1997

Molecular activity prediction from voltammetric measurements

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MOLECULAR ACTIVITY PREDICTION FROM VOLTAMMETRIC MEASUREMENTS

A Thesis
Presented to
The Faculty of the Department of Chemistry
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
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May, 1997

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ABSTRACT

MOLECULAR ACTIVITY PREDICTION FROM VOLTAMMETRIC MEASUREMENTS

by Dongqing, Li

The electrochemical behavior of antiinflammatory and analgesic agents. which are members of the class of arylacetic acid compounds. has been studied. Specifically, three commercially available (ketoprofen. tolmetin and suprofen) and two synthesized arylacetic acids were studied with cyclic voltammatry at a static mercury drop electrode. All five compounds are reducible. giving rise to one or two reduction waves on mercury electrodes . The second peak is less sensitive and less pH dependent. and is used for routine analysis. Voltammetric characteristics of these compounds were examined with respect to their different bioactivities. The voltammetric method is simple. sensitive and rapid. and shows promise for characterization of a series of similar arylacetic acid compounds associated with antiinflammatory therapy.

ACKNOWLEDGMENTS

I am extremely grateful to my advisor, Dr. Sam Perone for his assistance and helpful suggestions all through my graduate studies. It was his inspiration, guidance and support which enabled me to complete my graduate work.

I would also thank to Dr. Roy K.Okuda and Dr. Joseph J. Pesek for acting as graduate committee members and for their valuable suggests on my thesis.

Thanks are extended to Dr. Okuda for providing de-ionized water and to Dr. Joseph Muchowski of Roche Bioscience (Syntex) for providing samples of arylacetic acids. Special thanks are extended to Ed Kaiser for providing ketorolac data and great helps.

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INTRODUCTION

1.1. Review of Previous Work

The use of electrochemical techniques in the analysis of drugs, pharmaceuticals and biological samples has been reported for over 50 years [1] . In 1983, the First International Symposium on drug analysis was held in Brussels. At that time, several reports were presented on the application of electrochemical techniques to the analysis of drugs and pharmaceuticals. Patriarche et al. [2] expounded on the use of some new, modified electrodes in the analysis of pharmacological active substances. Bersier [3] dealt with the ways in which polarography and voltammetry could be used in industrial pharmaceutical laboratories. He pointed out that frequently these techniques were overlooked when, in actual fact, they offer rapid and sensitive means for the measurement of drugs in pure solutions, dosage forms, bulk materials and biological fluids. At the same conference, based on some of his own work as well as the experience of others, Chatten [4] presented a report on recent applications of various electrochemical techniques to the analysis of pharmaceuticals.

Chatten and co-workers employed differential pulse polarography extensively in their development of analytical procedures for a variety of drugs and pharmaceutical dosage forms. The non-steroidal anti-inflammatory agent, zomepirac sodium was one of the substances that they [5] studied and for which they developed a method of analysis. Britton-Robinson buffer at pH 11.0 containing 5% V/V of methanol served as the

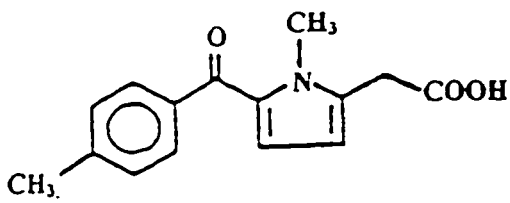
supporting electrolyte. The peak potential occurred at -1.50 V and the analytical recoveries were in excellent agreement with those of the manufacturer.

Another substance investigated by Chatten et al. was ketoprofen [6]. Electrochemical studies on ketoprofen revealed that the Ep occurred at -1.15 V in a supporting electrolyte of Britton-Robinson buffer (pH 6.0) containing 5% V/V methanol. The two-electron reduction gave recoveries that were in excellent agreement with those provided by the manufacturer's laboratory.

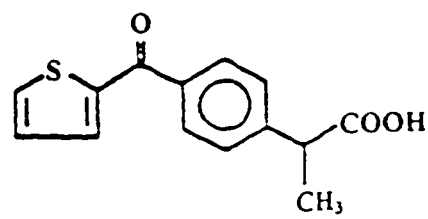
The manufacturer's method for the determination of ketoprofen in dosage forms involves extraction of the active ingredient from the tablet mass into methanol, followed by UV spectrophotometric measurement at 254 nm [7]. Reported methods for its determination in biological samples include HPLC [8-10], TLC or paper chromatography [11,12], GLC after derivatisation [13,14], spectrophotometry [11,15], radioisotope tracing [12] and colorimetry or polarography [13]. Most of these methods are either tedious or too cumbersome to be adapted for the routine analysis of dosage forms. The official method of analysis in the US Pharmacopoeia [16] is similar to that of the manufacturer's method [17].

In our work, cyclic voltammetry has been selected for the study of a set of arylacetic acids, not only because of the analytical implications, but also because it offered a way of characterizing the variability in redox behavior which might relate to anti-inflammatory or analgesic properties of these compounds. Cyclic voltammetry was applied to four arylacetic acid analgesic compounds including: tolmetin, suprofen (Fig. 1)

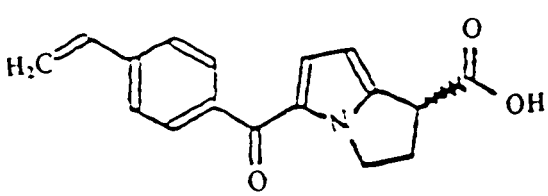
Figure 1. Structure of Four Compounds Studied



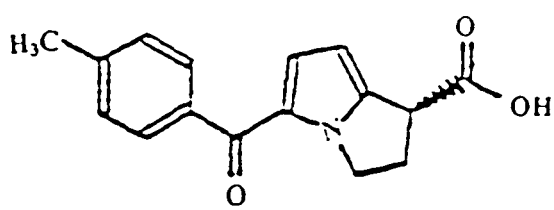
Tolmetin



Suprofen



rs49574



rs37629

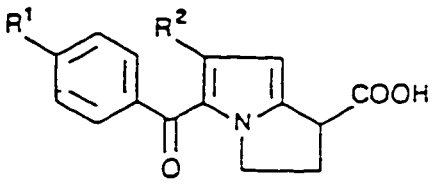
and two compounds synthesized and characterized by Roche Bioscience referred to as rs49574 and rs37629 (Fig. 1). These compounds were studied in Britton- Robinson buffer of pH 7.0 and 11.0, containing 2.5% and 10% methanol, at concentration 0.1 mM and 0.5 mM. A fractional factorial design [18] of seven different experimental variables was followed. All compounds were reduced between -1.010 v to -1.750v. Factor effects of pH; analyte concentration; voltage scan rate; methanol concentration; mercury drop hang time ; switching potential; and no. of cycles were investigated in this thesis.

1.2. Compounds

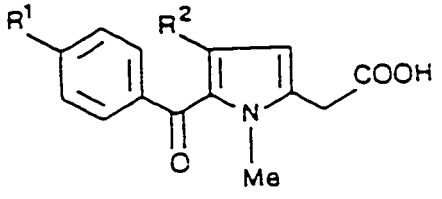
Analgesics can be broadly classified as acting centrally (narcotic) or peripherally (non-narcotic). The centrally acting analgesics are very effective in a variety of clinical situations but, in addition to relieving pain, cause an array of side effects that limit their clinical utility. The peripherally acting analgesics also have anti-inflammatory, antipyretic and antithrombotic properties. This class of agents has been developed in the past two decades primarily for the purpose of improving anti-inflammatory activity and have generally been classified as nonsteroidal anti-inflammatory drugs (NSAID). With the greater clinical exposure of these newer anti-inflammatory compounds, more attention has been given to their analgesic action. Recent clinical testing has demonstrated these newer agents to be not only more potent than some of the narcotic analgesics , but, in certain clinical situations, more efficacious. This finding has stimulated research to find new, safer and more effective peripherally acting agents capable of relieving pain [19].

In the early part of the 1970s, a wide ranging program was initiated to develop new high-potency nonsteroidal antiinflammatory agents that elicited fewer side effects than those in use (e.g., indomethacin)[20] . The new compounds comprise a series of 5-aroyl-1,2-dihydro-3H-pyrrolo[1,2-a]pyrrole-1-carboxylic acids and the homologous pyridine and azepine derivatives (Fig. 2). The parent member of this group of compounds I (R1=R2=H, ketorolac) is an inhibitor of prostaglandin synthesis. It possesses greater systemic analgesic than anti-inflammatory potency. Ketorolac survives the selection criteria of high potency, good therapeutic ratio, synthetic accessibility, etc., and was chosen for evaluation as an analgesic agent in humans [21].

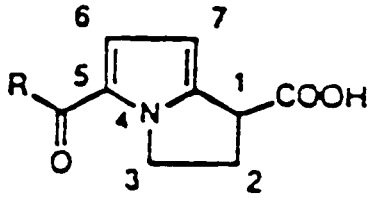
Figure 2. The structure of 5-Aroyl-1,2-dihydro-3H-pyrrolo[1,2-α] pyrrole-1-carboxylic acids



I

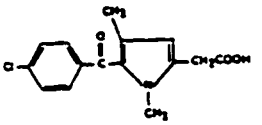
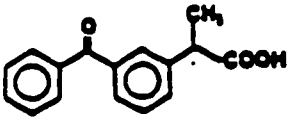
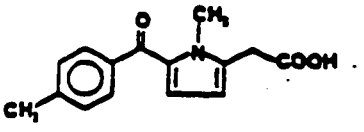
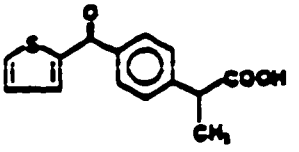
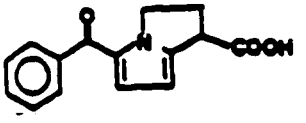
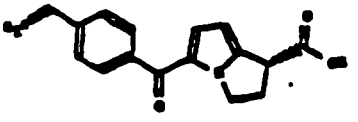
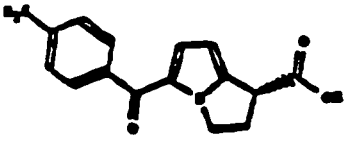


II



III

Table 1.* Arylacetic Acids and Related Compounds

Entry	Structure	Chemical name	Antiinflam m (rat paw) phenylbutaz one = 1**	Analgesic (mouse writh) aspirin = 1***	Ref.
I		5-(4-chlorobenzoyl)-1,4-dimethylpyrrol-2-ylethanoic acid (Zomepirac)	26	36	23
II		a-Methyl-3-benz-oylbenzeneacetic acid (Ketoprofen)	16	60	24
III		1-methyl-5-p-toluoylpyrrole-2-acetic acid (Tolmetin)	24	200	25
IV		a-methyl-4-(2-thienylcarbonyl)-benzeneacetic acid (Suprofen)	26	36	26
V		5-Benzoyl-2,3-di-hydro-1H-pyrrol-izine-1-carboxylic acid (Ketorolac)	55	347	27
VI		rs49574	127	200	27
VII		rs37629	39	165	27

* Table 1 from reference 22

** ED_{30} of phenylbutazone = 3 mg/kg

*** ED_{50} of aspirin = 70 mg/kg (ED_{50} is the dosage which gives a pharmacological effect equal to 50% of the maximum possible effect.)

The data obtained for several of the more interesting agents are shown in Table 1. [22]. Among this variety of drugs, some (I, II) have already been studied by differential-pulse polarography at dropping mercury electrodes, as reported in the literature [23,24]. No data concerning the electrochemical behavior of the other compounds have been reported until now. The antiinflammatory and analgesic activities of the compounds shown in Table 1 were first determined by using the carrageenan rat paw edema and mouse phenylquinone writhing assays, respectively [23-27]. From these data it is obvious that particularly high analgesic and antiinflammatory activities reside in the 1,2-dihydro-3H-pyrrolo[1,2-a]pyrrole-1-carboxylic acids bearing an aroyl group at the 5-position (see Fig. 2 III). Alteration of this basic structure was invariably associated with a major reduction in potency in both test systems [28].

Table 1 indicates the analgesic potency of ketorolac and some other compounds relative to aspirin in the mouse writhing assay, where the potency of aspirin is assigned as 1.0. The relative potency (weight for weight) of Ketorolac was 347 [27,29,30] whereas values for some other non-steroidal anti-inflammatory drugs (NSAIDs) were 36 for suprofen [26], 200 for rs49574 [27], and 165 for rs37629 [27]. The anti-inflammatory activity in Table 1 shows that ketorolac was 55 times more potent (weight for weight) than phenylbutazone, while other values were 24 for tolmetin [25], 26 for suprofen [26], 127 for rs49574 [27], and 39 for rs37629 [27] in suppressing carrageenan-induced paw edema in rats.

Tolmetin (Fig. 1 and Table 1. III) is a pyrrole derivative that has a structural resemblance to indomethacin but an action and toxicity more like those of the propionic

acid derivatives. Like other NSAID's, tolmetin inhibits prostaglandin synthesis, and this inhibition is postulated as the mechanism for its antiinflammatory effects. Tolmetin is used in the treatment of rheumatoid arthritis [31] and pain [32,33]. The drug is available in tablets of 100 and 200 mg. It was selected as one class of arylacetic acids, among several others [34-36], as lead compounds for structural manipulation.

rs49574 and rs37629 (Fig. 1 and Table 1.VI, VII) are powerful antiinflammatory agents being equipotent with or more potent than indomethacin.They were subjected to extensive pharmacological evaluation in animals as possible antiinflammatory and analgesic agents and were eliminated as clinical candidates because of their liability to precipitate gastrointestinal irritation and /or because of other deleterious effects[27]. The 4-vinylbenzoyl compound rs49574, which was the most active agent in the acute and chronic models of inflammation, is also undergoing extensive pharmacological evaluation in animals.

Suprofen is an orally effective, non-narcotic analgesic with a rapid onset and 4-hr duration of activity. Suprofen is 50 times more potent than acetaminophen, five times more potent than codeine and equipotent to the new peripheral analgesic, zomepirac [37].

Ketoprofen (Table 1. IV) is as potent as indomethacin in the tests for anti-inflammatory and analgesic activity [38].

1.3. Fractional Factorial Design

The seven variables which were investigated in this work are listed in Table 2. Performing a series of experiments in which one parameter is varied while all others are held constant at some arbitrary level would give a very narrow view of the effects of each parameter if there was any possibility of interaction between variables. To get a broader view of variable effects, we conducted the experiments by use of a fractional factorial design [39]. The Plackett-Burman (P-B) design (Table 3.) was developed by Plackett and Burman in 1946 for screening a large number of factors, i.e., reducing a large number of factors to a smaller set of important factors for subsequent experimentation. The obvious advantage of P-B designs is the limited number of runs to evaluate large numbers of factors. Table 4 is the use of the Plackett-Burman design in this study.

Table 2. Seven Experimental Variable Levels

variable no.	variable	low level(-)	high level(+)
X_1	pH	11.0	7.0
X_2	analyte conc.	0.1 mM	0.5 mM
X_3	% methanol	2.5%	10%
X_4	scan rate	250 mV/sec	1000 mV/sec
X_5	no. of cycles	1	2
X_6	drop hang time	1 sec	30 sec
X_7	switching potential	50 mV	100 mV

Table 3. Plackett-Burman (P-B) 8 Run Design

	Factors						
Run	A	B	C	D	E	F	G
1	+	−	−	+	−	+	+
2	+	+	−	−	+	−	+
3	+	+	+	−	−	+	−
4	−	+	+	+	−	−	+
5	+	−	+	+	+	−	−
6	−	+	−	+	+	+	−
7	−	−	+	−	+	+	+
8	−	−	−	−	−	−	−

(+) = high factor level; (-) = low factor level

Table 4. (P-B) 8 Run Design for Voltammetry Experiment of Analgesic Compounds

experiment no.	**pH**	**Conc**	**%MeOH**	**Scan R**	**#Cycle**	**Hang T**	**Switching E**
	X1	X2	X3	X4	X5	X6	X7
1	+	-	-	+	-	+	+
2	+	+	-	-	+	-	+
3	+	+	+	-	-	+	-
4	-	+	+	+	-	-	+
5	+	-	+	+	+	-	-
6	-	+	-	+	+	+	-
7	-	-	+	-	+	+	+
8	-	-	-	-	-	-	-

1.4. Aim of work

The purpose of this work is to demonstrate an effective voltammetric procedure for producing a large body of electrochemical data for antiinflammatory / analgesic agents and for determining the best measurements to be made for later pattern recognition analysis. Electroanalytical techniques are useful analytical tools for and elucidating the natural bioactivity or metabolism of many biological molecules. Several methods have been published on the analysis of drugs, pharmaceuticals and biological samples. They are: Coulometry Potentiometry, Polarography and Differential pulse polarography. On the other hand little attention has been paid to the characterizing information contained in cyclic voltammetry, which is the focus of our attention here.

Chapter 2

EXPERIMENTAL

2.1. Instrumentation and Software

A **Model 270** Electrochemical Analysis System combined with **EG&G PARC's** Model 273 Potentiostat/Galvanostsat, and Head Start Creative Electrochemistry Software was used for all experiments. The Model 270 software automates all of the sub-tasks involved in running an experiment. Under control of the menu selections, it automatically controls the potentiostat, acquires the data, and plots it on the display screen [40]. Default parameter values allow performance of a productive experiment with minimal entry of setup values. Features such as cyclic voltammetry, iR compensation, and control of the Model 303A Static Mercury Drop Electrode are among the experimental parameters selected in this work.

In all electrochemical cells, there is some resistance between the tip of the reference electrode and the outside of the double layer. This resistance is referred to as the uncompensated resistance. The Model 270 software uses the positive feedback method of iR compensation. iR compensation, or potential error correction, is a method of correcting the potential error that results from the uncompensated resistance between the reference and working electrodes. When the uncompensated resistance gets high or the current is large, the potential error can be significant and must be addressed to ensure reliable data.

2.2. Basic Procedures

2.2.1. Reagents

The standard tolmetin, suprofen and ketoprofen were obtained from Sigma , and the purified samples of rs49574, rs37629 were obtained from Roche Bioscience. All reagents were analytical reagent grade and were used without further purification. A stock solution of each compound (0.020 M) was prepared in anhydrous methanol and stored in a refrigerator when not in use.

2.2.2. Britton - Robinson buffer solution

The buffer solution is composed of a mixture of boric acid, sodium acetate, dibasic potassium phosphate, and potassium chloride, each 0.04 M, in distilled, deionized water. To obtain the desired pH, 0.2 M NaOH is added to the solution and the pH is checked with a pH meter [41,42].

2.2.3. Standard solution

Stock solutions of rs 49574, rs37629 , tolmetin and suprofen (2.0×10^{-2} M) were prepared in anhydrous methanol. Two test solutions of varying concentrations, 0.1 mM and 0.5 mM, were prepared by appropriately diluting the stock solution with Britton - Robinson buffer. In the total sample volume of exactly 20 ml, the amount of methanol was maintained at either 2.5% or 10%.

2.2.4. iR compensation

The iR compensation procedure was run before each cyclic voltammetry experiment. Figure 3 illustrates a typical output during an IR compensation procedure.

This is a plot of the current spikes obtained for a square wave voltage pulse. The internal program examines the shape of the current spikes and adjusts the appropriate settings for positive feedback to correct for a large function of the uncompensated resistance. The iR setup for a typical experiment is shown in Table 5. The value of uncompensated resistance from the iR compensation measurement (see Table 5) was entered in the corresponding cyclic voltammetry set up, which is shown in Table 6.

Table 5. IR Compensation / Positive Feedback set up for rs49574 run #5

Pulse Height	5.00E-03 V
Compensation Level	85 %
Undershoot	10 %
Rise Time	high speed
Working Electrode	HMDE
Electrode Area	9.600E-3cm^2
Measurement Potential	-1.400V
Current Range	1mA
IR Mode	measured
Uncompensated Resistance	638.0 ohms *
Estimate Total Ru	753.1 ohms *
Remaining Ru	115.1 ohms *
Reference Electrode	AgCl222.0E-3V
Open Circuit	-69.00E-3V

* to be changed for different solutions

Figure 3. Representative iR graph for rs49574 run # 5

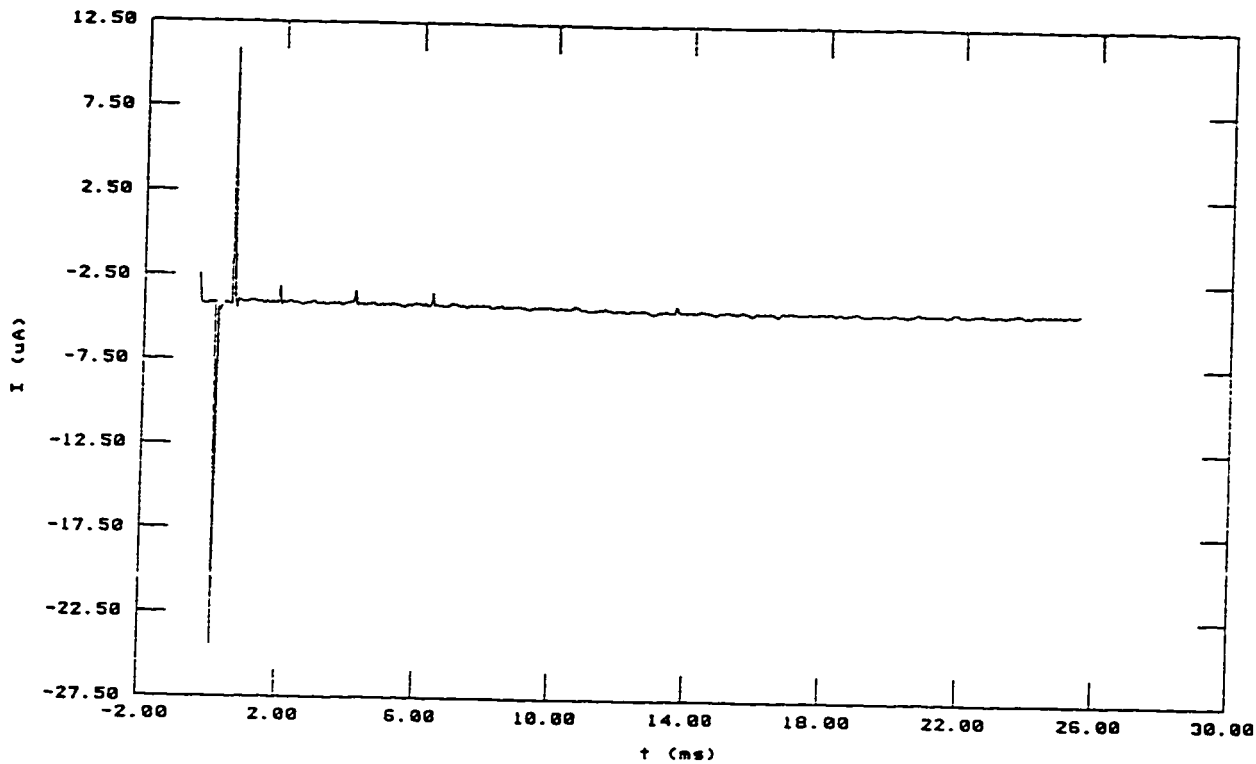


Table 6. Cycle Voltammetry Experimental Setup for rs49574 Run # 5

Initial potential	-1.025 V
Vertex 1 Potential	-1.537 V
Veryex Delay	pass
Vertex 2 Potential	pass
Final Potential	-1.025 V
Scan Rate	1000 mV/s
Scan Increasment	2.000 mV
Step Time	2.000E-3 s
No. of Points	1025
Acq. Mode	4/4
Current Range	1uA
No. of Cycles	2
Store Cycle	2
IR Mode	entered
Uncomp. Res.	597.0 ohms
Purge Time	240 s
Cond. Time	pass
Cond. Pot.	pass
Dept. Time	pass
Equil Time	1 s
Rise Time	high speed
Working Electrode	HMDE
Electrode Area	1.000 cm^2
Open Circuit	-59.00E-3 V
Filter	off
Reference Electrode	User 0.000V
AUX A/D	no

2.2.5. Cyclic Voltammetry

All voltammetric measurements were carried out with a three-electrode cell at 28 ± 1^0C. The reference electrode was a saturated calomel electrode, the counter electrode was a platinum wire, and the working electrode was a static mercury drop electrode (EG & G model 303A). All samples were purged with pure nitrogen for 4 min. prior to each run for removal of oxygen, and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Each compound was observed under eight different conditions which are shown in Table 7. Table 7 is a re-organized table of (P-B) 8 run design (Table 3.). The re-organization is for the convenience of experimental procedure (1&5, 2&3, 6&4, 8&7). Each design was run nine times and resulted in an averaged, smooth, blank- corrected data set (see 2.2.6). The experimental procedure is shown in Table 8.

Run #1 solution (see table 8) was a mixture of 8.0 ml pH 7 buffer; 1.75 ml distilled water, 0.05 ml of 0.02M standard solution (see 2.2.3.) and 0.2 ml MeOH. The total cell volume was 10 ml containing 2.5% methanol. Run #5 solution was obtained by adding 8.0 ml pH 7 buffer, 0.25 ml distilled water, 0.05 ml of 0.02M standard solution, and 1.70 ml MeOH to Run #1 solution to the final cell volume of 20 ml containing 10% methanol. The same procedure was used to prepare solutions for Run 2&3, 8&7,6&4 (see Table 8).

Table 7. Experimental Design

Run #	pH	Conc.	MeOH	Scan R (mV/s)	# Cyc	Hang T	Switch E
1	7	0.1 mM	2.5 %	1000	1	30 sec	100 mV
5	7	0.1 mM	10 %	1000	2	1 sec	50 mV
2	7	0.5 mM	2.5 %	250	2	1 sec	100 mV
3	7	0.5 mM	10 %	250	1	30 sec	50 mV
8	11	0.1 mM	2.5 %	250	1	1 sec	50 mV
7	11	0.1 mM	10 %	250	2	30 sec	100 mV
6	11	0.5 mM	2.5 %	1000	2	30 sec	50 mV
4	11	0.5 mM	10 %	1000	1	1 sec	100 mV

Table 8. Experimental procedure of making compound solutions

Run #	Buffer pH7 (ml)	Distilled H_2O (ml)	Stock solution 0.02M (ml)	MeOH (ml)	Total Cell Volume (ml)
1	8.00	1.75	0.05	0.20	10.00
additional volume to 1	8.00	0.25	0.05	1.70	10.00
5	16.00	2.00	0.10	1.90	20.00
2	8.00	1.75	0.25	0.00	10.00
additional volume to 2	8.00	0.25	0.25	1.50	10.00
3	16.00	2.00	0.50	1.50	20.00
Run #	Buffer pH11 (ml)	Distilled H_2O (ml)	Stock solution 0.02M (ml)	MeOH (ml)	Total Cell Volume (ml)
8	8.00	1.75	0.05	0.20	10.00
additional volume to 8	8.00	0.25	0.05	1.70	10.00
7	16.00	2.00	0.10	1.90	20.00
6	8.00	1.75	0.25	0.00	10.00
additional volume to 6	8.00	0.25	0.25	1.50	10.00
4	16.00	2.00	0.50	1.50	20.00

* The solution of Run 5, 3, 7, 4 were get by the additional volume to the solution of run 1, 2, 8, 6 respectively

2.2.6. Experimental Measurements

The experimental parameters were set up for each run as shown in Table 7 (except the switching E) and the sample solutions were prepared as instructed in Table 8. First, a rough potential range which was wide enough (-1.1 - -1.8 v) to observe all the reduction peaks was pre-selected for each run. The voltammetric measurements were carried out under this pre-selected potential range for three times and the second peak potential was obtained through these experimental runs. Second, the potential range of each run was selected by adding the switching potential, 50 mV or 100 mV (according to Table 7 experimental design), to the second peak potential as the vertex 1 potential and obtain the total points of 1024 for a complete cyclic voltammogram. Finally, each sample was run nine times and saved in an average, Savitsky-Golay linear smooth, Ascii mode.

2.2.7. Voltammetric Data Processing

Requirements. The processing of voltammetric data requires two basic functions: recognition and precise location of reduction peaks and precise measurement of peak heights. Unfortunately, the background current is generally not linear, therefore, it is assumed that any voltammetric data processed would be corrected for background current first. Only two kinds of base lines need be considered to make peak height measurements: linear base lines, and the base line afforded by the extension of the diffusion-controlled reduction peak. These two regions are specified in Figure 4. The linear region corresponds to potentials preceding any reduction steps. The second kind of base line indicated in Figure 4 is really the extension of the tail of a preceding reduction wave. This tail should follow a current-time function which decays with $1/(t)^{1/2}$, where t is

time [43, 44]. The diffusion-limited $1/(t)^{1/2}$ current on the tail of a peak is a general and well defined characteristic of voltammetric reduction waves.

Mathematical Approaches. The computer program for handling the extrapolated diffusion current base line established by a preceding reduction peak is computed by an iterative approach. This approach simply assumes that the data in the region shortly after the first peak follows a $1/(t)^{1/2}$ dependence as shown in Equation 1. The only unknown in the equation is t_o. (i_o = current at t_o; t = time past t_o.) The initial estimated of t_o is selected by the operator.

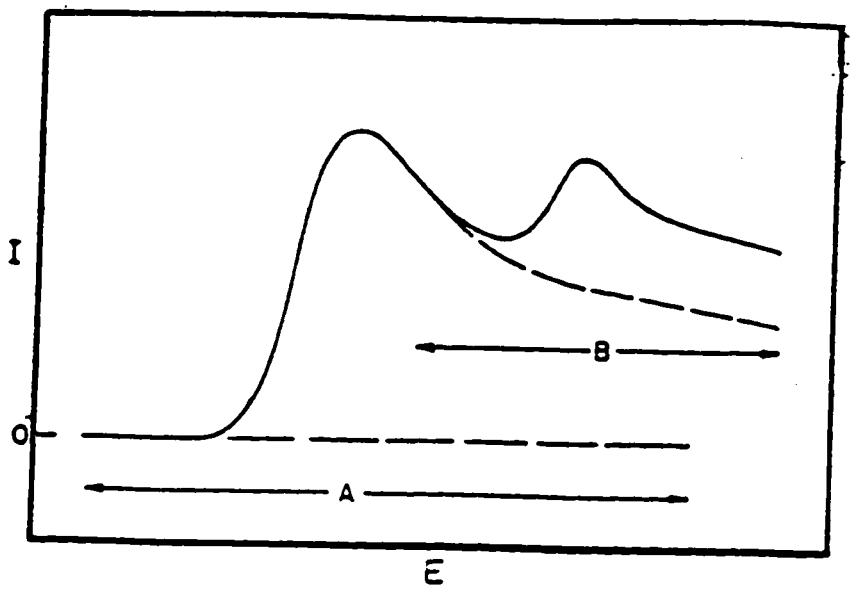
$$i_{(t+to)} = i_o [t_o/(t+t_o)]^{1/2} \qquad (1)$$

The program then attempts to fit the data to the $1/(t)^{1/2}$ function by finding the correct t_o to obtain an adequate fit [45]. The criterion for selection of t_o involves establishing a fit within 1%, to the latter data points in the region, based on the values of the earlier data points in the region. If the data do not follow a $1/(t)^{1/2}$ dependence, the iterative procedure will not converge rapidly, if at all. Thus , the operator will know immediately if the $1/(t)^{1/2}$ dependence does not fit the data.

2.2.8. Partial Least Squares Analysis

Partial least squares analysis was applied to examine multivariate relationships between electrochemical properties and pharmacological activities. Software for these studies was MATLAB® v.4.2c. 1, Chemometrics Toolbox, the Math Works, Inc., Natick, MA.

Figure 4. Types of base lines for voltammetric data
A. Linear region
B. $1/(t^{1/2})$ extrapolation region



Chapter 3

Results and Discussion

3.1. General Voltammetric Behavior

The five arylacetic acids (tolmetin, suprofen, ketoprofen, rs49574, rs37629) were studied, first, to characterize the general pH dependence of cyclic voltammetric properties. Then the experimental design defined in table 7 was applied to all compounds except ketoprofen. The following is an overall summary of observed behavior.

3.1.1. rs49574

rs49574 was studied at pH 7, 8 & 11. The results for twelve voltammograms are shown in Table 9 and Figures 5 A-F. Two peaks are observed at pH 7 & 8 . while only one peak occurs at pH 11. The first peak potential appears to shift cathodic with increasing pH. The second peak potential at pH 7 and 8 is about -1.487 v, and at pH 11 is about -1.531 v . The difference (-0.044 v) might be affected by the merging of the pH-dependent first peak with the second peak. The numerical values of the expression $Ip/C^*V^{1/2}$ were calculated for the second peak and are included in Table 9. This expression is obtained from the Randles-Sevcik Equation [46]:

$$ip = 269n^{3/2} AD^{1/2}v^{1/2} C^b \qquad (2)$$

where ip = peak height (amp)
n = number of electrons
A = area (cm^2)
D = Diffusion coefficient (cm^2sec^{-1})
v = scan rate (volts sec^{-1})
C^b = concentration in the bulk of solution (molar)

The value of the expression $ip/C^{b*}v^{1/2}(=269n^{3/2}AD^{1/2})$ should be constant for uncomplicated diffusion-limited electrode processes. We will refer to this quantity as K. The fact that this quantity, K, appears to be dependent on experimental condition suggests that the electrode process may involve complications such as surface adsorption and/or coupled chemical reactions. This will be discussed later. The anomalous pre-peak (preceding the first peak) that appears only at high analyte concentration for pH 7 solutions, is probably due to product adsorption.

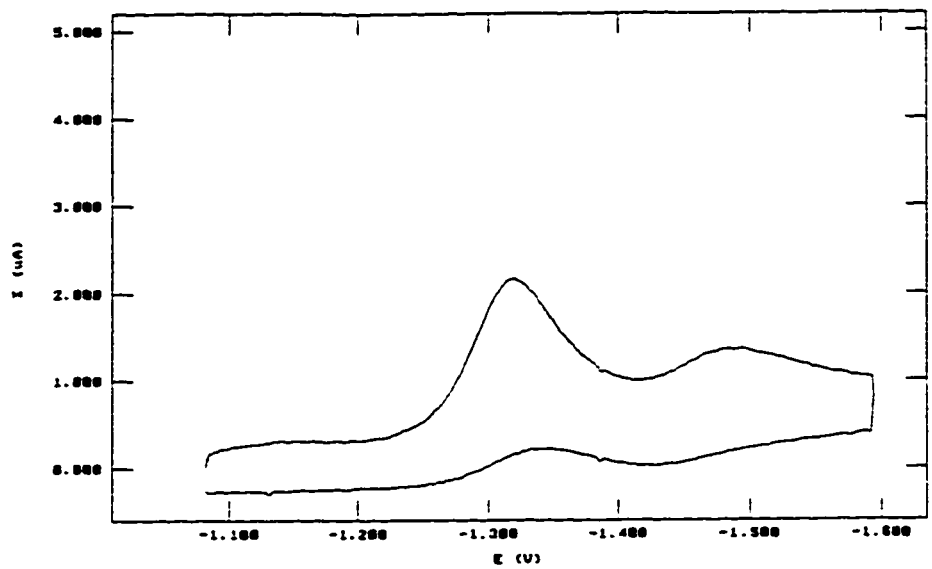
Table 9. Voltammetry data of rs49574 at pH 7, 8 , 11 , 1st and 2nd Peak

Run #	pH	Conc. (mM)	MeOH (%)	Scan R (mv/s)	E p(1) (v)	Ip(1) (uA)	Ep(2) (v)	Ip(2) (uA)	Ip(2)/C*V^1/2 (K)
1	7	0.1	2.5	1000	-1.319	2.162	-1.494	0.509	161
5	7	0.1	10	1000	-1.325	1.425	-1.487	0.506	160
2	7	0.5	2.5	250	-1.305	2.925	-1.479	0.496	15.7
3	7	0.5	10	250	-1.316	2.641	-1.482	0.378	12.0
6	11	0.5	2.5	1000			-1.531	5.200	328.9
4	11	0.5	10	1000			-1.535	4.987	315.4
8	11	0.1	2.5	250			-1.523	0.570	90.1
7	11	0.1	10	250			-1.531	0.568	89.8
6'	8	0.5	2.5	1000	-1.339	3.361	-1.491	3.077	194.6
4'	8	0.5	10	1000	-1.340	2.477	-1.496	3.579	226.4
8'	8	0.1	2.5	250	-1.331	0.486	-1.473	0.283	44.7
7'	8	0.1	10	250	-1.339	0.406	-1.475	0.293	46.3

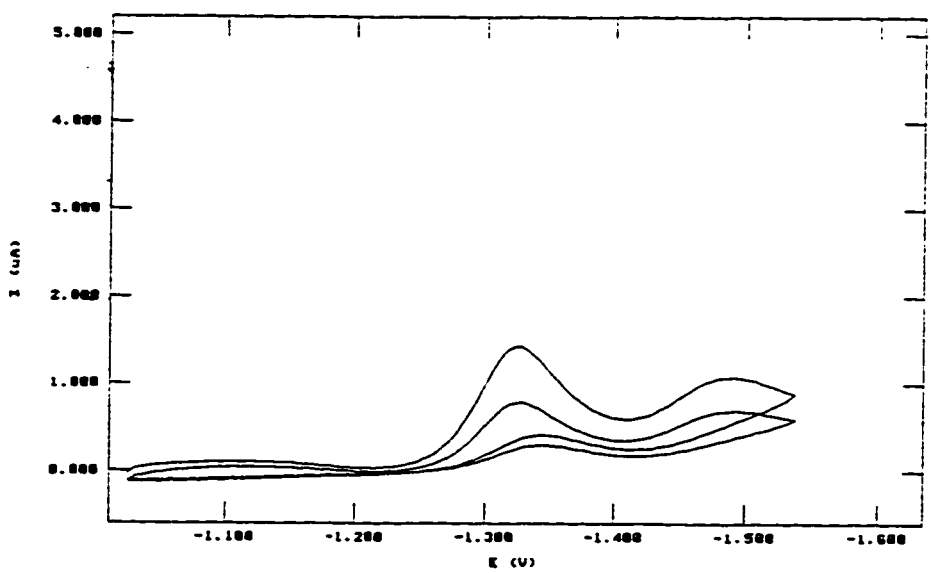
* Run # is referred to (P-B) 8 RUN # shown in Table 2.
* Run 6'-8'is using the same design as run 6-8 except using pH 8 instead of pH 11.
* Ip is peak current; C is concentration, V is scan rate.

Figure 5A. Cyclic voltammogram for 0.1 mM rs49574 in Britten-Robinson buffer of pH7 Run 1 & 5

A



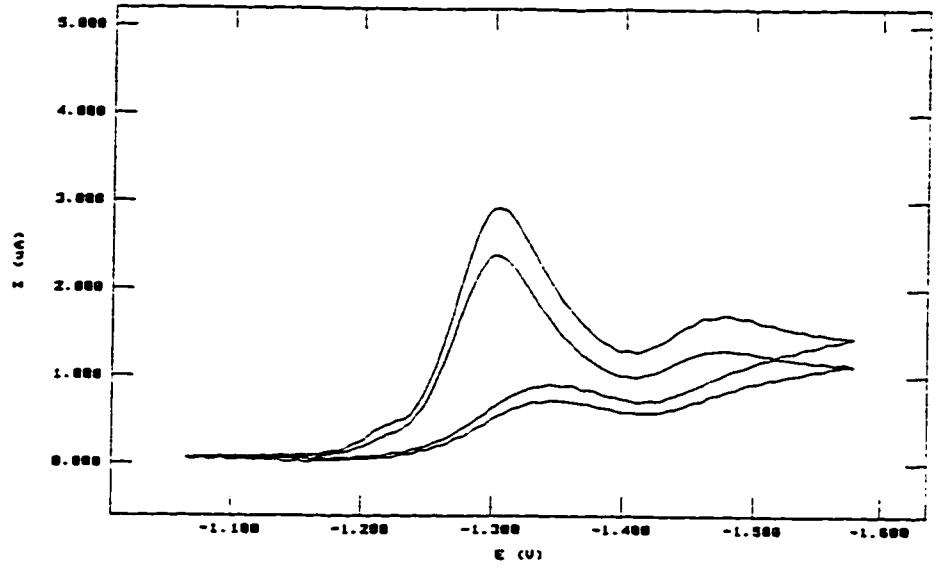
pH 7 Run # 1



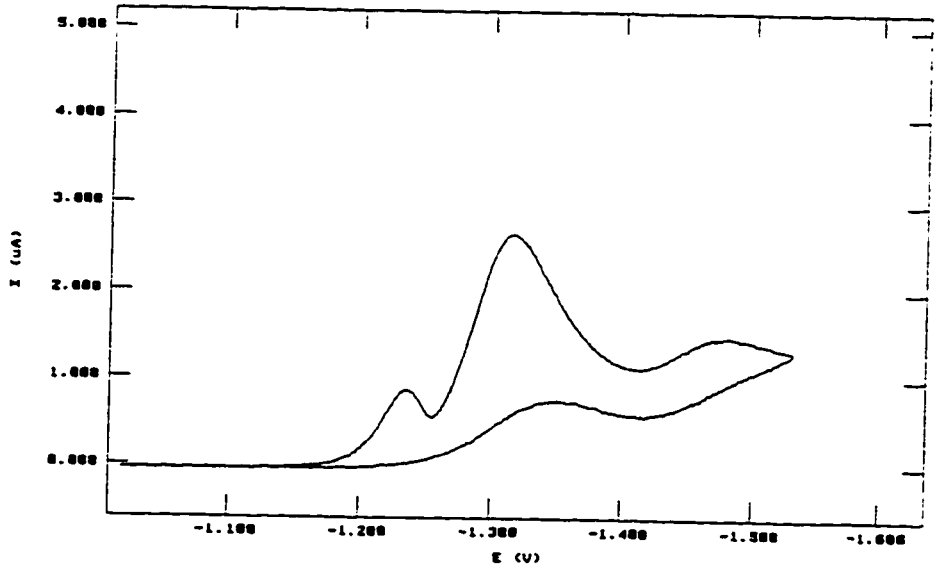
pH 7 Run # 5

Figure 5B. Cyclic voltammogram for 0.5 mM rs49574 in Britten-Robinson buffer of pH7 Run 2 & 3

B



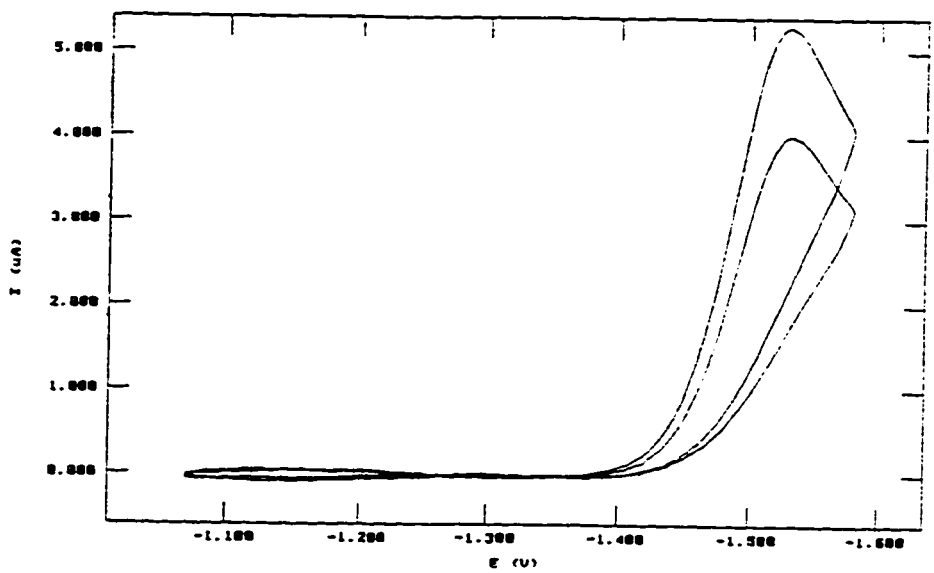
pH 7 Run # 2



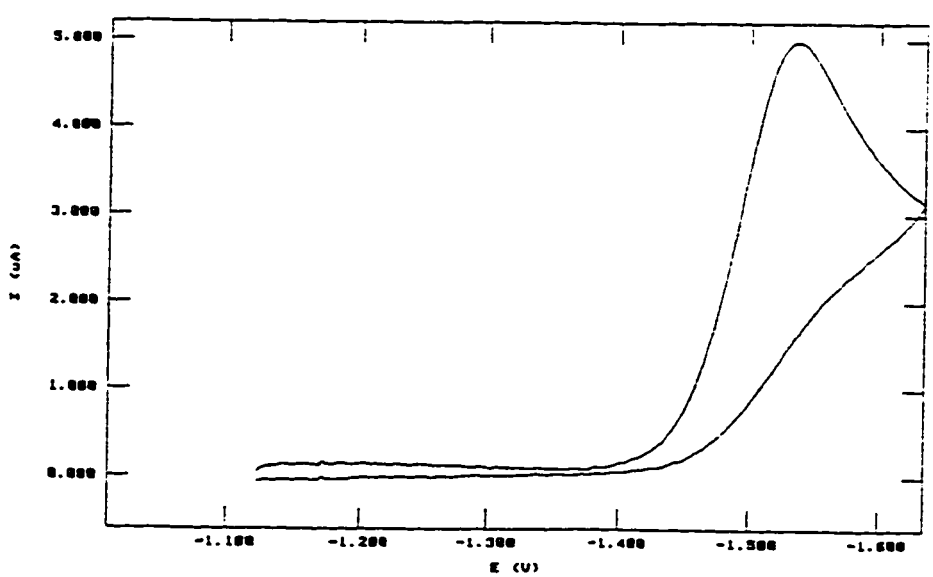
pH 7 Run # 3

Figure 5C. Cyclic voltammogram for 0.5 mM rs49574 in Britten-Robinson buffer of pH11 Run 6 & 4

C



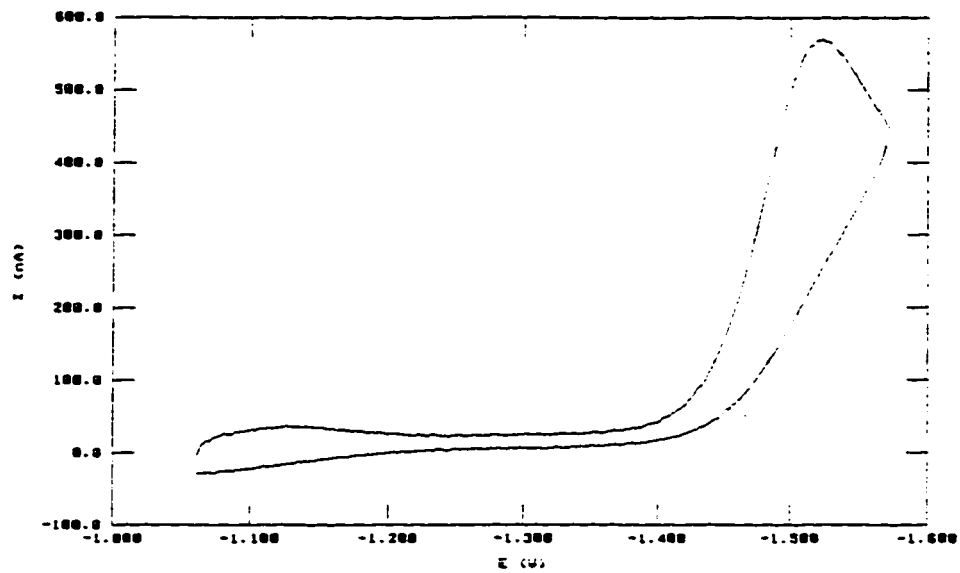
pH 11 Run # 6



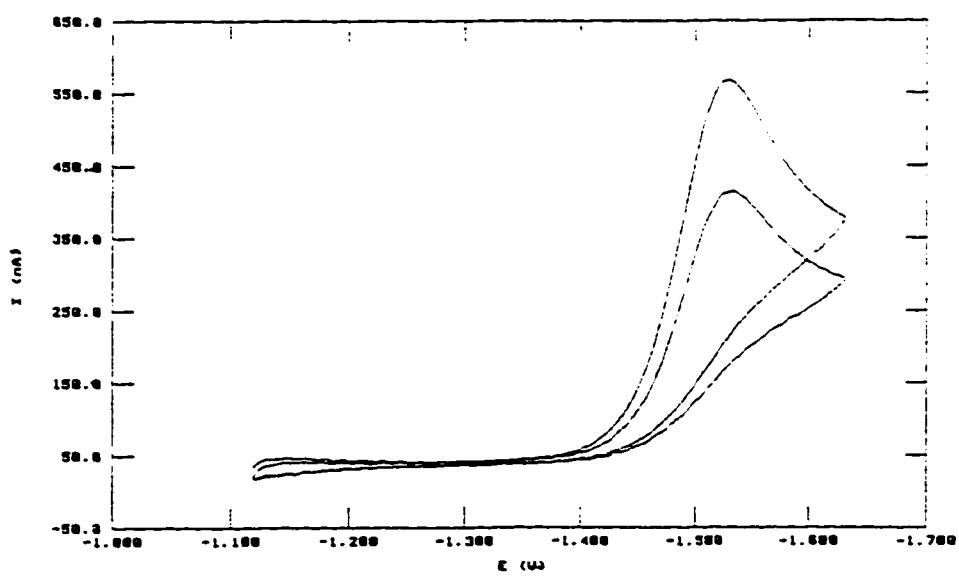
pH 11 Run # 4

Figure 5D. Cyclic voltammogram for 0.1 mM rs49574 in Britten-Robinson buffer of pH11 Run 8 & 7

D



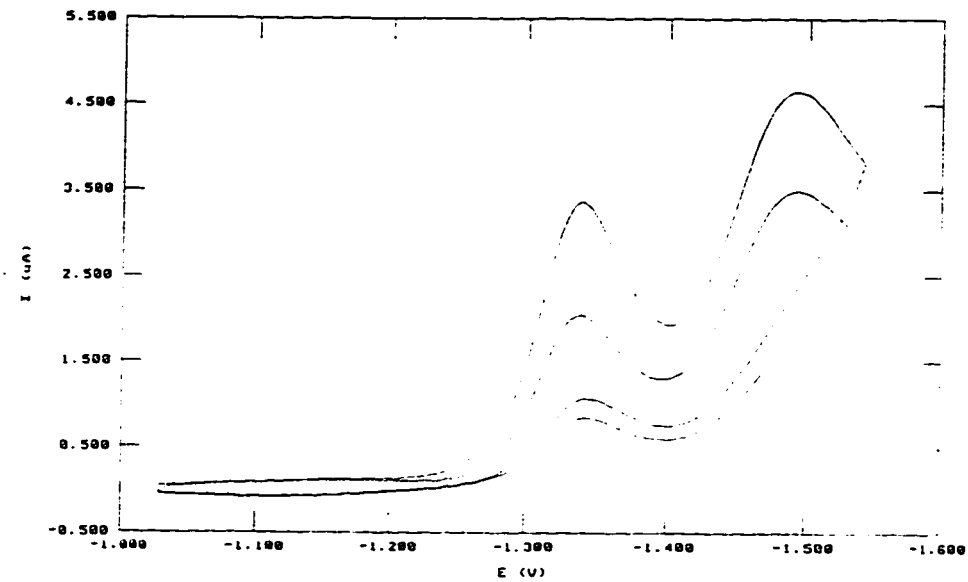
pH 11 Run # 8



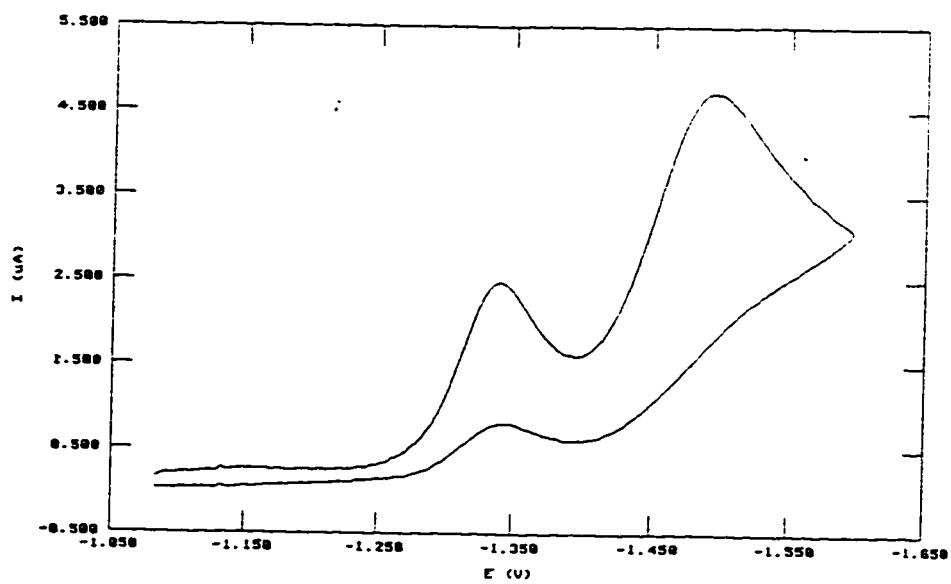
pH11 Run # 7

Figure 5E. **Cyclic voltammogram for 0.5 mM rs49574 in Britten-Robinson buffer of pH8 Run 6' & 4'**

E



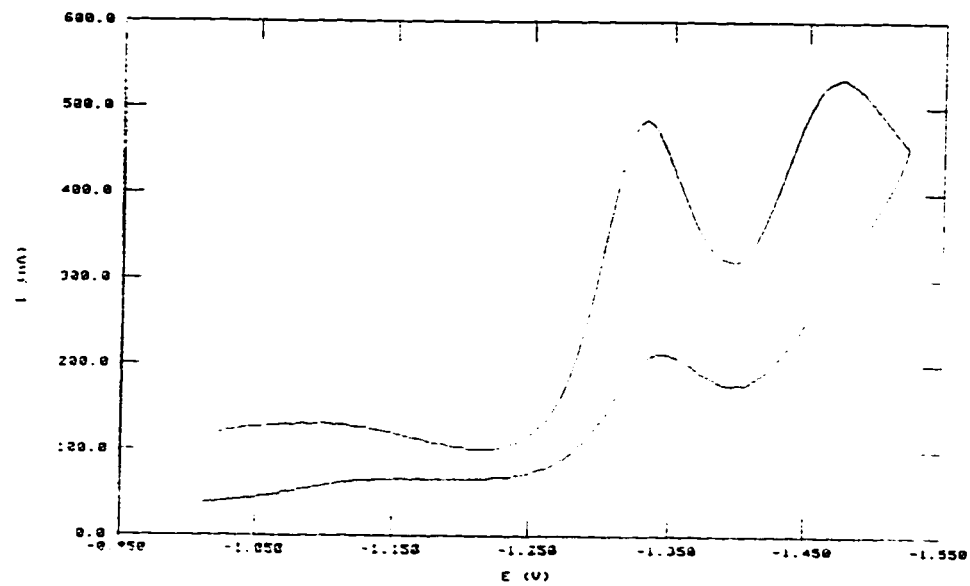
pH 8 Run # 6'



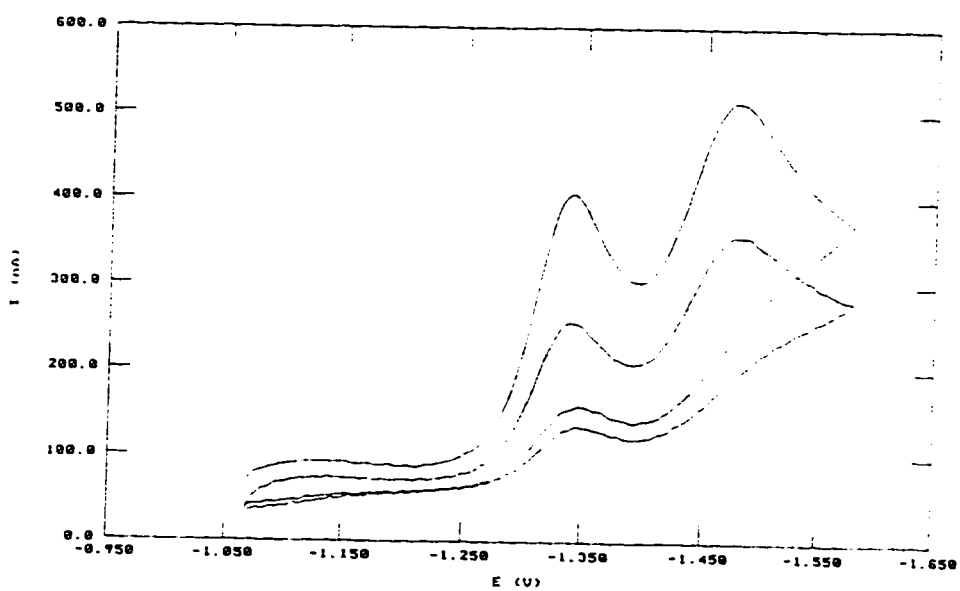
pH8 Run # 4'

Figure 5F. **Cyclic voltammogram for 0.1 mM rs49574 in Britten-Robinson buffer of pH8 Run 8' & 7'**

F



pH 8 Run # 8'



pH8 Run # 7'

3.1.2. rs37629

rs37629 shows two peaks at pH 7 and one peak at pH 11.The first peak potential is pH dependent. The second peak potential occurs between -1.615 and -1.640 v through the eight runs. The overall behavior and trends are similar to those observed for rs49574, including the appearance of the adsorption pre-peak with high analyte concentration at pH 7. It is also observed at low analyte concentration, but for a high scan rate at pH 7 (see Fig.6 A-D)

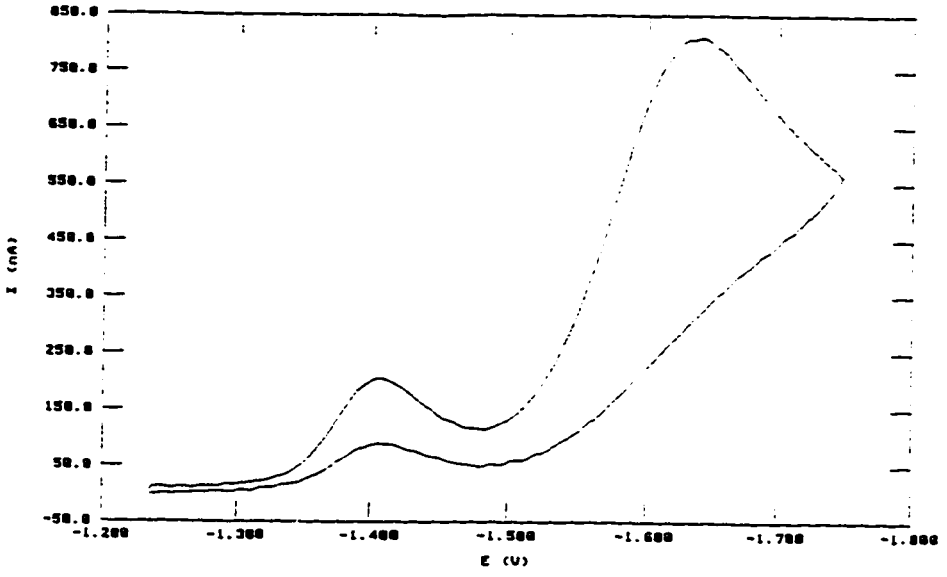
Table 10. Voltammetry data of rs37629 at pH 7 & 11 1st and 2nd Peak

Run #*	pH	Conc. (mM)	MeOH (%)	Scan R (mv/s)	Ep(1) (v)	Ip(1) (uA)	E p(2) (v)	Ip(2) (uA)	Ip(2)/C*V^1/2 (K)
1	7	0.1	2.5	1000	-1.407	0.204	-1.640	0.715	226.1
5	7	0.1	10	1000	-1.407	0.158	-1.635	0.722	228.3
2	7	0.5	2.5	250	-1.397	0.937	-1.619	1.875	59.3
3	7	0.5	10	250	-1.406	0.725	-1.616	1.815	57.4
6	11	0.5	2.5	1000			-1.637	4.805	303.9
4	11	0.5	10	1000			-1.637	4.636	293.2
8	11	0.1	2.5	250			-1.615	0.533	84.3
7	11	0.1	10	250			-1.619	0.522	82.7

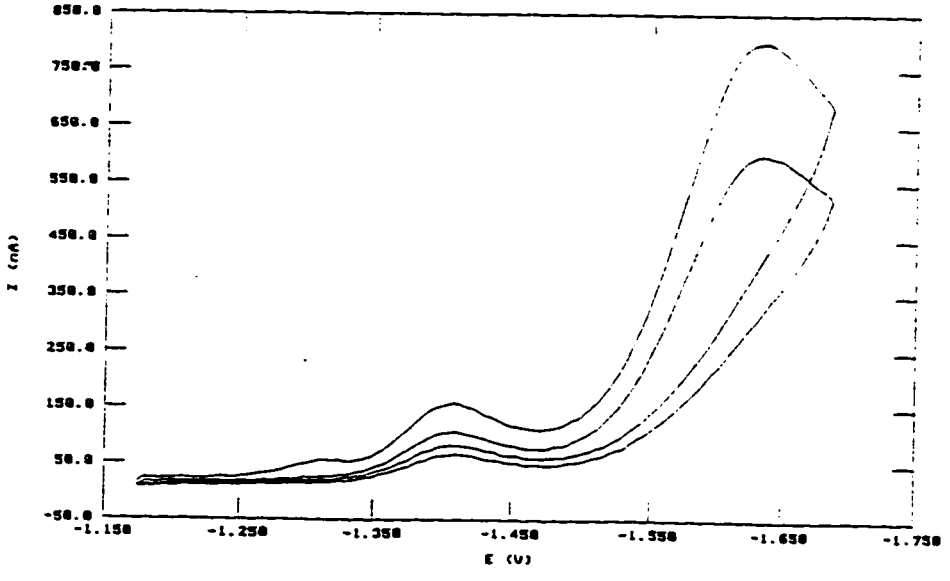
* Run # is referred to (P-B) 8 RUN # shown in Table 2.
** Ip is peak current; C is concentration, V is scan rate.

Figure 6A. Cyclic voltammogram for 0.1 mM rs37629 in Britten-Robinson buffer of pH7 Run 1 & 5

A



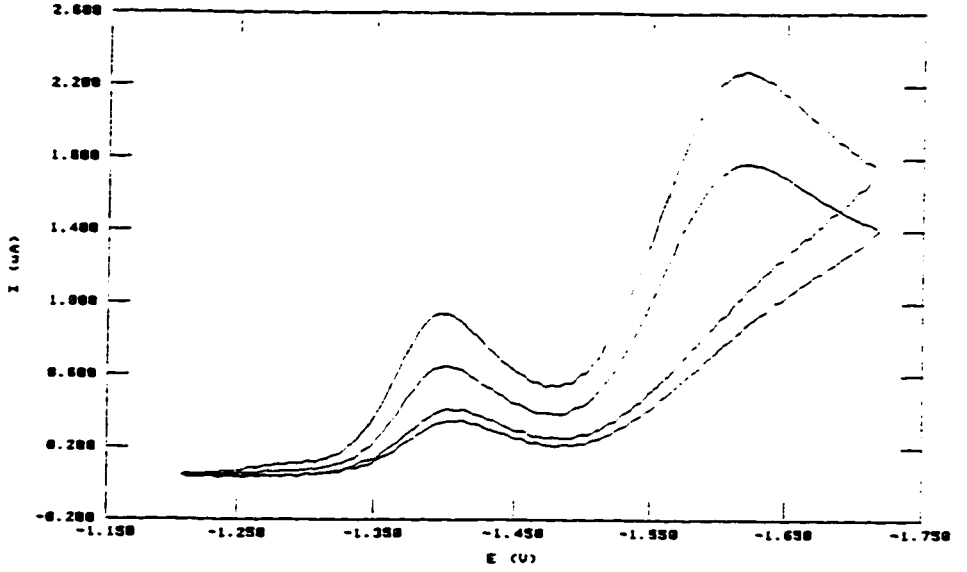
pH 7 Run # 1



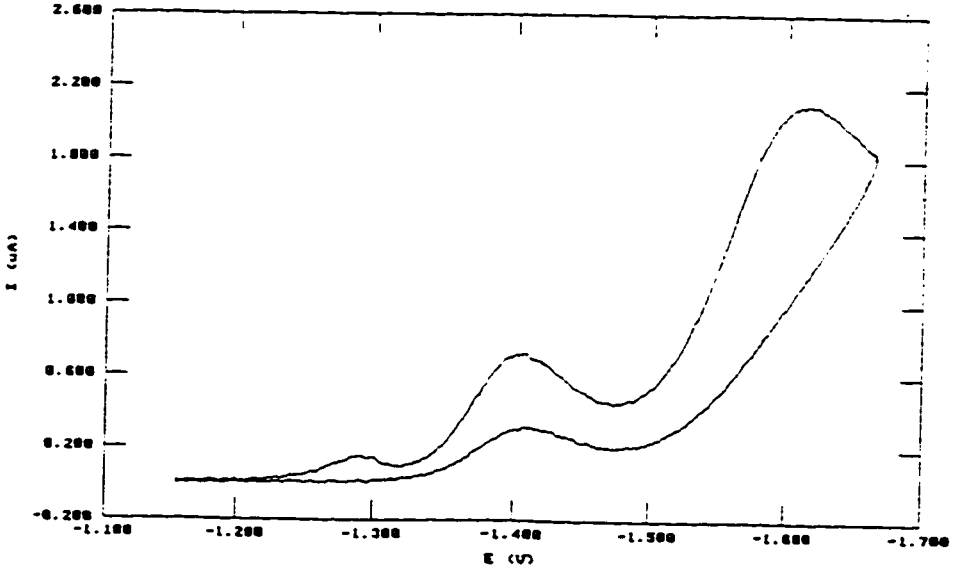
pH7 Run # 5

Figure 6B. Cyclic voltammogram for 0.5 mM rs37629 in Britten-Robinson buffer of pH7 Run 2 & 3

B



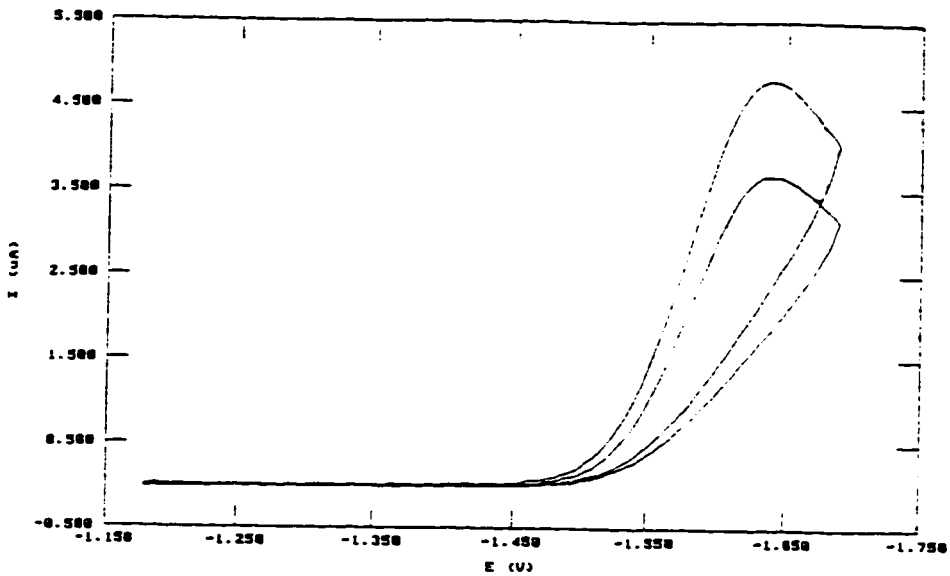
pH 7 Run # 2



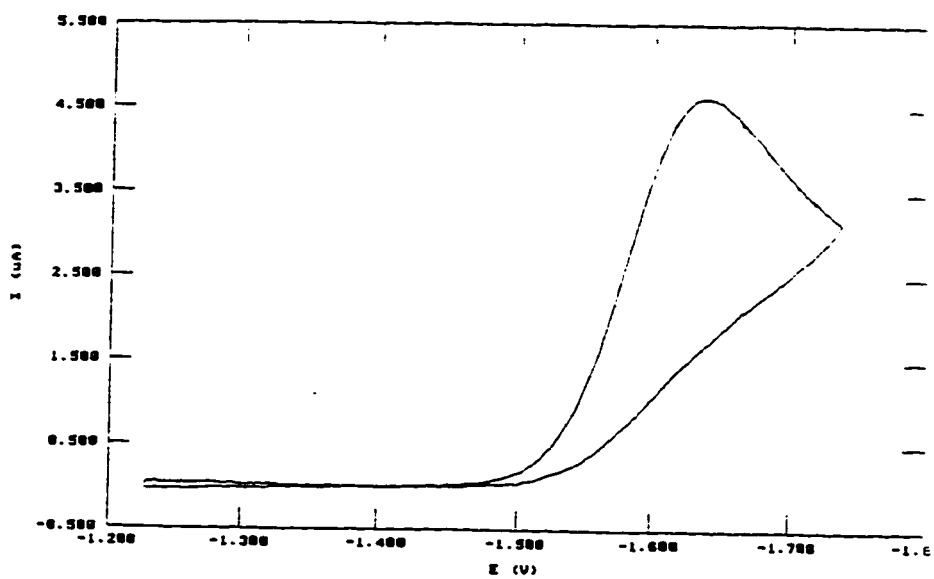
pH7 Run # 3

Figure 6C. Cyclic voltammogram for 0.5 mM rs37629 in Britten-Robinson buffer of pH11 Run 6 & 4

C



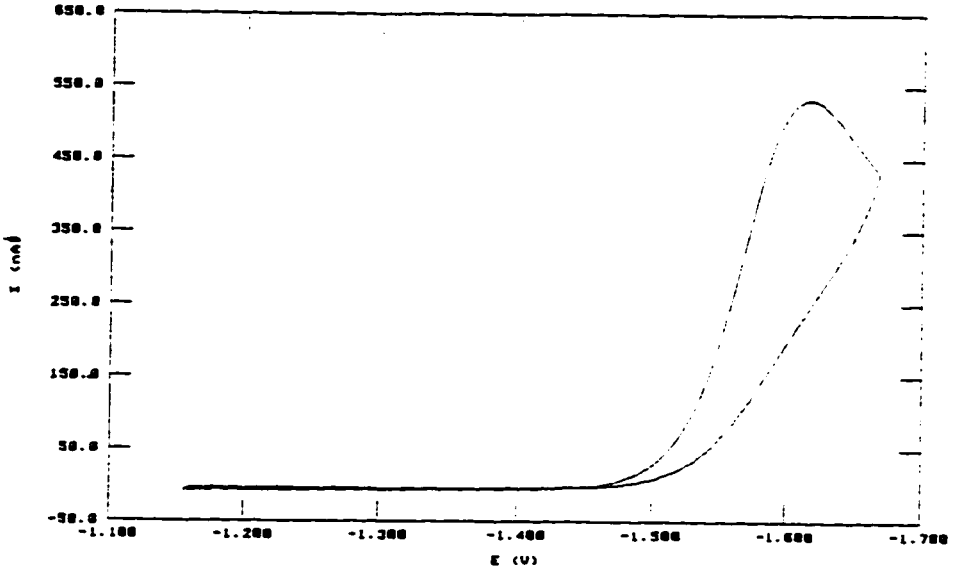
pH 11 Run # 6



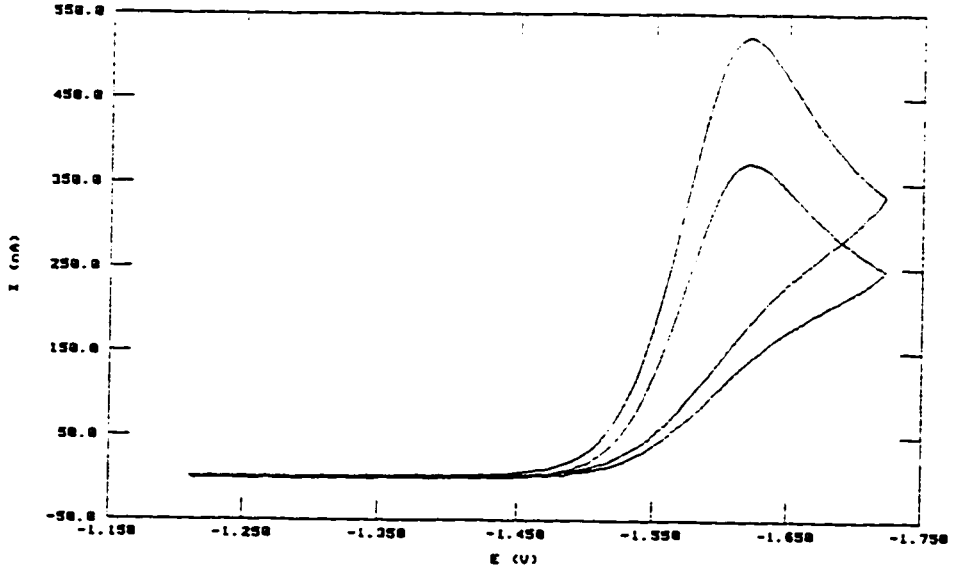
pH111 Run # 4

Figure 6D. Cyclic voltammogram for 0.1 mM rs37629 in Britten-Robinson buffer of pH11 Run 8 & 7

D



pH 11 Run # 8



pH11 Run # 7

3.1.3. Tolmetin and Ketoprofen

Tolmetin gave results similar to those obtained for rs37629 and rs49574. It also shows two peaks at pH 7 and one peak at pH 11.The first peak potential is pH dependent. The second peak occurs between -1.613 - -1.638 v. No adsorption pre-peak is observed. The data and graphs for tolmetin are shown in Table 11 and Figures 7 (A-D).

A study of the pH effect on the peak potential of tolmetin was examined and is illustrated in Figure 8. For pH values between 6 and 11, with scan rate 100 mv/s, concentration 2 x 10^{-4} M, scanning range from -1.1v - -1.8v, tolmetin shows only one peak at pH 6. The potential of the peak is -1.395v . At pH 7, the second process appears with a peak potential approximately 200 mV more negative than the first. This second peak is much smaller at pH 7 with a 100 mv/s scan rate than shown in Fig. 7 for 250 or 1000 mv/s scan rates. This suggests the second peak may be adsorption dependent.

The same pH study was done on Ketoprofen. It exhibited a similar behavior to tolmetin. The result is shown in Figure 9. From an independent study [47] it was obvious that Ketorolac also has pH behavior similar to Tolmetin. Although a detailed pH study was not done on rs49574 and rs37629, it is expected that they should have similar behavior.

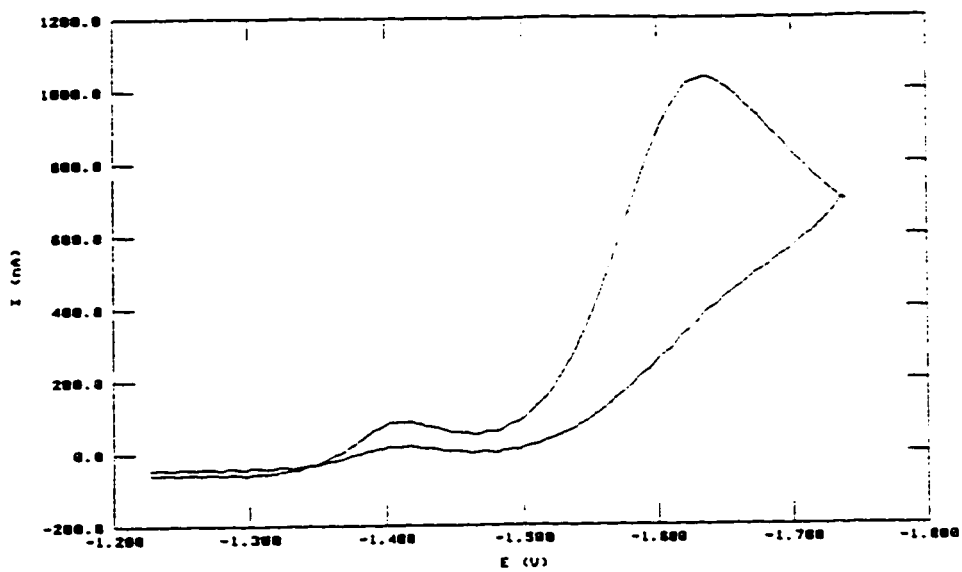
Table 11. Voltammetry data of tolmetin at pH 7 & 11 1st and 2nd Peak

Run* #	pH	Conc. (mM)	MeOH (%)	Scan R (mv/s)	Ep(1) (v)	Ip(1) (uA)	Ep(2) (v)	Ip(2) (uA)	Ip(2)/C*V^1/2** (K)
1	7	0.1	2.5	1000	-1.411	0.087	-1.638	1.000	316.2
5	7	0.1	10	1000	-1.414	0.123	-1.630	1.060	335.2
2	7	0.5	2.5	250	-1.411	0.777	-1.613	2.957	93.5
3	7	0.5	10	250	-1.419	0.421	-1.618	2.841	89.8
6	11	0.5	2.5	1000			-1.630	4.419	279.5
4	11	0.5	10	1000			-1.632	4.463	282.3
8	11	0.1	2.5	250			-1.624	0.643	101.7
7	11	0.1	10	250			-1.632	0.620	98.0

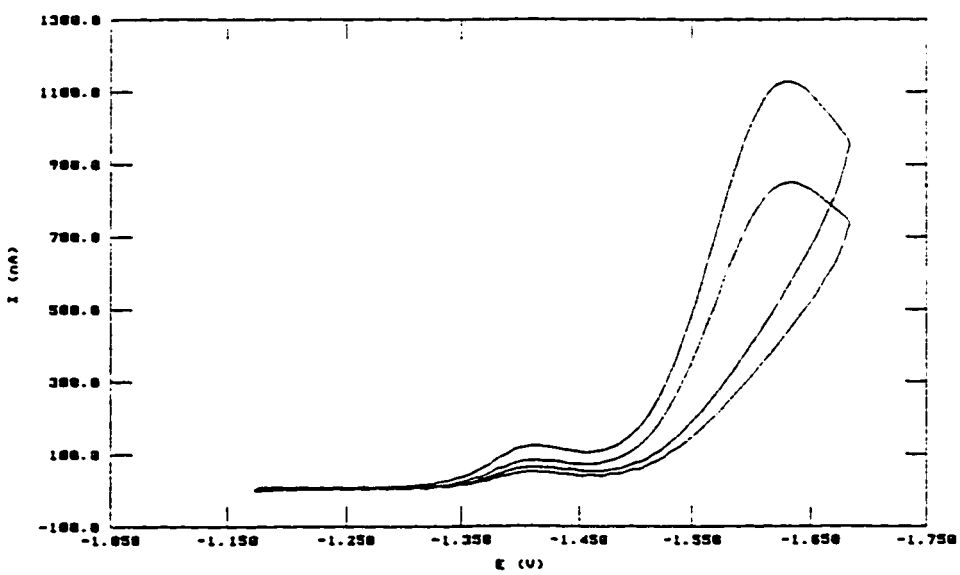
* Run # is referred to (P-B) 8 RUN # shown in Table 2.
** Ip is peak current; C is concentration, V is scan rate.

Figure 7A. Cyclic voltammogram for 0.1 mM Tolmetin in Britten-Robinson buffer of pH7 Run 1 & 5

A



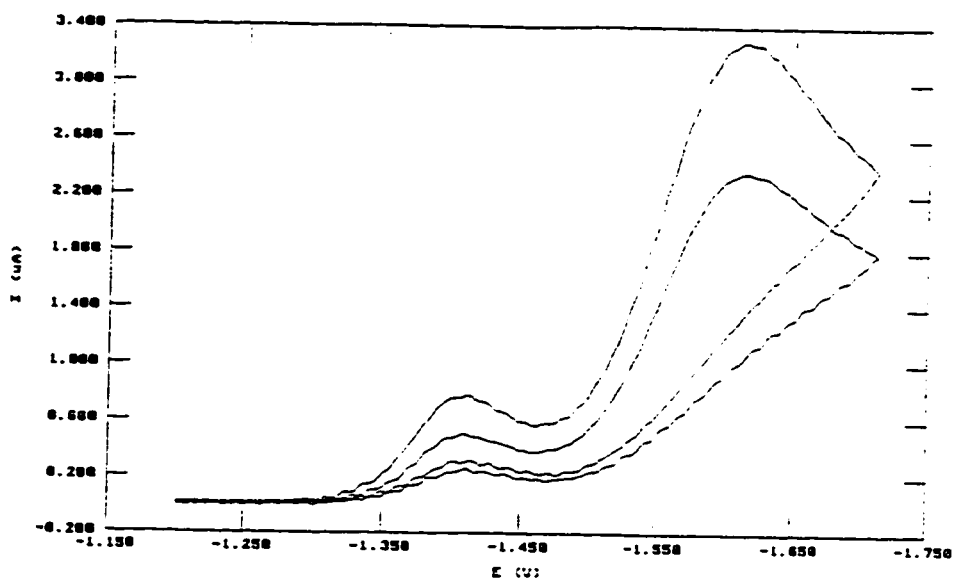
pH 7 Run # 1



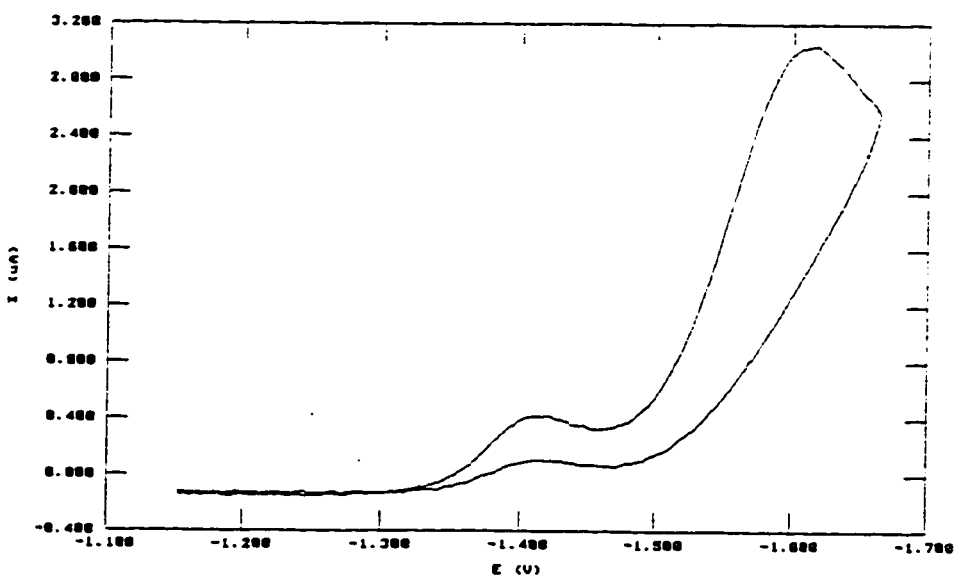
pH7 Run # 5

Figure 7B. Cyclic voltammogram for 0.5 mM Tolmetin in Britten-Robinson buffer of pH7 Run 2 & 3

B



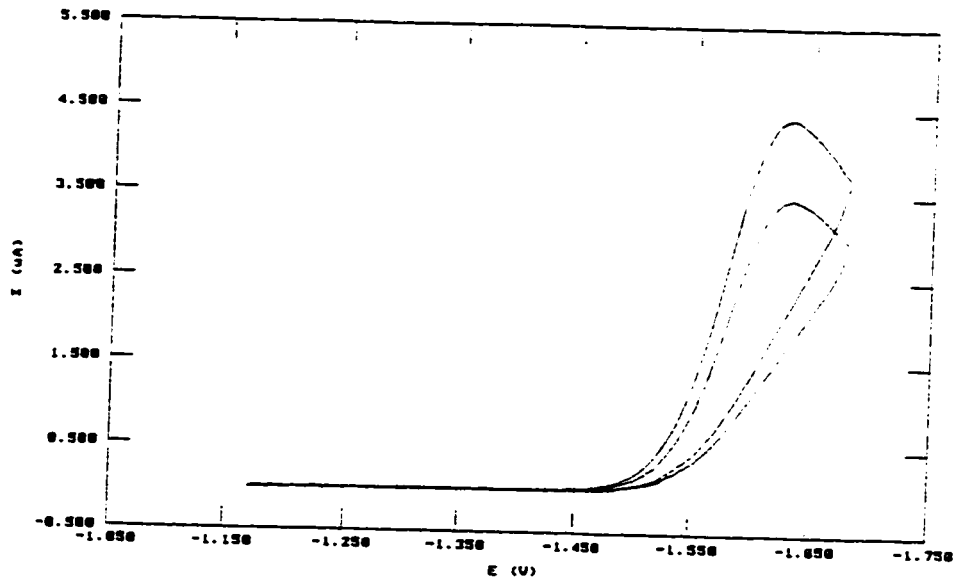
pH 7 Run # 2



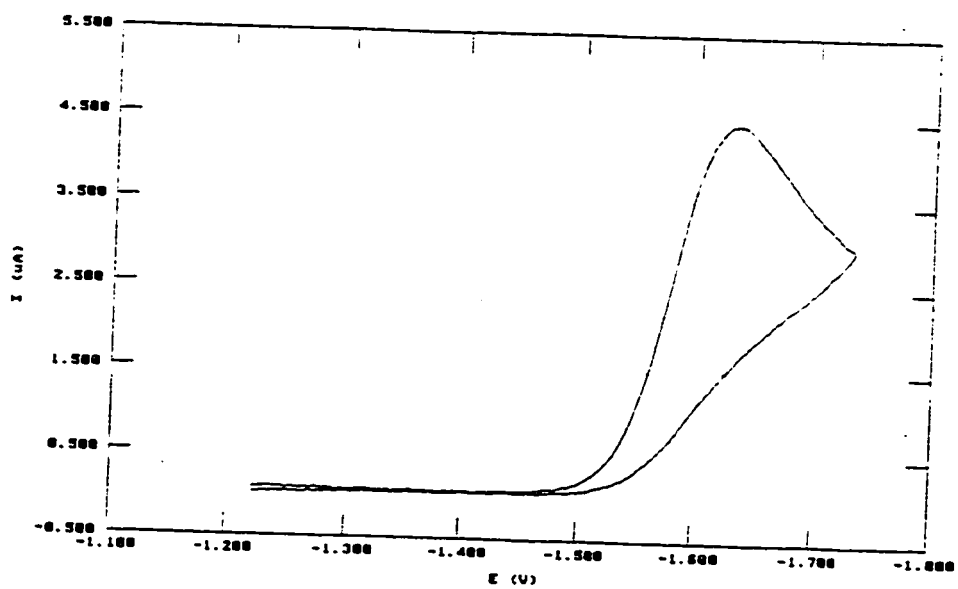
pH7 Run # 3

Figure 7C. Cyclic voltammogram for 0.5 mM Tolmetin in Britten-Robinson buffer of pH11 Run 6 & 4

C



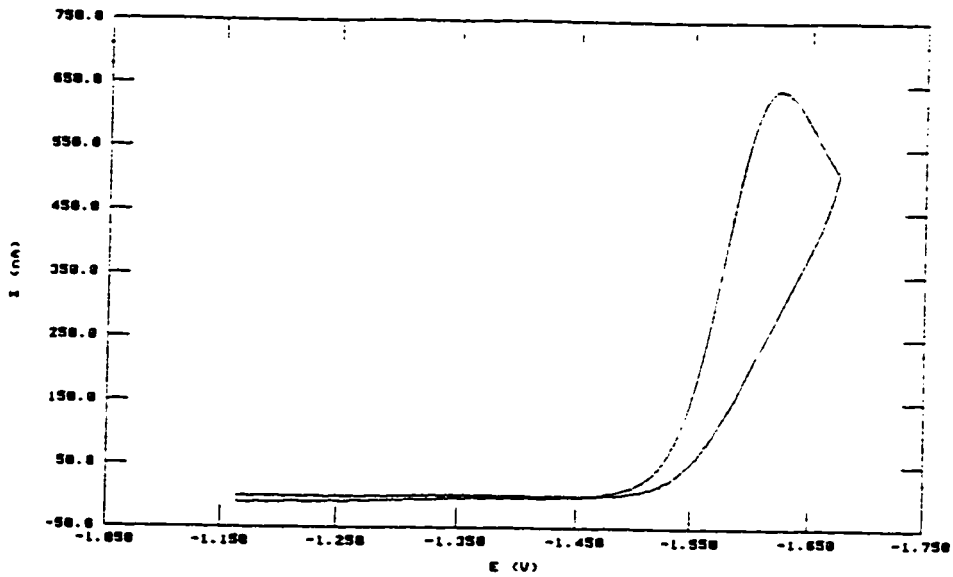
pH 11 Run # 6



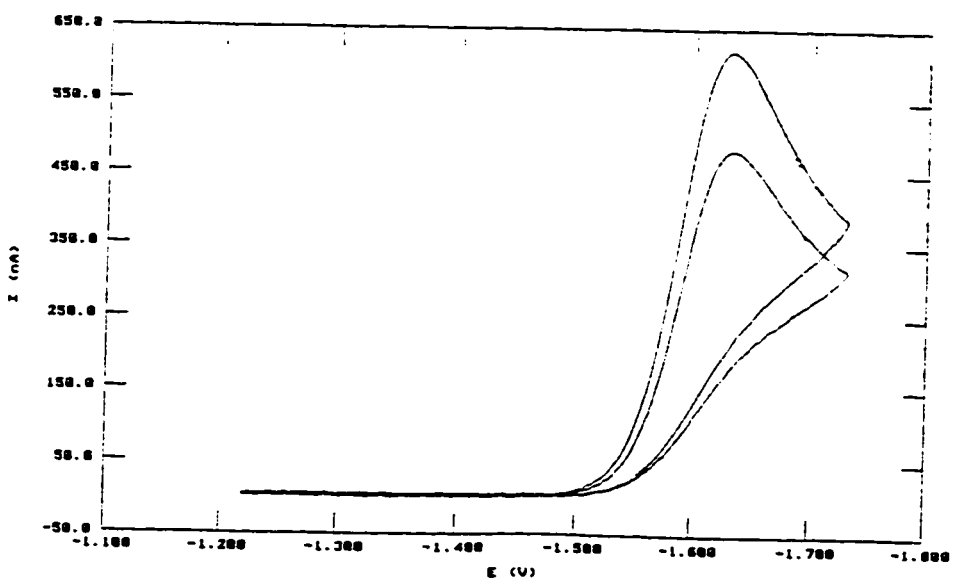
pH11 Run # 4

Figure 7D. Cyclic voltammogram for 0.1 mM Tolmetin in Britten-Robinson buffer of pH11 Run 8 & 7

D



pH 11 Run # 8



pH11 Run # 7

Figure 8. An overlay graph of pH 6 - 11 for Tolmetin

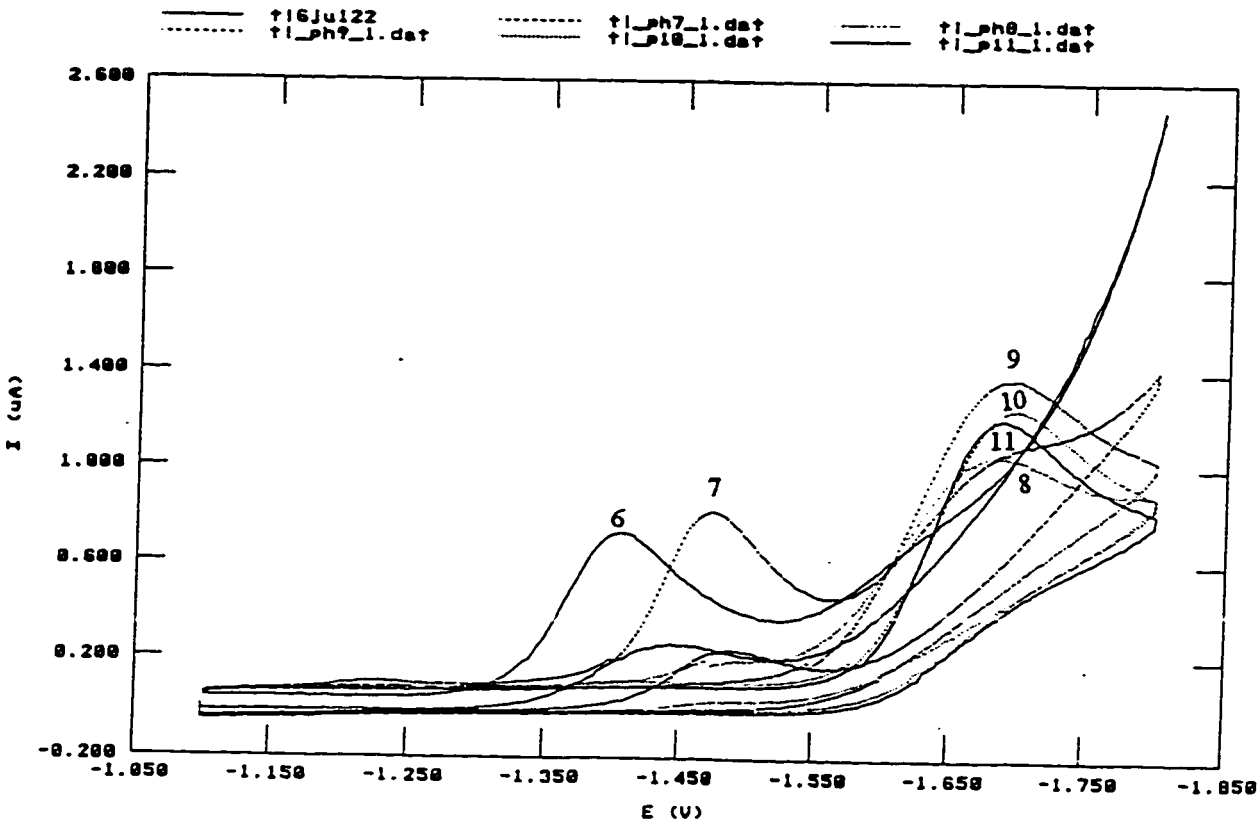
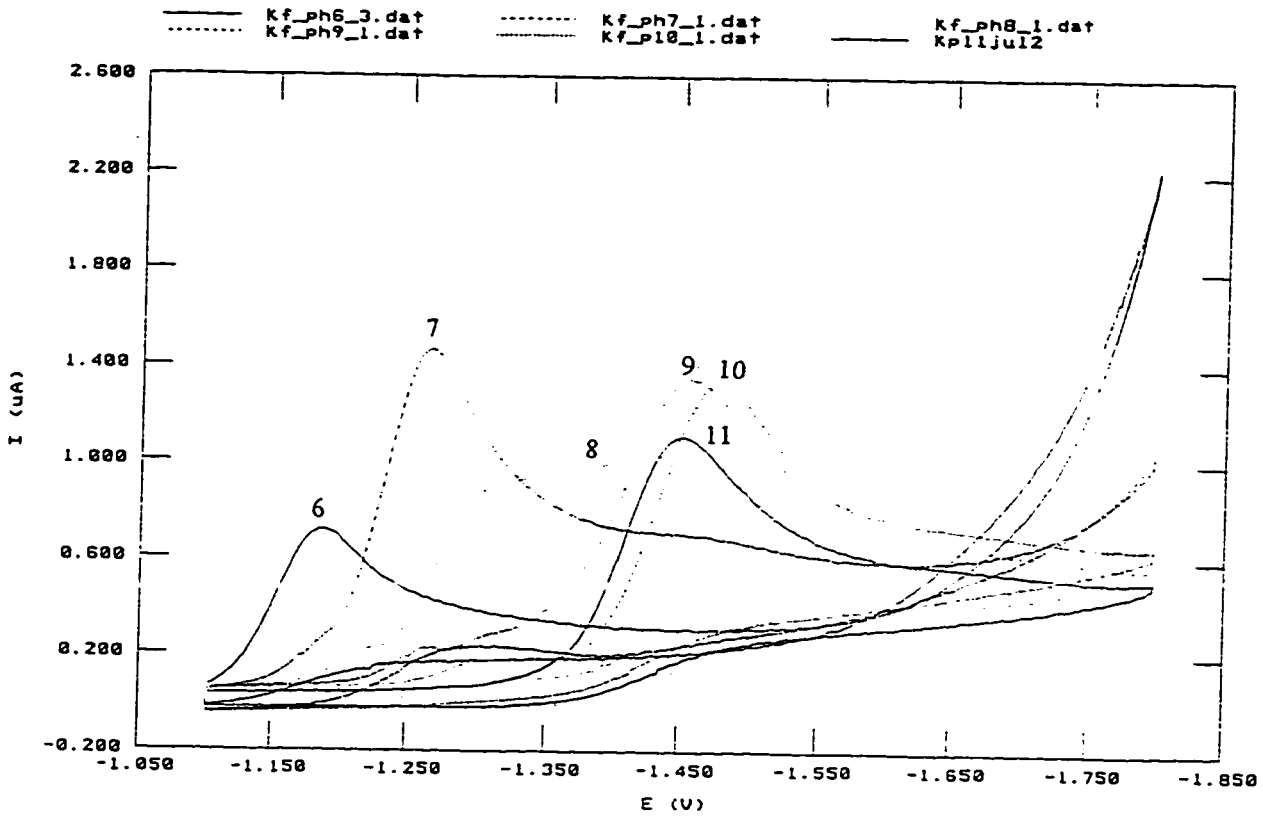


Figure 9. An overlay graph of pH 6 - 11 for Ketoprofen



3.1.4. Suprofen

A remarkable pH effect was observed with Suprofen (see Fig. 10). Only one pH-dependent peak is observed from pH 6 to 9 (with a small pre-peak under some conditions) The peak potential move cathodically with increasing pH. Thus for runs # 1, 2, 3 and 5 at pH 7 in the P-B study, Fig 11, the main peak is the same as the first peak for the previous compounds studied, and no data are acquired for the second peak, which does not appear. The main peak at pH 7 is at -1.200 V, and the peak potential at pH 11 is about -1.364 V , indicating a catholic shift of about - 40 mv/pH.

Figure 10. An overlay graph of pH 6 - 11 for Suprofen

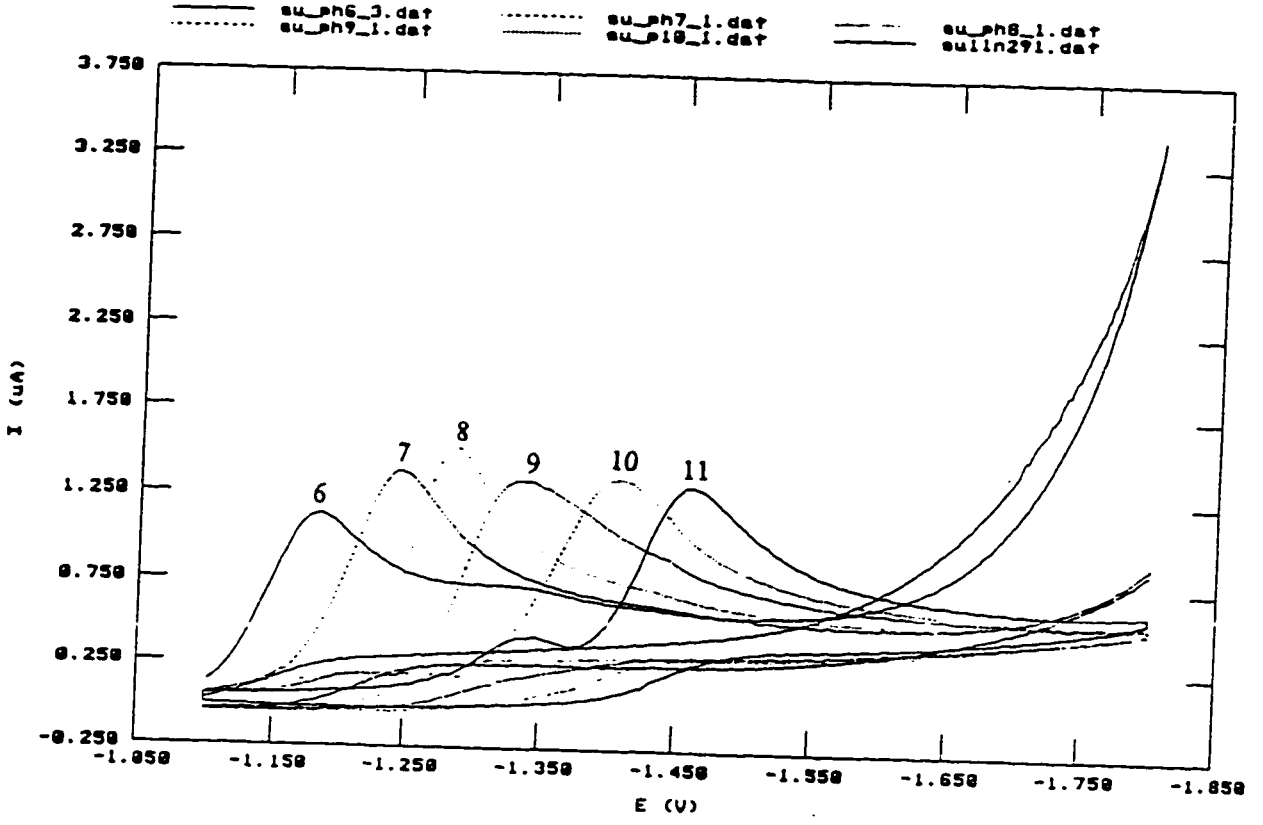


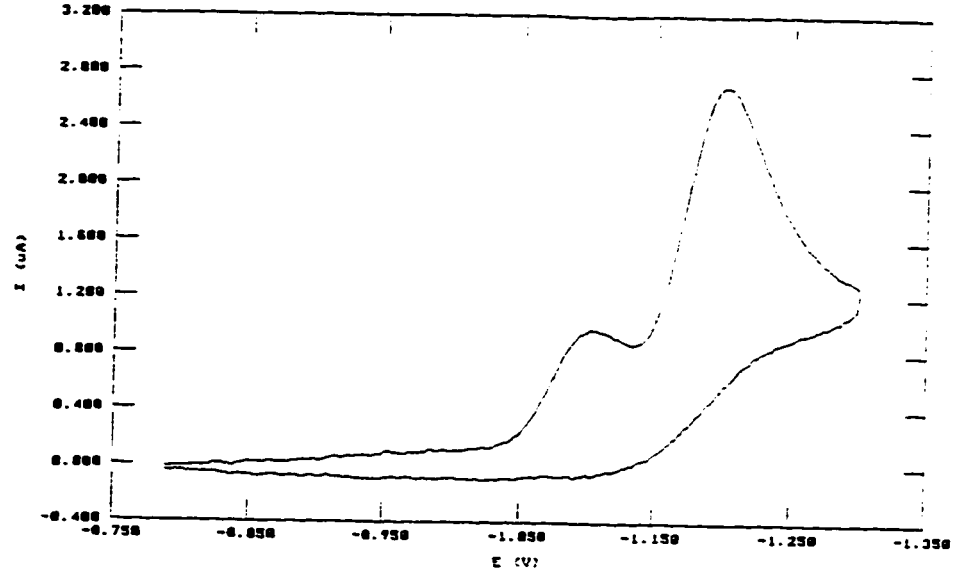
Table 12. Voltammetry data of suprofen at pH 7 & 11 2nd Peak

Run* #	pH	Conc. (mM)	MeOH (%)	Scan R (mv/s)	Ep(2) (v)	Ip(2) (uA)	Ip(2)/C*V^1/2 (K)
1	7	0.1	2.5	1000	-1.200	0.018	5.7
5	7	0.1	10	1000	-1.208	0.035	11.1
2	7	0.5	2.5	250	-1.181	0.280	8.9
3	7	0.5	10	250	-1.200	0.034	1.1
6	11	0.5	2.5	1000	-1.358	5.535	350.1
4	11	0.5	10	1000	-1.369	4.989	315.5
8	11	0.1	2.5	250	-1.363	0.617	97.6
7	11	0.1	10	250	-1.364	0.573	90.6

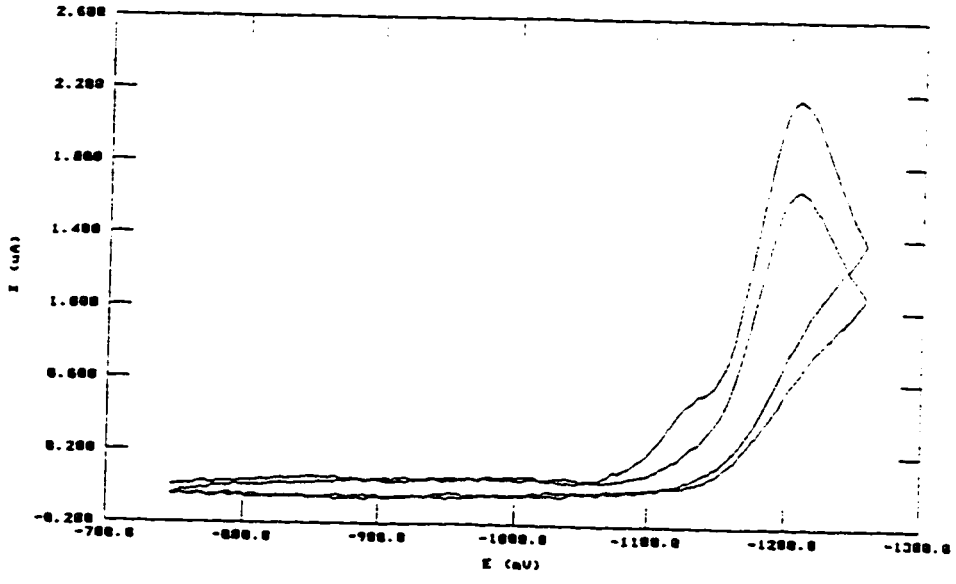
* Run # is referred to (P-B) 8 RUN # shown in Table 2.
** Ip is peak current; C is concentration, V is scan rate.

Figure 11A. Cyclic voltammogram for 0.1 mM Suprofen in Britten-Robinson buffer of pH7 Run 1 & 5

A



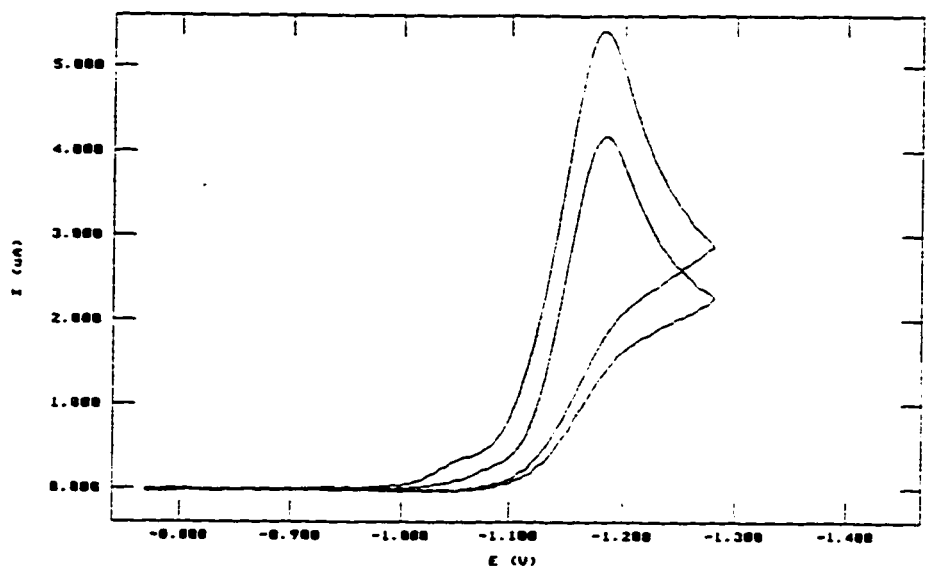
pH 7 Run # 1



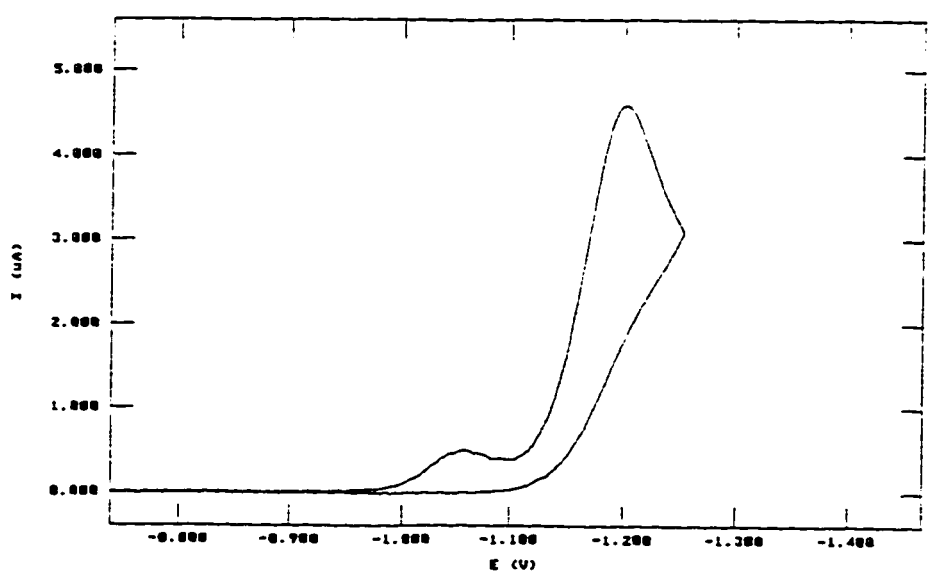
pH7 Run # 5

Figure 11B. Cyclic voltammogram for 0.5 mM Suprofen in Britten-Robinson buffer of pH7 Run 2 & 3

B



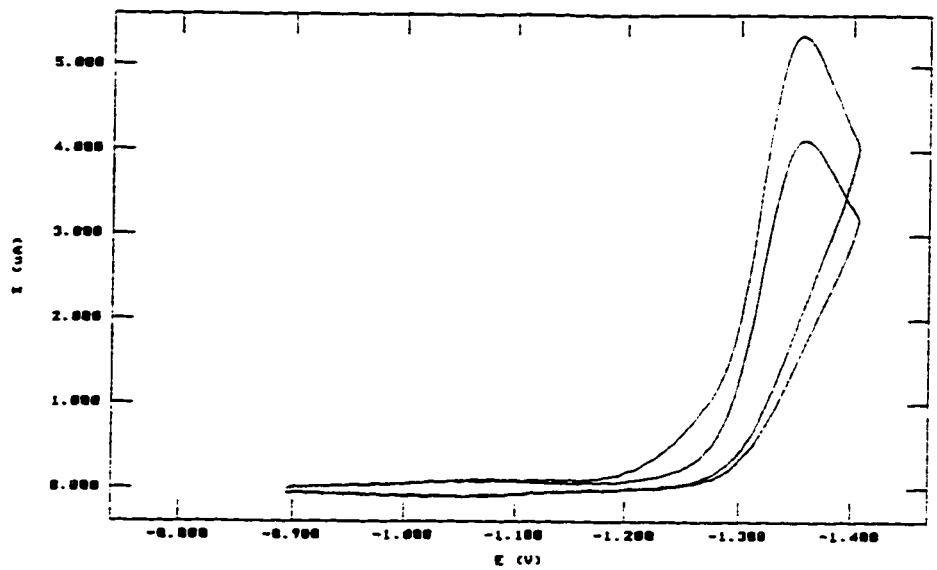
pH 7 Run # 2



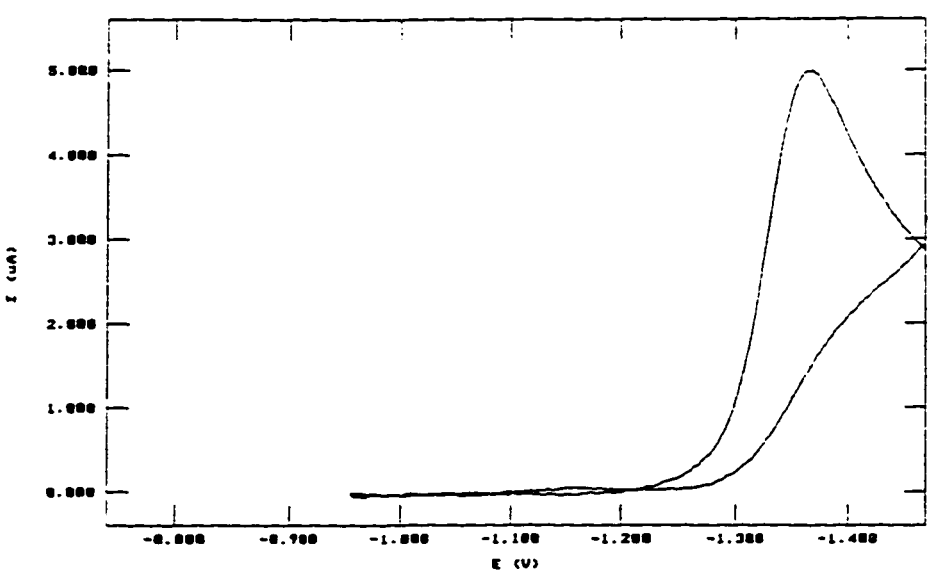
pH7 Run # 3

Figure 11C. Cyclic voltammogram for 0.5 mM Suprofen in Britten-Robinson buffer of pH11 Run 6 & 4

C



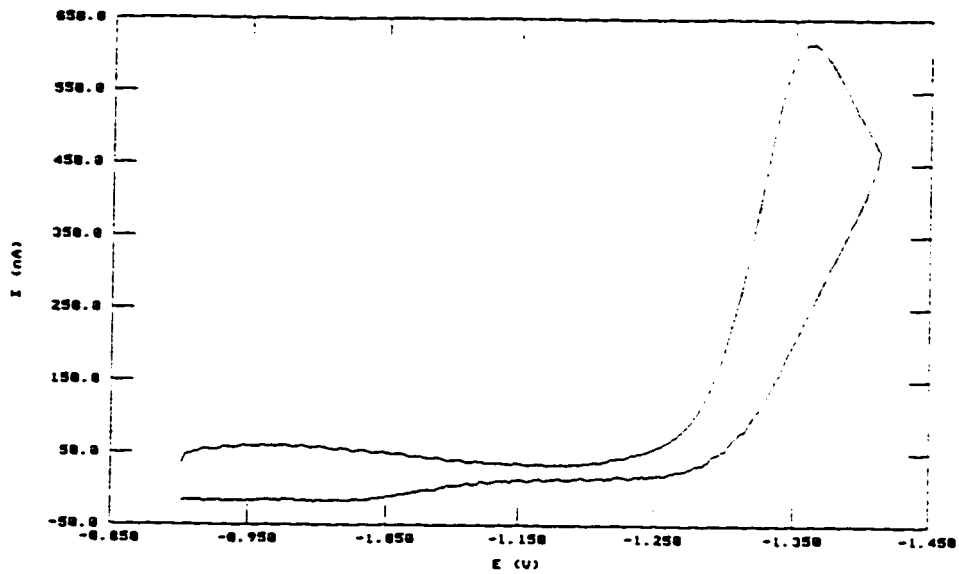
pH 11 Run # 6



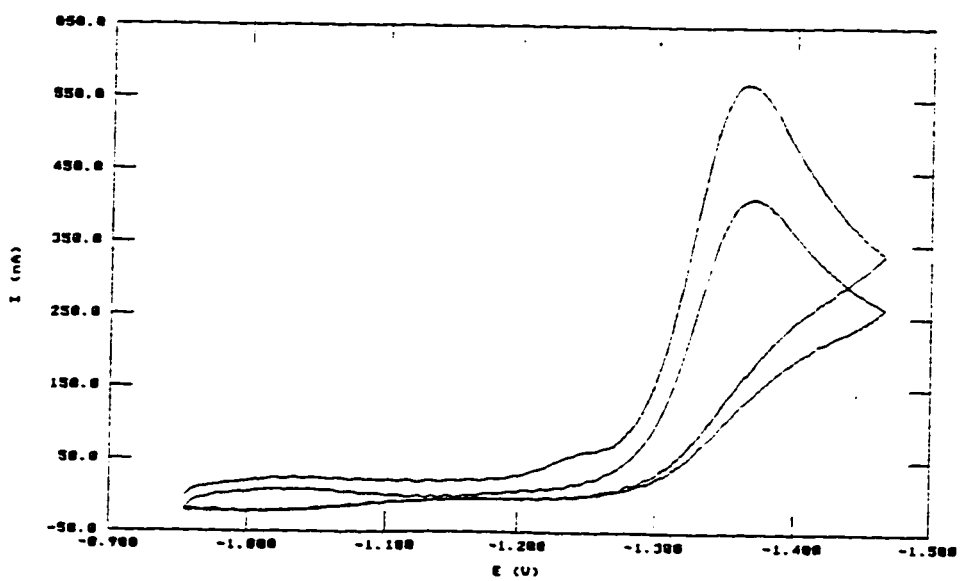
pH11 Run # 4

Figure 11D. Cyclic voltammogram for 0.1 mM Suprofen in Britten-Robinson buffer of pH11 Run 8 & 7

D



pH 11 Run # 8



pH11 Run # 7

Table 13. Voltammetry data of ketorolac at pH 7&11 1st and 2nd Peak

Run #	pH	Conc. (mM)	MeOH (%)	Scan R (mv/s)	Ep(1) (v)	Ip(1) (uA)	Ep(2) (v)	Ip(2) (uA)	Ip(2)/C*V^1/2 (K)
1	7	0.1	2.5	1000	-1.369	0. 589	-1.575	1.175	371.6
5	7	0.1	10	1000	-1.378	0.386	-1.576	1.104	349.1
2	7	0.5	2.5	250	-1.366	2.740	-1.562	2.727	86.1
3	7	0.5	10	250	-1.369	1.637	-1.562	2.546	80.5
6	11	0.5	2.5	1000			-1.580	7.866	497.5
4	11	0.5	10	1000			-1.601	7.170	453.5
8	11	0.1	2.5	250			-1.575	0.911	144.0
7	11	0.1	10	250			-1.580	0.634	100.2

* Run # is referred to (P-B) 8 RUN # shown in Table 2.
* Ip is peak current; C is concentration, V is scan rate.
* Data received from Edward Kaiser

3.2. Electrochemical Processes

The general scheme for the electroreduction of arylacetic acids is illustrated in Figure 12 for Tolmetin [23]. The first step is the reduction of the keto group of the benzoyl substituent by a one-electron process to give a free-radical anion. In the second step the radical anion undergoes a further one-electron reduction to the dianion, which subsequently becomes protonated to give the alcohol. These two steps correspond to the first and second voltammetric peaks. (see Figs. 7 and 8).

In acidic medium the radical anion produced at the first peak undergoes dimerization to the pinacol. This peak is pH sensitive because the radical anion is in equilibrium with the protonated radical, and the E_p moves cathodically with increasing pH (peak A in Fig 13). At higher pH, 6-8 , the second reduction becomes observable, and is better developed above pH 8. It was found that the second wave is irreversible in nature, and is attributed to the well known two-electron reduction of the keto group to the alcohol. In Fig. 13, at higher pH, the two waves shown in B merge to form one wave C. This single peak at high pH is well-suited to analytical work.

Figure 12. Pathway for the electro-reduction process of tolmetin

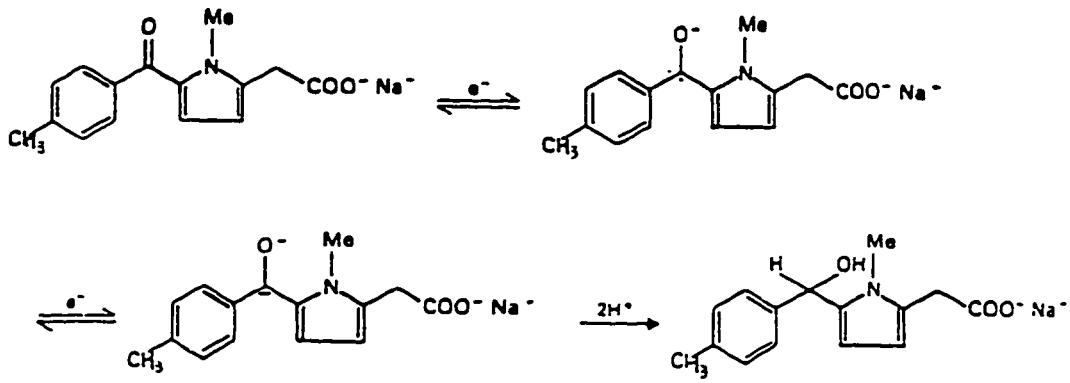
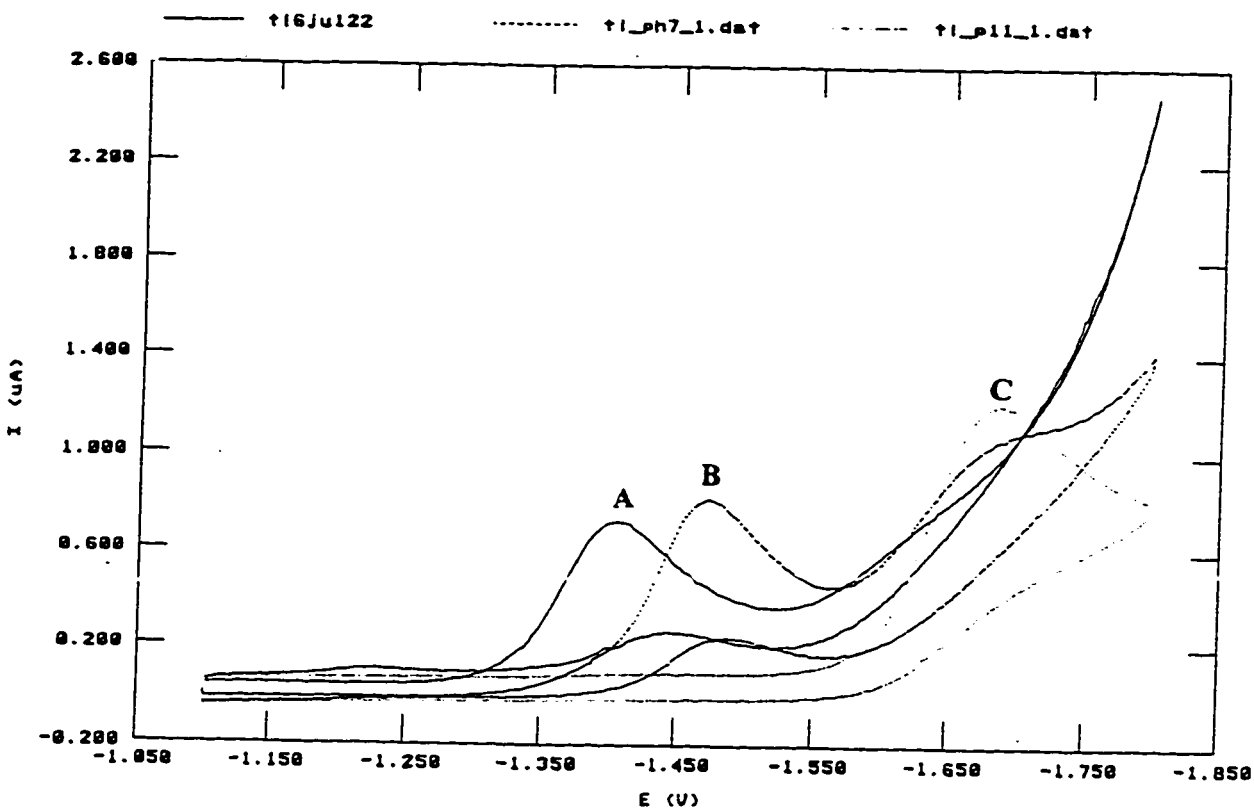


Figure 13. Cyclic voltammatric waves of tolmetin (1.0×10^{-4} M) in Britton-Robinson buffer. A, pH 6.0; B, pH 7.0; C, pH 11.0.



3.3. Factor Effects

The main effects of experimental parameters were evaluated by processing the data obtained in the factorial designed studies for each compound (see Tables 2-4). The effect of each factor is evaluated by summing the response parameter (either K or Ep) when the variable was at its high level, and then subtracting all responses when the variable was at its low level. This approach is analogous to the method reported in Reference 47 for extracting the contribution of each variable, free from the effects of other variables. The results of this factor effect computation for each compound studied are listed in Table 14. The statistical evaluation of these results is provided below.

Most factor effect data are computed for the second peak, as it is normally observable at both extremes of pH considered in this study. For some compounds it was also possible to observe both peaks at two pH levels, and some of these factor effect data are also included in Table 14. To evaluate the results in Table 14, consider that the performance indicator, K, removes the normal effects of concentration and scan rate. Thus, for all factors examined, there is no expected change in the value of K, unless a given factor influences the fundamental nature of the electrode process.

3.3.1. Effect of Scan Rate

Scan rate has a major effect on the second peak for all experimental compounds. The highest effect is for ketorolac at 1260.9. It has a similar effect on rs49574, rs37629, and tolmetin. There is also a large effect on the first peak for rs49574.

3.3.2. Effect of pH

pH is the second major factor that affects all experimental compounds. The highest effect is on rs49574 at -475.5. Tolmetin is exceptional in that it has a small positive effect, 73.2. There is also a large positive effect on the first peak of rs49574.

3.3.3. Factor Effect Patterns

Figures 14 to 18 illustrate graphically the factor effects reported numerically in Table 14. These visual patterns may be useful in identifying different fundamental properties in more than one dimension for this set of compounds.

Table 14. Effects of Current Function K for compounds studied

Compound	Δ Ip/c*v^1/2 (=K)				
A. Ph7 & 11 2nd peak	**pH**	**Conc.**	**MeOH**	**Scan R**	**Hang T**
rs49574	-475.5	171.1	-18.5	757.7	10.5
rs37629	-193	92.4	-12	767.8	5.0
tolmetin	73.2	-106	14.4	830.2	-29.2
suprofen	-827	470.6	-44	484.2	-285.6
ketorolac	-307.9	152.7	-115.9	1260.9	17.1
B. pH 7 & 8 2nd peak					
rs49574**	-163.3	36.7	28.7	623.3	-32.9
c. pH 7 & 8 1st peak					
rs49574***	800	-730	-310.6	1186.6	267.4

* factor effects are calculated from experimental results summarized in tables 9-13
** Δ Ip/c*v^1/2 for pH 7 & 8 2nd peak
*** Δ Ip/c*v^1/2 for pH7 & 8 1st peak

Table 15. Effects of ΔEp(2) for compounds studied

Compound	ΔEp(2)				
A. Ph7 & 11 2nd peak	**pH**	**Conc.**	**MeOH**	**Scan R**	**Hang T**
rs49574	0.178	0.008	-0.008	-0.032	-0.014
rs37629	-0.002	0	0.004	-0.08	-0.006
tolmetin	0.019	0.031	-0.007	-0.043	-0.019
ketorolac	0.061	0.001	-0.027	-0.053	0.017
B. pH 7 & 8 2nd peak					
rs49574**	-0.007	-0.019	-0.003	-0.059	-0.007
c. pH 7 & 8 1st peak					
rs49574***	0.084	0.014	-0.026	-0.032	-0.012

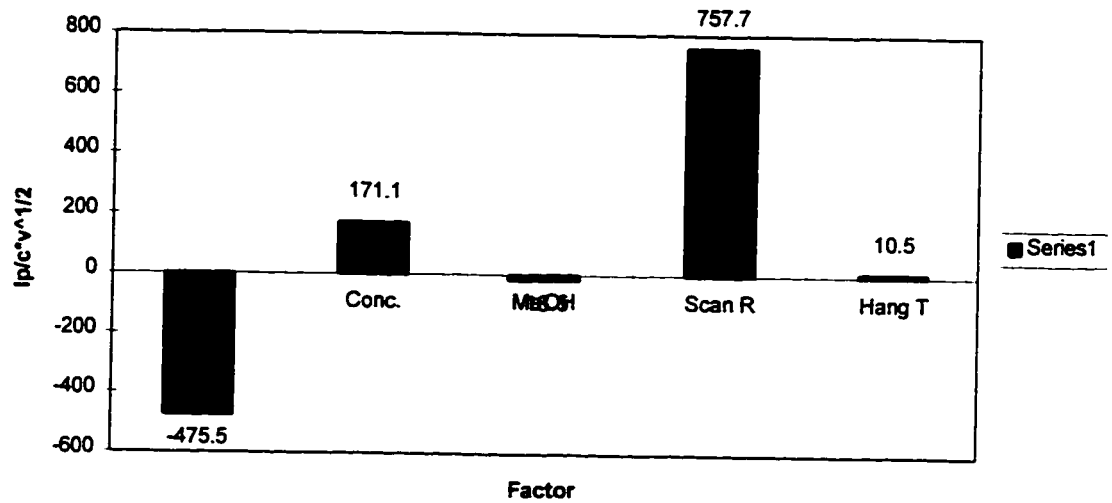
* factor effects are calculated from experimental results summarized in tables 9-13

** Δ Ep(2) for pH 7 & 8 2nd peak

*** Δ Ep(2) for pH7 & 8 1st peak

Figure 14. Effects of K for rs49574 2nd peak

A. pH 7 & 11 2nd Peak



B. pH 7 & 8 2nd Peak

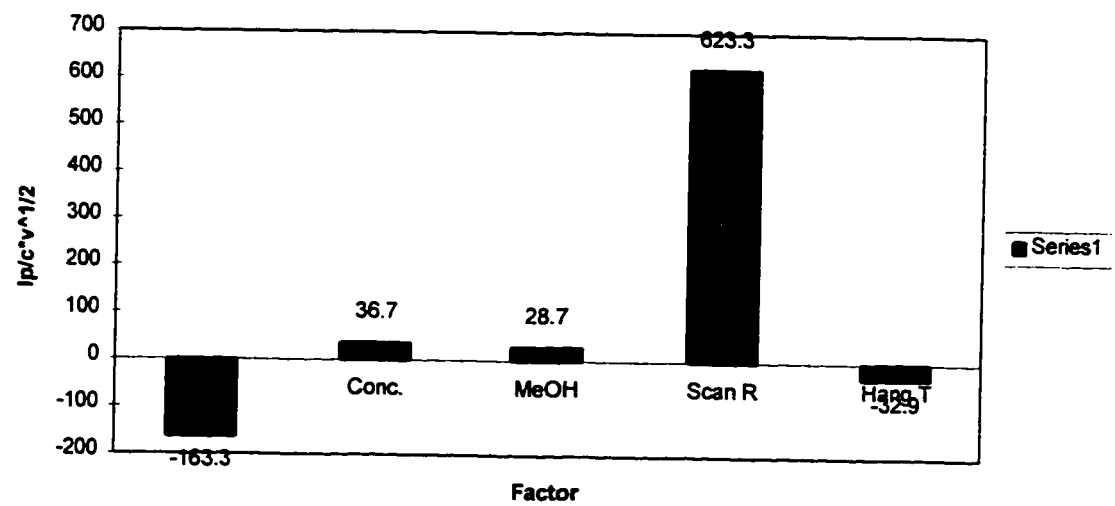


Figure 15. Effects of K for rs49574 pH7 & 8 1 st peak

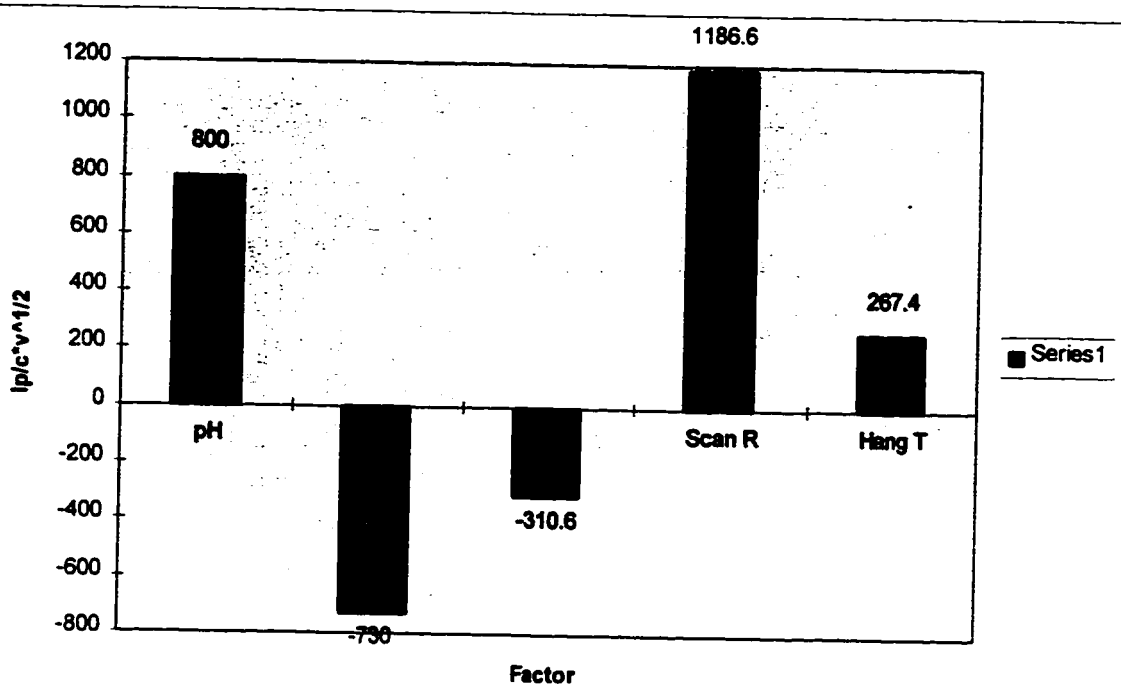


Figure 16. Effects of K for rs37629 pH 7 & 11 2nd Peak

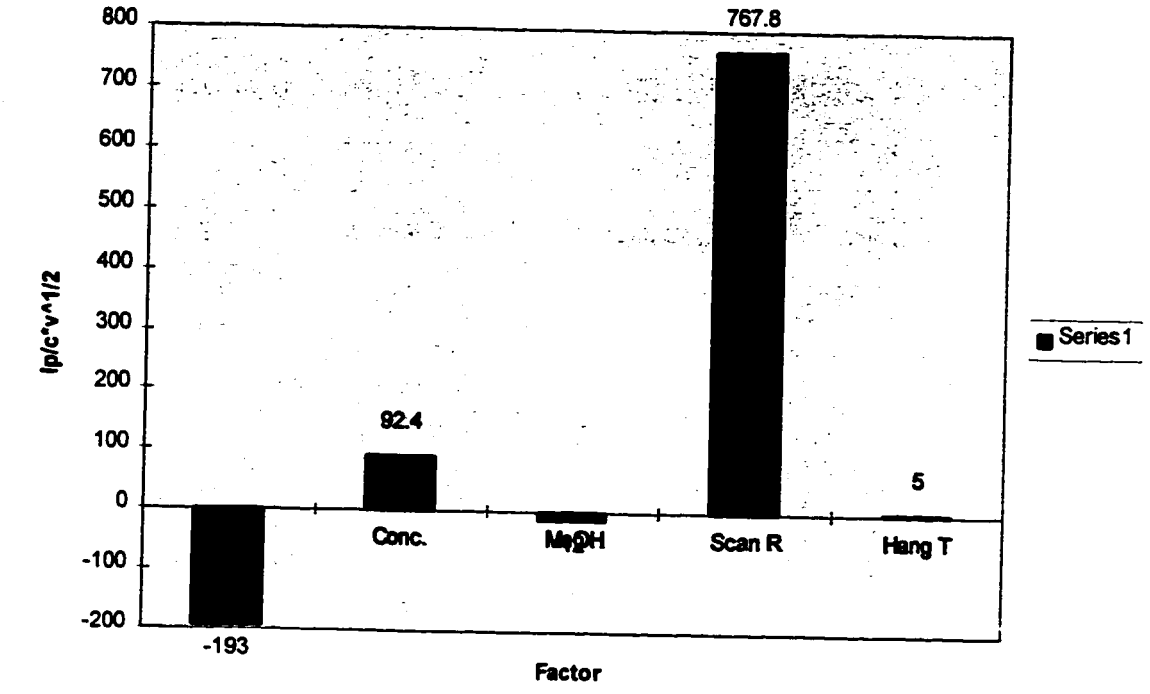


Figure 17. Effects of K for Tolmetin pH7 & 11 2nd Peak

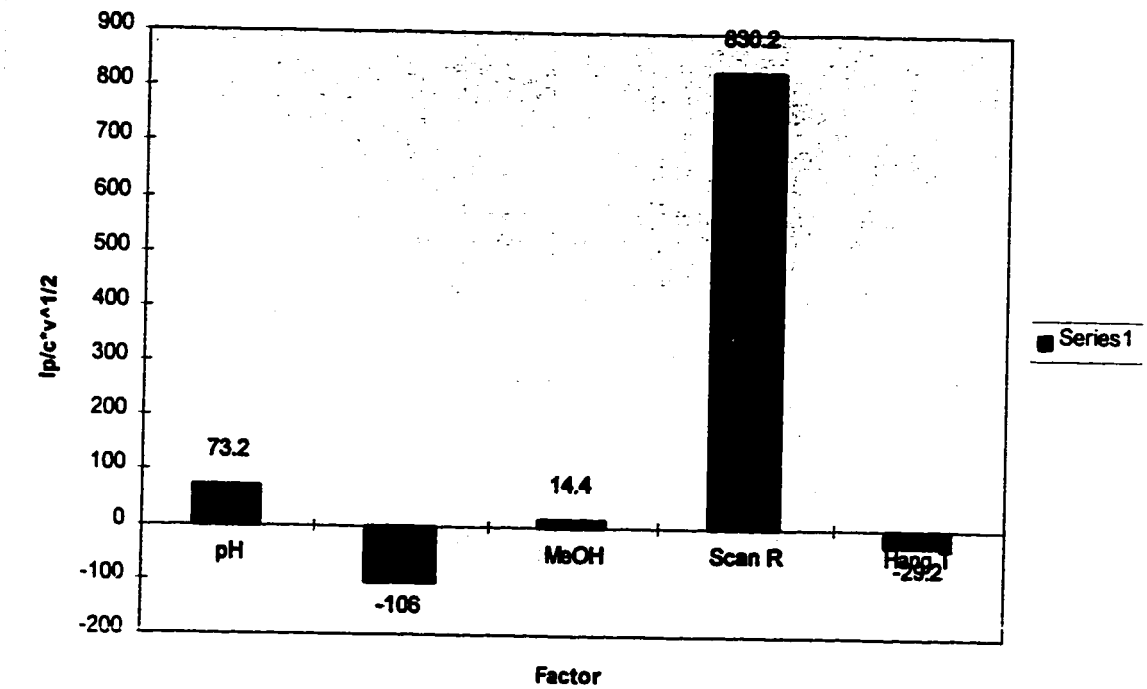
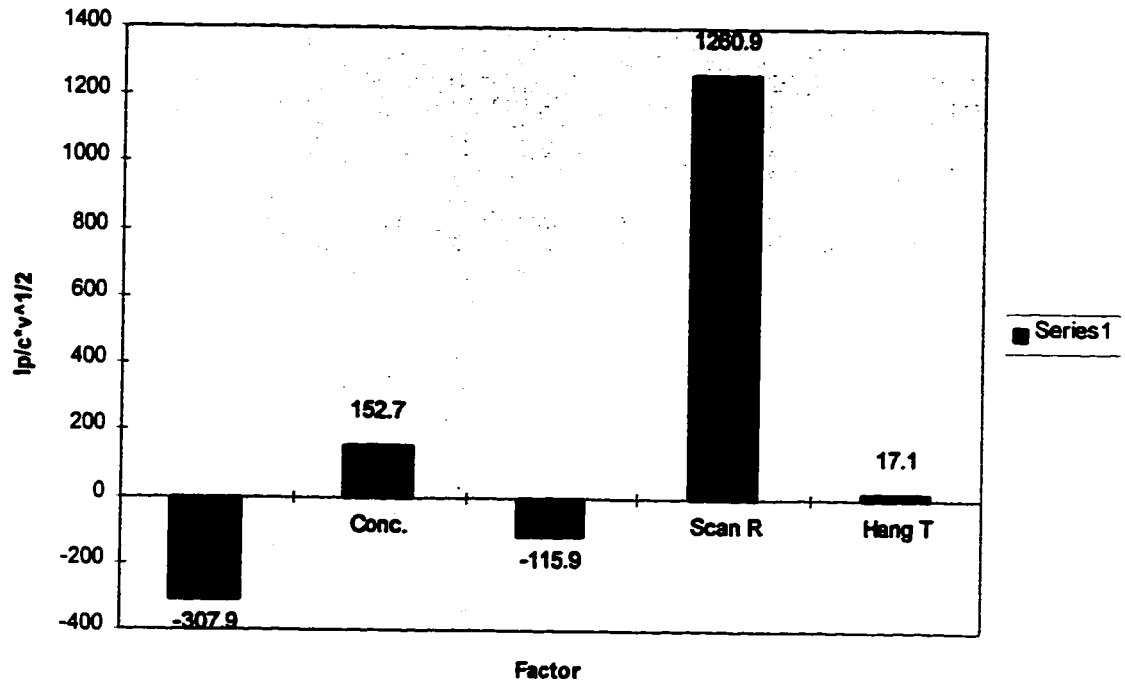


Figure 18. Effects of K for Ketorolac pH 7 & 11 2nd Peak



3.4. Analysis of Variance

Analysis of variance (ANOVA) can be used [48] to assess the statistical significance of the measured factor effects discussed in the previous section. We applied ANOVA to the factor effect data obtained for the seven experimental variables to determine which had a significant effect on peak potential or current function, K, for the second voltammetric peak.

The ANOVA procedure [48] is discussed below:

The average response and variance for each run, r, in the design matrix are found by using Equation 3 and Equation 4 respectively.

$$\bar{y}_r = \sum_{1}^{n_r} \frac{y_i}{n_r} \qquad (3)$$

$$S_r^2 = \sum_{1}^{n_r} \frac{(y_i - \bar{y}_r)^2}{(n_r - 1)} \qquad (4)$$

y_i is the ith response and n_r is the number of response values per variable at a given value of that variable.

For the parameter, pH, and using data from Table 8 (rs49574), the average response at pH 7 is obtained from Equation 3 as:

$$\bar{y}_1 = \frac{161 + 160 + 15.7 + 12}{4} = 87.2$$

and the related variance is:

$$S_1^2 = \frac{(161-87.2)^2 + (160-87.2)^2 + (15.7-87.2)^2 + (12-87.2)^2}{3} = 7171.2$$

The variance from each run could be used to estimate the overall σ^2; however, a better estimate will be obtained by pooling each of the run S_r^2 values. This pooled estimate of run variances is referred to as the mean square error (MSE) and is found as shown in equation 5, where each sum, Σ, is over all design matrix runs.

$$MSE = \frac{\sum (df_r)(S_r^2)}{\sum (df_r)} = \frac{\sum (n_r - 1)(S_r^2)}{\sum (n_r - 1)} \qquad (5)$$

Another estimate for σ^2 called mean square between (MSB) can be found using Equation 6.

$$MSB = \frac{N}{4}(\Delta^2) \qquad (6)$$

where N is the total number of response values obtained in the experimental matrix. The Δ in Equation 6 represents the difference in response average for pH at pH 7 minus the response average at pH 11 (i.e., $y_{pH7} - y_{pH11}$). So for pH the Delta average is:

$$87.2 - 206.1 = -118.9$$

Both MSE and MSB are independent estimates of σ^2 and their observed ratio, $F_o = \frac{MSB}{MSE}$ has an F distribution. To determine whether or not there is a significant effect of a given experimental parameter, the ratio, F_o, is determined and compared to the tabulated value, Fc , predicted for the specified no. of degrees of freedom. For the 2^8 fractional factorial design used here, the no. of degrees of freedom for MSB = 1; MSE = (8 - 2) = 6. Thus, at the 95% probability level, Fc = F (.95,1,6) = 5.99. Thus, if $F_o > F_c$, it can be concluded that the particular factor has a significant effect on the response parameter examined. In our studies the response parameters were the

voltammetric current function, K, and the voltammetric peak potential, Ep. ANOVA calculations were done for all experimental compounds studied, and the results are listed in Table 15 A & B & C & D. Some detailed ANOVA calculations are shown for rs49574 in Table 16.

From the F-test results in table 15A, scan rate has a significant effect on the second peak current function for all the experimental compounds, while pH, concentration, %MeOH, and drop hang time do not have statistically significant effects.

Table 15B lists the ANOVA results for current function effects on the first voltammetric peak at pH 7 and 8 for rs49574. Only the scan rate has a significant effect but a much more smaller effect compared to the rs49574 second peak in 15A. Table 15C and 15D list the ANOVA results for factor effects on the second voltammetric peak potential. pH has a significant effect on the second peak potential at pH7-11 for rs49574 and also the first peak potential at pH7-8 for rs49574 , while scan rate has significant effects on the second peak potential at pH7-11 for rs37629 and the second peak potential at pH7-8 for rs49574.

Table 16. F-test Results

A. For the 2nd peak current function K at pH7 & 11

Compound	F Observed	pH7 & 11 2nd peak current function K			
	pH	Conc.	MeOH	Scan rate	Hang T
rs49574	2.25	0.22	0.00	13.45	0.00
rs37629	0.37	0.08	0.00	76.41	0.00
tolmetin	0.05	0.10	0.00	227.48	0.01
ketorolac	0.35	0.08	0.05	70.30	0.00
	pH 7 & 8 2nd peak current function K				
rs49574	0.41	0.02	0.01	72.55	0.02
	F(.95,1,6) Tabulated				
	5.99	5.99	5.99	5.99	5.99

B. For the 1st peak current function at pH7 & 8

Compound	F Observed	pH7 & 8 1st peak K			
	pH	Conc.	MeOH	Scan rate	Hang T
rs49574	1.77	1.40	0.21	6.02	0.16
	F(.95,1,6)Tabulated				
	5.99	5.99	5.99	5.99	5.99

C. For the second peak potential E at pH 7 & 11

Compound	F Observed		pH7 & 11 2nd peak E		
	pH	Conc.	MeOH	Scan rate	Hang T
rs49574	115.92	0.01	0.01	0.19	0.04
rs37629	0.00	0.00	0.01	188.24	0.03
tolmetin	0.00	0.55	0.39	2.73	0.00
ketorolac	4.77	0.00	0.57	3.02	0.21
	pH 7 & 8 2nd peak E				
rs49574	0.07	0.56	0.01	27.55	0.07
	F(.95,1,6)Tabulated				
	5.99	5.99	5.99	5.99	5.99

D. For the first peak potential at pH 7 & 8

Compound	F Observed		pH7 & 8 1st peak E		
	pH	Conc.	MeOH	Scan rate	Hang T
rs49574	20.08	0.13	0.48	0.75	0.10
	F(.95,1,6)Tabulated				
	5.99	5.99	5.99	5.99	5.99

Table 17. F-test Calculation Table for rs49574 pH 7 & 11 2nd Peak Current Function K

RUN	pH		Conc.		MeOH %		Scan Rate		Hang Time	
#	+	-	+	-	+	-	+	-	+	-
	pH 7	pH 11	0.5 mM	0.1 mM	10%	2.50%	1000mV/Sec	250mV/Sec	30 Sec	1 Sec
1	161			161		161	161		161	
5	160			160	160		160			160
2	15.7		15.7			15.7		15.7		15.7
3	12		12		12			12	12	
6		328.9	328.9			328.9	328.9		328.9	
4		315.4	315.4		315.4		315.4			315.4
8		90.1		90.1		90.1		90.1		90.1
7		89.8		89.8	89.8			89.8	89.8	
Total	348.7	824.2	672	500.9	577.2	595.7	965.3	207.6	591.7	581.2
Net	-118.9		42.775		-4.625		189.425		2.625	
Avg.	87.2	206.1	168.0	125.2	144.3	148.9	241.3	51.9	147.9	145.3
Grand Avg		146.6		146.6		146.6		146.6		146.6
Delta Avg		-118.9		42.8		-4.6		189.4		2.6
s	84.7	134.2	178.1	40.7	129.1	133.8	93.5	44.0	135.1	127.8
s^2	7171.2	18002.7	31715.6	1659.3	16665.1	17915.4	8740.8	1932.7	18259.0	16331.1
MSB		28263		3659.4		42.7813		71763.661		13.7813
MSE		12587		16687		17290.2		5336.7413		17295.1
F Observed		2.25		0.22		0.00		13.45		0.00
F (.95,1,6)										
Calculated		5.99		5.99		5.99		5.99		5.99
Significant ?										
Fo>Fc		No		No		No		Yes		No

3.5. Electrochemical property - Activity Relationship

Table 17 summarizes the relationships between electrochemical properties and analgesic activity for the compounds studied. The univariate graphic representations for Table 17 are shown in Figures 19 A-E. These figures do not show well defined correlations between any single electrochemical property and activity. However, when a multivariate calibration method is applied (Partial Least Squares, PLS) [49-51], a reasonable correlation is obtained in the six-dimensional parameter space defined by the three peak potentials and three current functions measured in Table 17. The PLS calibration plots (property/activity) are shown in Figures 20 and 21. Better correlation with antiinflammatory activity is obtained, but both correlations are reasonably good, even when as few as one or two factors are used for the PLS plot.

Table 18. Comparisons of Electrochemical Properties and Analgesic Activity for Compounds Studied

	DATA for Run # 2				Data for run # 7			Activity (from table 1)	
Compound	Ep(1), pH7	K(1), pH7	Ep(2), pH7	K(2), pH7	Ep(2), pH11	K(2), pH11	F.E.(2),SR Ph7-11	antiinflamm phenylbutazone=1** * Rat Paw	Analgesic aspirin=1**** Mouse writhing
rs49574	-1.305	92.5	-1.479	15.7	-1.531	89.8	757.7	127	200
rs37629	-1.397	29.6	-1.619	59.3	-1.619	82.7	767.8	39	165
tolmetin	-1.411	24.6	-1.613	93.5	-1.632	98	830.2	24	150
ketorolac*	-1.366	86.6	-1.562	86.1	-1.58	100.2	1260.9	55	347
ketoprofen**	-1.266	73.2	-1.266	73.2	-1.452	55.3		16	60

* reference to Ed Kaiser MS Thesis, SJSU, Dec,1996.

** Leslie G. Chatten, Stanley Pons and Lawrence Amankwa, Analyst, August, 1983, Vol. 108, pp. 997-1002

*** DeClerck, F., Vermylen, J., and Reneman, R., Effects of suprofen, and inhibitor of prostaglandin biosynthesis on platelet function, plasma coagulation and fibrinolysis. In vivo experiments, Arch. Int. Pharmacodyn., 217, 68, 1975

**** Muchowski, J. M. et al., Synthesis and antiimflammatory and analgesic activity of 5-aroyl-1,2-dihydro-3H-pyrrolo[1,2-a]pyrrole-1-carboxylic acids and related compounds, J. Med. Chem., 28,1037,1985.

Figure 19A. Electrochemical Property/Activity Plot Ep(1), pH7

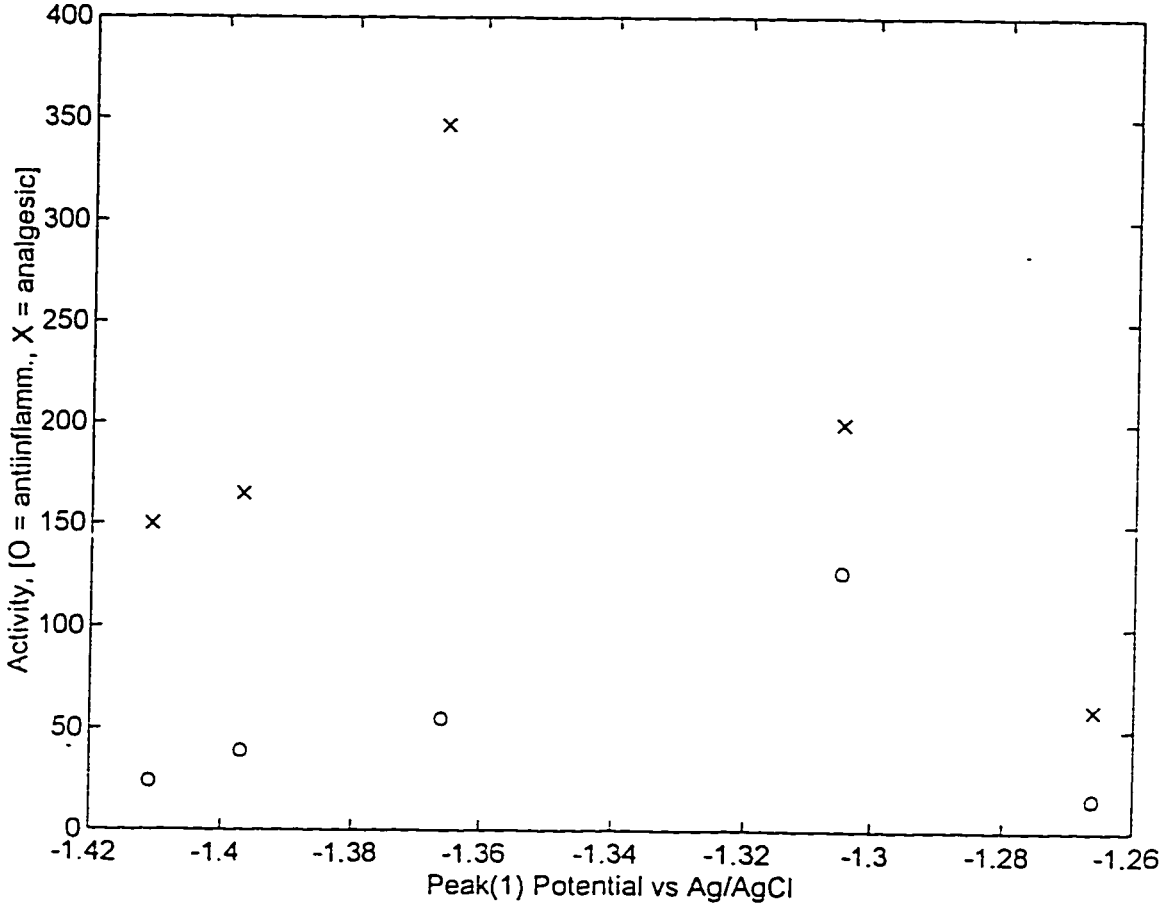


Figure 19B. Electrochemical Property/Activity Plot Ep(2), pH7

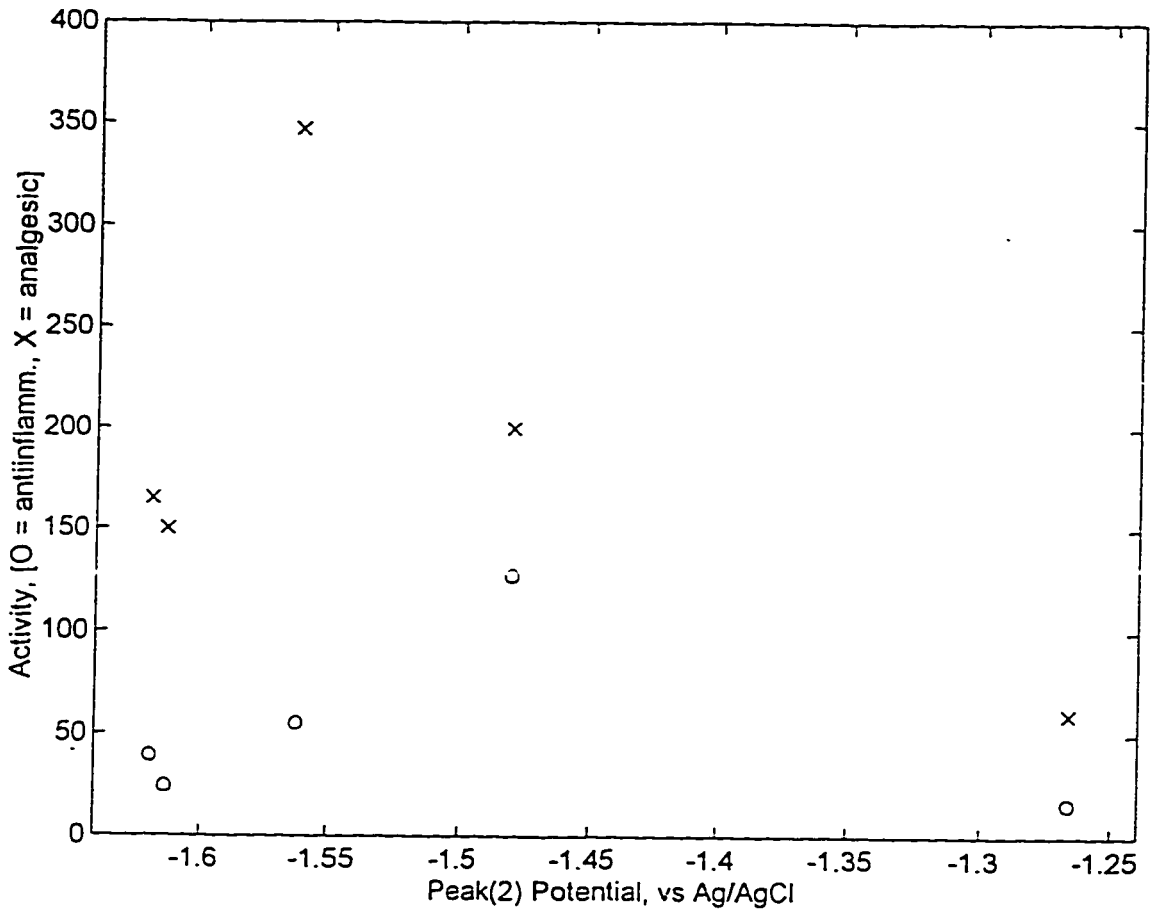


Figure 19C. Electrochemical Property/Activity Plot Ep(2), pH11

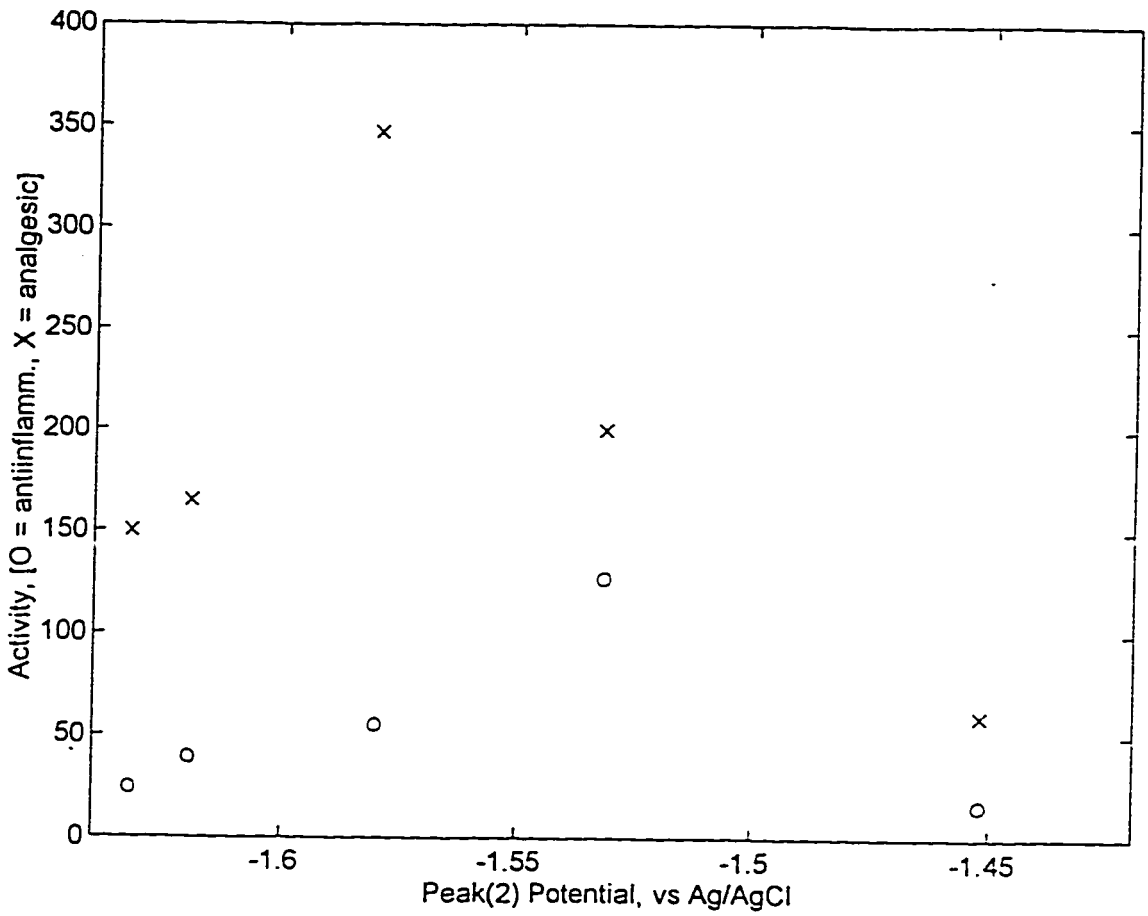


Figure 19D. Electrochemical Property/Activity Plot K(1), pH7

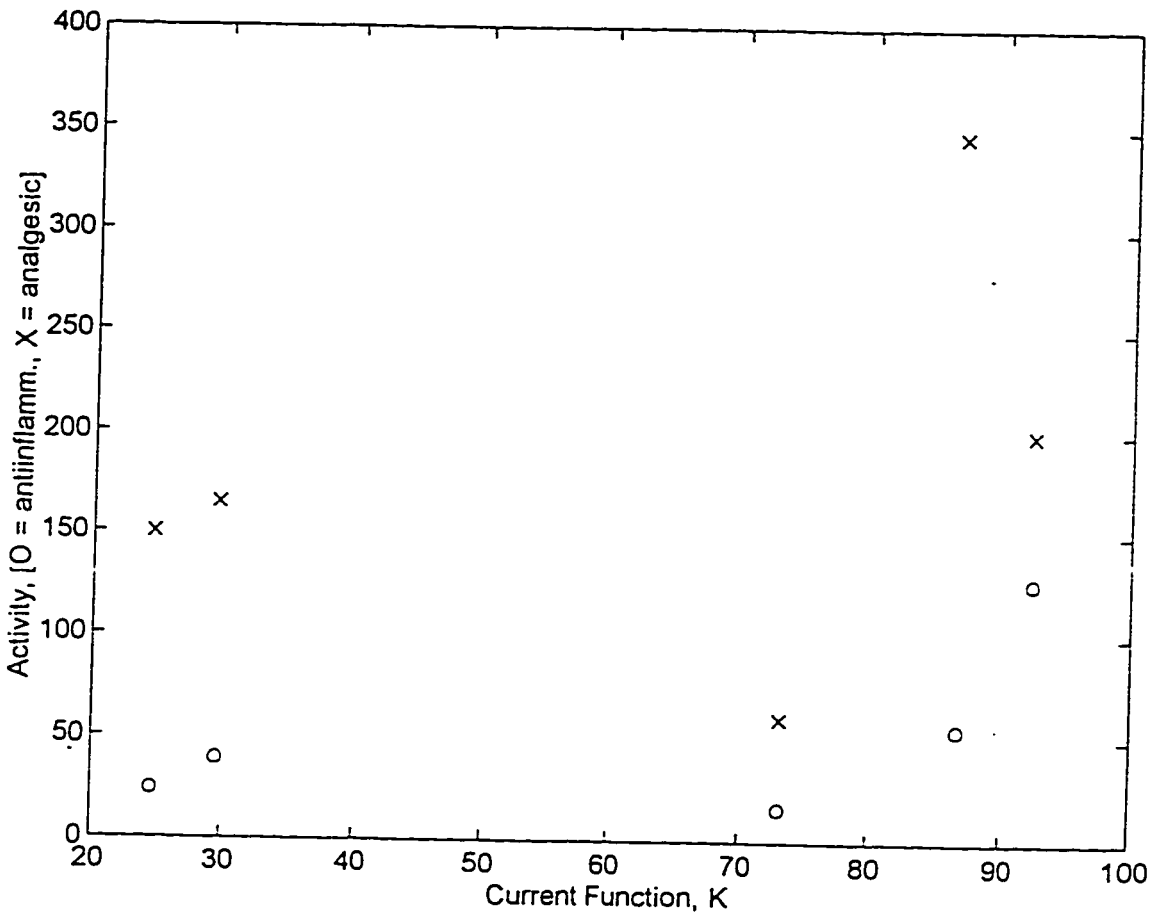


Figure 19E. Electrochemical Property/Activity Plot K(2), pH7

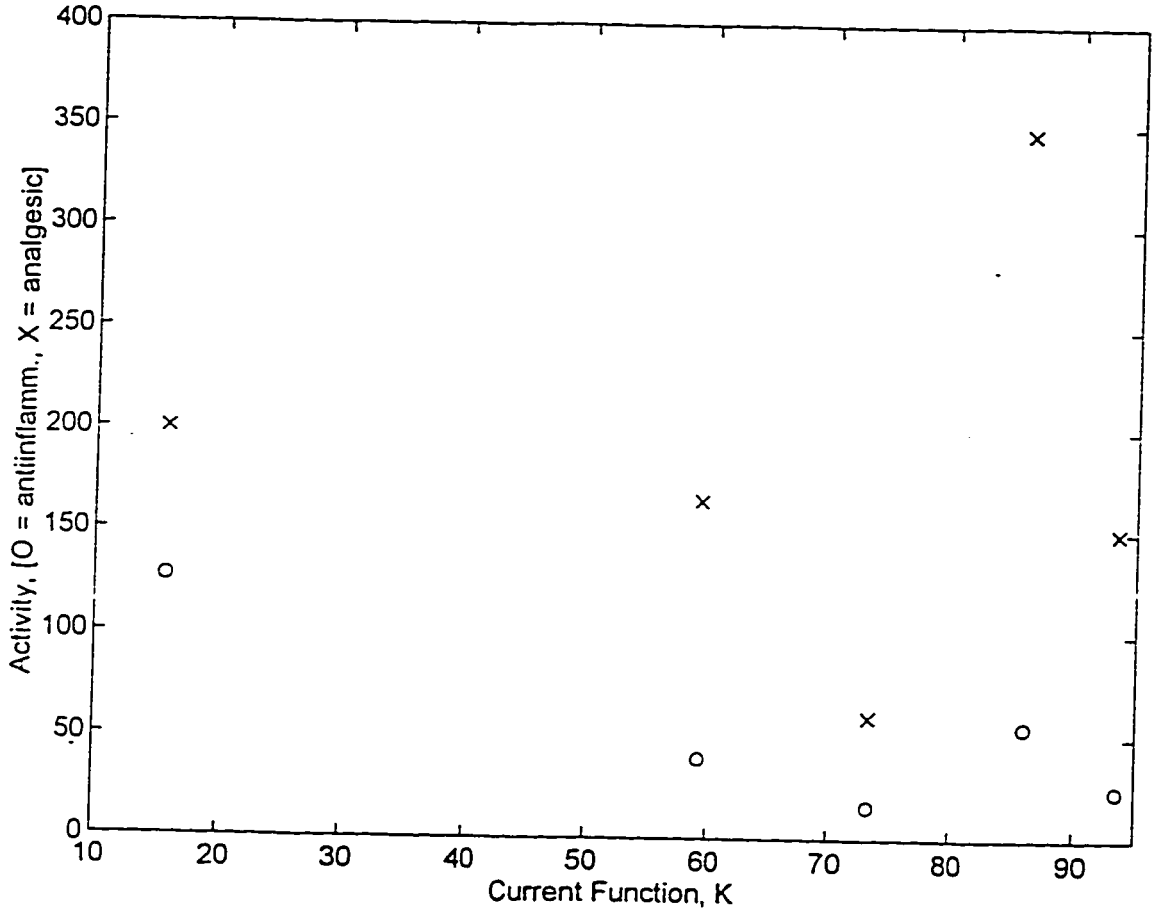


Figure 19F. Electrochemical Property/Activity Plot K(2), pH11

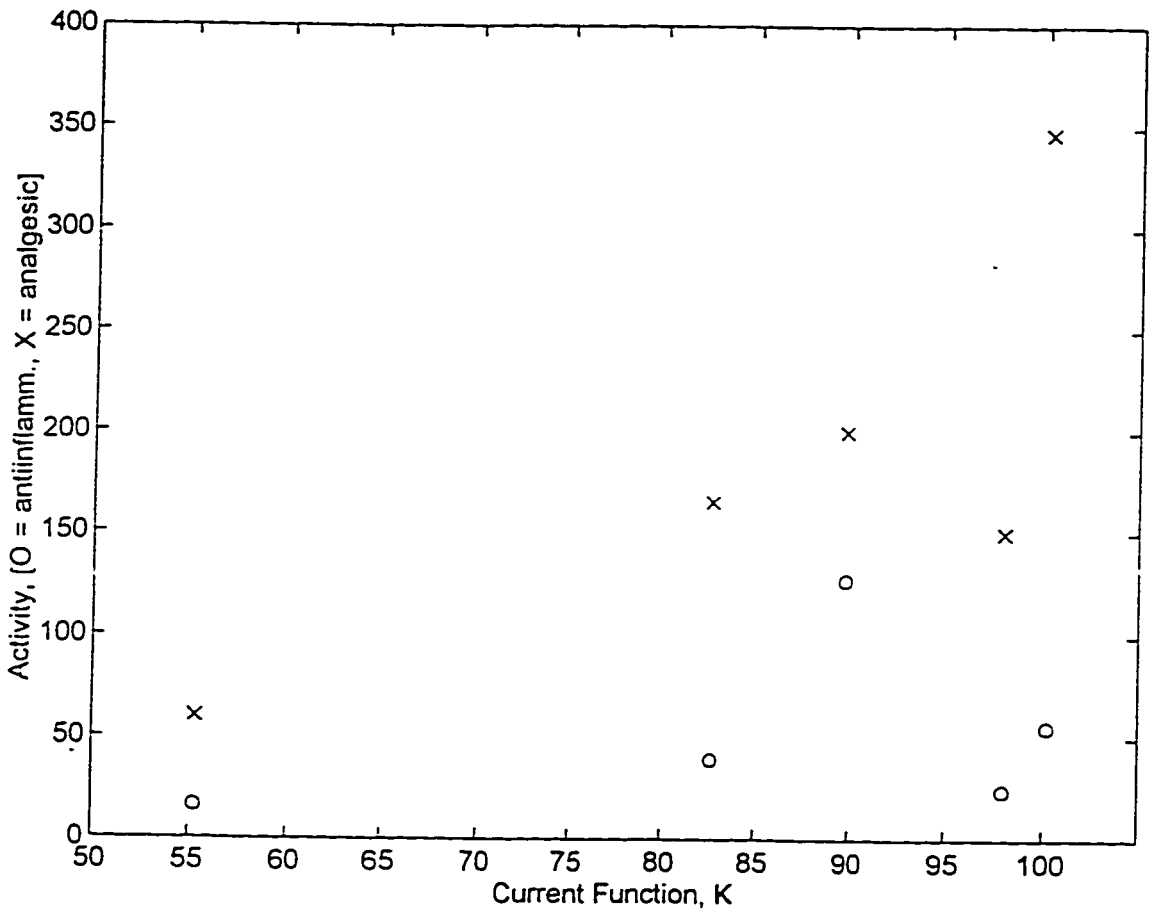


Figure 20. PLS for six voltammetric parameters and antiinflamm. property (suprofen omitted)

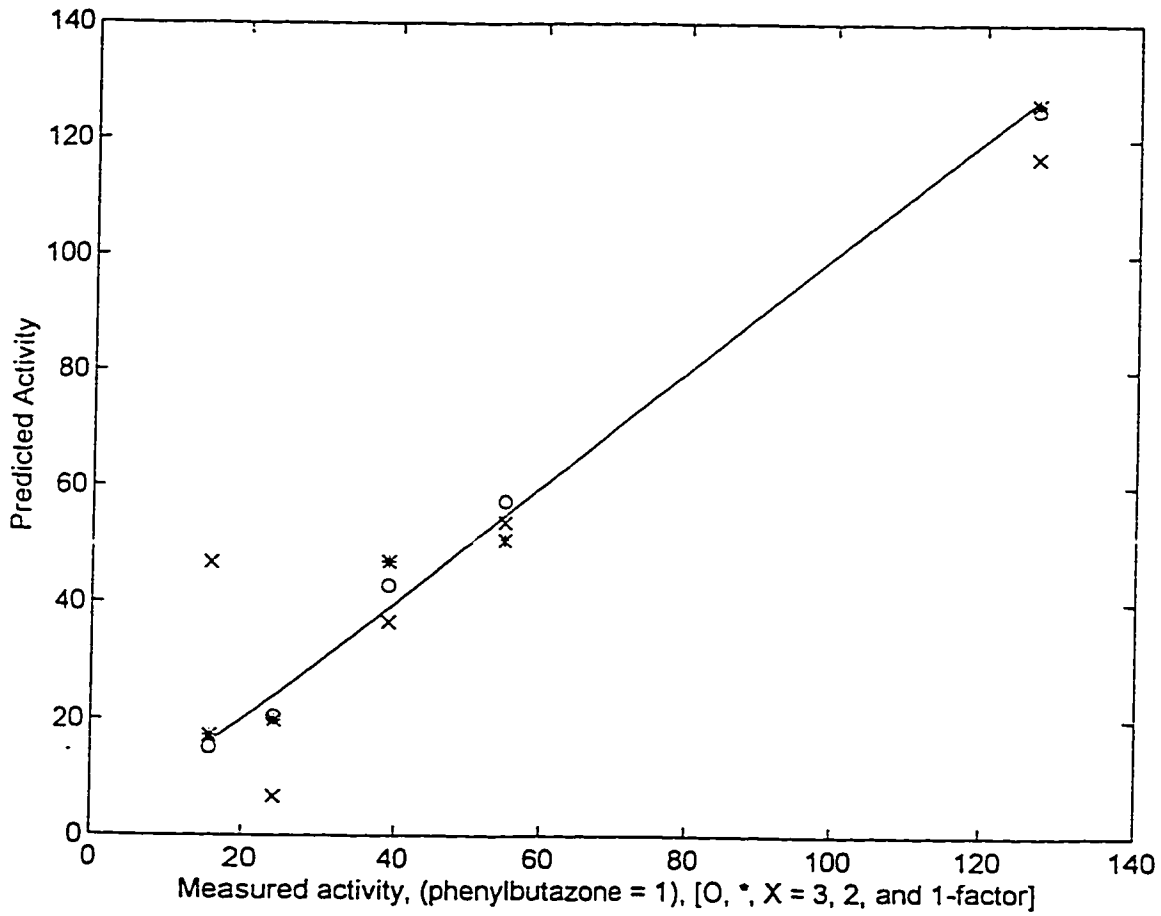
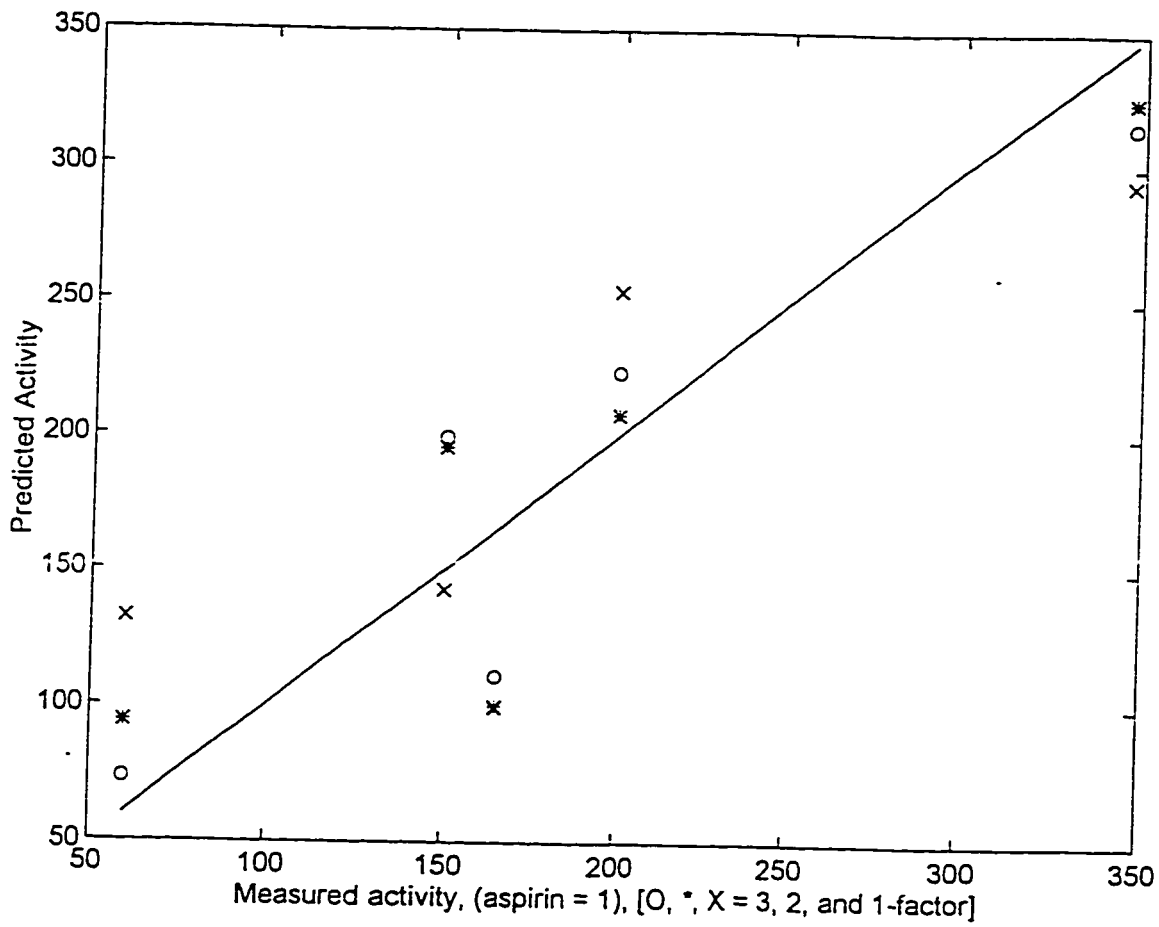


Figure 21. PLS for six voltammetric parameters and analgesic property (suprofen omitted)



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CONCLUSIONS

From these studies, it is evident that cyclic voltammetry is useful for investigating the electrochemical characteristics of the arylacetic acid family of compounds. In fact, voltammetry would appear to be an effective tool for routine analytical procedures.

With regard to the primary objective of these studies (to determine if correlations might be observed between electrochemical properties and pharmacological properties of the arylacetic acid compounds), substantial progress has been made. Regarding the search for significant fundamental effects of various voltammetric parameters on the electrode process, the factorial designed experiments established that scan rate impacted each of the observed peaks under almost all conditions studied for both performance indicators determined (peak potential and current function). The pH effect was next in importance, but was rarely statistically significant.

More important than the magnitude of factor effects associated with each voltammetric parameter are the multivariate patterns described by the voltammetric properties themselves. These are shown visually in Figures 14 to 18. But the more useful result was obtained when partial least squares (PLS) analysis was applied to the raw voltammetric data. The results shown graphically in Figures 20 and 21 suggest a meaningful correlation between the six voltammetric properties and pharmacological activities of the five compounds studied.

It is important to point out, however, that the apparent multivariate correlations

observed here should be taken with a great deal of caution. Because of the limited size of the set of compounds studied, the observed correlations may be fortuitous. Nevertheless, there is some cause for optimism; and it certainly appears worthwhile to extend these studies to a large compound set. It would be particularly useful to have a set large enough to provide some blind unknown for prediction.

APPENDIX I. Table of Ru and Potential Range for rs49574

Run #	Ru	Potential Range (mV)
1	571	-1.082 - -1.594
2	563	-1067 - -1.579
3	611	-1.020 - -1.532
4	476	-1.123 - -1.635
5	597	-1.025 - -1.537
6	460	-1.069 - -1.581
7	446	-1.119 - -1.631
8	409	-1.061 - -1.573
4'	611	-1.084 - -1.596
6'	573	-1.029 - -1.541
7'	600	-1.067 - -1.579
8'	552	-1.011 - -1.523

APPENDIX II. Table of Ru and Potential Range for rs37629

Run #	Ru	Potential Range (mV)
1	564	-1.236 - -1.748
2	560	-1.207 - -1.719
3	587	-1.154 - -1.666
4	507	-1.227 - -1.739
5	560	-1.175 - -1.687
6	468	-1.179 - -1.691
7	460	-1.211 - -1.723
8	428	-1.156 - -1.668

APPENDIX III. Table of Ru and Potential Range for tolmetin

Run #	Ru	Potential Range (mV)
1	468	-1.227 - -1.739
2	444	-1.201 - -1.713
3	481	-1.153 - -1.665
4	510	-1.223 - -1.735
5	492	-1.172 - -1.684
6	458	-1.170 - -1.682
7	490	-1.220 - -1.732
8	450	-1.164 - -1.676

APPENDIX IV. Table of Ru and Potential Range for suprofen

Run #	Ru	Potential Range (mV)
1	510	-0.788 - -1.300
2	559	-0.769 - -1.281
3	562	-0.741 - -1.253
4	374	-0.957 - -1.469
5	532	-0.746 - -1.258
6	358	-0.894 - -1.406
7	381	-0.954 - -1.466
8	356	-0.901 - -1.413

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