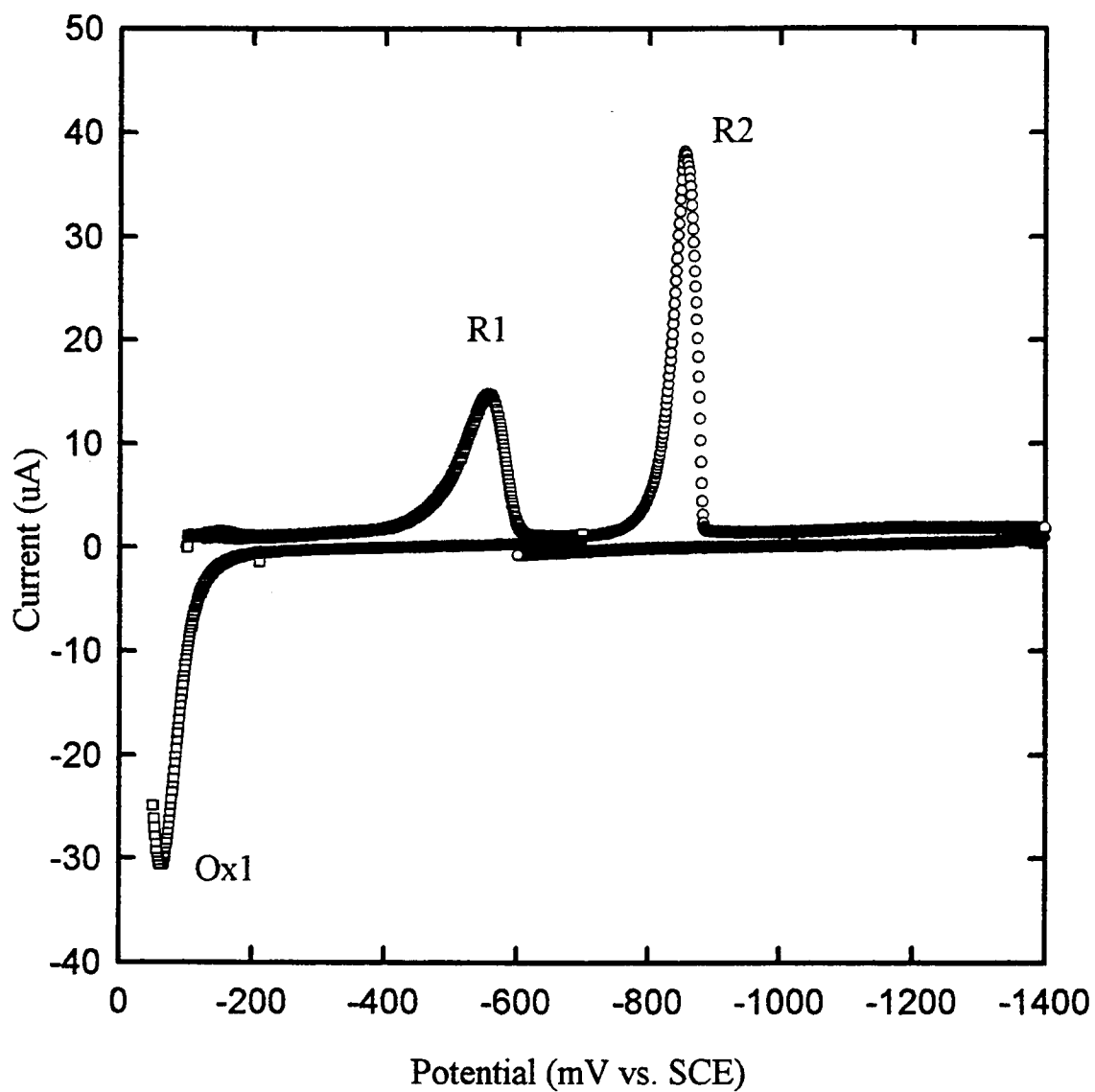
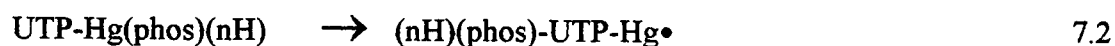


Figure 7-2: Voltammograms of  $7.0 \times 10^{-5}$  M UTP-HgX in 0.1 M potassium phosphate buffer, pH=7.5, with a  $0.0225 \text{ cm}^2$  HMDE and a scan rate of 10 V/s.

transfer. For example, the peak potential for R1 varies 45 mV per pH unit until reaching a pH region (pH=9) which is consistent with the  $pK_a$  of the ionizable amine proton on the ring nitrogen of the base (9.6). The peak potential of R2 also varied with pH until reaching a plateau region at pH values greater than 7. At pH values less than 4, the peak potential varies 90 mV per pH unit and between 4 and 7, the peak potential varies 15 mV per pH unit. It has been shown previously that the R1 wave is due to an one-electron transfer step followed up by decomplexation, and that the R2 wave is due to an electron transfer step followed by neutralization. If the rates of these follow-up reactions are pH dependent throughout the range of study, the peak potentials would contain a kinetic term for these reactions. Thus, caution must be exercised in assigning specific protonation steps from slopes of the lines. However, one can speculate on the origins of the proton involvements. For example, the variation of the R1 wave at pH values less than 9 may result from protonation of phosphate arm as shown in equations 7.1 and 7.2 (the value n is the number of protons involved). This is consistent with literature presented in Chapter I where the phosphate arm was shown to be totally deprotonated at pH values in the vicinity of 9 and greater. Hence, a plateau region would be predicted as shown in Figure 7-1. In this region the phosphate arm is not protonated.



The variation of the peak potential of wave R2 with pH also illustrates the involvement of protons with this electron transfer, but a plausible explanation has not been found. It will only be noted here that at pH values less than 8 the reduced form of the  $\text{RHg}\bullet/[\text{RHg}]^-$  couple is protonated.

Except for the degree of involvement of protons during electron transfer, this pH study has demonstrated that the voltammetric behavior of the UTP-HgX in the phosphate medium remains essentially the same as that in 0.1 M KCl. However, in acidic solutions (HCl) a major change in the voltammetry was observed. Hence, a pH study in 0.1 M phosphate buffers with the addition of 0.2 M KCl was conducted.

*Voltammetric behavior of the mercurated UTP in potassium phosphate buffers with 0.2 M KCl.* The kinetic peak potential diagram illustrated previously for the UTP-HgX was also constructed using the 0.1 M phosphate buffers with 0.2 M KCl. This diagram is illustrated in Figure 7-3. Figure 7-4 illustrates a typical cyclic voltammogram obtained at a pH of 7.5 and used in the construction of the kinetic peak-potential diagram (the potential sweep in this voltammogram and next two figures was switched before the R2 wave). This voltammogram is very consistent with previous voltammograms, although, there does appear to be more charge under the anodic wave than the cathodic wave ( $Q_{\text{R1}} = 0.34 \text{ uC}$ ;  $Q_{\text{Ox1}} = 0.40 \text{ uC}$ ). This may be the result of some diffusional component in the R1 wave.

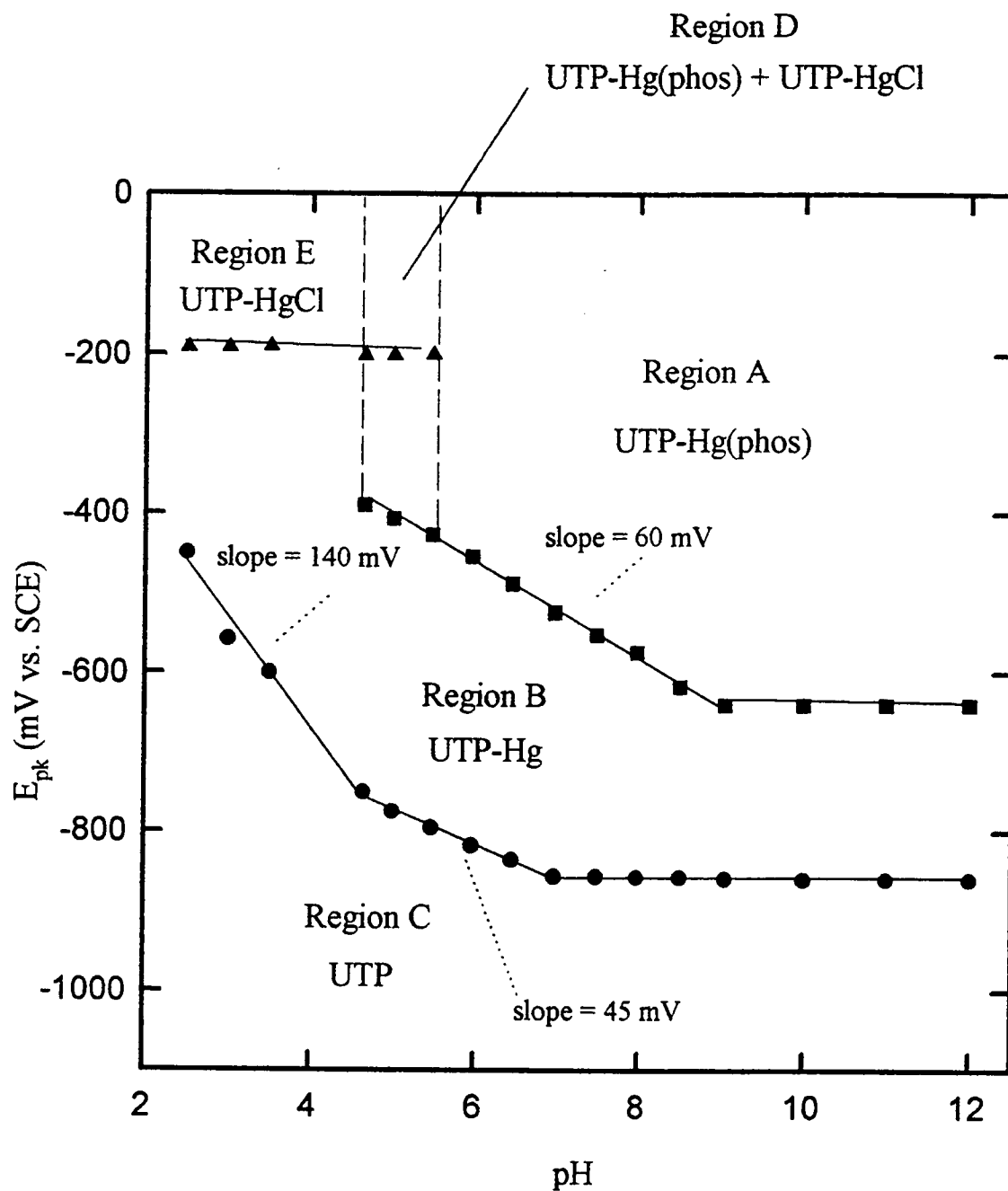


Figure 7-3: Kinetic peak-potential diagram for the UTP-HgX in 0.1 M potassium phosphate buffers with 0.2 M KCl.  $C_{bulk} = 0.07$  mM; 10 V/s;  $0.0225 \text{ cm}^2$  HMDE.

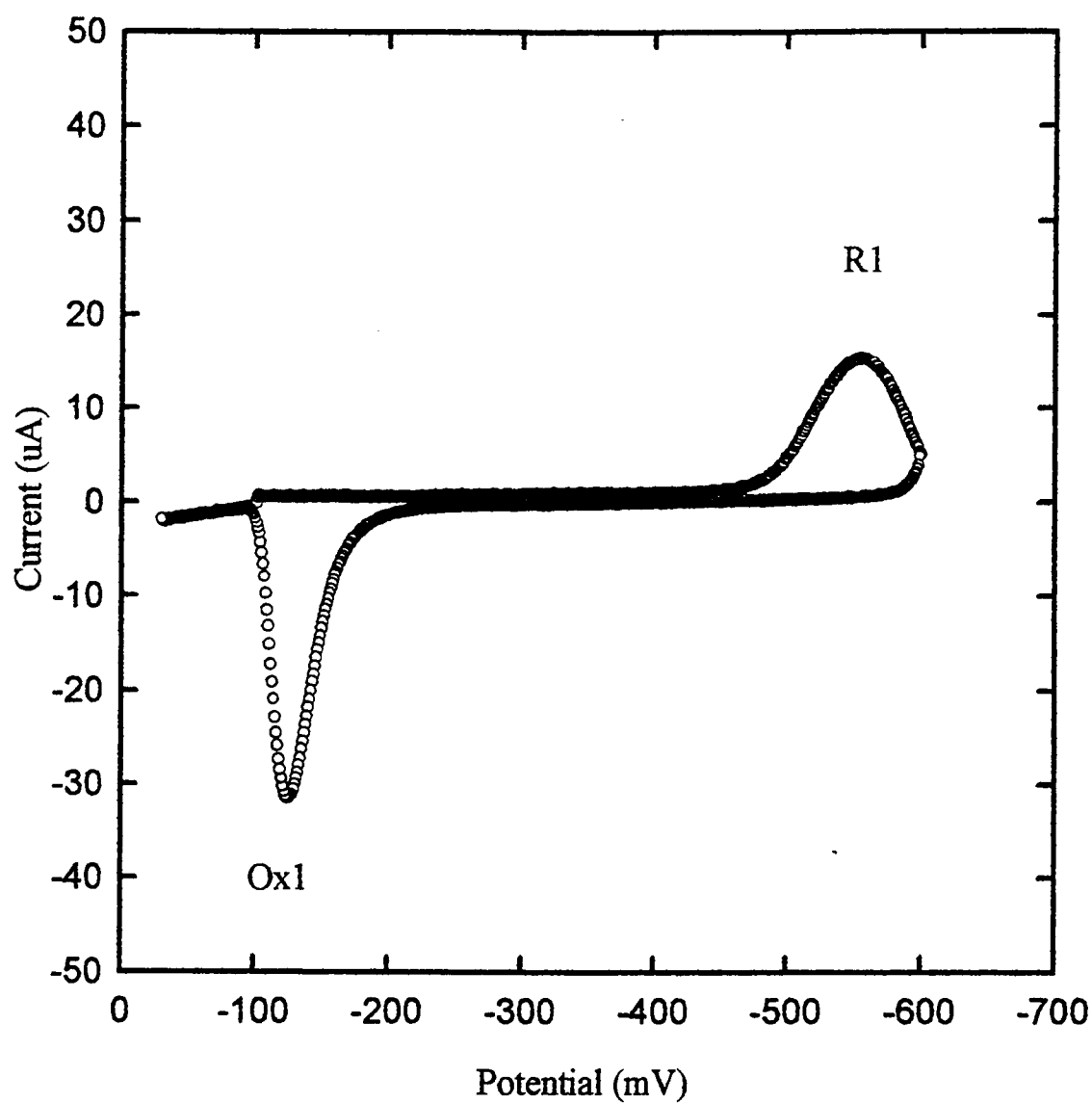


Figure 7-4: Cyclic voltammogram of 0.07 mM UTP-HgX in 0.1 M potassium phosphate buffer, pH=7.5, and 0.2 M KCl. 0.0225 cm<sup>2</sup> HMDE; 5 V/s.

Figure 7-5 shows a typical voltammogram obtained at a pH of 5.0. Evident from this figure is a new wave R1' which appears to form a reversible couple with the Ox1 wave. Although the coulombic charge under R1 was not equal to Ox1, it was determined that the total coulombs under both reduction process R1 and R1' was approximately equal to the charge under Ox1 ( $Q_{R1} = 0.17$ ;  $Q_{R2} = 0.16$ ;  $Q_{Ox1} = 0.35$  uC). Figure 7-6 shows typical voltammograms of the UTP-HgX at a pH of 3 at various scan rates. Here the R1 wave has completely disappeared, but the coulombic charges under R1' and Ox1 are equivalent.

To gain an understanding of this voltammetric behavior, a chloride concentration study was conducted at a pH of 5.5. Table 18 illustrates that the ratio of the coulombic charges ( $Q_{R1'}/Q_{R1}$ ) increased with chloride concentration. For example, in a solution containing 0.1 M potassium phosphate and 0.05 M KCl, the ratio ( $Q_{R1'}/Q_{R1}$ ) was found to be 0.06 and in a solution containing 0.1 M potassium phosphate and 0.30 M KCl the ratio was found to be 0.94. This result is also demonstrated in Figure 7-7 where at low chloride concentration the R1 wave predominates and at high chloride concentration the R1' wave predominates. The above results suggest that the following equilibrium is occurring (equation 7.3),

Table 18: Dependence of the coulombic charge ratios of  $Q_{R1'}$  to  $Q_{R1}$  for the mercurated UTP with chloride concentration.

| KCl   | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 |
|-------|------|------|------|------|------|------|------|------|
| Ratio | 0.06 | 0.12 | 0.21 | 0.30 | 0.58 | 0.94 | 1.10 | 2.01 |

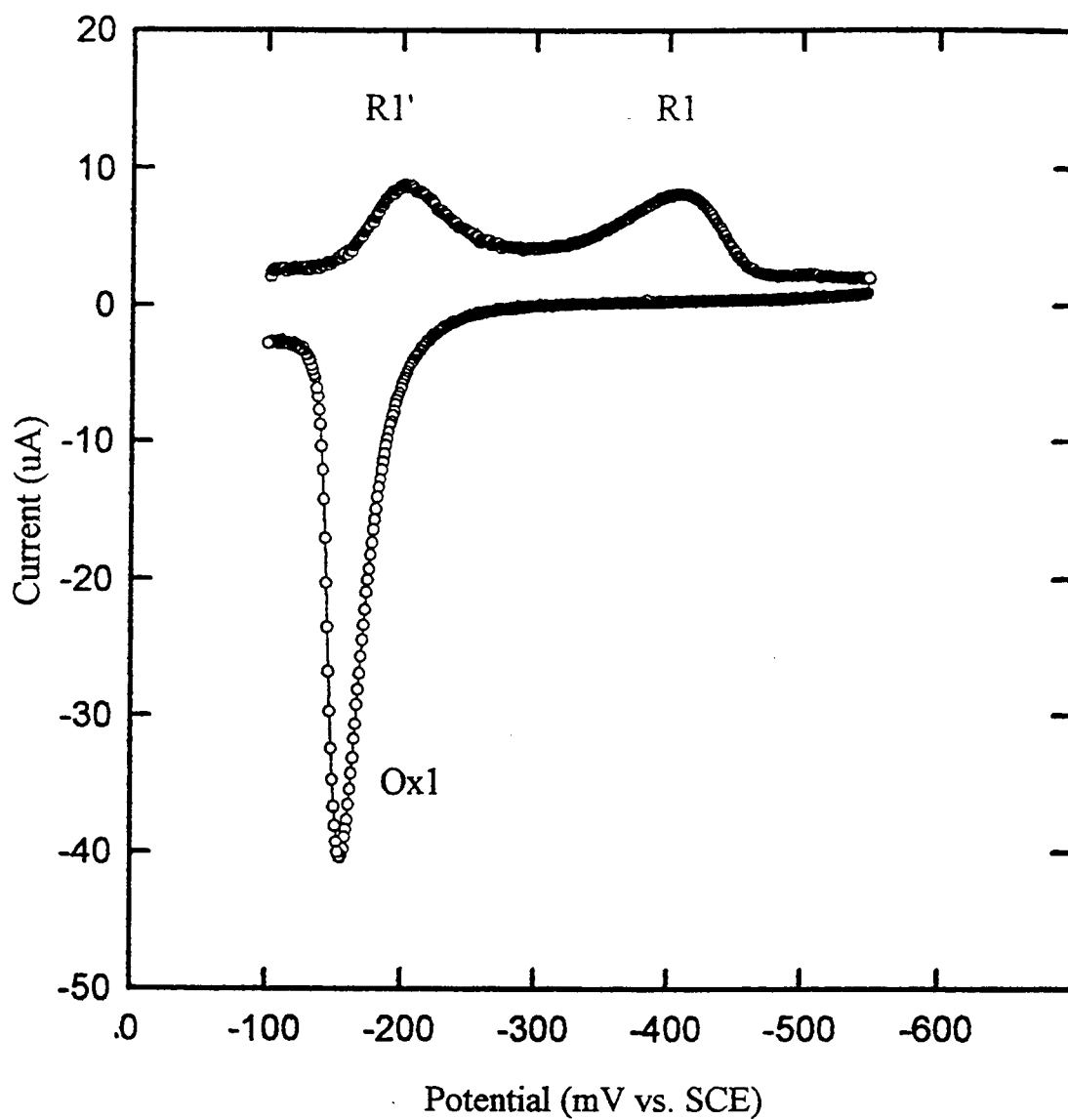


Figure 7-5: Cyclic voltammogram of 0.07 mM UTP-HgX in 0.1 M potassium phosphate buffer, pH=5, and 0.2 M KCl. 0.0225 cm<sup>2</sup> HMDE; 5V/s.

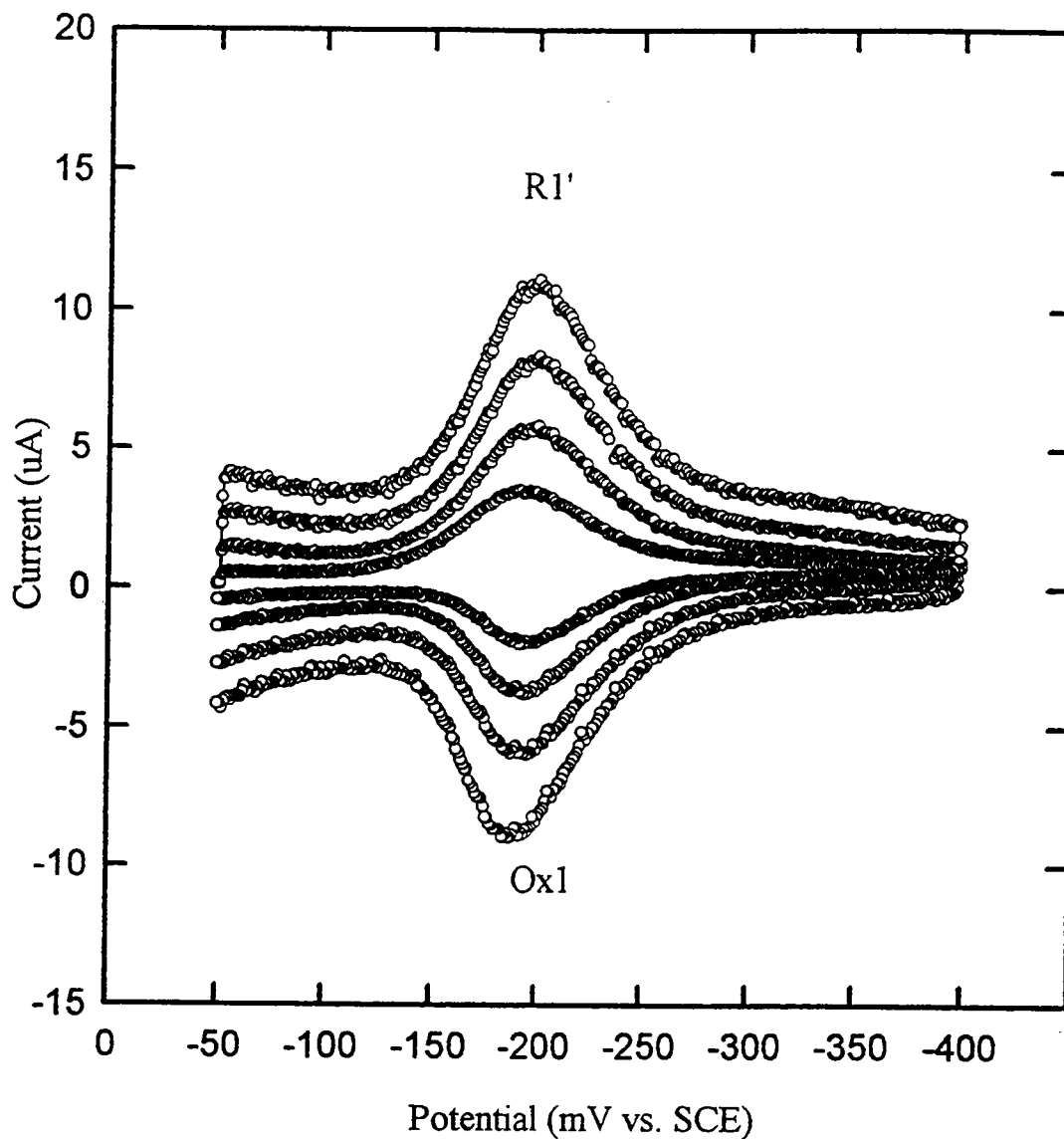


Figure 7-6: Cyclic voltammograms of  $7 \times 10^{-5}$  M UTP-HgX in 0.1 M potassium phosphate buffer, pH = 3, with 0.2 M KCl.  $0.0225 \text{ cm}^2$  HMDE; scan rates of 2, 3, 4 & 5 V/s.

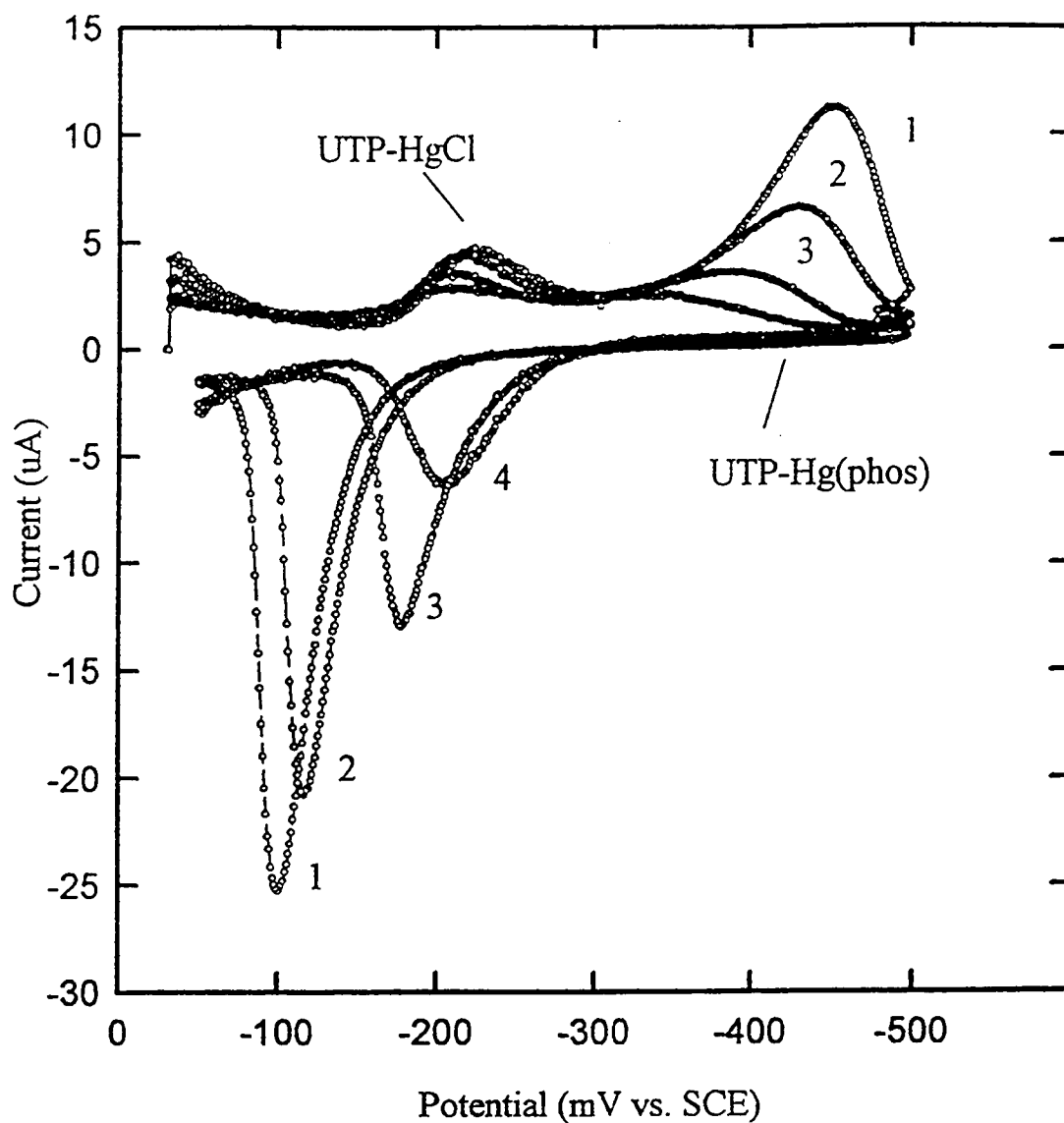
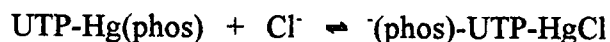


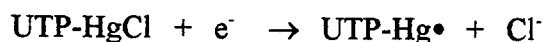
Figure 7-7: Cyclic voltammograms of  $7 \times 10^{-5}$  M UTP-HgX in 0.1 M potassium phosphate buffers, pH = 5.5, with a scan rate of 5 V/s and chloride concentrations of (1) 0.1 (2) 0.2 (3) 0.3 (4) 0.4 M.



7.3

where the phosphate arm, intramolecularly complexing the mercury substituent, is replaced by chloride. As illustrated above, an increase in chloride concentration or a decrease in pH drives equation 7.3 to the right. Decreasing the pH has the effect of reducing the ligation strength of the phosphate by protonation of the phosphate arm. Thus, in the new region D of the kinetic peak potential diagram (Figure 7-3), where both the R1 and R1' wave are present, both the intramolecularly complexed phosphate and chloride species are present. At lower pH values only the R1' wave is present (equation 7.4), thus the chloride complexed species has been assigned to region E. The species present in the other regions, A-C, are the same as those assigned earlier in the first kinetic peak potential diagram (UTP-Hg(phos), UTP-Hg, and UTP).

It should be noted that the above explanation requires that establishment of the equilibrium (equation 7.3) is sluggish on the time scale of the voltammetry. In chapter VIII, additional evidence for the change in complexation state of the mercury atom will be provided by comparing calculated and experimental voltammograms. The calculated voltammograms contain kinetic terms for equation 7.3. Excellent agreement will be demonstrated.



7.4

*<sup>1</sup>H-NMR evidence of the change in complexation of the mercurated UTP*

*with pH.* The voltammetric pH study demonstrated a major change in the voltammetry. This change was contributed to a change in complexation state of the mercury substituent. Hence, a proton NMR study was conducted to observe possible change in complexation state of the mercurated UTP. The aromatic region of the NMR spectra of the nucleotides was shown previously to be diagnostic for the presence or absence of the mercury substituent on the pyrimidine ring. Here it will be shown that the aromatic region is also diagnostic for the nature of the complexation state of the mercury atom. The chemical shift of interest, corresponds to the H-6 proton adjacent to the mercury atom on the pyrimidine ring.

Table 19 illustrates that the chemical shift for the H-6 proton varies with pH. For example, in 0.1 M phosphate buffers with 0.2 M KCl a chemical shift of  $7.82 \pm 0.04$  ppm was observed at higher pH values (#5). In the lower pH solutions, a chemical shift of  $8.24 \pm 0.03$  ppm was seen (#1 and #2). At higher pH values the intramolecularly complexed species has been shown to be present. Thus, the chemical shift of  $7.82 \pm 0.04$  for the H-6 proton corresponds to the intramolecularly phosphate complexed mercurated UTP. At lower pH values, the R1' wave appears as a result of chloride complexation. Hence, the chemical shift of  $8.24 \pm 0.04$  ppm can be assigned to a chloride complexed species. At pH values of 7 and 9 (#3 and #4) both chemical shifts were observed. In this region, it is believed that both the phosphate complexed and chloride complexed species are present.

Table 19: Proton NMR shifts for the H-6 proton of the pyrimidine ring of the mercurated UTP in various solutions. An A states that the UTP-HgCl species is present and B states that the UTP-Hg(phos) species is present.

|     |                                                   |           |     |
|-----|---------------------------------------------------|-----------|-----|
| #1  | 0.2 M KCl + 0.1 M Phosphate    pH = 3             | 8.25      | A   |
| #2  | 0.2 M KCl + 0.1 M Phosphate    pH = 5             | 8.24      | A   |
| #3  | 0.2 M KCl + 0.1 M Phosphate    pH = 7             | 8.30/7.81 | A,B |
| #4  | 0.2 M KCl + 0.1 M Phosphate    pH = 9             | 8.23/7.83 | A,B |
| #5  | 0.2 M KCl + 0.1 M Phosphate    pH = 11            | 7.82      | B   |
| #6  | 0.2 M KClO <sub>4</sub> + 0.1 M Phosphate pH = 3  | 8.39      | B   |
| #7  | 0.2 M KClO <sub>4</sub> + 0.1 M Phosphate pH = 5  | 8.39      | B   |
| #8  | 0.2 M KClO <sub>4</sub> + 0.1 M Phosphate pH = 7  | 8.37/7.82 | B   |
| #9  | 0.2 M KClO <sub>4</sub> + 0.1 M Phosphate pH = 9  | 8.30/7.83 | B   |
| #10 | 0.2 M KClO <sub>4</sub> + 0.1 M Phosphate pH = 11 | 7.81      | B   |
| #11 | 0.1 M Phosphate    pH = 3                         | 8.40      | B   |
| #12 | 0.1 M Phosphate    pH = 7                         | 8.39/7.81 | B   |
| #13 | 0.1 M Phosphate pH = 11                           | 7.80      | B   |
| #14 | 0.1 M KCl                                         | 7.82      | B   |

In 0.1 M phosphate buffers containing no additional electrolyte or 0.2 M noncomplexing perchlorate similar results are seen with a change in chemical shift with pH. However, in these media the chemical shift for the H-6 proton is not consistent with that assigned to the chloride complexed species. The chemical shift,  $8.40 \pm 0.03$  ppm (#6, #7, #11), is probably the result of the different environment seen by the H-6 proton due to protonation of the phosphate arm or the nitrogen of the pyrimidine ring. In fact, even at a pH of 3, with no chloride in solution, the voltammetric behavior is consistent with intramolecular phosphate complexation. This was previously illustrated in the first kinetic peak potential diagram (Figure 7-2).

It is interesting to note that in the chloride medium (#14), the square scheme voltammetry is present. Table 19 illustrates that the chemical shift of the H-6 proton in 0.1 M KCl solution is consistent with that previously assigned to the phosphate complexed species. From the buffered solution results, one would expect to have a mixture of the phosphate and chloride complexed UTP-HgX because 0.1 M KCl is neutral. However, due to the fact that solutions were prepared with the tri-sodium or tri-lithium salt of the nucleotide in the absence of buffer, the prepared solutions containing only chloride were in fact basic ( $\text{pH} = 8.5 \pm 0.5$ ).

*Effect of pH on the adsorption equilibria of the mercurated UTP.* It should be noted that the surface coverage of the mercurated UTP varies with pH. The variation in surface coverage of the mercurated UTP appears to be related to the replacement

of the intramolecularly phosphate complexed species with the chloride complexed species. Figure 7-8 illustrates this effect via the isotherms at pH values of 9, 7, 5, and 3. From Figure 7-8 it is evident that at pH=9, where the intramolecularly complexed species predominates, the surface coverage at saturation is  $1.5 \times 10^{-10}$  mol/cm<sup>2</sup> and at pH=3, where the chloride complexed species predominates, the surface coverage is  $0.5 \times 10^{-10}$  mol/cm<sup>2</sup>. These values correspond to 1.1 and 3.3 nm<sup>2</sup>/molecule. Hence, the chloride complexed species appears to occupy three times as much surface area as the phosphate complexed species. This result does not appear to be only the result of reorientation of the molecule with a change in complexation because the flattest possible orientation of the phosphate complexed species was found to be in the vicinity of 1 nm<sup>2</sup>/molecule with a simple molecular drawing/minimizing computer program. The larger area per molecule must be the result of the phosphate arm extending from the uridine molecule after decomplexation with hydration and some repulsive interaction between the adjacent molecules.

To determine if repulsive interactions contribute to the above results, the adsorption equilibria were studied in the manner previously described in chapter V. The results for the Langmuir plot for the mercurated UTP at a pH of 9 gave great linear correlation with a linear coefficient of 0.9996. Figure 7-9 illustrates the experimental and theoretical Langmuir isotherm at this pH value. As shown, the experimental and theoretical isotherms also demonstrate excellent correlation. Hence, identical to the results in just 0.1 M KCl, the adsorption equilibrium of the

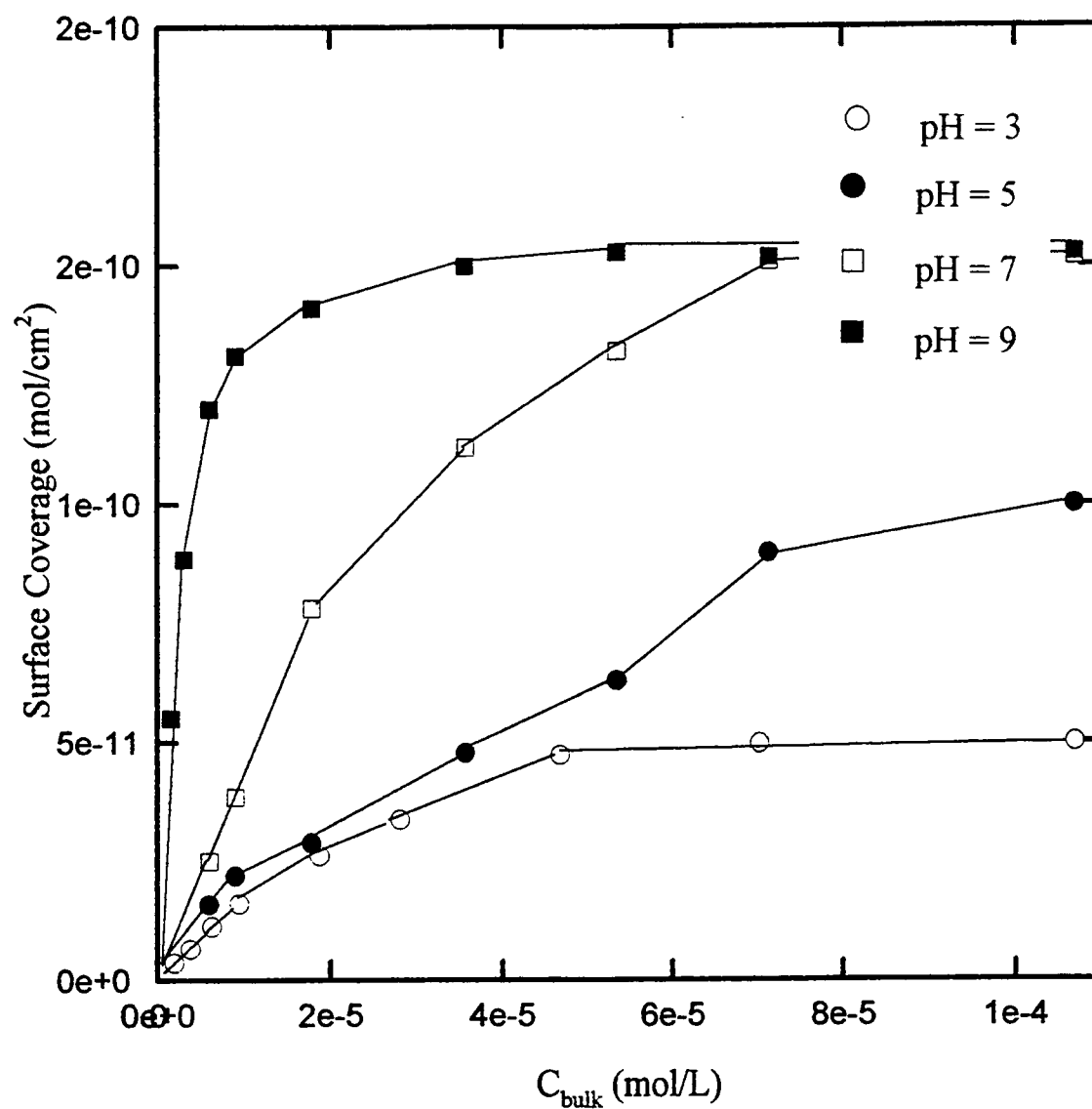


Figure 7-8: UTP-HgX isotherms at pH values of 3, 5, 7, and 9.

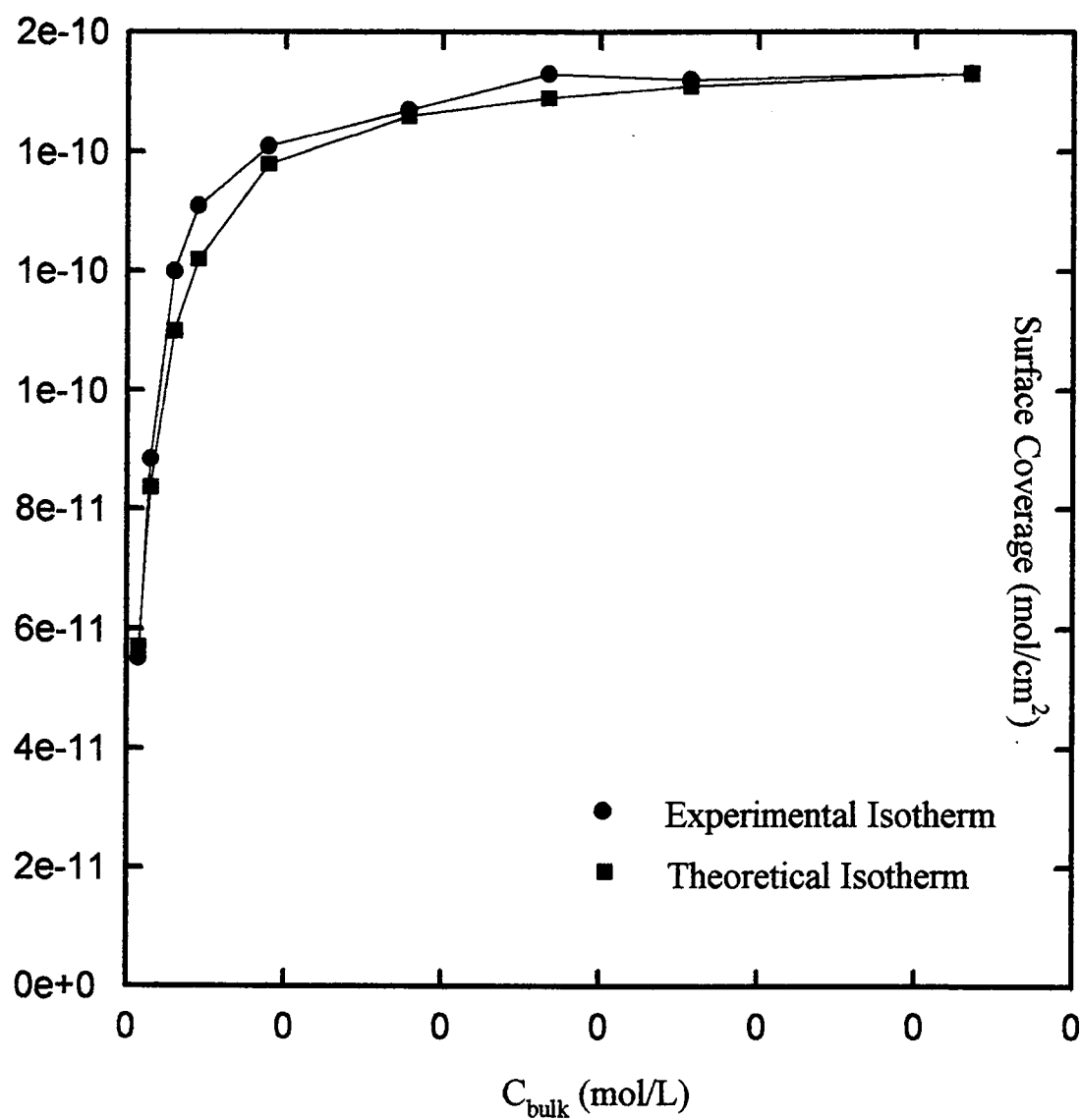


Figure 7-9: Experimental isotherm and theoretical Langmuir isotherm for the mercurated UTP in 0.1 M potassium phosphate, pH=9, and 0.1 M KCl.

mercurated UTP, at a pH value of 9, obeys Langmuir behavior with no indication of interactions between adsorbed molecules.

The Langmuir plot for the mercurated UTP at a pH value of 3 also illustrated linearity, but the linear coefficient was notably lower with a value of 0.9862. Figure 7-10 illustrates the experimental and theoretical isotherm for the mercurated UTP at a pH of 3. The results demonstrate that the experimental isotherm gives a negative shift from true Langmuir behavior. This indicates that some repulsive interactions between adsorbed molecules exist. These repulsive interactions may contribute to the larger surface area encompassed by the chloride complexed species.

*Effect of pH on the voltammetric behavior of the mercurated UDP.* The pH study previously demonstrated for the UTP-HgX was also conducted with the UDP-HgX. The results were almost identical to that of the mercurated UTP. Hence only the peak potential diagram of the UDP-HgX in 0.1 M potassium phosphate with 0.2 M KCl will be presented here. Figure 7-11 illustrates the diagram. This diagram demonstrates that the UDP-HgX also undergoes the change in complexation at lower pH values. Hence, the intramolecularly complexed mercurated UDP has been assigned to region A, UDP-Hg• has been assigned to region B, UDP has been assigned to region C, UDP-HgCl has been assigned to region E, and both the intramolecularly complexed and chloride complexed species have been assigned to region D.

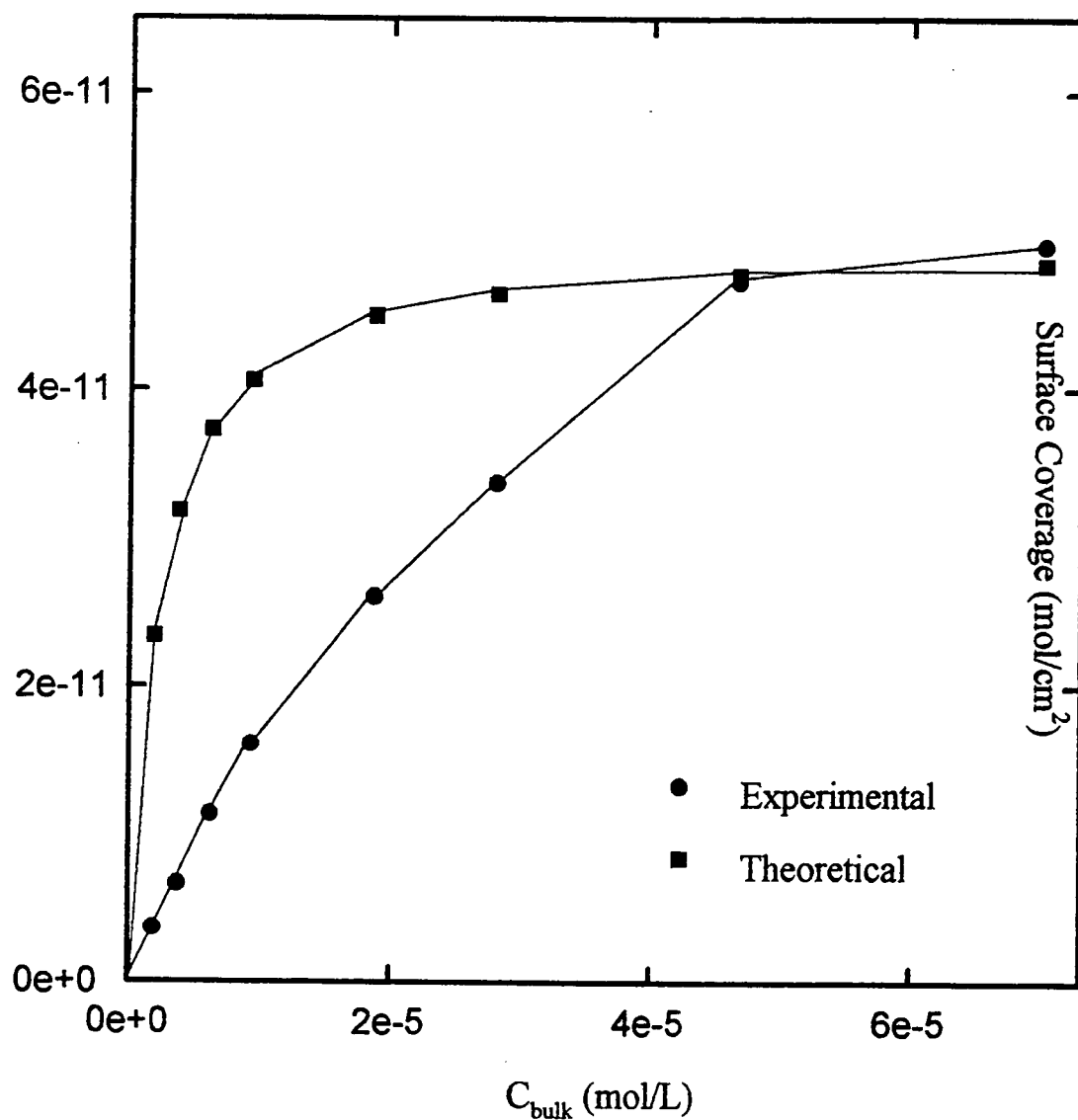


Figure 7-10: Experimental isotherm and theoretical Langmuir isotherm for the mercurated UTP in 0.1 M potassium phosphate, pH=3, and 0.1 M KCl.

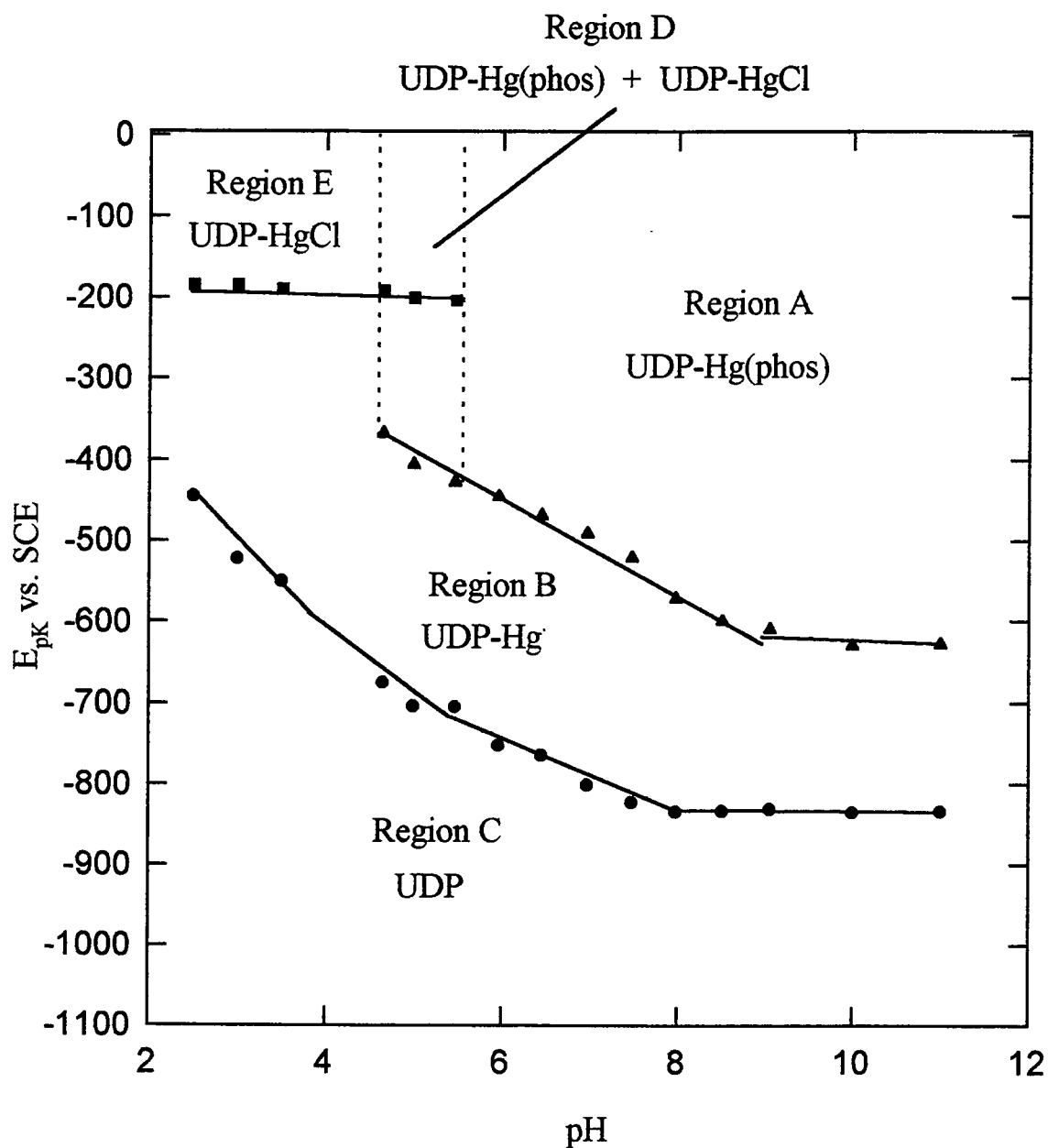


Figure 7-11: Kinetic peak-potential diagram for the UDP-HgX in 0.1 M potassium phosphate buffers with 0.2 M KCl.  $C_{bulk} = 0.07$  mM; 10 V/s;  $0.0225 \text{ cm}^2$  HMDE.

*Effect of pH on the voltammetric behavior of the mercurated UMP.* Figure 7-12 illustrates the kinetic peak potential diagram for the mercurated UMP in 0.1 potassium phosphate buffers containing 0.2 M KCl (assignments will be explained below). This diagram is very similar to the mercurated UTP and UDP diagrams with the peak potential of R1 decreasing with pH until a new wave appears. However, it is interesting to note that the peak potential for the R1 wave does not attain a plateau value in the vicinity of the  $pK_a$  of the nitrogen ( $pK_a=9.6$ ). It was previously concluded that the R1 wave in 0.1 M KCl consisted of two species; UMP-Hg(phos) and  $[PO_4^-R-Hg^+-PO_4^-R-Hg^+]_n$  or  $[R-Hg--N-R-Hg--N]_n$ . Hence, the R1 wave did not adhere to the theoretical peak width of 66 mV for a fast first order chemical reaction (decomplexation) following electron transfer. The variation in peak potential for the R1 wave at high pH may be the result of the intermolecular polymer complex that results from complexation between the mercury substituent and the N-3 nitrogen of an adjacent UMP-HgX molecule. The equilibrium equation between the intramolecular and intermolecular complexed UMP can now be written as shown in equation 7.5. With a  $pK_a$  of 9.6 for the 3-N nitrogen of UMP, this equilibria should be strongly dependent on pH. Hence, the effect of pH on the voltammetric waveshape of R1 was examined. Figure 7-13 illustrates typical R1 waves obtained at pH values of 7.0, 8.5, and 10.0. The pH value of 8.5 is in the pH vicinity of the 0.1 M KCl/UMP-HgX solutions and once again the peak width at half the maximum current was found to be 91 mV at a scan rate of 5 V/s. As shown in Figure 7-12,

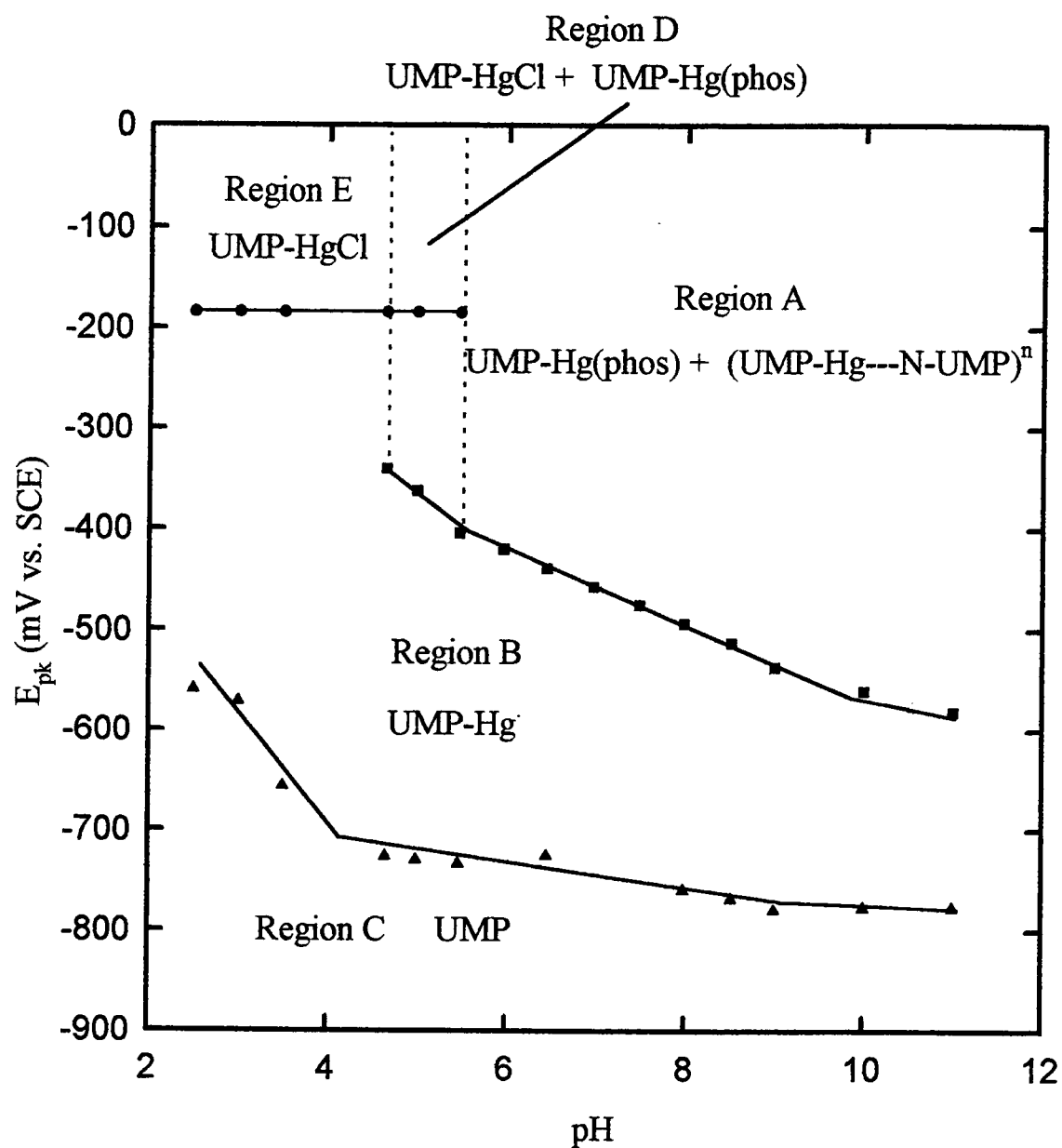


Figure 7-12: Kinetic peak-potential diagram for the UMP-HgX in 0.1 M potassium phosphate buffers with 0.2 M KCl.  $C_{bulk} = 0.07$  mM; 10 V/s;  $0.0225 \text{ cm}^2$  HMDE

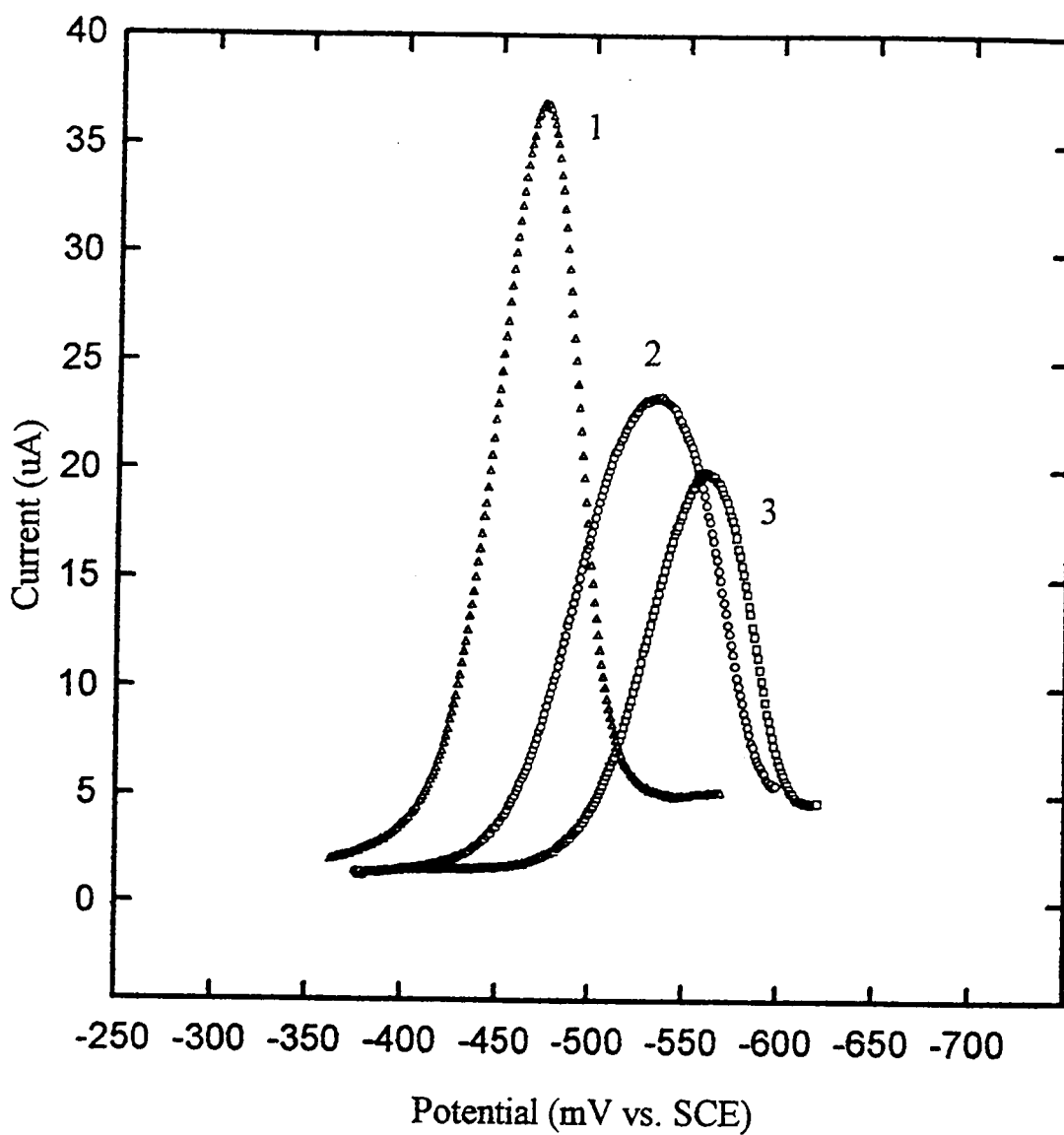
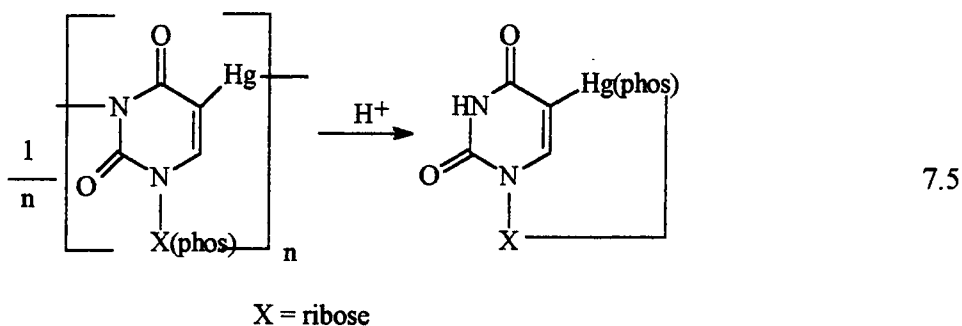


Figure 7-13: Variation of the R1 wave of UMP-HgX with pH. 1: pH = 7.0; 2: pH = 8.5; 3: pH = 10.0;  $C_{\text{bulk}} = 7 \times 10^{-5} \text{ M}$ ;  $\nu = 5 \text{ V/s}$ ; HMDE =  $0.0225 \text{ cm}^2$ .



decreasing or increasing the pH has a dramatic effect on the R1 wave. At a pH of 7.0, the R1 wave has a peak width of 60 mV and at a pH of 10 the peak width is 65 mV. These values are consistent with Laviron's theory for a fast first-order chemical reaction following the electron transfer and demonstrate the change in complexation with pH. It can now be concluded that upon lowering the pH, the nitrogen is protonated and the equilibrium equation (equation 7.5) is shifted to the right. With a peak width value of 60 mV, it appears that at a pH of 7 intramolecular complexation completely predominates. Increasing the pH has the opposite effect upon equation 7.5 and shifts it to the left where the intermolecular polymer complex predominates.

plot for the data in Figure 7-14 show strong linear correlation ( $r^2 = 0.9989$ ), but the theoretical Langmuir isotherm generated from the experimentally determined energy parameter and surface coverage also showed excellent correlation with the experimental isotherm (Figure 7-14). Unlike the results in 0.1 M KCl or at higher pH values, where a positive deviation from the Langmuir plot suggested some attractive interaction, these results demonstrate that in the vicinity of pH=7, there appears to be minimal interactions between adsorbed UMP-HgX species.

From the above results, the intramolecularly phosphate complexed UMP-HgX species and the intermolecular polymer type complex have been assigned to region A of the kinetic peak potential diagram (Figure 7-12). However, the intramolecularly phosphate complexed species predominates on the left hand side of region A and the intermolecularly complexed species predominated on the right hand side. In addition, the radical has been assigned to region B and the parent compound, UMP, has been assigned to region C.

As with the mercurated UTP and UDP, voltammetric data have demonstrated the replacement of the phosphate as the ligand with chloride at lower pH values. Hence, the chloride complexed species has been assigned to region E and both the phosphate and chloride complexed species have been assigned to region D. Evidence supporting these latter assignments will be illustrated shortly.

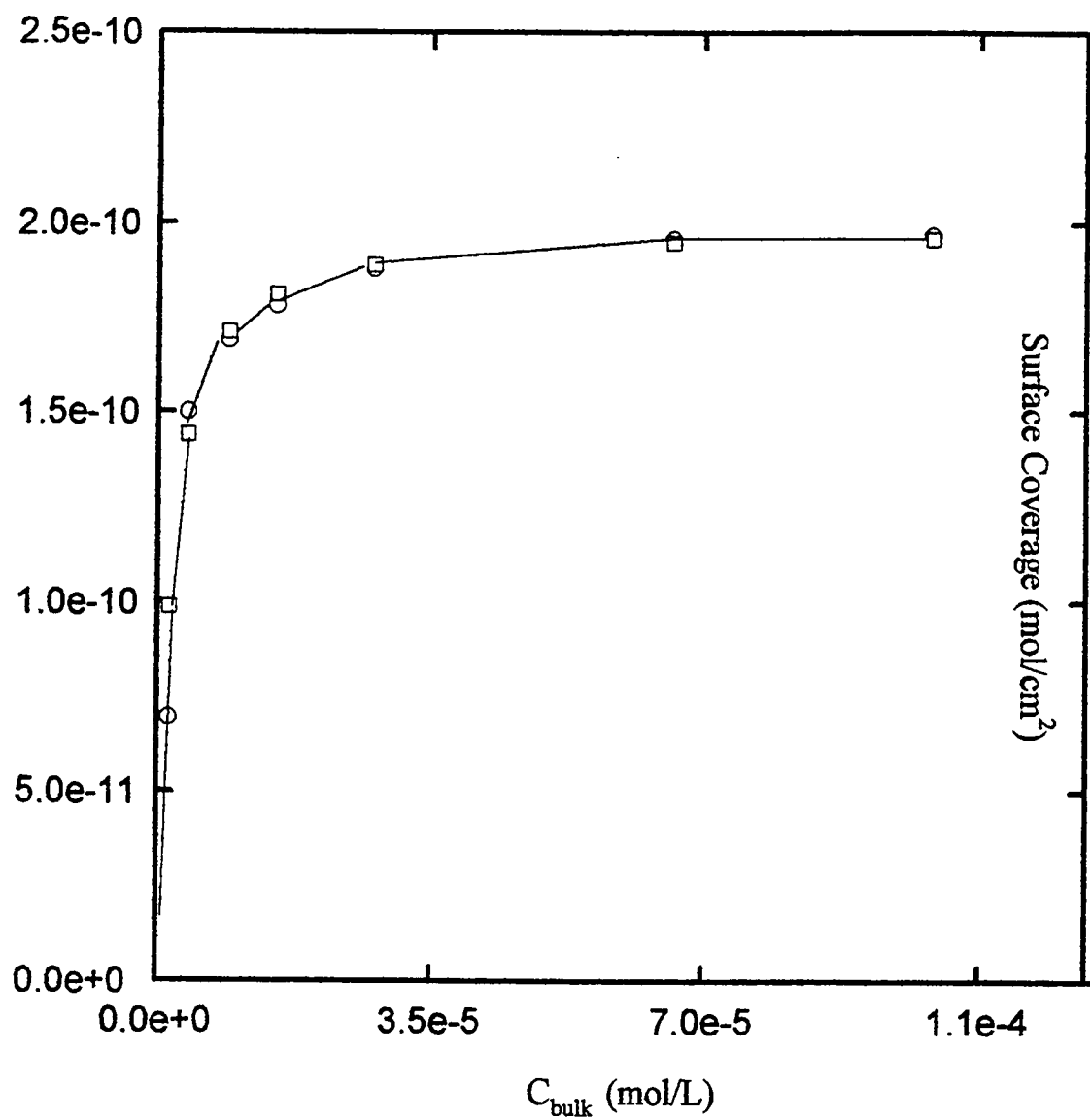


Figure 7-14: Experimental isotherm and theoretical Langmuir isotherm for UMP-HgX in 0.1 M potassium phosphate, pH=7, and 0.2 M KCL.

*Potentiometric titration of the mercurated UMP.* It was shown in the experimental section (page 41) that  $7.21 \times 10^{-5}$  equivalent protons were required to titrate  $3.57 \times 10^{-5}$  moles of the mercurated UMP. Hence, 2x the expected amount of protons were required. This result can now be explained. The large excess of protons results from the decomplexation of the intermolecular complexed species in solution as shown in equation 7.5. Thus, in addition to protonation of the phosphate arm, additional protons were required to protonate the nitrogen of the pyrimidine base. For example, if the intermolecular complex consisted of 4 UMP monomer units,  $6.70 \times 10^{-5}$  equivalent protons would be required (using a molecular weight of 2331g/mol for the polymer). If the complex consisted of 8 UMP monomer units,  $7.18 \times 10^{-5}$  equivalent protons would be required (using a molecular weight of 4662 g/mol for the polymer). Another explanation of the above result may be the presence of two pKa's, however, the shape of the titration curve (Figure 3-5) is not consistent with this conclusion. The above explanation (polymer titration) suggests that the intermolecular complexed species is in the vicinity of 8 units or greater in length.

*Effect of pH on the cyclic voltammetry of the mercurated UMP.* Figure 7-15 shows a cyclic voltammogram of the mercurated UMP-HgX in 0.1 M potassium phosphate and 0.2 M KCl, pH=8. This voltammogram is very similar to the voltammetry obtained in unbuffered KCl and results from having both the intermolecularly complexed and intramolecularly complexed species on the surface. Figure 7-16 shows a voltammogram at a pH of 7. At this pH, the voltammetry

looks very similar to that of the mercurated UTP and UDP with the coulombic charge under both R1 and Ox1 being equivalent. Previously (also evident in figure 7-15), it was shown that the coulombic charges under the R1 and Ox1 waves of the mercurated UMP were not equivalent because of disproportionation (dimer formation). This result was obtained at higher pH values where both the intermolecular and intramolecular complexed species reside on the surface. In Figure 7-16, only the intramolecular complexed species resides on the surface. Hence, it appears that the disproportionation seen with the mercurated UMP occurs only with the intermolecular complexed species. In fact, cyclic voltammograms conducted at pH values greater than 9 showed no anodic current which could be assigned to the Ox1 wave even at faster scan rates (50 V/s).

Figure 7-17 illustrates a cyclic voltammogram obtained in 0.1 M potassium phosphate with 0.2 M KCl at a pH of 5.0. At this pH the voltammetry looks very similar to that of the mercurated UTP and UDP with the appearance of a R1'/Ox1 couple. This R1'/Ox1 couple, consistent with the behavior of the mercurated UTP and UDP, is the result of chloride complexation. In fact, a chloride concentration study showed this to be true. This study was conducted at a pH of 5.5 and a chloride concentration varying from 0.05 to 0.40 M. Table 20 shows the coulombic charge ratios of  $Q_{R1'}$  to  $Q_{R1}$  at each chloride concentration. The result, identical to that of the mercurated UTP, showed that as the chloride concentration was increased, the coulombic charge under R1' increased while that under R1 decreased. Hence, the

Table 20: Dependence of the coulombic charge ratios of  $Q_{R1'}$  to  $Q_{R1}$  with chloride concentration

|       |      |      |      |      |      |      |      |      |
|-------|------|------|------|------|------|------|------|------|
| KCl   | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 |
| Ratio | 0.09 | 0.17 | 0.26 | 0.41 | 0.66 | 1.05 | 1.18 | 2.21 |

voltammetry of the mercurated UMP appears to be identical to the mercurated UTP and UDP in acid medium. In fact, Figure 7-18 illustrates a cyclic voltammogram of the mercurated UMP carried out with a pH of 3. Here the R1 wave has completely disappeared and the R1' wave predominates.

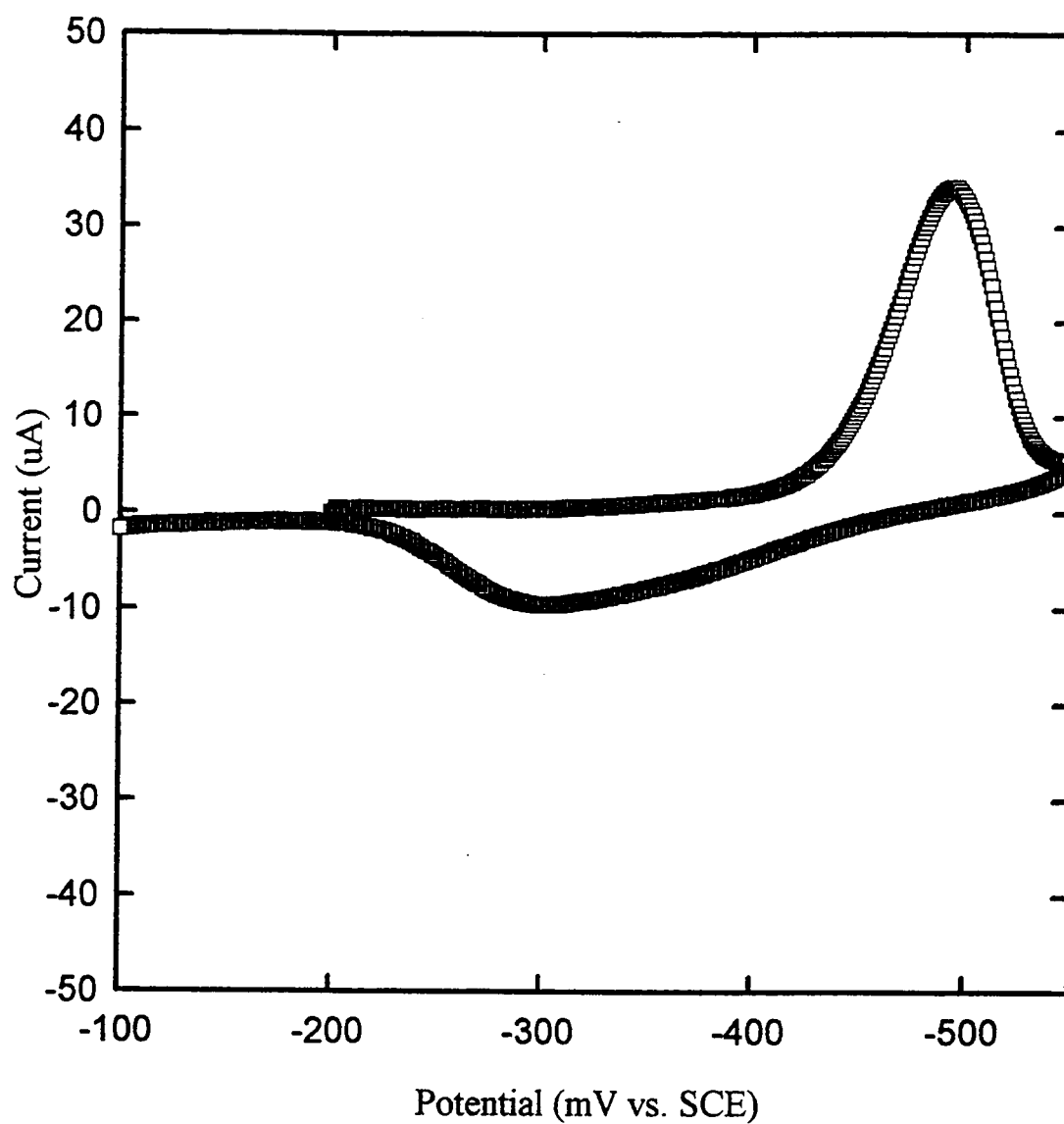


Figure 7-15: Cyclic voltammogram of  $7 \times 10^{-5}$  M UMP-HgX in 0.1 M phosphate at a  $0.0225 \text{ cm}^2$  HMDE; pH = 8.0;  $v = 5 \text{ V/s}$ .

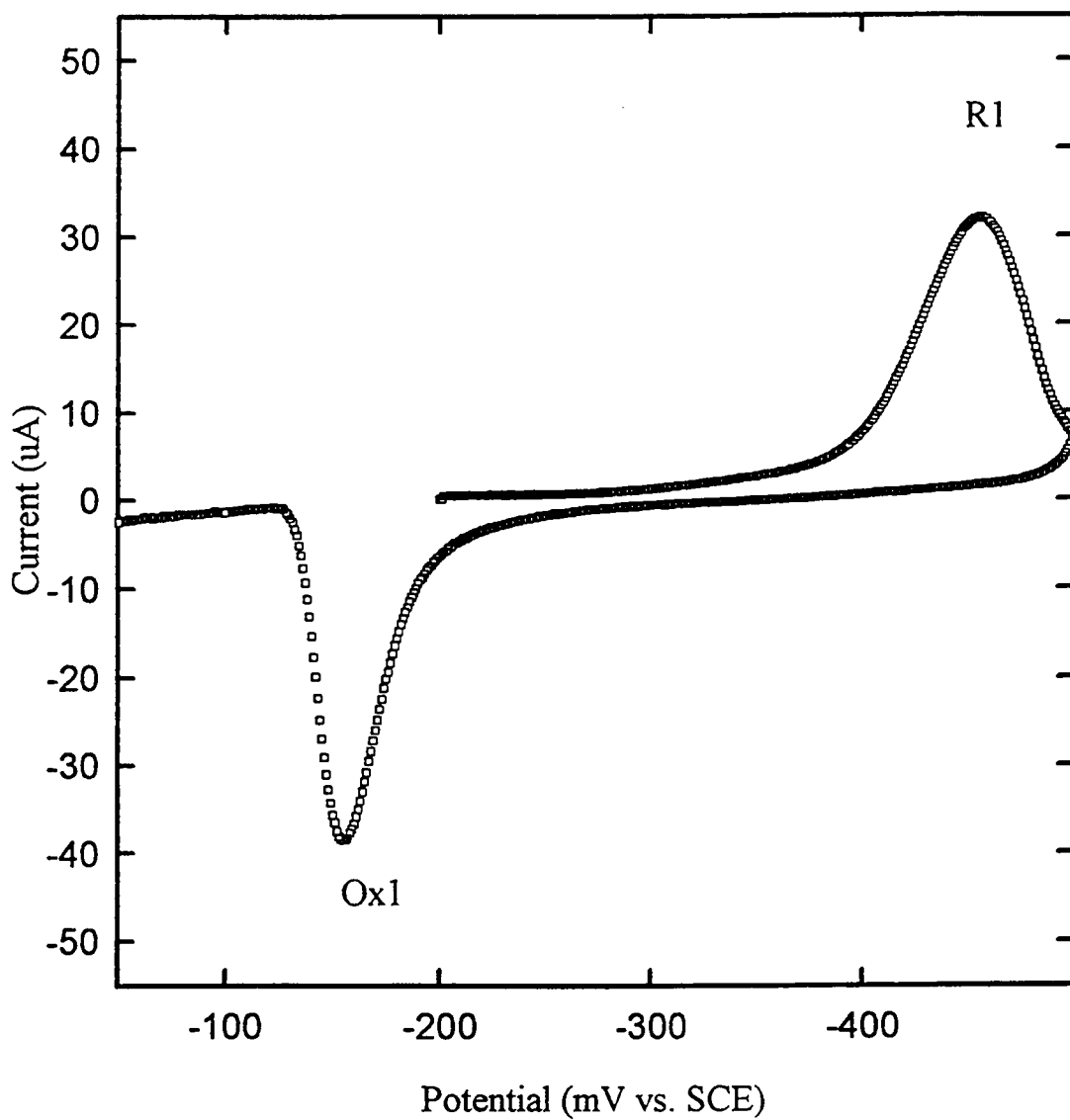


Figure 7-16: Cyclic voltammogram of  $7 \times 10^{-5}$  M UMP-HgX in 0.1 M phosphate at a  $0.0225 \text{ cm}^2$  HMDE; pH = 7.0;  $v = 5 \text{ V/s}$ .

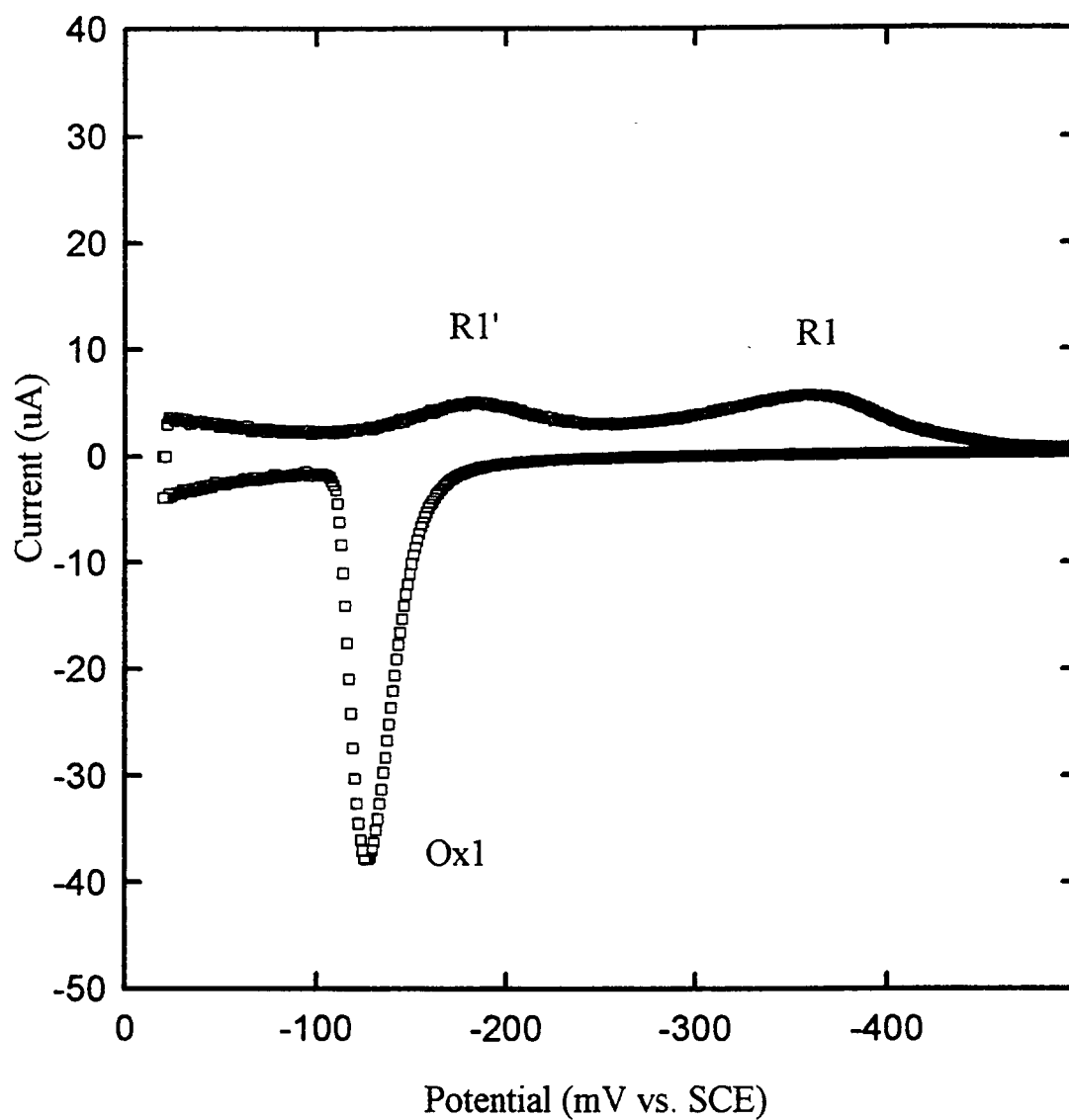


Figure 7-17: Cyclic voltammogram of  $7 \times 10^{-5}$  M UMP-HgX in 0.1 M phosphate with 0.1 M KCl at a  $0.0225 \text{ cm}^2$  HMDE; pH = 5.0;  $v = 5 \text{ V/s}$ .

## Chapter VIII

### Mechanistic Summary of the Voltammetric Behavior of the Mercurated Uridine Nucleotides

#### Introduction

As described in the previous chapter, the surface voltammetry of the mercurated uridine nucleotides is quite complex. In this chapter a mechanistic review of the voltammetry will be provided. In addition, selected voltammograms will be calculated assuming specific electrode mechanisms. These calculated cyclic voltammograms were calculated by a Quick Basic program that called on Nernst and kinetic subroutines after small incremental potential steps (46). The Nernst subroutine satisfied the Nernst condition for each redox couple, i.e.  $\Gamma_{\text{ox}}/\Gamma_{\text{r}} = \exp(F/RT(E-E^0))$ , and the kinetic subroutine adjusted the surface coverages for first-order kinetic decay, i.e.  $\Gamma_i = \Gamma_i^0 \exp(-k \Delta t)$ , where  $\Delta t$  is equal to the potential step divided by the scan rate. The program was checked by comparison to voltammograms calculated from Laviron's theory (32) for a simple case of a reversible electron transfer followed by a first-order chemical reaction (EC mechanism). By comparing the calculated and experimental voltammograms, conclusions can be made on the validity of the proposed mechanisms. It should be noted that these calculations assumed that the total surface coverage of nucleotide was constant and neglected

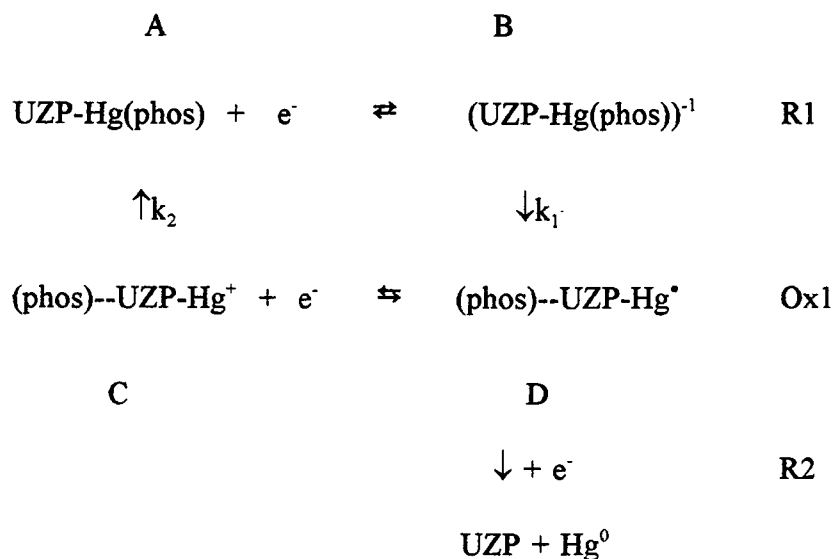
diffusional contribution to the current.

## Results and Discussion

### *Square scheme behavior of the mercurated uridine nucleotides.*    Cyclic

voltammetry of the mercurated UTP and UDP in 0.1 M KCl produced the R1 wave on the cathodic sweep and the Ox1 wave on the anodic sweep. In chapter V the R1 wave was found to result from the one-electron reduction of the surface confined nucleotide to the surface confined radical (equations 5.3) and in chapter VI the Ox1 wave was found to result from the one-electron oxidation of the surface confined radical (equation 6.1). It was also shown in these chapters that the irreversible, non-ideal behavior of the R1 and Ox1 waves was the result of decomplexation and complexation of the mercury substituent after electron transfer (equation 5.4 and 6.2). This behavior was also obtained with the mercurated UMP with solutions having a pH in the vicinity of 7.0 (figure 7-16). The combination of equations 5.3 and 5.4 with 6.1 and 6.2 yields a square scheme. This square scheme mechanism seen with the mercurated UTP, UDP, and UMP is illustrated in scheme 2 where the second reduction process, R2, has been included for clarity. Labels (A-D) have also been added to present subsequent material more clearly.

The significance of this "square scheme" mechanism is that it illustrates that the R1 and Ox1 waves are not directly coupled and each wave has its own  $E^0$  value. Hence, it may be possible to increase the potential sweep and see a wave assignable to the oxidation of  $(\text{UZP-Hg(phos)})^{-1}$  (B) to  $\text{UZP-Hg(phos)}$  (A) or the reduction of



**Scheme 2**

(phos)--UZP-Hg<sup>+</sup> (C) to (phos)--UZP-Hg<sup>•</sup> (D). Experimentally, this result was not obtained even at scan rates greater than 2000 V/s. This result demonstrates that the rate constants  $k_1$  and  $k_2$  are very fast and are consistent with Laviron's theory. Laviron's theory states that as  $k_1$  and  $k_2$  approach infinity, the peak width of the surface wave decreases to 66 mV (32). Experimentally, the R1 waves of the mercurated nucleotide were found to contain an average peak width of 69 mV.

Calculation of the square scheme behavior was conducted to verify the mechanism and determine the minimum values of  $k_1$  and  $k_2$ . The analysis of the CV's will be illustrated for UTP-HgX in 0.1 M KCl. The details of the calculation at a scan rate of 50 V/s are given below. The first step in the analysis was to

determine the minimum rate constants for decomplexation ( $k_1$ ) after electron transfer. At 50 V/s, reduction of this species gave an irreversible wave (R1) at -0.76 V. At this scan rate there was no indication of a reverse wave that could be assigned to reversible oxidation of a (UTP-Hg(phos))<sup>-1</sup> species when the potential sweep was reversed at -0.85 V. Calculation of this voltammogram, in which the square scheme was assumed, allowed a minimum value of  $k_1$  to be set. Input parameters included the electrode area, double layer capacity, initial potential, switching potential, surface coverage, scan rate,  $k_1$  and  $k_2$ , and  $E^0$  values. Initially, the  $E^0$  values were set equal to the peak potentials. Refinement of the  $E^0$  values was undertaken throughout the calculations by visual comparison of the calculated and experimental voltammograms until the reported values were obtained (Table 21).

The rate constant  $k_1$  was first arbitrary selected to be 1200 s<sup>-1</sup>. The resulting calculated voltammogram showed anodic current for oxidation of species B in the square scheme. Thus, the value of  $k_1$  was increased until there was no indication of any anodic current for the oxidation of B. A value of 2000 s<sup>-1</sup> was obtained.

Further refinement of this value is possible. As shown in the introduction, theoretically for a first-order chemical reaction following electron transfer, the peak width and peak current are a function of Laviron's  $b$  parameter (equation 2.20). As  $b$  varies from 0 to infinity for this mechanism, the peak width decreases from 90 to 66 mV, the peak current increases by 47%, and the wave shape becomes more asymmetric. At  $k/v = 40$  ( $k = 2000$  s<sup>-1</sup>;  $v = 50$  V/s), where these parameters have not quite reached their limiting values, it was observed that the fit of the calculated

and experimental voltammograms was improved by increasing  $k_1$ . Thus,  $k_1$  was increased until no further improvement was obtained. The  $k_1$  value obtained was  $8000\text{ s}^{-1}$ . The limiting value of the rate constant for complexation of the  $\text{UTP-Hg}^+$  species was determined to be  $1 \times 10^4\text{ s}^{-1}$  in a similar manner.  $k_2$  was determined to be  $1 \times 10^4\text{ s}^{-1}$ . These values are the minimum values for the rate constants. Figure 8-1 illustrates the experimental and calculated voltammograms for the  $\text{UTP-HgX}$  with the above rate constants at  $50\text{ V/s}$ . These voltammograms are in good agreement. The only significant deviation between the calculated and experimental voltammograms results is for the anodic wave shape where the experimental current is larger at the foot of the wave. Figures 8-3 and 8-4 illustrate experimental and calculated voltammograms at  $30$  and  $10\text{ V/s}$ . At  $30\text{ V/s}$ , the experimental and calculated voltammograms are in even better agreement than at  $50\text{ V/s}$ , but at  $10\text{ V/s}$  the experimental anodic wave is sharper and shifted to more negative potentials and the experimental cathodic wave is shifted slightly to more positive potentials. The deviation in the potential may be the result of uncompensated IR drop in the electrode cell, since there was no instrumental compensation made for IR drop. However, the experimentally obtained sharper anodic wave does show real deviation from theory. In fact, this deviation was seen previously at  $5\text{ V/s}$ . As stated in chapter VI, the smaller peak width seen for the anodic wave, Ox1, could result from interactions between adsorbed radical molecules.

Table 21 gives the parameters used during the above calculations. It is evident from this table that the only parameter significantly varied was the scan rate.

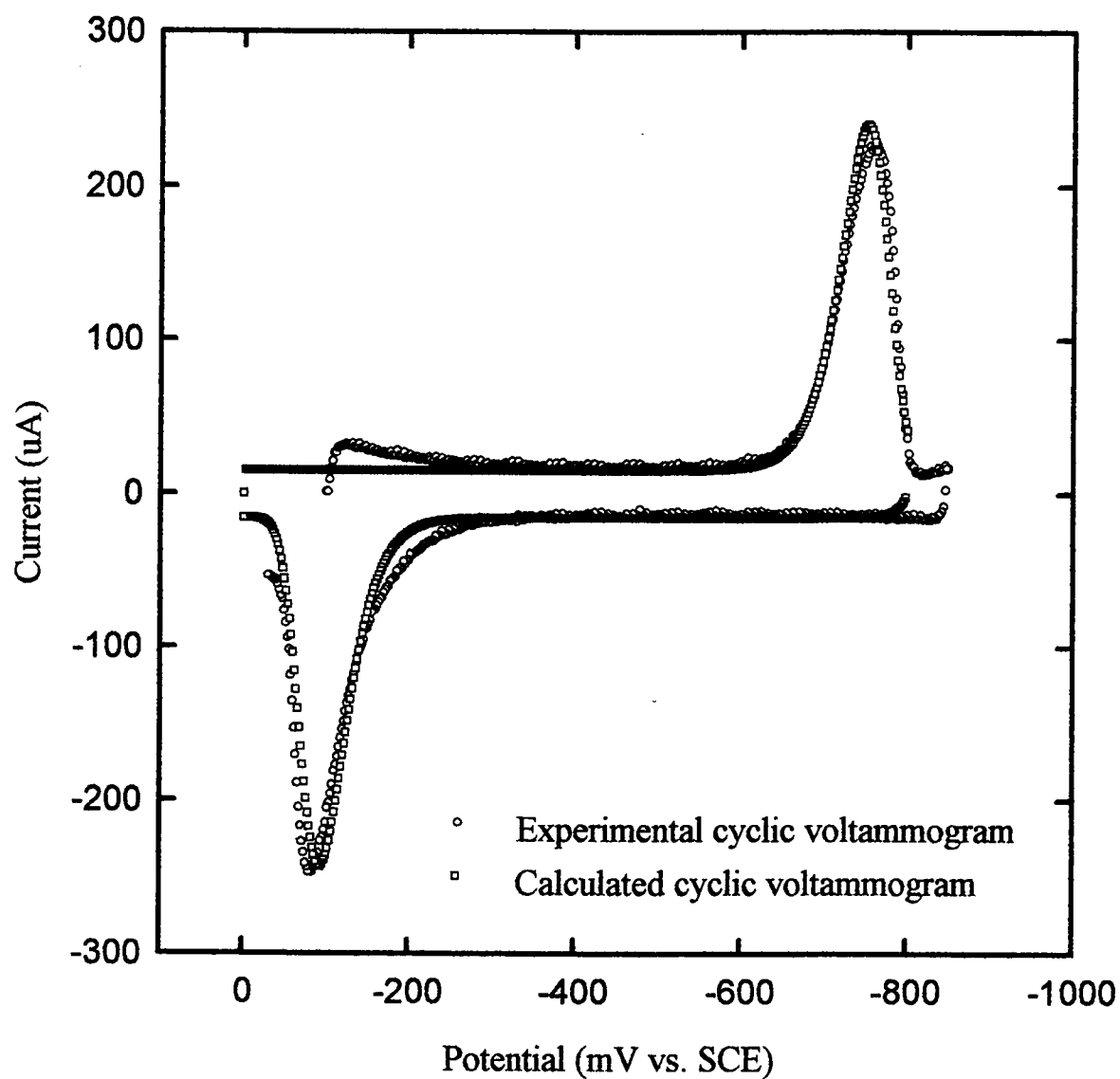


Figure 8-1: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M KCl.  $k_1 = 8000 \text{ s}^{-1}$ ;  $k_2 = 1 \times 10^4 \text{ s}^{-1}$ ;  $v = 50 \text{ V/s}$ .

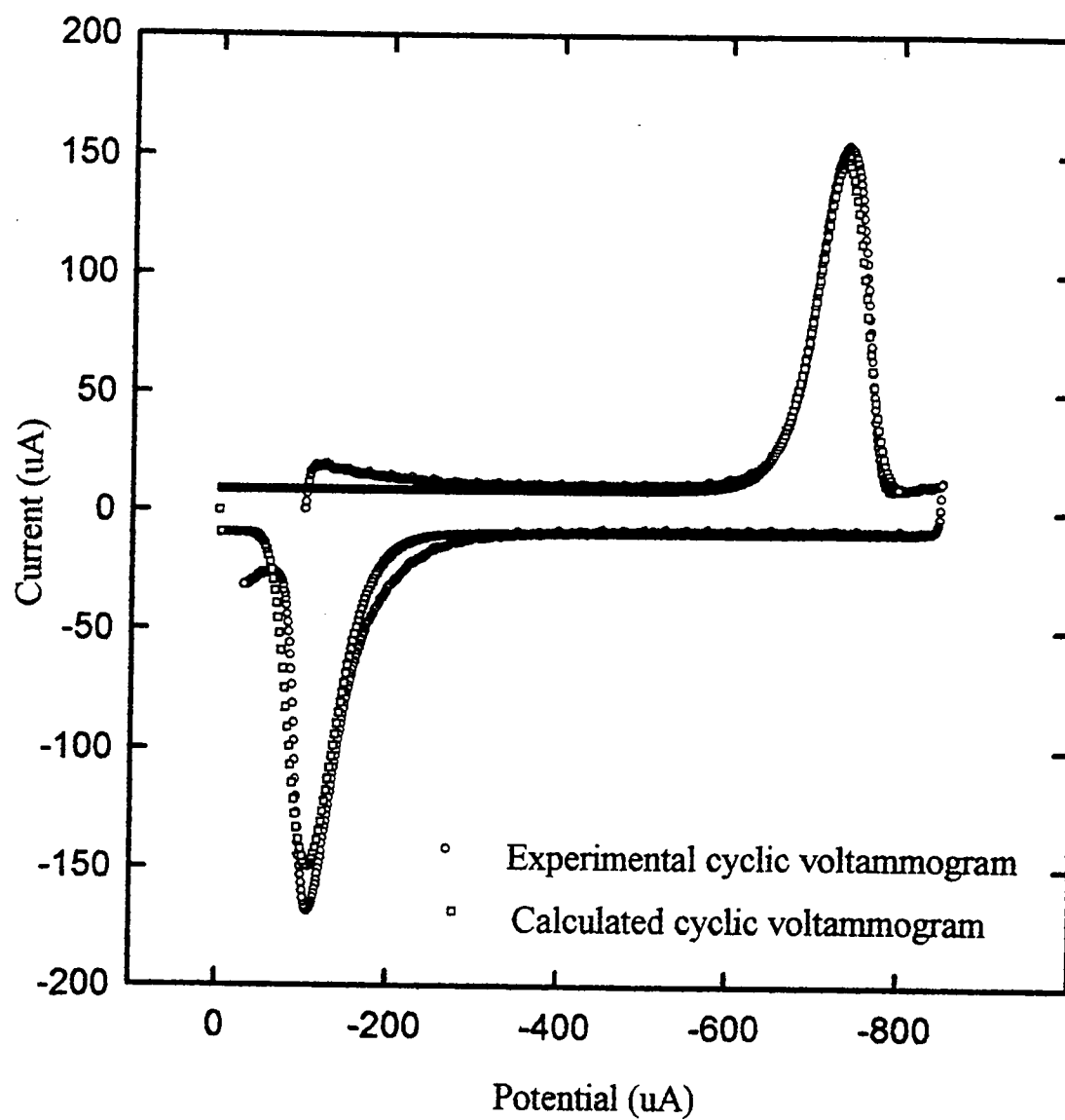


Figure 8-2: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M KCl.  $k_1 = 8000 \text{ s}^{-1}$ ;  $k_2 = 1 \times 10^5 \text{ s}^{-1}$ ;  $v = 30 \text{ V/s}$ .

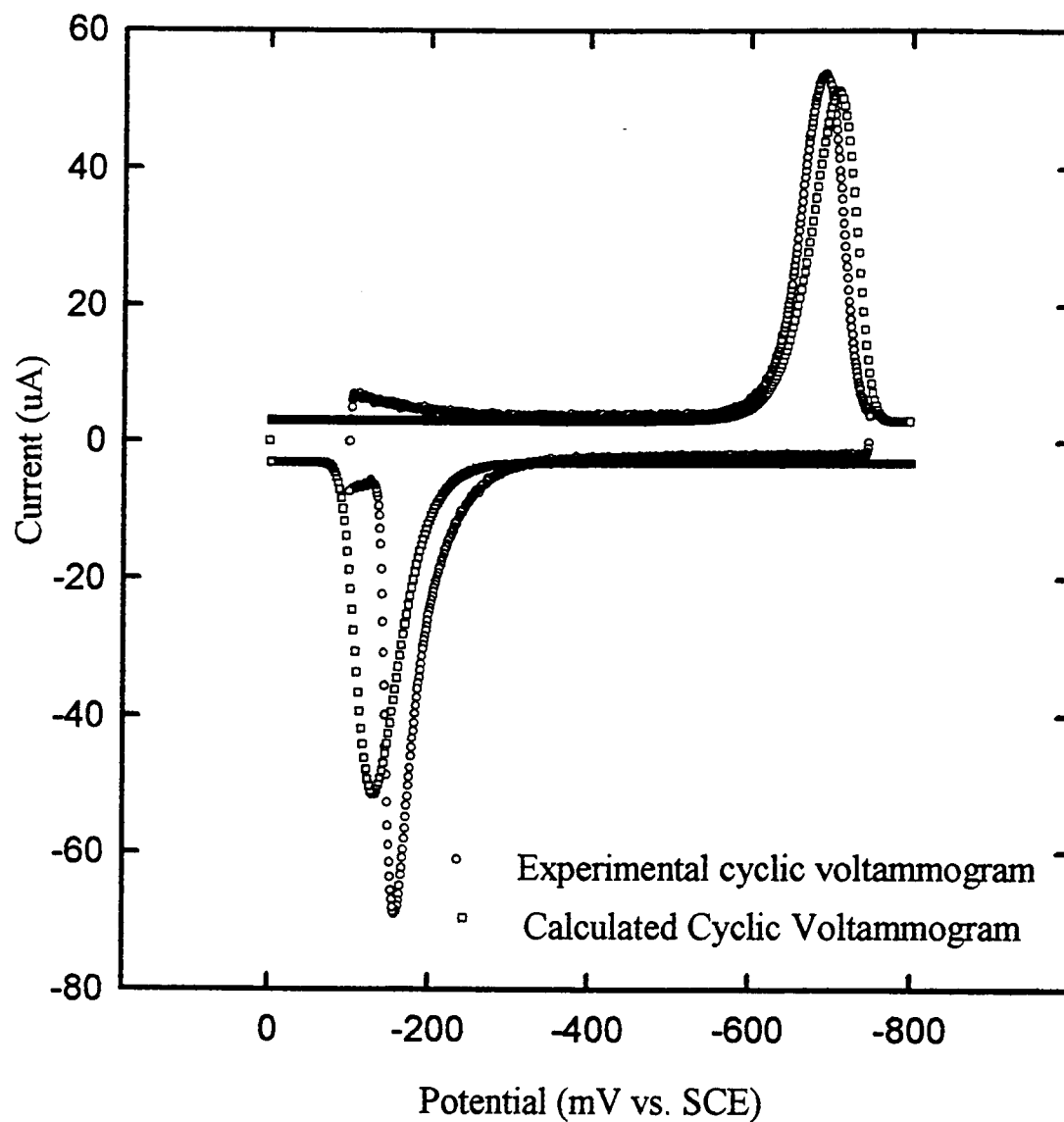


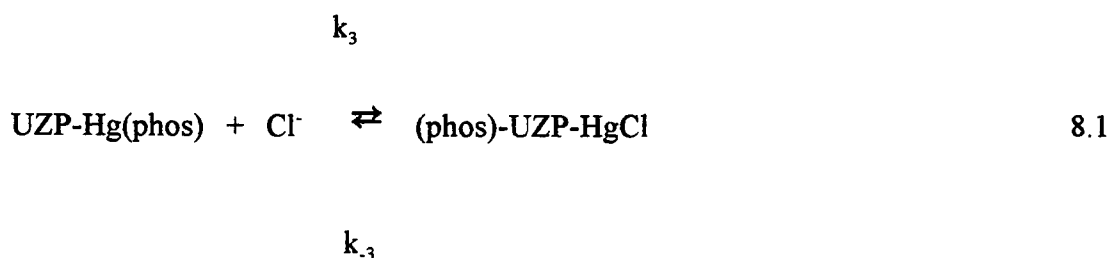
Figure 8-3: Experimental and calculated cyclic voltammetry for the UTP-HgX in 0.1 M KCl.  $k_1 = 8000 \text{ s}^{-1}$ ;  $k_2 = 1 \times 10^4 \text{ s}^{-1}$ ;  $v = 10 \text{ V/s}$ .

Table 21: Input parameters utilized in the Quick Basic program to calculate the square scheme voltammograms of UTP-HgX at 10, 30, and 50 V/s.

| Parameters                                          | Figure 8-1 | Figure 8-2 | Figure 8-3 |
|-----------------------------------------------------|------------|------------|------------|
| Electrode area ( $\text{cm}^2$ )                    | 0.225      | 0.225      | 0.225      |
| Double layer capacity ( $\mu\text{F}/\text{cm}^2$ ) | 13.6       | 13.6       | 13.6       |
| Initial potential (V)                               | 0          | 0          | 0          |
| Switching potential (V)                             | -0.8       | -0.8       | -0.8       |
| Surface Coverage ( $\mu\text{C}$ )                  | 0.34       | 0.37       | 0.32       |
| Scan Rate (V/s)                                     | 50         | 30         | 10         |
| $k_1$ ( $\text{s}^{-1}$ )                           | 8000       | 8000       | 8000       |
| $k_2$ ( $\text{s}^{-1}$ )                           | 10000      | 10000      | 10000      |
| $E^0_{\text{A/B}}$ (mV)                             | -785       | -785       | -785       |
| $E^0_{\text{C/D}}$ (mV)                             | -50        | -50        | -50        |

Theoretically, only the scan rate should change (the surface coverages were obtained from the experimental voltammograms and will vary slightly). Hence, the square scheme calculation program appears to simulate the experimental voltammograms with good accuracy. This provides strong support for the square scheme mechanism outlined above. It should be emphasized that the program assumed Nernstian behavior and  $n=1$  for the electron transfer step. The excellent agreement of the experimental and calculated wave shapes provides strong support for the one-electron  $n$ -value assumed throughout this thesis for the R1/Ox1 couple.

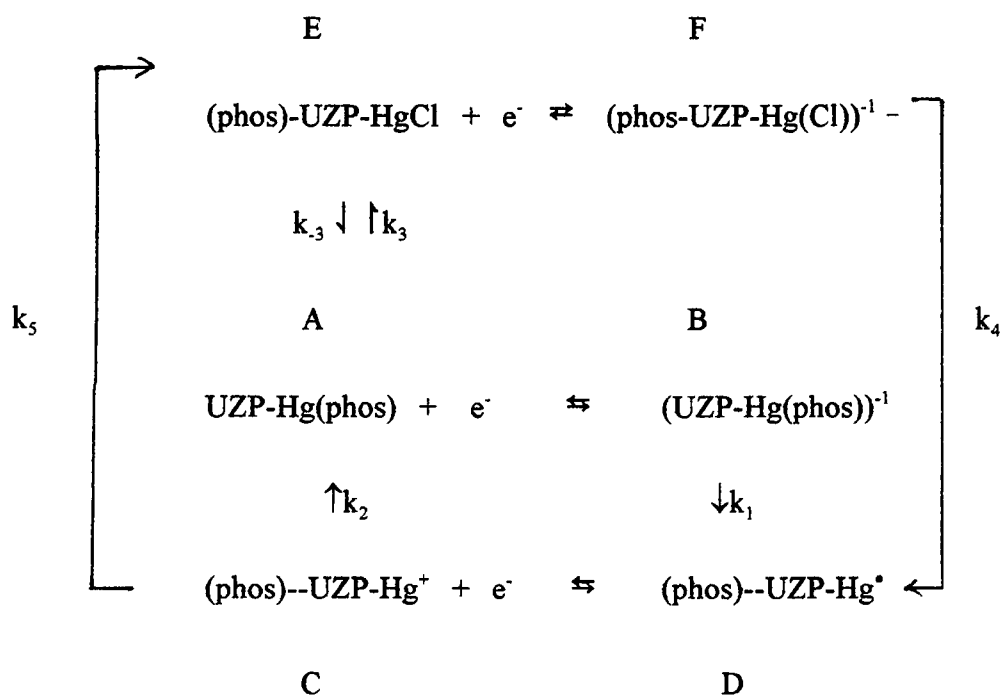
*Ladder scheme behavior of the mercurated uridine nucleotides.* In chapter VII it was shown that upon lowering the pH, the complexation state of the mercury substituent on the uridine nucleotides changes from the phosphate arm to chloride. The result is a new wave in the voltammetry, R1', which was assigned to the reduction of the chloride complexed species. Scheme 2 for the mercurated nucleotides can now be extended to include the R1' process by the addition of the E/F couple where the chloride complexed species is reduced to the radical (scheme 3). The rate constants  $k_3(k_{-3})$ ,  $k_4$ , and  $k_5$  have also been added to the scheme. The presence of two separate waves on the cathodic segment of the voltammogram and the assignment of to different complexation states of the mercury substituent requires that the equilibrium between the species is slow on the voltammetric time scale. This equilibrium can be written as shown in equation 8.1, where  $K = k_3/k_{-3}$ .



Hence,  $k_3$  describes the rate of phosphate displacement by the chloride and  $k_{-3}$  describes the rate of chloride displacement by the phosphate. The rate at which decomplexation of the chloride ion occurs after electron transfer is given by  $k_4$  and  $k_5$  describes the rate of chloride complexation of the mercury substituent after oxidation of the radical. In this scheme, the R1' wave is not associated directly with the

anodic wave Ox1. From the voltammograms (Figures 7-5 and 7-6) R1' appears to form a reversible couple with Ox1, but simulation of this voltammogram was best obtained with scheme 3.

As for the four-membered square scheme behavior, a Quick Basic program, which incorporated the  $k_3/k_{-3}$  equilibrium the kinetic subroutine, was used to calculate the voltammograms of the mercurated UTP. Particularly, the voltammetry of the UTP-HgX in 0.1 M potassium phosphate (pH=5.5) was calculated as a function of the chloride concentration. In this manner the equilibrium between the chloride and



**Scheme 3**

phosphate complexed could be examined. Input parameters included the electrode area, double layer capacity with adsorbed UTP-HgX, double layer capacity with the adsorbed radical, initial potential, switching potential, surface coverage, electrode area,  $k_1$ ,  $k_2$ ,  $k_3$ ,  $k_{-3}$ ,  $k_4$ ,  $k_5$ , scan rate, and chloride concentration. Two double layer capacity's were used in this calculation because experimentally the double layer capacity was observed to change between the oxidized and reduced surface. Figures 8-4 through 8-9 illustrate experimental and calculated voltammograms with chloride concentrations ranging from 0.05 to 0.30 M. Table 22 tabulates the calculation parameters. Although there does appear to be a small variation in wave shape with wave R1 at higher chloride concentrations, the results for the R1' and R1 waves show good consistency between experimental and calculated voltammograms. The greatest deviation between the experimental and calculated voltammograms is seen with the Ox1 wave. There appears to be more coulombic charge under the Ox1 wave experimentally than in the calculated CVs. The surface coverage was an input parameter in the simulation and was determined experimentally from the area under both the R1 and R1' waves. However, experimentally the area under Ox1 is greater. This is because of a small diffusional component during the cathodic sweep and is evident in the voltammogram as a sloping background. In fact, during simulation a cathodic background faradaic current was added to improve the match between experimental and calculated voltammograms, but this charge was not added to Ox1.

From the tabulated calculation parameters it is evident that the  $E^\circ$  value were adjusted throughout the above calculations. This represents a deviation from theory

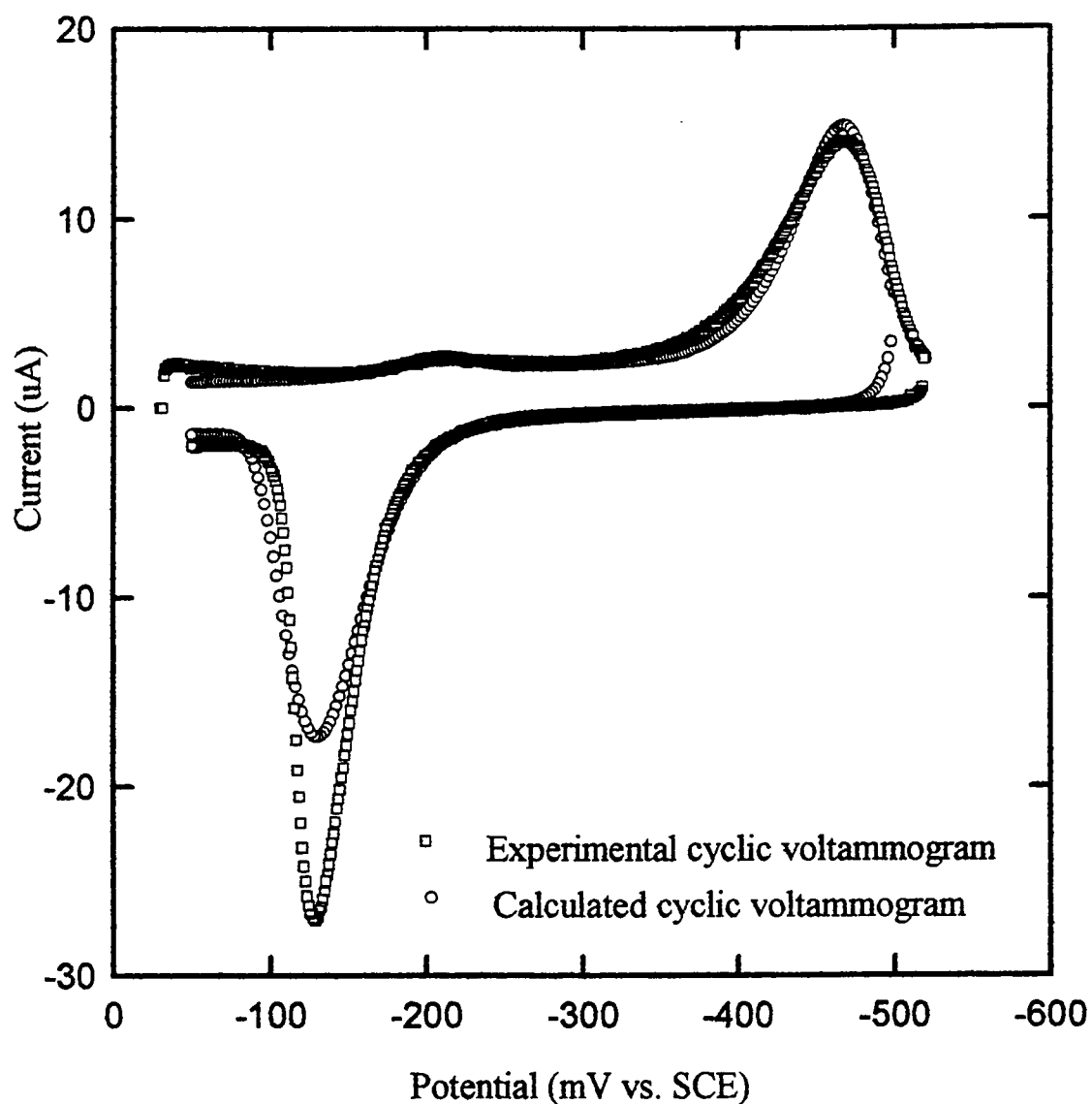


Figure 8-4: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5.5, with 0.05 M KCl.

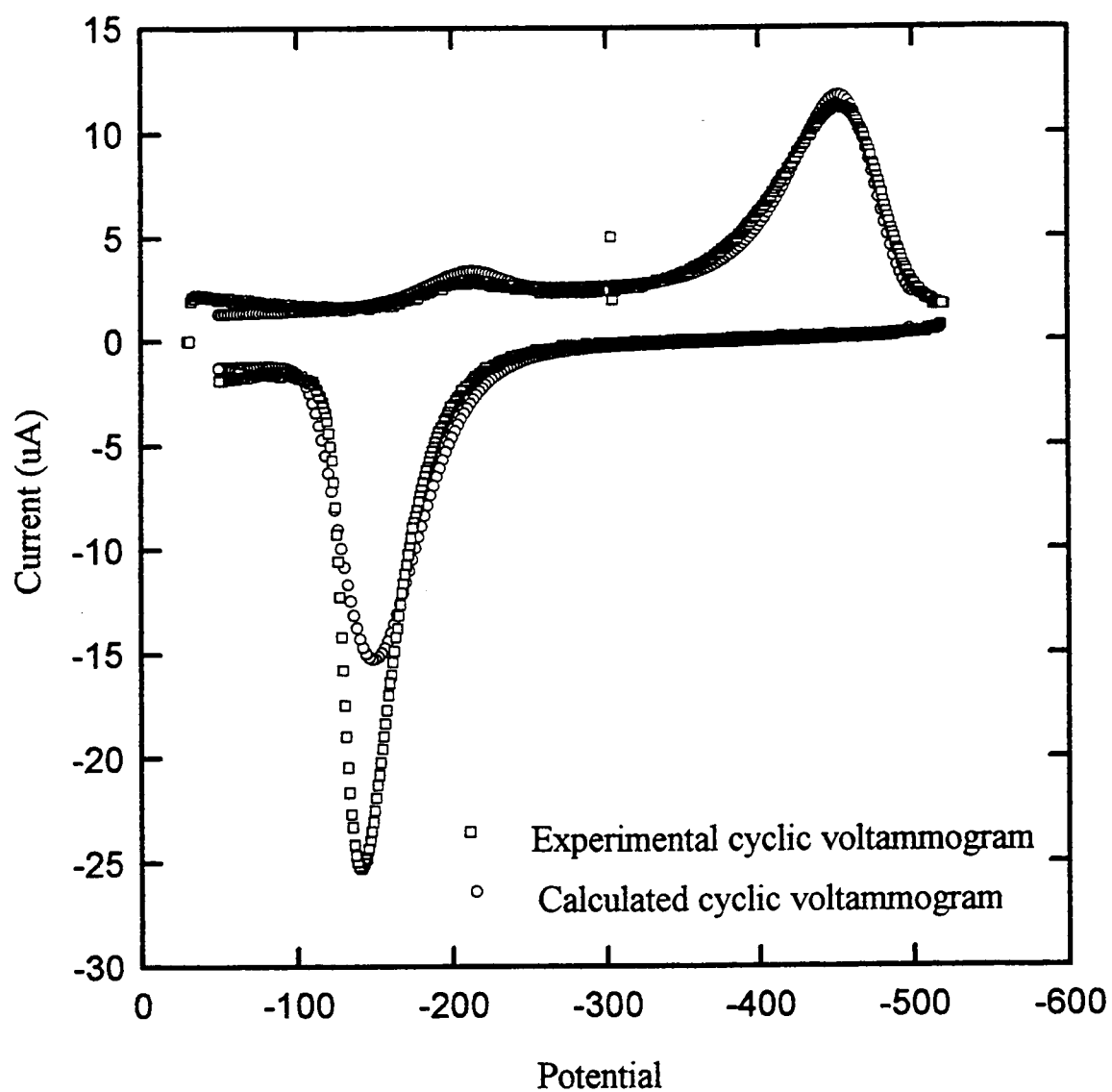


Figure 8-5: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5.5, with 0.1 M KCl.

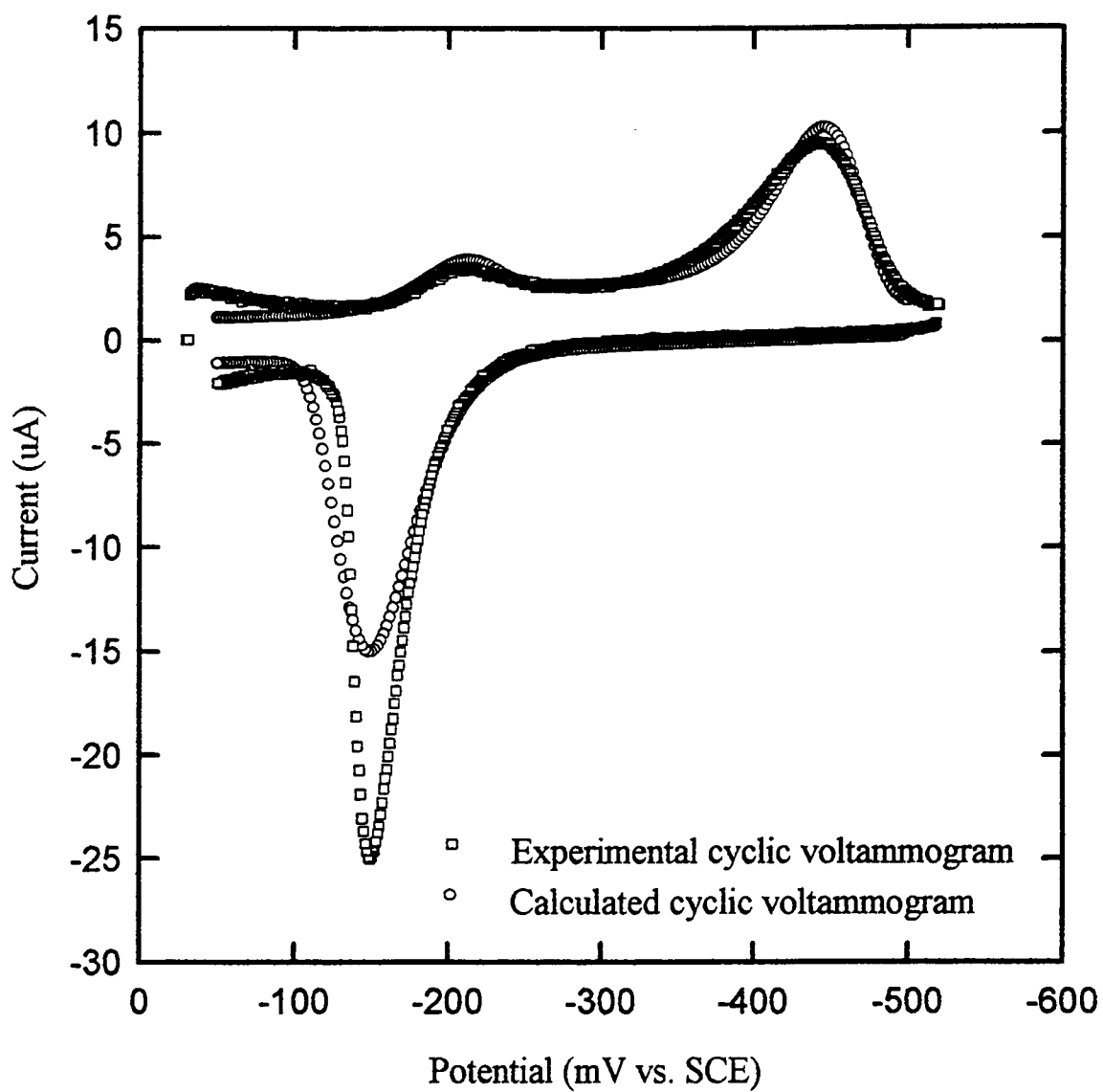


Figure 8-6: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5.5, with 1.5 M KCl.

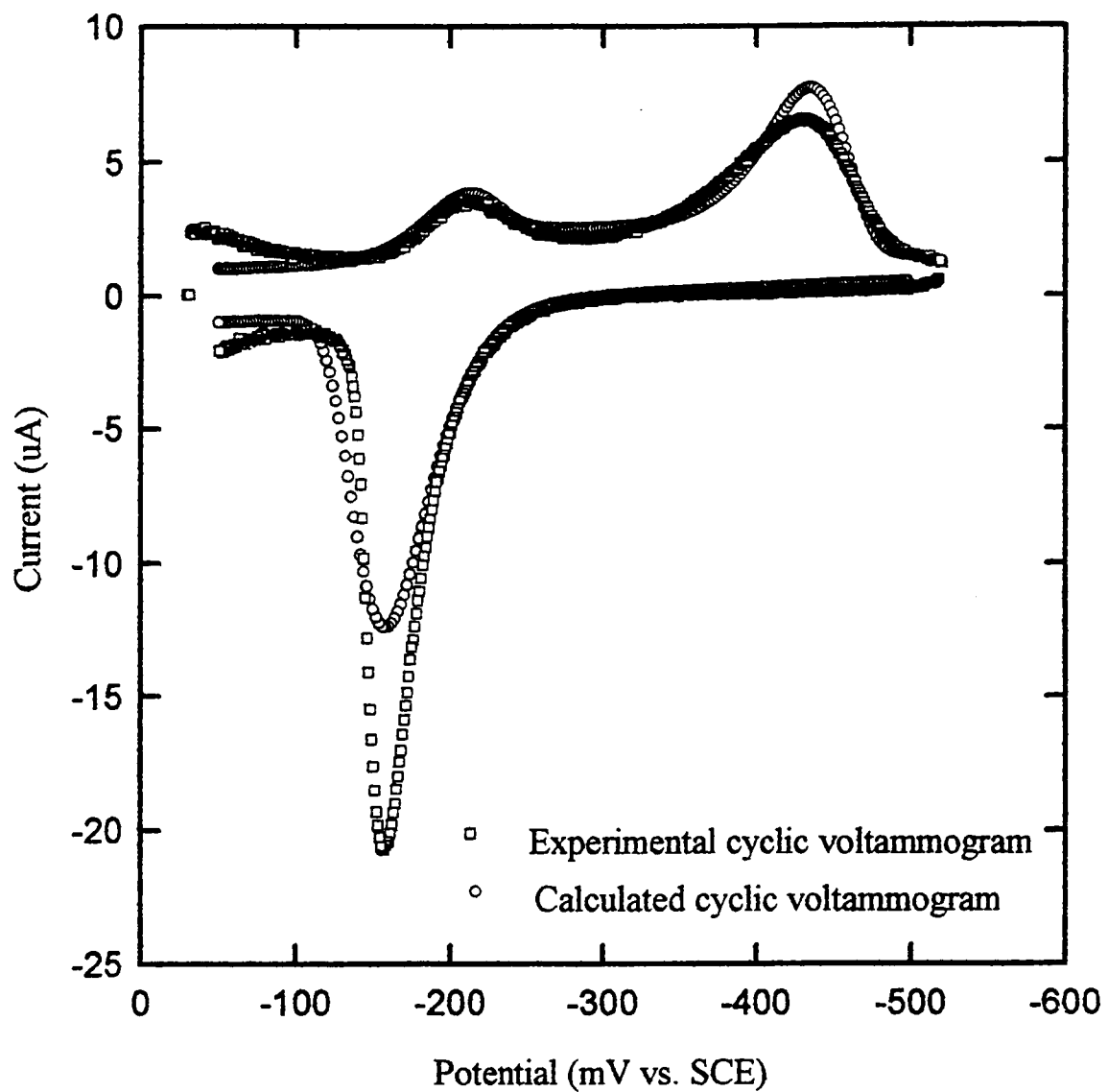


Figure 8-7: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5.5, with 0.20 M KCl.

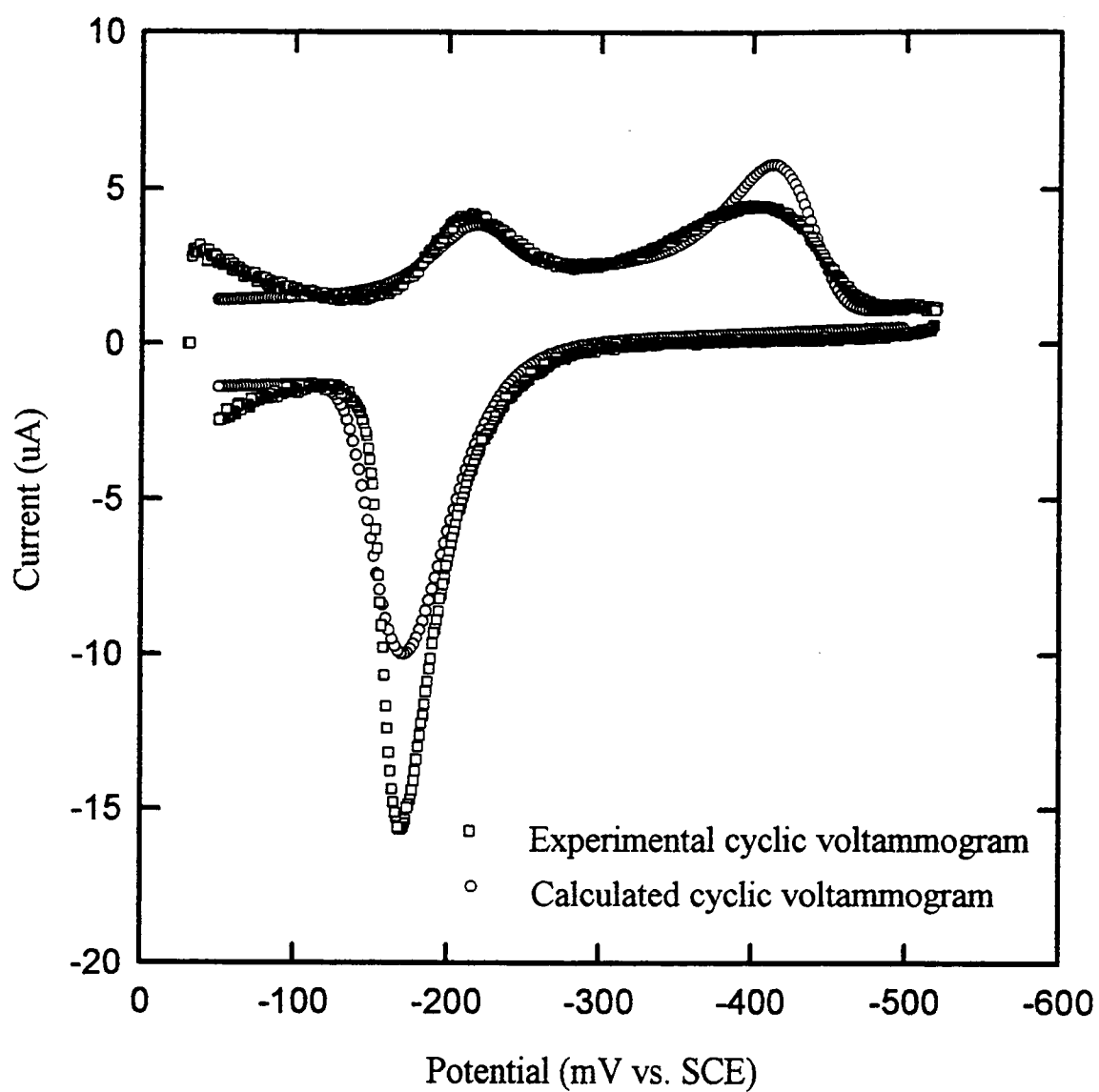


Figure 8-8: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5.5, with 0.25 M KCl.

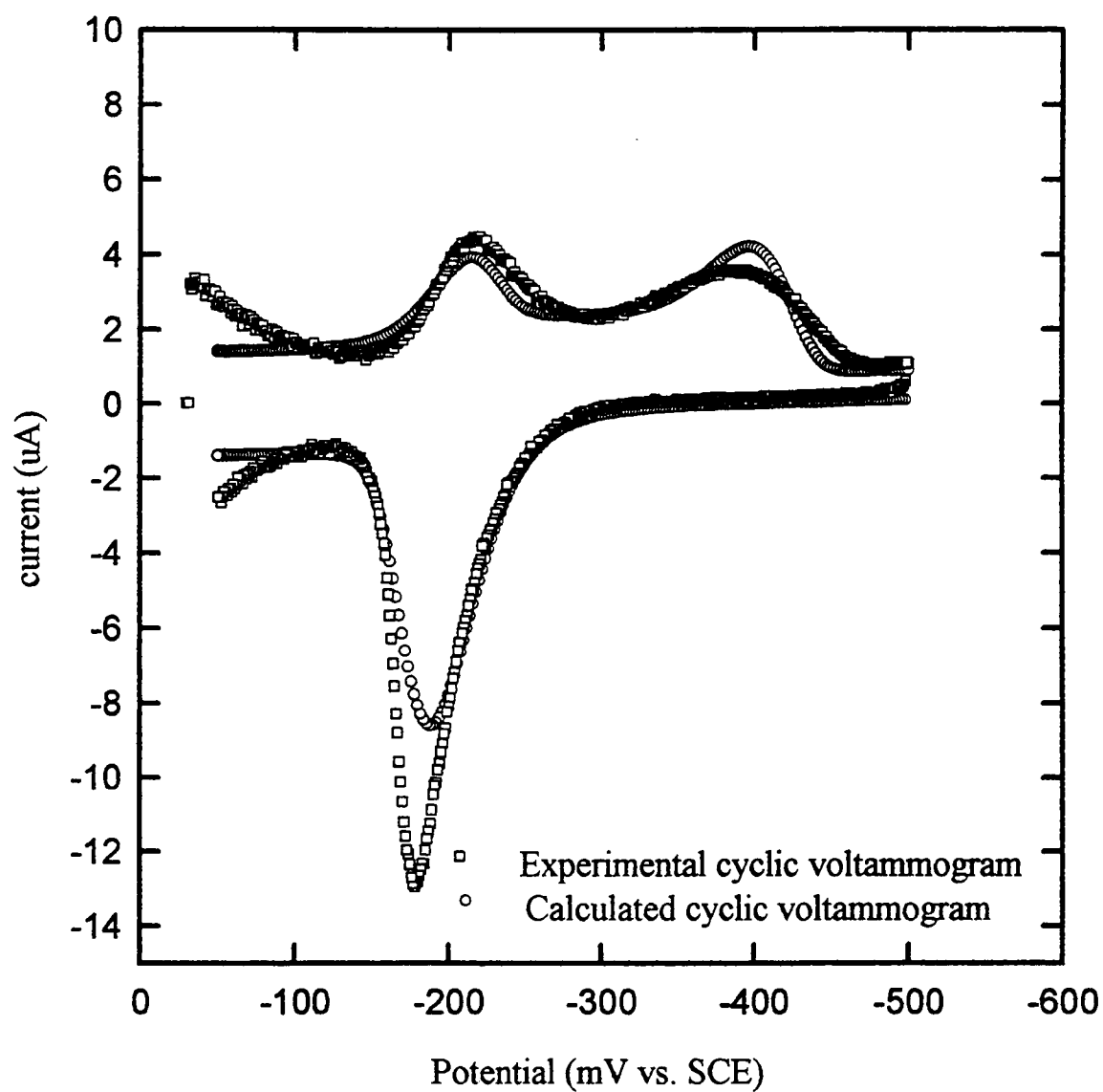


Figure 8-9: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5.5, with 0.30 M KCl.

Table 22: Input parameters utilized in the Quick Basic program to calculate the Ladder scheme voltammograms of UTP-HgX as a function of chloride concentration.

| Parameter                                       | Fig 8-4 | Fig 8-5 | Fig 8-6 | Fig 8-7 | Fig 8-8 | Fig 8-9 |
|-------------------------------------------------|---------|---------|---------|---------|---------|---------|
| KCl (mol/L)                                     | 0.05    | 0.10    | 0.15    | 0.20    | 0.25    | 0.30    |
| DL capacity Ox<br>( $\mu\text{F}/\text{cm}^2$ ) | 14      | 13      | 11      | 10      | 14      | 14      |
| DL capacity R<br>( $\mu\text{F}/\text{cm}^2$ )  | 7       | 8       | 8       | 5       | 3       | 4       |
| Cath background ( $\mu\text{A}$ )               | 0.8     | 1       | 1       | 1       | 0.8     | 0.5     |
| Initial potential (mV)                          | -50     | -50     | -50     | -50     | -50     | -50     |
| Switching potential<br>(mV)                     | -500    | -500    | -500    | -500    | -500    | -500    |
| $E^0$ A/B (mV)                                  | -545    | -530    | -524    | -515    | -495    | -480    |
| $E^0$ C/D (mV)                                  | -50     | -70     | -70     | -80     | -95     | -105    |
| $E^0$ E/F (mV)                                  | -284    | -278    | -278    | -278    | -284    | -278    |
| Sweep rate (V/s)                                | 5       | 5       | 5       | 5       | 5       | 5       |
| Surface Coverage ( $\mu\text{C}$ )              | 0.22    | 0.19    | 0.19    | 0.16    | 0.12    | 0.10    |
| Electrode area ( $\text{cm}^2$ )                | 0.199   | 0.199   | 0.199   | 0.199   | 0.199   | 0.199   |
| $k_1$ ( $\text{s}^{-1}$ )                       | 8000    | 8000    | 8000    | 8000    | 8000    | 8000    |
| $k_2$ ( $\text{s}^{-1}$ )                       | 10000   | 10000   | 10000   | 10000   | 10000   | 10000   |
| $k_4$ ( $\text{s}^{-1}$ )                       | 8000    | 8000    | 8000    | 8000    | 8000    | 8000    |
| $k_5$ ( $\text{s}^{-1}$ )                       | 8000    | 8000    | 8000    | 8000    | 8000    | 8000    |
| $k_3$ ( $\text{s}^{-1}\text{m}^{-1}$ )          | 49.5    | 49.5    | 49.5    | 49.5    | 49.5    | 49.5    |
| $k_{-3}$ ( $\text{s}^{-1}$ )                    | 42.5    | 42.5    | 42.5    | 42.5    | 42.5    | 42.5    |

because theoretically the  $E^0$  values should remain constant with varying chloride concentration. However, this deviation is small with the greatest variation being 65 mV. It should be noted that the values of the rate constants were constant throughout the calculations. These values should remain constant. This result gives strong support for the one-electron ladder scheme outlined above.

Calculated ladder scheme voltammograms were also obtained for the mercurated UTP at various scan rates. Figures 8-10, 8-11, and 8-12 show the calculated voltammograms (using the parameters in Table 23) and experimental voltammograms at 10, 5, and 1 V/s. The experimental voltammograms were obtained at a pH of 5 and a chloride concentration of 0.2 M KCl.

The calculated voltammograms showed excellent agreement at 10 and 5 V/s. However, at 1 V/s the experimental voltammogram contains the R1 and R1' wave in almost equal proportions. The calculated voltammogram, in contrast, contains only the R1' wave at this scan rate. The calculated voltammogram contains only the R1' because at this scan rate (1 V/s) equation 8.1 is not sluggish on the time scale of the voltammetry and approaches equilibrium before the potential sweep has had time to reach the potential region where UTP-Hg(phos) is reduced. Hence, while the potential sweep is in the region of the R1' wave, the concentration of the UTP-HgCl species approaches zero and the UTP-Hg(phos) species is rapidly converted to UTP-HgCl and reduced. The presence of the two waves (R1 and R1') with the experimental voltammogram may be a result of diffusion of the UTP-Hg(phos) species to the surface during the potential sweep.

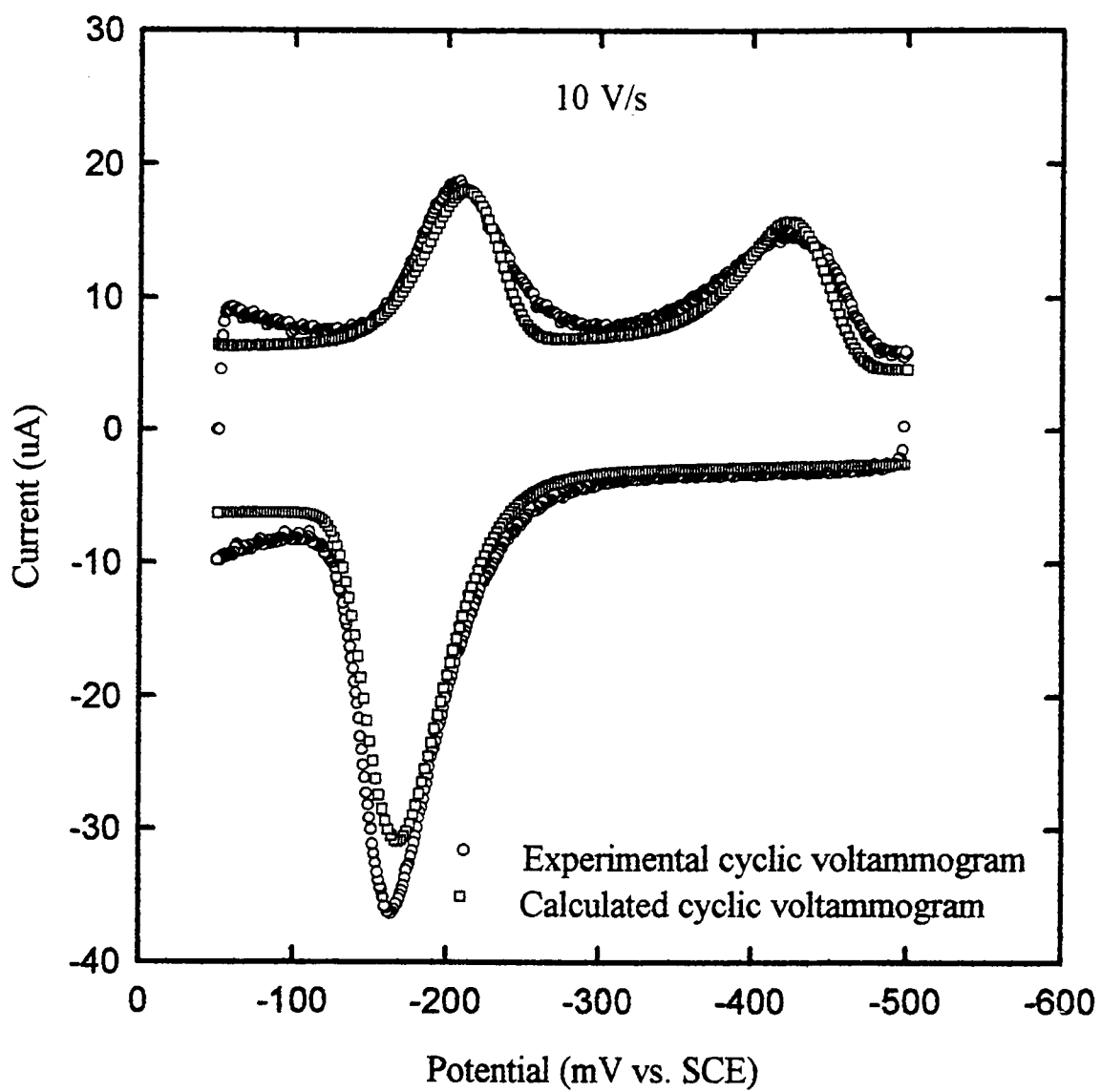


Figure 8-10: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5, with 0.20 M KCl.

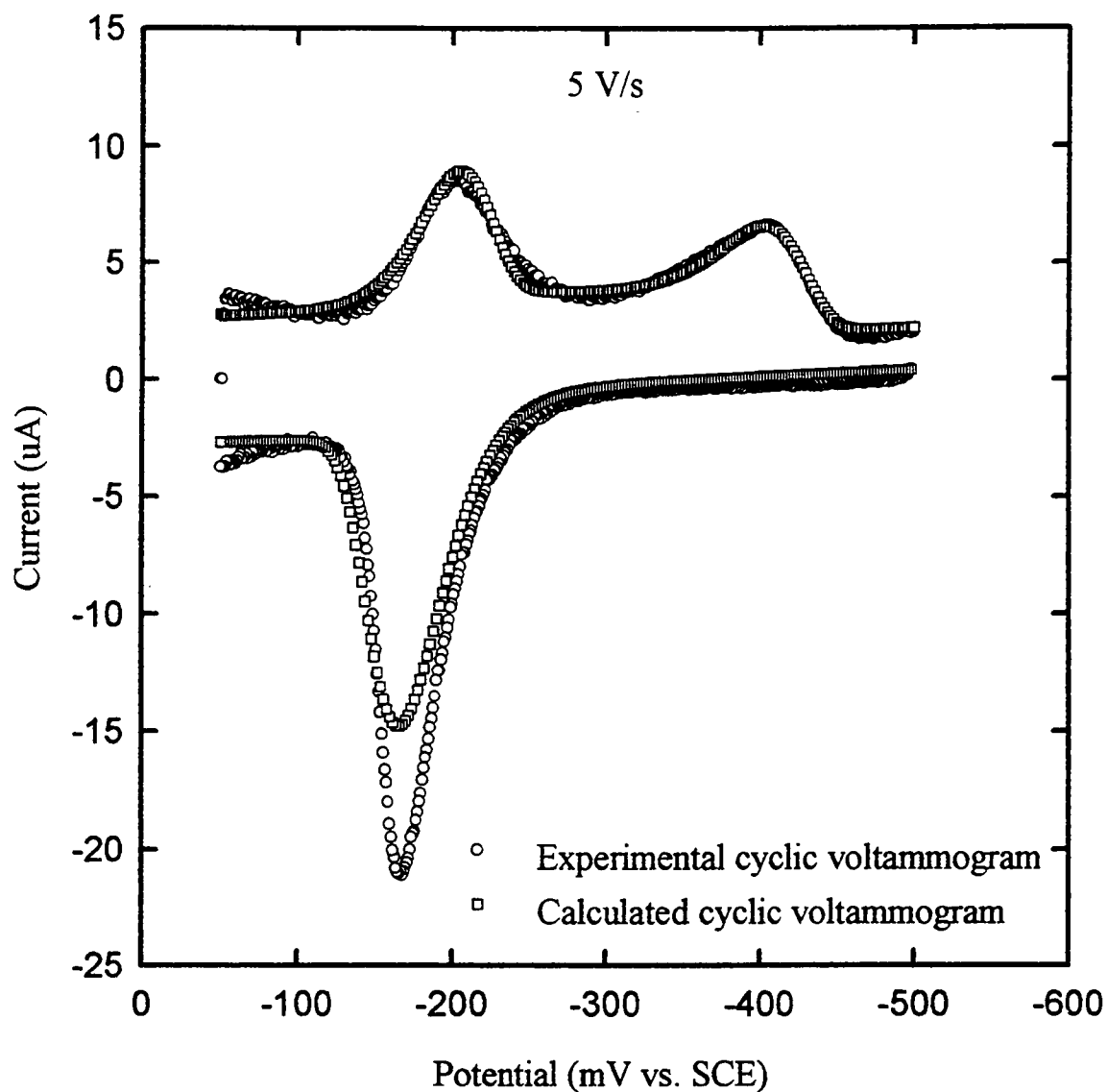


Figure 8-11: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5, with 0.20 M KCl.

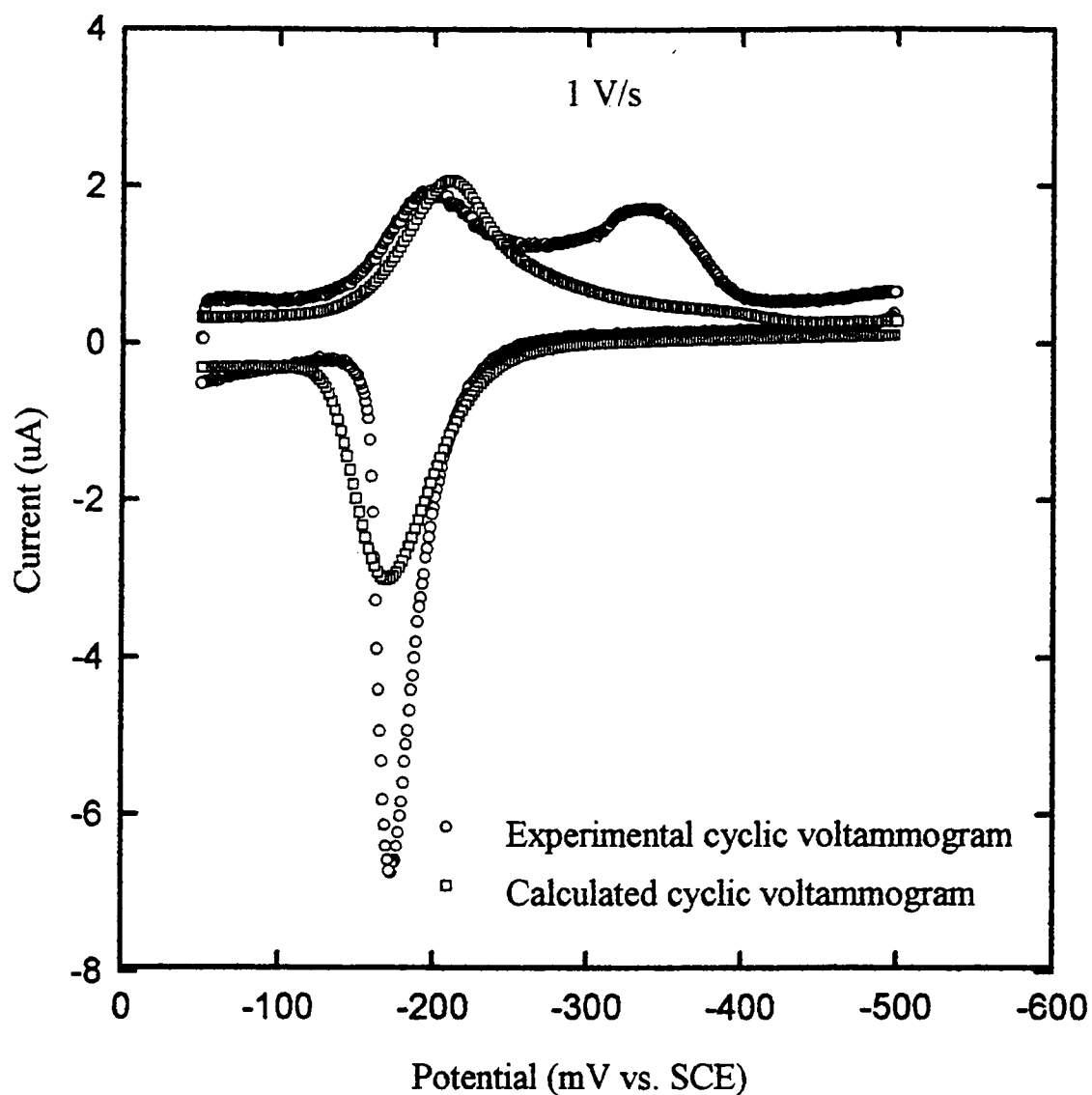


Figure 8-12: Experimental and calculated cyclic voltammograms of the UTP-HgX in 0.1 M potassium phosphate, pH = 5, with 0.20 M KCl.

Table 23: Input parameters utilized in the Quick basic program to calculate the Ladder scheme voltammograms of UTP-HgX at various scan rates.

| Parameter                                    | Fig 8-10 | Fig 8-11 | Fig 8-12 |
|----------------------------------------------|----------|----------|----------|
| KCl (mol/L)                                  | 0.20     | 0.20     | 0.20     |
| DL capacity Ox ( $\mu\text{F}/\text{cm}^2$ ) | 28       | 24       | 14       |
| DL capacity R ( $\mu\text{F}/\text{cm}^2$ )  | 16       | 8        | 4        |
| Cath background ( $\mu\text{A}$ )            | 1        | 1.3      | .2       |
| Initial potential (mV)                       | -50      | -50      | -50      |
| Switching potential (mV)                     | -500     | -500     | -500     |
| $E^0$ A/B (mV)                               | -485     | -485     | -485     |
| $E^0$ C/D (mV)                               | -95      | -95      | -95      |
| $E^0$ E/F (mV)                               | -274     | -274     | -274     |
| Sweep rate (V/s)                             | 10       | 5        | 1        |
| Surface Coverage ( $\mu\text{C}$ )           | 0.17     | 0.17     | 0.18     |
| Electrode area ( $\text{cm}^2$ )             | 0.199    | 0.199    | 0.199    |
| $k_1$ ( $\text{s}^{-1}$ )                    | 8000     | 8000     | 8000     |
| $k_2$ ( $\text{s}^{-1}$ )                    | 10000    | 10000    | 10000    |
| $k_4$ ( $\text{s}^{-1}$ )                    | 8000     | 8000     | 8000     |
| $k_5$ ( $\text{s}^{-1}$ )                    | 8000     | 8000     | 8000     |
| $k_3$ ( $\text{s}^{-1} \text{M}^{-1}$ )      | 77.5     | 77.5     | 77.5     |
| $k_{-3}$ ( $\text{s}^{-1}$ )                 | 14.5     | 14.5     | 14.5     |

*Chloride complexed behavior at low pH.* It was illustrated in chapter VII that upon lowering the pH below 5 with 0.2 M KCl, the chloride complexed species predominates and the R1 wave disappears leaving only R1' and Ox1 waves (the R2 wave was also present if the potential sweep is not reversed after the R1 wave). It is interesting to note that these waves appeared reversible and did not at first appear to result from a square scheme. This would suggest that decomplexation of chloride occurs slowly on the time scale of the experiment and that the product,  $\text{UZP-HgCl}^{1-}$ , is the species being oxidized in the Ox1 wave. However, the peak widths for the R1' waves of the mercurated UTP, UDP, and UMP, were found to have an average value of 66 mV. This is consistent with a square scheme and not with a reversible one-electron surface wave.

To determine if the low pH behavior is consistent with a square scheme, calculated voltammograms using both reversible and square scheme subroutines were compared to experimental voltammograms. The calculations using reversible kinetics did not simulate the voltammetry in any respect. However, the calculated voltammograms using square scheme subroutines simulated the voltammetry consistently. for example, Figures 8-13, 8-14, and 8-15 illustrate the experimental and calculated voltammograms for the mercurated UTP, UDP, and UMP at a pH of 3 and a scan rate of 5 V/s. Table 24 gives the calculation parameters. Although not as conclusive as previous calculations, these figures show that the R1' / Ox1 voltammetry is consistent with square scheme behavior. The major source of deviation between the calculated and the experimental voltammograms appears to be

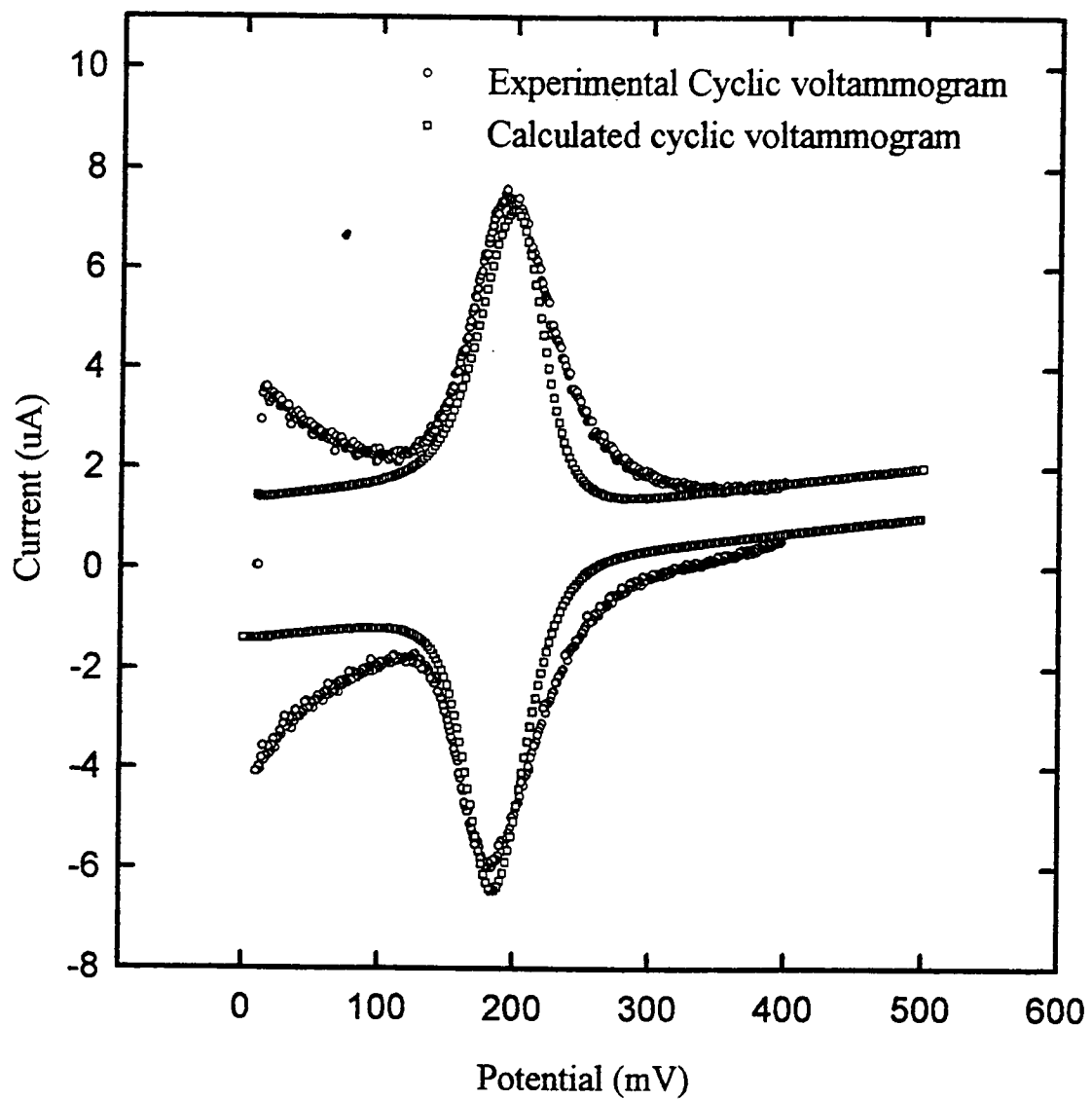


Figure 8-13: Experimental and calculated voltammogram of the UTP-HgX in 0.1 M potassium phosphate (pH = 3) and 0.2 M KCL.

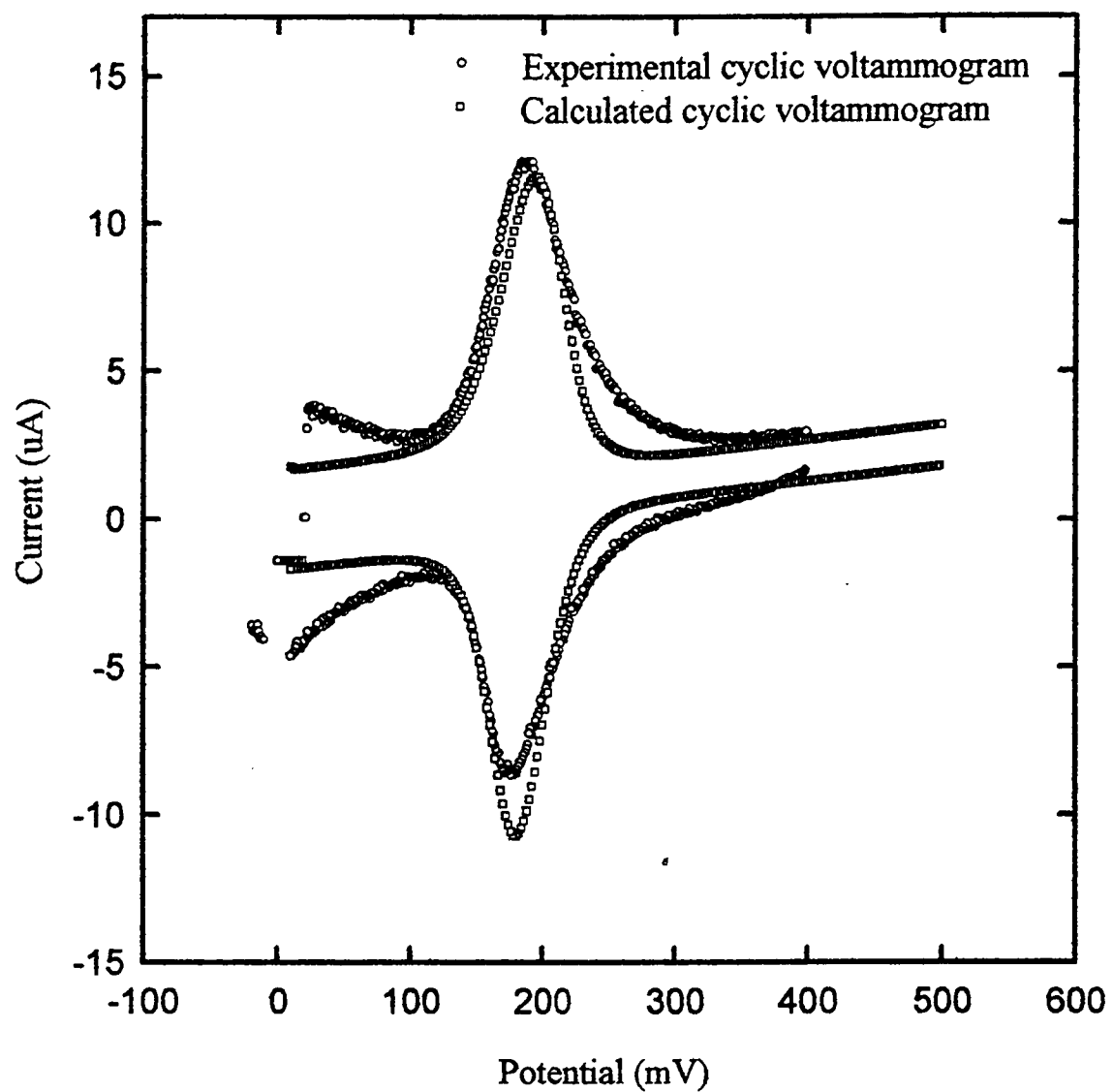


Figure 8-14: Experimental and calculated voltammogram of the UDP-HgX in 0.1 M potassium phosphate (pH = 3) and 0.2 M KCL.

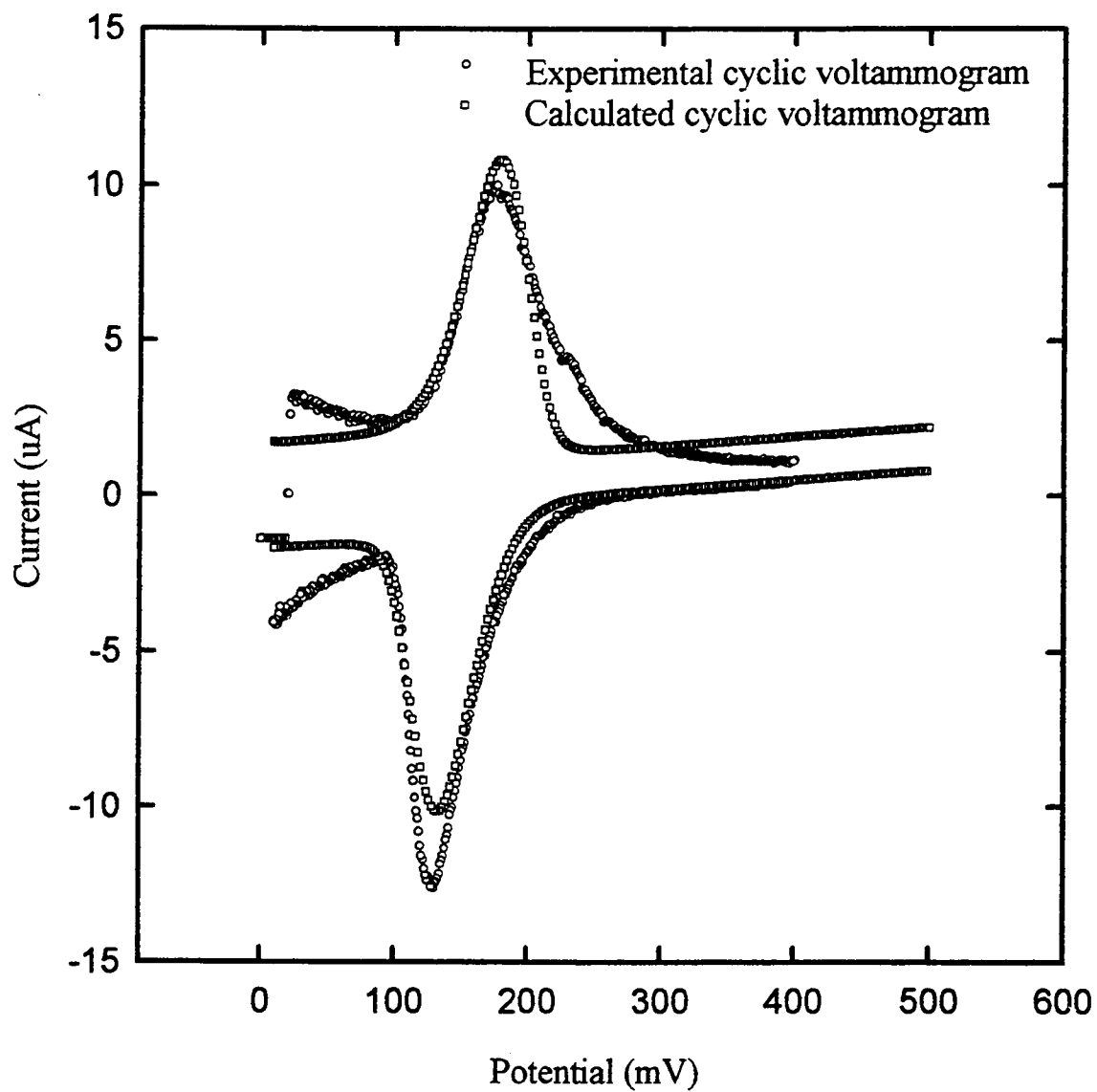


Figure 8-15: Experimental and calculated voltammogram of the UMP-HgX in 0.1 M potassium phosphate (pH = 3) and 0.2 M KCL.

Table 24: Input parameters utilized in the Quick Basic program to calculate the voltammetry of the mercurated nucleotide in 0.1 M potassium phosphate with 0.1 M KCl.

|                                      | Fig 8-13 | Fig 8-4 | Fig 8-15 |
|--------------------------------------|----------|---------|----------|
| Parameters                           | UTP-HgX  | UDP-HgX | UMP-Hg   |
| Electrode area (cm <sup>2</sup> )    | 0.0199   | 0.0199  | 0.0199   |
| DL capacity Ox (uF/cm <sup>2</sup> ) | 14       | 17      | 17       |
| DL capacity R (uF/cm <sup>2</sup> )  | 5        | 7       | 7        |
| Cath background (uA)                 | 1.5      | 2.5     | 1.5      |
| Initial potential (mV)               | -10      | -10     | -10      |
| Switching potential (mV)             | -500     | -500    | -500     |
| Surface Coverage (uC)                | 0.07     | 0.12    | 0.11     |
| Scan Rate (V/s)                      | 5        | 5       | 5        |
| k <sub>1</sub> (s <sup>-1</sup> )    | 8000     | 8000    | 8000     |
| k <sub>2</sub> (s <sup>-1</sup> )    | 10000    | 10000   | 10000    |
| E <sup>0</sup> <sub>A/B</sub> (mV)   | -235     | -230    | -250     |
| E <sup>0</sup> <sub>C/D</sub> (mV)   | -138     | -130    | -60      |

with the changing double layer capacity with the experimental voltammograms. Only a double layer capacity for the oxidized and reduced surface was incorporated into the calculation.

*Unique mechanism obtained with the mercurated UMP.* Although the voltammetry of the mercurated UMP appeared to differ significantly from that of the mercurated UTP and UDP voltammetry described in Chapters V and VI, the results in Chapter VII demonstrated that the voltammetry of the mercurated UMP was more similar than dissimilar to that of UTP-HgX and UDP-HgX. For example, it was shown in the pH region of 7, the square scheme was present (intramolecular complexation). and at lower pH values the chloride complexed species began to predominate. The only difference between the voltammetry of the mercurated UMP and the mercurated UTP + UDP is at pH values greater than 7.5 where intermolecular complexation is important. It was shown in Chapter VII that this intermolecular complexation occurs between the nitrogen on the pyrimidine ring and the mercury substituent. The voltammetry of the mercurated UMP can be summarized by the addition of G/H couple and the dimer step to scheme 3. This is shown in scheme 4 where the rate constants  $k_6$ ,  $k_7$ ,  $k_8$  have also been added where  $k_6$  is the rate constant for intermolecular complexation of the UMP-Hg• species,  $k_7$  is the rate constant for decomplexation of the reduced intermolecularly complexed species, and  $k_8$  is the rate constant for the formation of the dimer ( I ).

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The dimer assignment shown in scheme 4 will be briefly explained. In chapter V it was shown that the coulombic charge under the R2 wave was dependent on the scan rate. This was explained in terms of the formation of a dimer (I). The scheme below shows that dimer formation occurs from species H, not D. This assignment was made with the aid of data in chapter VII which illustrated that in the pH vicinity of 7, the square scheme was present. Figure 7-16 illustrated that the charges under the R1 and Ox1 were equivalent during this square scheme behavior. Hence, the (phos)-UMP-Hg• species (D) was shown to be stable on the mercury surface. It was also shown in chapter VI that two anodic wave were present when there was a mixture of [UMP-Hg-UMP-Hg] and UMP-Hg(phos) on the surface (Figure 6-8). However, there was no evidence of an anodic wave during sweep reversal when only the [UMP-Hg-UMP-Hg] species resided on the surface (Figure 7-10) (Chapter VII). The absence of anodic waves was determined to be the result of dimer formation with the product and suggest that  $k_8 \gg k_7$ . Hence, the reduced intermolecular complexed species (H), can be considered activated for dimer formation. The two anodic waves (Ox1 and Ox2) will now be explained in terms of scheme 4. It was illustrated in Chapter VI that two anodic waves were obtained at moderate scan rates and that only the Ox1 wave was obtained at faster scan rates. (Figure 6-9). The Ox1 wave was assigned to the oxidation of D to form C with fast intramolecular complexation ( $k_2$ ) and the Ox2 wave was assigned to the oxidation of D to C with intermolecular complexation following electron transfer ( $k_6$ ). The Ox2 wave cannot be the result of the oxidation of species H because faster scan rates

would increase the coulombic charge under the Ox2 wave. The opposite was obtained, Ox2 disappeared. Hence, the previous assignments appear to hold, but an explanation of why the Ox1 wave predominates at higher scan rates has not been found. The difficulty in constructing a Quick Basic program to calculate or simulate this unique voltammetry has prevented its presentation here. Future work may entail this work.

## CHAPTER IX

### Conclusion

This thesis has outlined the voltammetric behavior of the mercurated uridine nucleotides at a mercury drop electrode in 0.1 M KCl and in potassium phosphate buffers with and without chloride. A significant finding in this work was the intramolecular complexation found to occur with the mercurated nucleotides. This intramolecular complexation resulted from the negatively charged phosphate arm attached to the ribose sugar. This intramolecular phosphate complexation was observed for all three nucleotides.

The surface voltammetry of the mercurated uridine nucleotides was found to be similar to that of previously studied organomercury compounds with two one-electron reduction steps with a radical intermediate and parent nucleotide product. However, this work has demonstrated that the mechanism of electron transfer is dependent on the complexation state of the mercury substituent. For example, when intramolecular phosphate complexation dominated, a square scheme mechanism was found to describe the voltammetry. This square scheme resulted from phosphate complexation and decomplexation after electron transfer, hence, making the electron transfers irreversible.

Another significant finding in this work was the effect of pH on the

intramolecular phosphate complexation. By lowering the pH, protonation of the phosphate arm occurs. This made the phosphate a weaker ligand. Hence, when chloride was present, the phosphate complexation was replaced by chloride complexation. This resulted in a new wave during the cathodic sweep which was assigned to the one-electron reduction of the mercurated nucleotide to the radical with chloride decomplexation following electron transfer. It should be noted that of the halogens, only the chloride was studied. Future work may involve the study of the voltammetric behavior of the mercurated nucleotides with the other halogens present in solution. The effect of pH on the voltammetric behavior of the mercurated UMP was very revealing and provided answers to why the voltammetry of the mercurated UMP differed so much from that of the mercurated UTP and UDP in unbuffered electrolyte solutions. Actually, the voltammetry of the mercurated UMP is almost identical to that of the mercurated UTP and UDP. Only in solutions with pH values in the vicinity of 8 and greater does the voltammetry differ. It was determined that at these pH values intermolecular complexation through the nitrogen of the pyrimidine occurs with the mercurated UMP. The result is a polymerization. This conclusion not only explained the voltammetric results but explained why such a large excess of protons were required to titrate the mercurated UMP. There was no significant evidence that this polymerization occurs with the mercurated UTP and UDP when adsorbed on the mercury surface. It was concluded that this may be the result of the phosphate arm rapping around the mercury substituent and preventing approach by an adjacent nucleotide molecule.

Another finding in this work was the phase transition nucleation found to accompany the R2 wave of the mercurated uridine nucleotides. Chronoamperometry of the three uridine nucleotides clearly illustrated the maximum in the current transients which is indicative of nucleation. However, no nucleation was observed with the mercurated UMP when intermolecular complexation predominated. Hence, polymerization prevented the nucleation. Future work may involve a more detailed study of this phase transition/nucleation phenomena observed with the mercurated nucleotides.

It has been shown in this work that the uridine nucleotides are mercurated under relatively mild conditions. Hence, this work may have an impact on the understanding of the effect of mercury introduced into biological systems. However, the immediate significance of this work lies in the combination of the theoretical and experimental voltammetry used to interpret the behavior of the mercurated uridine nucleotides. To the authors knowledge, a similar study of surface confined species has not been presented.

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## VITA

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