1-1-1971

Polarographic reduction of D-glucurono-gamma-lactone in nonaqueous media.

Karl G. Blass
University of Windsor

POLAROGRAPHIC REDUCTION OF D-GLUCURONO-γ-LACTONE IN NONAQUEOUS MEDIA

BY

KARL G. BLASS

A Thesis
Submitted to the Faculty of Graduate Studies through the
Department of Chemistry in Partial Fulfillment
of the Requirements for the Degree of
Master of Science at the
University of Windsor

Windsor, Ontario

1971

Approved:

Roger J. Richard

William J. Abbot

ABSTRACT

The nonaqueous polarographic reduction of D-glucurono-γ-lactone (apparent $\bar{E}_{1/2} = -1.94$ V versus mercury pool) is analytically applicable over the concentration range of 50-300 μg/ml. A number of other lactones have also been examined in aqueous and nonaqueous media. Methods of recrystallizing TBI and TECl have been improved. Constant potential reductions were carried out, but the reduction product has not been identified.

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to Dr. R. J. Thibert without whose patient and guided direction this work could not have been completed.

I also wish to acknowledge the support of the National Research Council of Canada who sponsored this study in part. Finally, I thank Mr. J. R. Johnston for his assistance in the polarographic studies, Mr. W. Eberhart for his work on the special glassware, and Mr. D. Hill for his technical help on the i.r. and n.m.r.

DEDICATION

TO MY PARENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

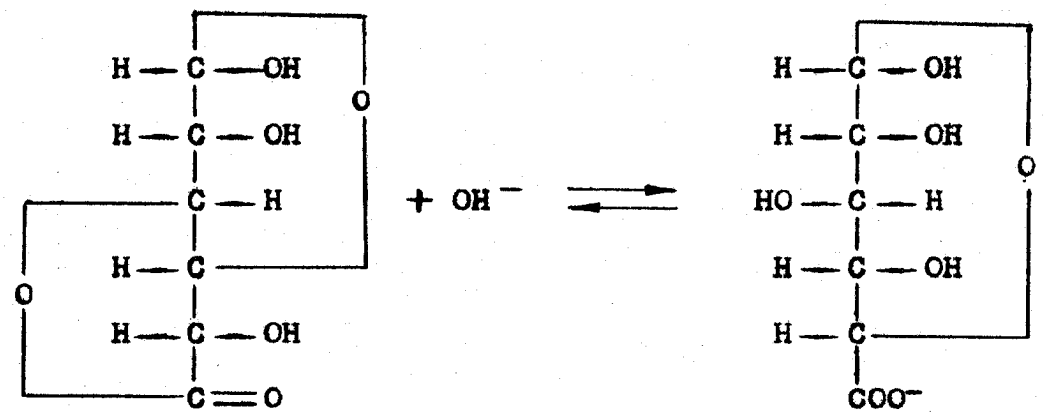
°C	degree centigrade
cm^2	square centimeter
D	diffusion coefficient
$E_{1/2}$	half-wave potential
$\bar{E}_{1/2}$	average half-wave potential
g	gram
i	diffusion current
mm	millimeter
M	molar
n.m.r.	nuclear magnetic resonance spectroscopy
i.r.	infrared spectroscopy
V	volt
µA	microampere
µg	microgram
µl	microliter
DME	dropping mercury electrode
DMF	dimethyl formamide
S.C.E.	saturated calomel electrode
TBCl	tetrabutylammonium chloride
TBI	tetrabutylammonium iodide
TBOH	tetrabutylammonium hydroxide
TEB	tetraethylammonium bromide
TECl	tetraethylammonium chloride
TEI	tetraethylammonium iodide

CHAPTER I

INTRODUCTION

Early attempts at the polarographic reduction of D-glucuronic acid and D-glucurono-γ-lactone by Ishidate and Shimozawa (1) did not produce reproducible waves. Later investigation by Thibert and Boyle (2) gave reproducible waves for D-glucurono-γ-lactone in an unbuffered medium. They examined the concentration range of 0-100 μg per ml which is the normal concentration found in biological fluids. This concentration range was extended to 6 mg per ml by Thibert and Johnston (3) while examining the quenching effect of phosphate on the lactone wave. The foregoing studies have led up to the question of what the lactone is reduced to. In general it has been assumed that the lactone is reduced to its corresponding sugar (4,5). One of the objects of this research was to find the reduction product of D-glucurono-γ-lactone.

In order to find the reduction product one must first locate the correct potential to reduce D-glucurono-γ-lactone. Thibert and Johnston (3) found a wave which was shown to obey Ilkovic's equation, but they were unable to distinguish between the half-wave potentials of hydrogen ion, D-glucurono-γ-lactone, and D-glucuronic acid. Another difficulty is that in an aqueous medium the lactone establishes an equilibrium with the acid form, the latter being the predominant species. In forming the acid, the lactone removes OH^- ions from solution, thus decreasing the pH:



Lactone equilibria (5-7) and changes in pH (6) due to lactone hydrolysis have been investigated. The equilibrium and the rate at which it is attained have been shown to vary with the concentration of the lactone, the temperature of the solution, and the buffer used in the system (6). Early investigations by Matheson et al. (5) showed that lactone waves decreased in height with respect to time as the height of the hydrogen waves increased. This was shown for a number of lactones by running a series of polarograms at various time intervals. Matheson et al. used 0.1 M tetraethylammonium chloride as their supporting electrolyte. This electrolyte enabled them to examine the range of 0 to -2.8 V versus mercury pool.

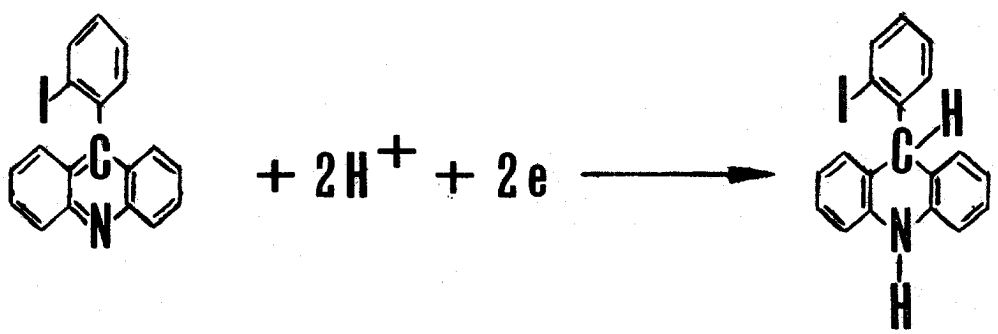
Preliminary investigation with respect to D-glucurono-γ-lactone confirmed the findings of Thibert and Johnston (3). The hydrolysis study of D-gluceno-γ-lactone by Matheson et al. was also confirmed. This latter investigation in 0.05 M aqueous lithium chloride showed that the reduction wave was above the decomposition of the KCl electrolyte used previously in this laboratory by Thibert et al. (3,8). Since it has been shown that certain lactones are reduced above -2.0 volts versus the mercury pool, it was of interest to examine the higher voltage range of -2.0 to -3.0 volts. At the same time it was thought to be desirable

to circumvent the complications of lactone hydrolysis and pH changes encountered in aqueous medium by using nonaqueous solvents. A number of solvents have been used in polarography of which some of the more common ones are: DMF; DMSO; dioxane; and methanol. Of these, methanol has been used in this laboratory in the polarographic reduction of beta-substituted phenylcystine derivatives (9). This solvent has the disadvantage of being very volatile, and thus changes in concentration occur during the long deoxygenation period which is needed for organic solvents.

Of the above solvents, DMF was selected as the medium for polarography. It was chosen because previous researchers (10) found it to be a superior solvent for compounds whose half-wave potentials are in the order of -2.0 to -2.5 volts versus S.C.E. Lambert (10) reported that DMF is easily purified by distillation, and that no maxima were observed in his reduction of halogenated aromatic compounds. He also reported that very few erratic drops occur and, although we found this to be true for low concentrations, it unfortunately was not the case for constant potential analysis at concentrations of D-glucurono-γ-lactone at levels of mg per ml. Lambert and Kobayashi (11) reported that 0.05 M tetrabutylammonium iodide in DMF has an apparent decomposition potential of approximately -2.85 volts vs S.C.E. In general, the tetrabutylammonium salts have a higher decomposition potential than the tetraethyl salts. The chloride, bromide, and iodide salts are commercially available, but in most cases must be purified by recrystallization. The iodide form is prefered for most studies because of its high reduction potential and solubility in most organic solvents. Since the iodide salt is not soluble in water, the chloride and bromide salts are employed in aqueous media. There are a number of procedures for the purification of these

quaternary salts (12-17). Most of these methods give low yields and must be repeated from three to five times in order to obtain a reagent suitably pure for polarography. The procedure of Silverman and Bradshaw (13) for the purification of tetrabutylammonium iodide gave very high yields and was found to be extremely efficient, upon modifying the procedure to employ a rotary evaporator, instead of allowing the solution to evaporate slowly overnight. Thus, three recrystallizations can easily be carried out in a matter of three hours.

Organic synthesis employing polarographic techniques is a very precise method of reducing (or oxidizing) organic molecules. In general, the procedure involves finding a polarographic reduction wave for the organic molecule. An electrolytic reduction is then carried out at constant potential, which is selected at an appropriate point on the diffusion current plateau of the polarogram. The separation of the products depends on the type of products obtained and on the starting materials. It is generally most convenient to eliminate contamination from material produced at the anode and cathode compartments. Lingane, Swain, and Fields (18) used a 68 cm^2 Hg pool cathode and a 20 cm^2 Hg pool anode separated by a connecting tube which had a coarse sintered glass disk of 4 cm in diameter sealed into the middle of the tube. This cell they used in the preparation of 9-(o-iodophenyl)-dihydroacridine by reduction of 9-(o-iodophenyl)-acridine, as follows:



This reduction shows the specificity of polarographically controlled syntheses. Chemical reduction was not applicable in the above reaction because, either reduction did not occur at all or complete reduction with the elimination of iodine resulted. Some of the advantages of the Lingane et al. (18) system include the low resistance of the electrolytic cell (approximately 180Ω) and the high molecular weight of the reacting species and product.

The reduction of aromatic ketones and aldehydes in acetonitrile and DMF mediums were examined by Wawzonek and Gunderson (19) and by Given et al. (20). Other studies in DMF have shown the existence of stable anion-free radicals in the reduction of aromatic hydocarbons (16,21). Wawzonek et al. (22) have shown the existence of semiquinone anions from the reduction of quinones in DMF.

Hoijtink et al. (23) have proposed two general possible mechanisms for hydrocarbon reduction at the dropping mercury electrode. At a voltage above the start of the first wave, the ion R^- formed by the reversible addition of one electron must either diffuse into the bulk of the liquid, where $R^- + H^+ \longrightarrow RH$, or it reacts with a proton to form a radical RH· which then adds an electron to become RH^-. The RH^- then reacts with another proton producing RH_2. The basicity and the availability of protons determines whether, one wave equivalent to a two-electron step, or two waves each corresponding to a one-electron step are observed. This second wave corresponds to the addition of a further electron to R^-. Fast proton addition results in one wave and slow proton addition (compared to diffusion from the electrode surface) results in two waves.

Hoijtink et al. (23) and Given (24) have found that the use of dioxane-water mixtures containing 25% water resulted in the exhibition of

two reduction waves for most hydrocarbons. Direct proof of fast proton addition was obtained by the addition of a proton donor which reduced the height of the second wave or caused it to disappear, while the first wave would increase without a large change of half-wave potential (23). Common proton donors that have been used in DMF are HI (4 mmoles per 1), water (3 moles per 1), and benzoic acid (4 mmoles/1).

Rausch, McEwen, and Kleinberg (25) have postulated that a stable di-negative ion of the structure $R_2C^- -O^-$ is formed in the reduction of ketones. If this ion were to capture a proton from the solvent or water that may be present, then a stable alkoxide ion, R_2CH-O^-, would be formed (16). Wawzonek et al. (16,22) showed that di-negative ions abstract protons from DMF and acetonitrile. The authors suggested that the negative solvent ion then decomposes or polymerizes.

DMF has been widely used and recommended in polarography (26-44). A commonly used electrolyte with DMF is TBI. Some of the advantages as stated by Lambert (10) are: few erratic drops occur; no slight imperfections in the envelope of the polarographic curve can be noted; and the capillary stays clean for months if stored in pure DMF. An additional advantage is that no maxima are observed with low concentrations of many aliphatic and aromatic halogen compounds. This eliminates the need for maximum suppressors.

In this thesis a nonaqueous medium was sought in order to eliminate the hydrolysis of the lactone and possible interference waves, like the catalytic hydrogen ion reduction wave. A thorough study of the Ilkovic relationship for D-glucurono-γ-lactone in a nonaqueous medium using a quaternary ammonium salt as the supporting electrolyte was carried out. Quaternary ammonium salts, because of their high decomposition poten-

tials at the dropping mercury electrode, were used to examine the possibility of a reduction wave above -1.80 volts versus the mercury pool.

CHAPTER II

EXPERIMENTAL

A. Polarographic Reduction of D-Glucurono-γ-Lactone

Materials, Methods, and Procedure

The Sargent (Sargent-Welch Scientific Company) Model XVI Polarograph was employed for this investigation. A ten milliliter Heyrovsky cell was used in the concentration study. The characteristics of the capillary (capillary 1) used were: $m=1.154$ mg sec^{-1}; $t=5.8$ sec; $m^{2/3}t^{1/6}=1.474$ $mg^{2/3}sec^{-1/2}$. The height of the mercury column was 48.0 cm. The cell was placed in a water bath maintained at $25 \pm 0.1°C$. The first solution to be analyzed was prepared by adding 0.09235 g of three times recrystallized TBI to the Heyrovsky cell followed by 4.8 ml of DMF. To this was added 0.1 ml of 0.02 M aqueous TBOH. The solution was deoxygenated for 10 min after which the mercury pool was added. This solution was then deoxygenated for another 20 min after which it was utilized for running blanks. To this blank solution 0.1 ml of a freshly-prepared solution of D-glucurono-γ-lactone in DMF was added, followed by 5 min of deoxygenation and mixing. The foregoing lactone solution was prepared by adding 0.125 g of D-glucurono-γ-lactone to a 25-ml volumetric which was brought up to volume with DMF. For other concentrations the volume of the lactone solution was increased as the initial DMF addition was decreased proportionally, thus always maintaining the total volume of DMF at 5.0 ml. Polarograms were run on duplicate samples for each concentration studied (polarograms of each sample were done in triplicate). Deoxygenation was carried out using

99.996 % nitrogen (Liquid Carbonic), however, it was passed through two wash bottles containing DMF, the first of which was at room temperature and the second was in the water bath at 25 ± 0.1°C. Even with these two DMF rinses, an increase of 30 min in the deoxygenation time gave slightly high results, the latter indicating a loss of solvent from the cell thereby increasing the solute concentration.

The DMF was obtained from Fisher Scientific Company and was redistilled by the procedure cited by Lambert (10), retaining only the middle fraction. The TBI from Eastman was recrystallized 3 times by a modified procedure of Silverman and Bradshaw (13). A rotary evaporator was employed to reduce the solvent volume at a faster rate, rather than allowing the solution to evaporate to half the volume overnight in a fume hood. This modification not only saved time but also allowed for greater control over the recrystallization volume. The D-glucurono-δ-lactone was obtained from Eastman Organic Chemicals. Triple distilled mercury was obtained from Engelhard Industries of Canada Ltd. Tetrabutylammonium hydroxide, 1.0 M aqueous solution, was obtained from Southwestern Analytical Chemicals, Inc., Austin, Texas. It was diluted with deionized-distilled water to 0.02 M. A volume of 0.1 ml was added in the concentration study. The latter concentration was found to eliminate the CO_2 impurity wave while at the same time not causing the blank solution to turn pink prior to the addition of the lactone.

B. Polarographic Reduction of Other Lactones

Materials, Methods, and Procedure for γ-Butyrolactone

In the aqueous study a Sargent (Sargent-Welch Scientific Company) Model XXI Polarograph was employed. The characteristics of the capillary (capillary 2) used were: $m = 7.53$ mg sec^{-1}; $m^{2/3}t^{1/6} = 1.623$ mg $^{2/3}sec^{-1/2}$. The height of the mercury column was 48.0 cm. An H-cell with a saturated calomel reference electrode (S.C.E.) was used. The supporting electrolyte was 0.2 M KCl. The blank was deoxygenated with nitrogen, which was purified by passing the gas through a series of wash bottles. The first bottle contained copper turnings and a 1:1 ammonium hydroxyde solution saturated with ammonium chloride. A glass wool trap was between this basic solution and the 1.0 N sulfuric acid tower. This acid tower was also followed by a glass wool trap. The nitrogen then passed through two towers which contained the same solvent and supporting electrolyte as was in the polarographic cell. Blank runs were made and the sample was then added using a microburet with a syringe needle tip which was permanently inserted through the rubber stopper of the polarographic cell. A 5-minute deoxygenation period followed in order to allow for proper mixing of the sample.

A nonaqueous study employing absolute CH_3OH with 0.2 M $NaC_2H_3O_2$ as the supporting electrolyte was carried out on γ-butyrolactone using a Sargent Model XVI Polarograph. The capillary was the same as used in the preceding aqueous study. Anhydrous methanol was prepared according to the method described by Fieser (45), except that iodine was employed as a catalyst. The lactone was added, either as in the aqueous study, or using a 100-μl pipet. Samples were deoxygenated for a period of 30

min with scrubbed nitrogen. The nitrogen was scrubbed by passing it through a train, like the one described above. The only variation was that concentrated sulfuric acid was used in the acid tower.

Materials, Methods, and Procedures for α-Methyl-γ-Butyrolactone

The Sargent Model XVI Polarograph was used. Studies were carried out with capillary 2. A 30-min deoxygenation with scrubbed nitrogen was performed as described under the aqueous γ-butyrolactone study. The solvent medium was anhydrous methanol prepared as previously stated. The electrolyte was 0.2 M $NaC_2H_3O_2$. The same cell as employed for γ-butyrolactone was used with a methyl calomel electrode (9).

Materials, Methods, and Procedures for D-Mannurono-γ-Lactone

The Sargent Model XVI Polarograph was used. Studies were carried out with capillary 2. A ten-milliliter Heyrovski cell was used. Deoxygenation was carried out over a period of 30 min. The solvent was DMF, purified as mentioned above, and the supporting electrolyte was 0.05 M tetraethylammonium chloride. The ethyl quaternary ammonium salt was recrystallized three times from a 1:3 methanol-acetone mixture to which an excess of cold acetone was added, as described in section D of CHAPTER II.

Materials, Methods, and Procedures for γ-Valerolactone

A study in aqueous media employing 0.2 M KCl in an H-cell with an S.C.E. was carried out. The Sargent Model XXI Polarograph was employed. The capillary characteristics are included in the aqueous investigation of γ-butyrolactone. Sample addition and deoxygenation procedures are the

same as in the aqueous γ-butyrolactone study. An identical investigation of the polarographic reduction of γ-valerolactone was made using the Sargent Model XVI Polarograph. All other conditions were the same as expressed above.

Anhydrous methanol with 0.2 M $NaC_2H_3O_2$ as the supporting electrolyte was also employed using the Sargent Model XVI Polarograph. The methods and procedures followed are the same as above with one exception, i.e., deoxygenation in a nonaqueous system takes much longer (for a methanol system this is usually 30 to 45 min).

Materials, Methods, and Procedures for δ-Valerolactone

The Sargent Model XXI Polarograph was used. Studies were carried out with capillary 2 and the supporting electrolyte was aqueous 0.2 M KCl. Samples were added as in the aqueous γ-butyrolactone study. The nitrogen deoxygenation time was extended to 10 min.

The Sargent Model XVI Polarograph was employed in the nonaqueous polarographic reduction of δ-valerolactone. The medium was anhydrous methanol and the supporting electrolyte was 0.2 M $NaC_2H_3O_2$. The cell and a procedure identical to that employed in the nonaqueous γ-butyrolactone study were used in this polarographic investigation of δ-valerolactone.

C. Constant Potential Electrolysis of D-Glucurono-γ-Lactone

Materials, Methods, and Procedures

The chemicals for the constant potential electrolyses are as follows: Hg (triple distilled) from Engelhard Industries of Canada, Ltd.; DMF purified according to procedure (10); TBI purified as described in the section on "Purification of Tetraalkylammonium Halides"; and D-glucurono-γ-lactone from Eastman Organic Chemicals.

Three separate electrolyses were carried out. For convenience they will be refered to as E-1, E-2, and E-3.

Electrolysis E-1 consisted of a 4000-ml beaker with a Hg pool (approximate area of 324 cm^2) covering the bottom. To this two liters of 0.1 N TBI and 20.0 g of D-glucurono-γ-lactone were added. A glass stirring rod and a nitrogen connected air stone were introduced to the cell. Both the stirring and the deoxygenation with N_2 were continuous throughout the first 5 days of this study, after which deoxygenation was discontinued. The top of the beaker was covered with a plastic wrap. A Sargent-Slomin Electrolytic Analyzer (Sargent-Welch Scientific Company) was utilized to maintain the potential of the cell at an apparent -2.1 ± 0.1 V. The D-glucurono-γ-lactone was electrolyzed for one week.

Electrolysis E-2 was similar to the first electrolysis with one major exception. The platinum anode was covered with Dialyzer Tubing (Arthur H. Thomas Company, Philadelphia, P., 19105, U.S.A.) diameter, 1 7/8 in, and thickness, 0.0016 in, which was employed as a semipermeable membrane. The volume of solution employed was one-half of that utilized in E-1. The voltage was maintained at an apparent value of

-2.3 ± 0.1 V.

Electrolysis E-3 was performed with the Sargent Model XVI Polarograph as a potentiostat. The cell was specially designed with a mercury overflow tube which allowed excess mercury to leave the cell while maintaining the surface area of the mercury pool constant (Figure 1). To the cell 15 ml of DMF, 0.5541 g TBI (final concentration 0.1 M), and 0.315 g of D-glucurono-γ-lactone (final concentration of 21.0 mg/ml) were added. The voltage drive of the polarograph was set at 76% which in the 0.0 to -3.0 V range gives a potential of -2.28 V versus the mercury pool. The solution was electrolyzed over a period of ten days. An initial sample, and additional samples removed over a two to three day interval, were immediately stored at -15°C.

Blank solutions were prepared in 25-ml volumetric flasks. These were deoxygenated for 45 minutes and then stoppered. Solutions were stored in the dark. The solutions contained similar volumes and concentrations to those employed in electrolysis E-3.

D. Purification of Tetraalkylammonium Halides

In Table I is a list of some of the tetraalkylammonium halides along with solvents and conditions needed for recrystallization. In most cases the purification of the salts is not described well in the literature. Many of the recrystallizations are difficult and must be repeated three to five times. The purification of TBCl according to the procedure of Kryukava and Tomilov (46) employing DMF was found to be very difficult to perform and resulted in a crystalline gel which essentially gave no purification beyond the initial filtration of the hot solution. Matheson et al. (5) showed an extensive study of lac-

FIGURE 1

THE CELL

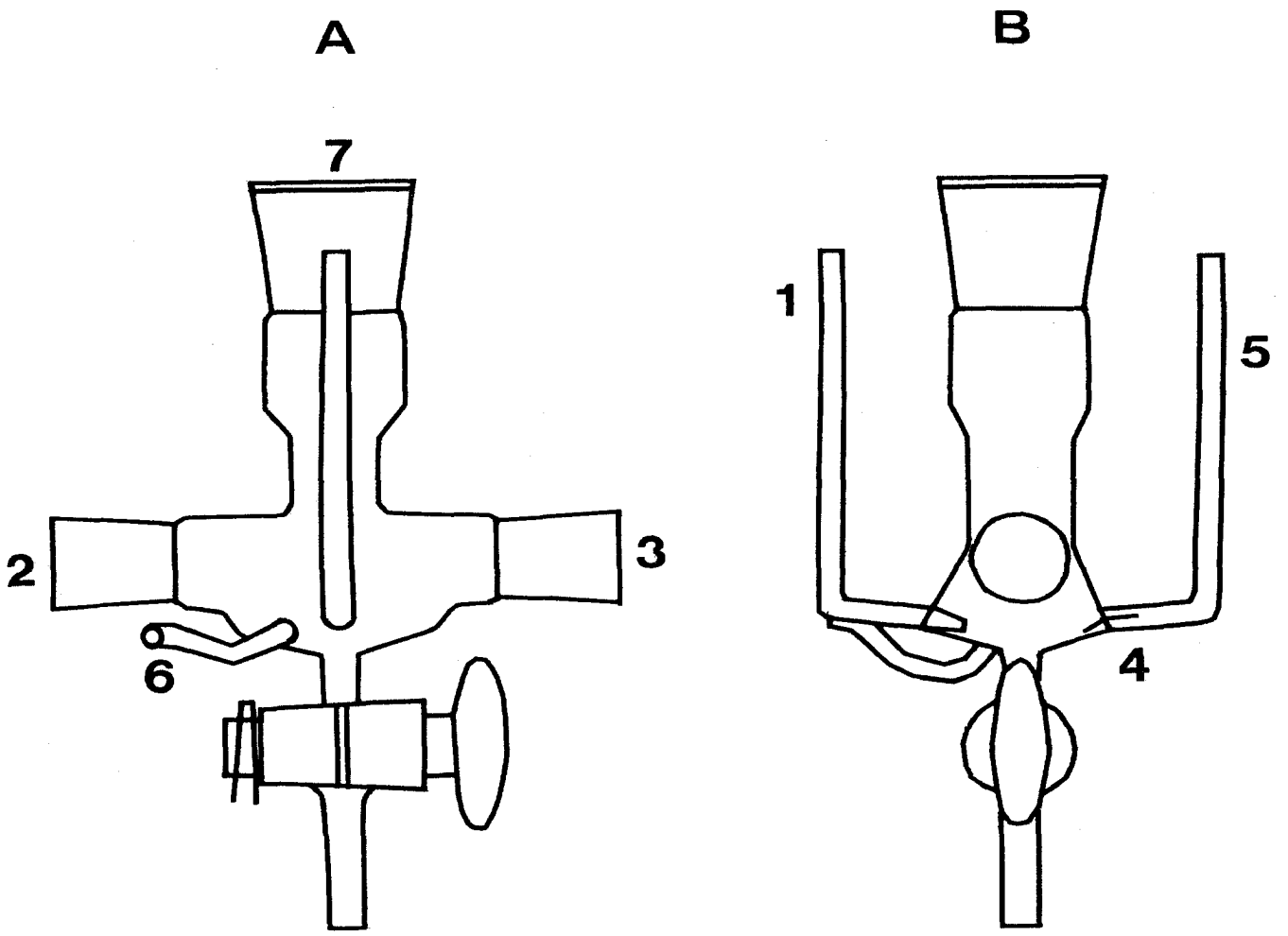
Legend

This is a specially designed cell that can be employed for constant potential electrolysis. The parts of the front (A) and side (B) view of this cell are as follows:

1. Nitrogen inlet
2. Position for a replaceable side arm electrode.
3. Same as 2.
4. Platinum wire
5. Mercury
6. Mercury overflow tube
7. Position for DME

FIGURE 1

THE CELL



tones in an aqueous system using TECl. They reported that the method which they employed would be published at a later date, but such was not found in the literature. They did report that a slight alkali metal wave which definitely increased with time could be detected if Pyrex cells are used. A similar finding was observed in the constant potential reduction of D-glucurono-γ-lactone. This wave was very small and could even have been attributed to other factors in system (e.g., reduction product, hydrogen wave).

Pickard and Neptune (17) used methanol in acetone as a recrystallization medium for the purification of tetramethylammonium chloride. About 10 grams are dissolved in 100 ml of hot 25% methanol in acetone. The hot solution is filtered, and 100 to 110 ml of acetone are added. The crystalline material is filtered from the cool solution. The salt is placed in a vacuum desiccator for about three days prior to its use. The yield is about 60 to 65%. This method is considered to be a superior procedure compared to the usual ethyl alcohol recrystallization medium. Three or four recrystallizations of the salt must be carried out in ethyl alcohol to achieve sufficient purity for use as a polarographic supporting electrolyte.

The Pickard and Neptune (17) recrystallization procedure was slightly modified and used to recrystallize tetraethylammonium chloride. A 25% methanol in acetone mixture (25 ml) is used to dissolve approximately 1.5 g of tetraethylammonium chloride. To this solution approximately 35 ml of acetone are added. The final crystallization must be carried out in an ice bath. The crystals are filtered off and placed in a vacuum desiccator for about three days prior to use.

The purification of TBI by the method of Silverman and Bradshaw (13)

TABLE I

PURIFICATION OF QUATERNARY AMMONIUM SALTS

Salt	Alternative Methods	Recrystallization medium
$(CH_3)_4NI$	1[a]	Double distilled H_2O
$(C_2H_5)_4NI$	1[a]	EtOH
$(C_3H_7)_4NI$	1[a]	Spectroquality Me_2CO
$(C_4H_9)_4NI$	1[a]	Spectroquality Me_2CO + Et_2O (75/25 by vol)
	2[b]	Me_2CO + MeOH followed by H_2O
	3[c]	Anhydrous ETOAc
$(CH_3)_4NBr$	1[a]	CH_3OH + EtOH (50/50 by vol)
	2[d]	H_2O + EtOH
$(C_2H_5)_4NBr$	1[a]	MeOH
$(C_3H_7)_4NBr$	1[a]	Me_2CO
$(C_4H_9)_4NBr$	2[e]	Anhydrous EtOAc
$(C_5H_{11})_4NBr$	1[a]	EtOAc
$(CH_3)_4NCl$	1[a]	MeOH + Et_2O (80/20 by vol)
	2[f]	Me_2CO + MeOH
$(C_2H_5)_4NCl$	1[a]	MeOH
	2[g]	Me_2CO + MeOH
$(C_3H_7)_4NCl$	1[a]	Same as for $(C_4H_9)_4NCl$ except for use of mixture 40% EtOH + 20% Et_2O + 40% EtOAc by vol.
$(C_4H_9)_4NCl$	1[a]	First recrystallization from EtOAc by adding chilled Et_2O to the soln. and then cooling to

Table (continued)

	-10 °C. Crystals washed well with Et_2O. Salt then recrystallized twice from 20% EtOH + 4% Et_2O + 40% EtOAc (vol %).

[a] Procedure from reference (12)

[b] Procedure from reference (13)

[c] Procedure from reference (14)

[d] Procedure from reference (15)

[e] Procedure from reference (16)

[f] Procedure from reference (17)

[g] Description in text.

makes use of the fact that the salt is insoluble in water. A 5-gram sample of TBI is dissolved in 50 ml of 3:1 acetone in CH_3OH. This solution is filtered and allowed to evaporate over night to concentrate to 25 ml. The TBI is precipitated by adding 5 ml of distilled water. The salt is filtered and dried in a vacuum desiccator. By modifying this procedure to employ a rotary evaporator, one is able to select the exact volume one wishes to evaporate. With this modification three recrystallizations can be carried out in a matter of three hours.

CHAPTER III

RESULTS

A. Polarographic Reduction of D-Glucurono-γ-Lactone

Effect of Concentration on Diffusion Current

To obtain a blank for the concentration study, a 0.01 M aqueous solution of TBOH had to be added in order to eliminate CO_2 interference. In Figure 2 the effect of adding aqueous TBOH can be seen. An addition of 0.1 ml of 0.02 M TBOH was found to be sufficient for eliminating the CO_2 interference while at the same time not discoloring the solution. At higher concentrations of TBOH the blank solution would become pink in color.

A linear relationship of the diffusion current to the concentration of D-glucurono-γ-lactone was observed in the range of 50-300 μg (Table II and Figure 3.) The apparent $E_{1/2}$ for D-glucurono-γ-lactone is -1.94 V (Table III).

The diffusion current was calculated from the following equations:

$$i_D = \text{Sens.} \times h$$

Where i_D = the diffusion current in μA

Sens. = the sensitivity in μA/mm

h = the wave height in mm

The wave height, h, was measured in mm. A typical polarogram for the reduction of D-glucurono-γ-lactone can be seen in Figure 4. A sample calculation for a 150 μg/ml D-glucurono-γ-lactone concentration can be

FIGURE 2

EFFECT OF AQUEOUS TETRABUTYLAMMONIUM HYDROXIDE ON THE REMOVAL OF THE CARBON DIOXIDE IMPURITY

Legend

At a sensitivity of 0.006 μA/mm the carbon dioxide impurity wave is off scale for this figure.

Wave 1. Addition of a final concentration of 0.4×10^{-4}M TBOH.

Wave 2. Addition of a final concentration of 1×10^{-4}M TBOH.

Wave 3. Addition of a final concentration of 4×10^{-4}M TBOH

FIGURE 2

EFFECT OF AQUEOUS TETRABUTYLAMMONIUM HYDROXIDE ON THE REMOVAL OF THE CARBON DIOXIDE IMPURITY

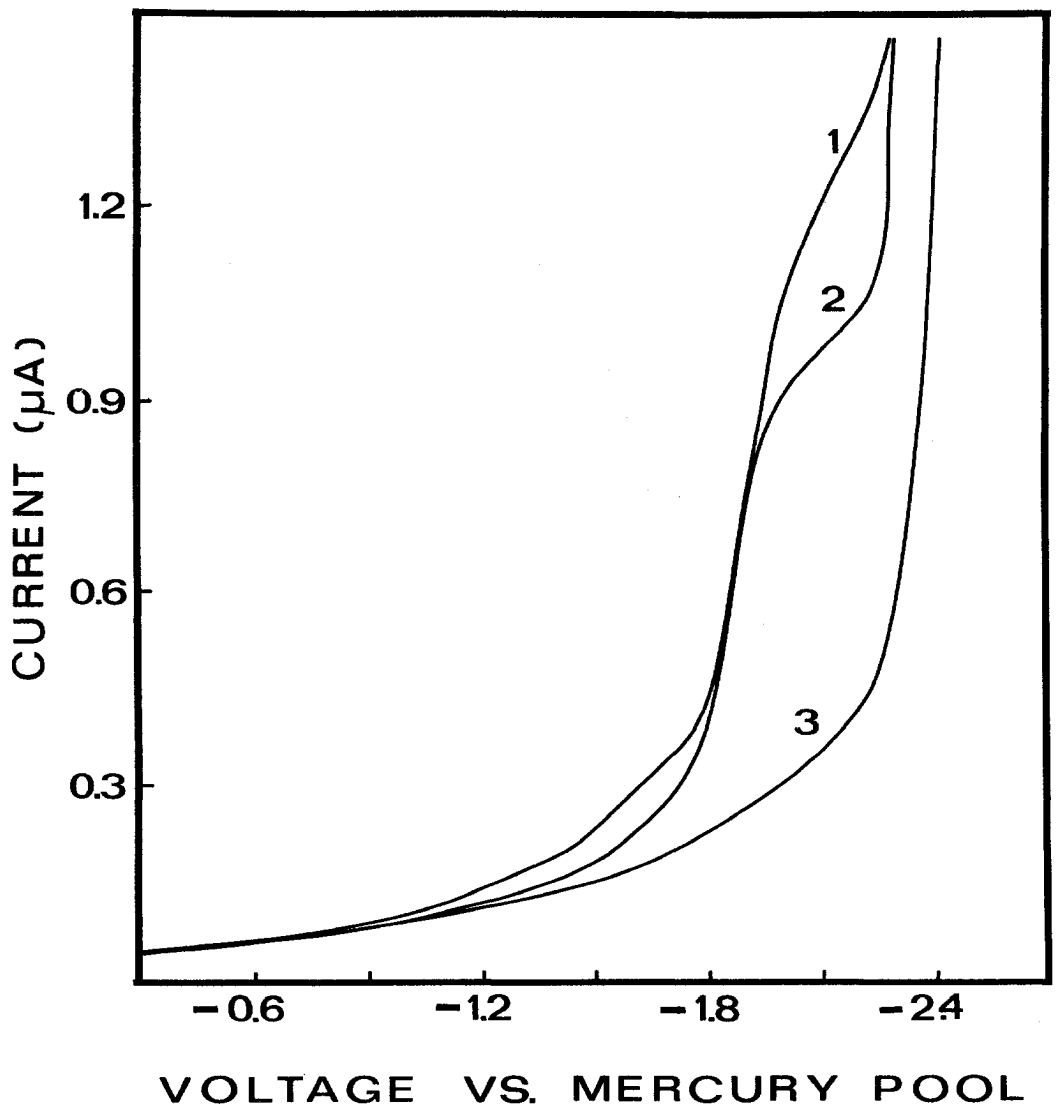


TABLE II

EFFECT OF D-GLUCURONO-γ-LACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The values reported represent an average of three samples at each concentration studied, with three polarograms run for each sample. All solutions were prepared fresh just prior to their analysis.

TABLE II

EFFECT OF D-GLUCURONO-γ-LACTONE CONCENTRATION ON DIFFUSION CURRENT

D- Glucurono-γ-lactone Concentration μg/ml	Diffusion current μA
50	0.151
100	0.310
150	0.457
200	0.629
250	0.791
300	0.930

FIGURE 3

EFFECT OF D-GLUCURONO-γ-LACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The values reported represent an average of three samples at each concentration studied, with three polarograms run for each sample. All solutions were prepared just prior to their analysis.

FIGURE 3

EFFECT OF D-GLUCURONO-γ-LACTONE CONCENTRATION ON DIFFUSION CURRENT

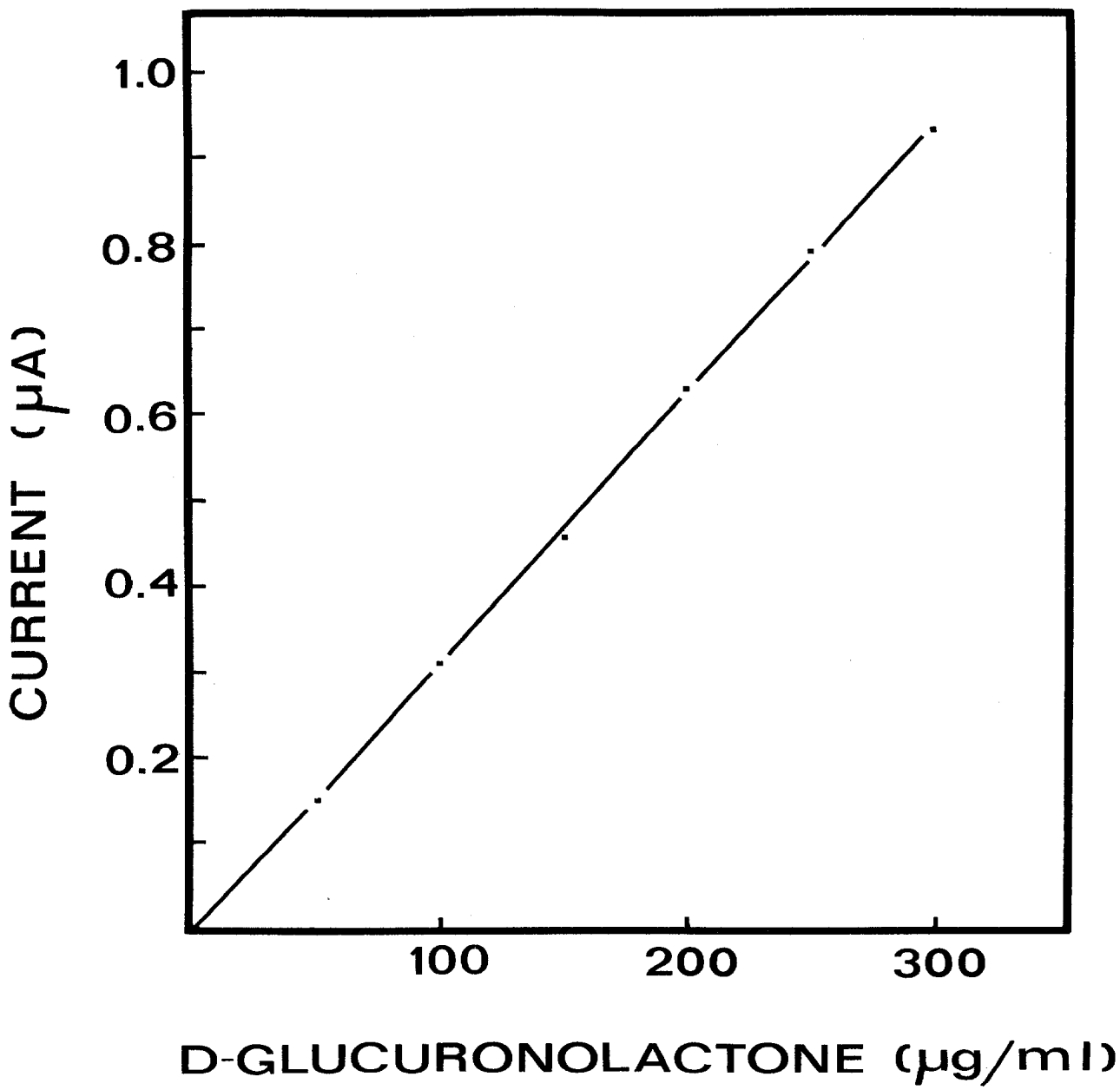


TABLE III

APPARENT HALF WAVE POTENTIALS OF LACTONES

Lactone investigated	Apparent $E_{1/2}$ in volts	Solvent-supporting electrolyte
D-Glucurono-γ-lactone	-1.94*	DMF-TBI
	-1.49**	H_2O-KCl
γ-Butyrolactone	-1.35**, First wave	H_2O-KCl
	-1.47**, Composite wave	H_2O-KCl
	-0.282***, First wave	CH_3OH-$NaC_2H_3O_2$
	-1.12***, Second wave	CH_3OH-$NaC_2H_3O_2$
α-Methyl-γ-butyro-lactone	-0.452***, First wave	CH_3OH-$NaC_2H_3O_2$
	-1.17***, Second wave	CH_3OH-$NaC_2H_3O_2$
D-Mannurono-γ-lactone	-1.75*, First wave	DMF-TECl
	-1.92*, Second wave	DMF-TECl
	-1.49****	H_2O-KCl
γ-Valerolactone	-1.51**, Composite wave	H_2O-KCl
	-0.484***, Composite wave	CH_3OH-$NaC_2H_3O_2$
δ-Valerolactone	-1.41**, First wave	H_2O-KCl
	-1.63**, Second wave	H_2O-KCl
	-0.473***, First wave	CH_3OH-$NaC_2H_3O_2$
	-1.22***, Second wave	CH_3OH-$NaC_2H_3O_2$

* Apparent $\bar{E}_{1/2}$ versus mercury pool.

** Apparent $\bar{E}_{1/2}$ versus S.C.E.

Table III (continued)

*** Apparent $\bar{E}_{1/2}$ versus Methanol Saturated Calomel Electrode (9).

**** Apparent $\bar{E}_{1/2}$ versus S.C.E. obtained from reference (8).

FIGURE 4

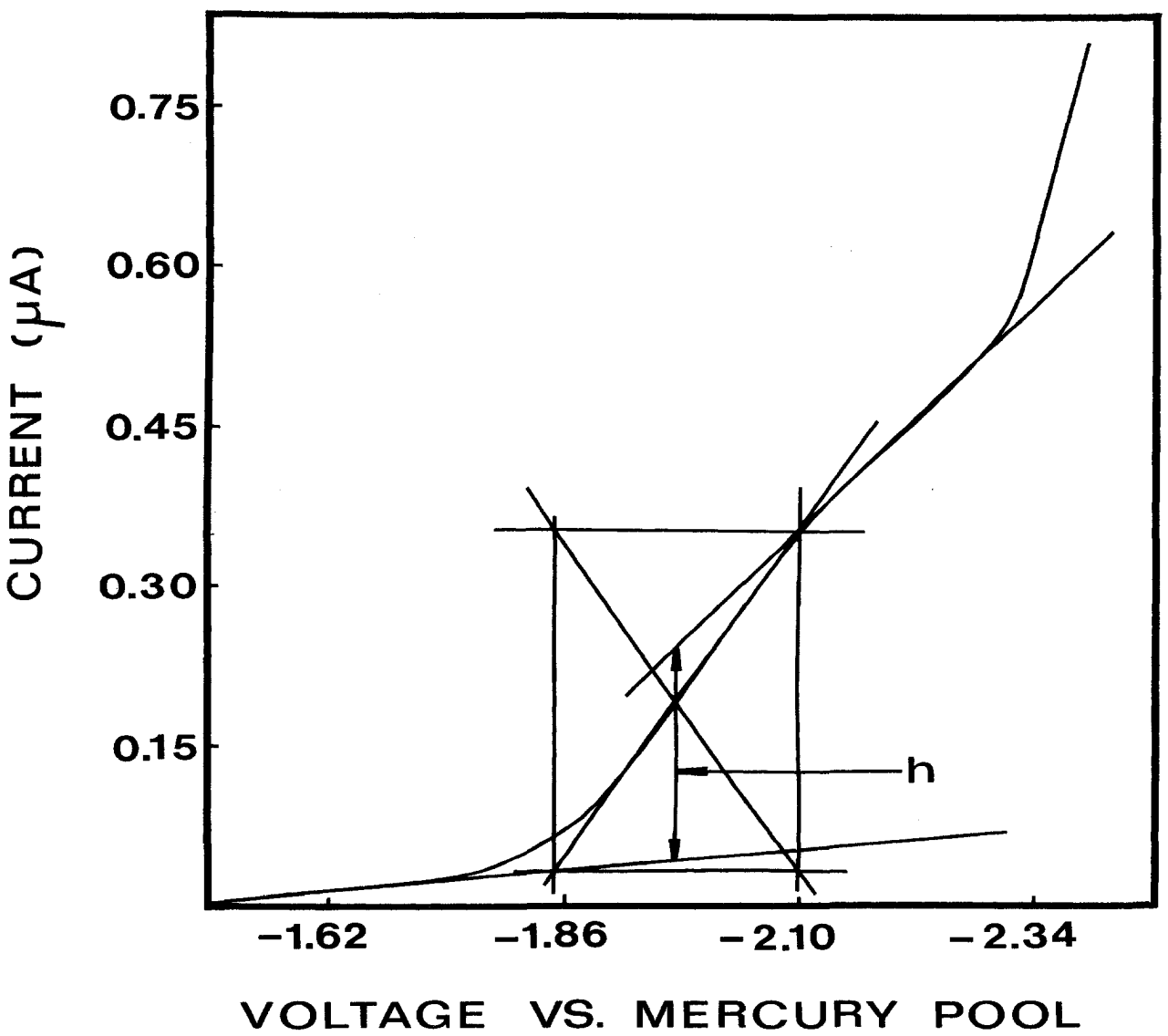
TYPICAL POLAROGRAM OF D-GLUCURONO-γ-LACTONE

Legend

This is a typical polarogram of D-glucurono-γ-lactone in DMF with TBI along with aqueous TBOH. Methods for measuring the diffusion current (h) and the apparent half-wave potential are depicted.

FIGURE 4

TYPICAL POLAROGRAM OF D-GLUCURONO-γ-LACTONE



calculated as follows:

$$\text{Sens.} = .008\ \mu A/mm$$

$$h = 57\ mm$$

$$i_D = 0.008 \times 57$$

The apparent half-wave potentials were calculated by measuring the distance in inches from the beginning of the polarogram to the midpoint of the diffusion current wave (47) as shown in Figure 2.

These distances are substituted into the following equations:

$$\text{apparent } E_{1/2} = (-3.0/25.0) \times d$$

Where apparent $E_{1/2}$ = the apparent half-wave potential

d = distance in inches

-3.0/25.0 = a constant for the polarograph.

The constant, -3.0/25.0, varies with the settings used on the Sargent Model XVI Polarograph. A polarogram is 25.0 inches long when the settings are medium and fast for the voltage drive and chart paper drive, respectively. The -3.0 represents the span of 0.0 to -3.0 volts with the negative sign indicating a negative increase in voltage. Thus every inch of the polarogram is a negative increase of 0.12 volts.

A polarogram for the D-glucurono-γ-lactone study wich was started at 0.0 volts and with d = 16.43 would yield:

$$E_{1/2} = -.12 \times 16.43$$

$$= -1.972 \text{ volt}$$

True half-wave potentials previously reported from this laboratory (1,3,8,9) have been calculated according to the method of Taylor and

Smith (48). The method is a manual arrangement which employs a Fluke Model 825A Differential Voltmeter. The voltage was applied to the cell by changing the voltage drive setting of the polarograph, and the voltage of the DME was measured versus the Hg pool. The current and voltage were measured at increments of 0.1 volt and greater prior to the reduction wave of D-glucurono-γ-lactone and also, similarly, on the diffusion current plateau. For the voltage of the reduction wave, increments of approximately 0.05 V were measured. The results were non-reproducible. The voltage was stable and could be read to four decimal places, but the current would vary with time. Readings were attempted at small standard time intervals, but these also gave irregular results. Finally, a 5-min deoxygenation with Hi-pure nitrogen was attempted for each point of the polarogram obtained manually. The latter also gave very unsatisfactory results. An alternate method employing a large number of polarograms (fifteen) and averaging their apparent $E_{1/2}$ values results in an average apparent $E_{1/2}$ expressed as apparent $\bar{E}_{1/2}$.

B. Polarographic Reduction of Other Lactones

γ-Butyrolactone

In the aqueous study a double wave was observed. The two waves were not well defined; however, measurements of diffusion current versus concentration were obtained for the first wave and for a composite of the two waves (Tables IV and V, and Figures 5 and 6). Apparent $E_{1/2}$ values for the first wave and the composite wave are -1.35 and -1.47 V, respectively.

The nonaqueous study of the polarographic reduction of γ-butyrolactone resulted in two reduction waves, which were well defined. In

TABLE IV

EFFECT OF γ-BUTYROLACTONE ON DIFFUSION CURRENT

γ-Butyrolactone concentration* in ml	Diffusion current in μA**
0.2 ml	0.202
0.3 ml	0.518
0.4 ml	0.788

* In this preliminary concentration study the lactone was added to 5 ml of aqueous 0.2 M KCl.

** Diffusion current values for the first wave of the polarographic reduction of γ-butyrolactone. Refer to text for details.

TABLE V

EFFECT OF γ-BUTYROLACTONE ON DIFFUSION CURRENT

γ-Butyrolactone concentration* in ml	Diffusion current** in μA
0.2	0.608
0.3	0.813
0.4	0.920
0.8	1.30

* In this preliminary concentration study the lactone was added to 5 ml of aqueous 0.2 M KCl.

** Diffusion current values for the compositewave of the polarographic reduction of γ-butyrolactone. Refer to text for details.

FIGURE 5

EFFECT OF γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The data for this plot was calculated from the diffusion current of the first aqueous reduction wave of γ-butyrolactone. These results are an average of two analyses for each concentration examined.

FIGURE 5

EFFECT OF γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT

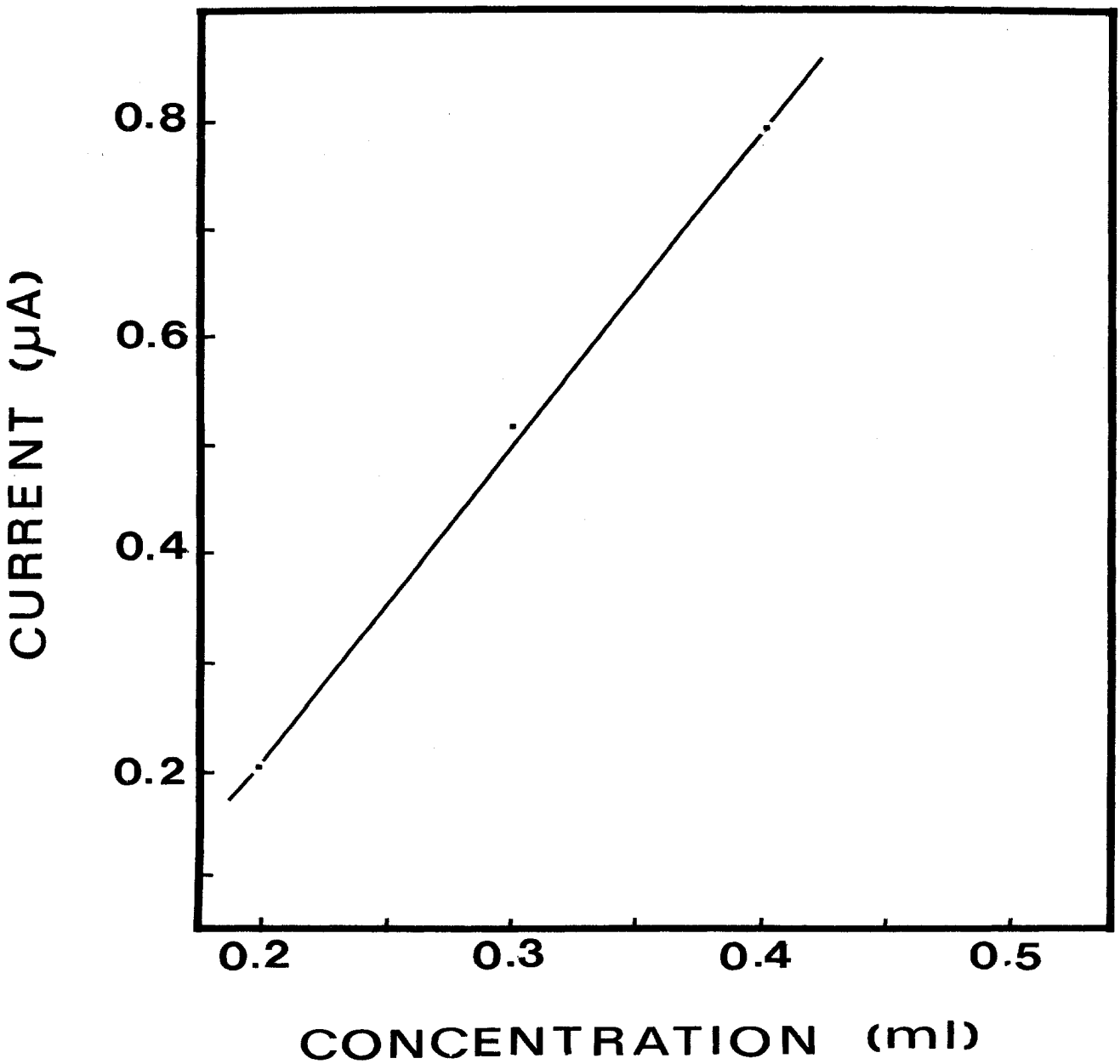


FIGURE 6

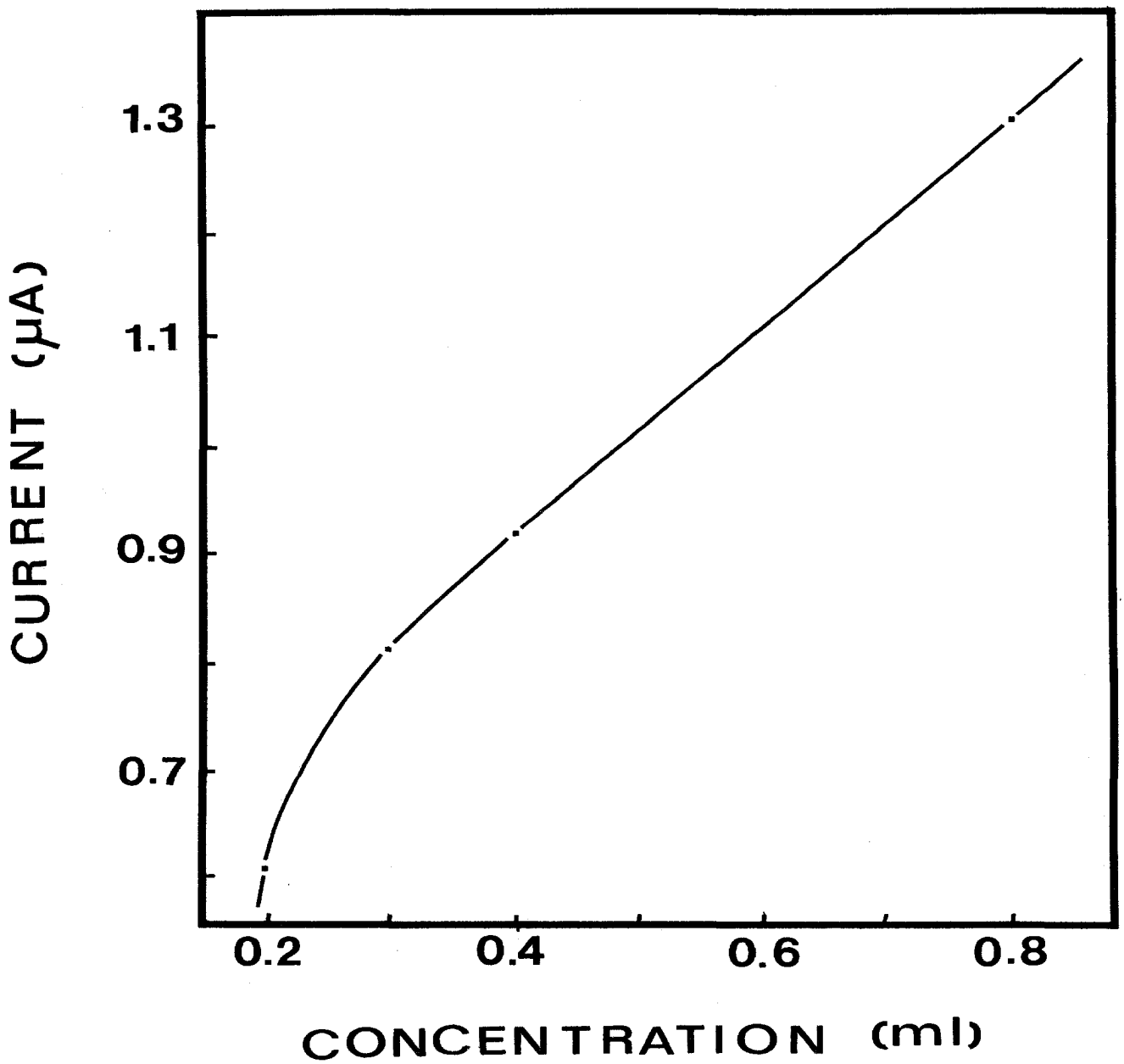
EFFECT OF γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The data for this plot was calculated from the diffusion current of the composite reduction wave of γ-butyrolactone. These results are an average of two analyses for each concentration examined.

FIGURE 6

EFFECT OF γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT



Tables VI and VII are the results of this study. Figure 7 is a plot of diffusion current versus concentration of γ-butyrolactone. This Figure shows that the diffusion current of both waves is similar for the various concentrations examined.

α-Methyl-γ-Butyrolactone

A well-defined double wave was observed for the polarographic reduction of α-methyl-γ-butyrolactone in methanol with 0.2 M $NaC_2H_3O_2$ as supporting electrolyte. The apparent $E_{1/2}$ values for the first and second waves were -0.452 and -1.17 volts respectively. The results of this preliminary study are recorded in Tables VIII and IX, and in Figure 8.

D-Mannurono-γ-Lactone

A double reduction wave was observed for the polarographic reduction of D-mannurono-γ-lactone in DMF with 0.05 M TEC1 supporting electrolyte. The first and second waves had apparent $E_{1/2}$ values of -1.75 V and -1.92 V, respectively.

γ-Valerolactone

In Table X are the results of a preliminary aqueous concentration study. An apparent $E_{1/2}$ = -1.51 volts versus an S.C.E. reference electrode was obtained for γ-valerolactone. In Figure 9 is a plot of diffusion current versus concentration for γ-valerolactone.

A typical aqueous γ-valerolactone polarogram (Figure 10A) is shown as a comparison to the nonaqueous methanol polarogram (Figure 10B). These typical polarograms were taken from a study run on the Sargent

TABLE VI

EFFECT OF γ-BUTYROLACTONE ON DIFFUSION CURRENT

γ-Butyrolactone concentration* in ml	Diffusion current in μA**
0.06	0.048
0.10	0.066
0.15	0.149
0.20	0.095
0.25	0.045
0.30	0.027

* In this preliminary concentration study the lactone was added to 10 ml of 0.2 M $NaC_2H_3O_2$ in methanol.

** Diffusion current values for the first wave of the polarographic reduction of γ-butyrolactone. Refer to text for details.

TABLE VII

EFFECT OF γ-BUTYROLACTONE ON DIFFUSION CURRENT

γ-Butyrolactone concentration* in ml	Diffusion current** in μA
0.10	0.064
0.15	0.161
0.20	0.096
0.30	0.023

* In this preliminary concentration study the lactone was added to 10 ml of 0.2 M $NaC_2H_3O_2$ in methanol.

** Diffusion current values for the second wave of the polarographic reduction of γ-butyrolactone. Refer to text for details.

FIGURE 7

EFFECT OF γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The data for this plot was calculated from the diffusion current of the first and second nonaqueous reduction waves of γ-butyrolactone. The results are an average of two analyses for each concentration examined.

A First reduction wave results ——————

B Second reduction wave results - - - - - - - - - - -

FIGURE 7

EFFECT OF γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT

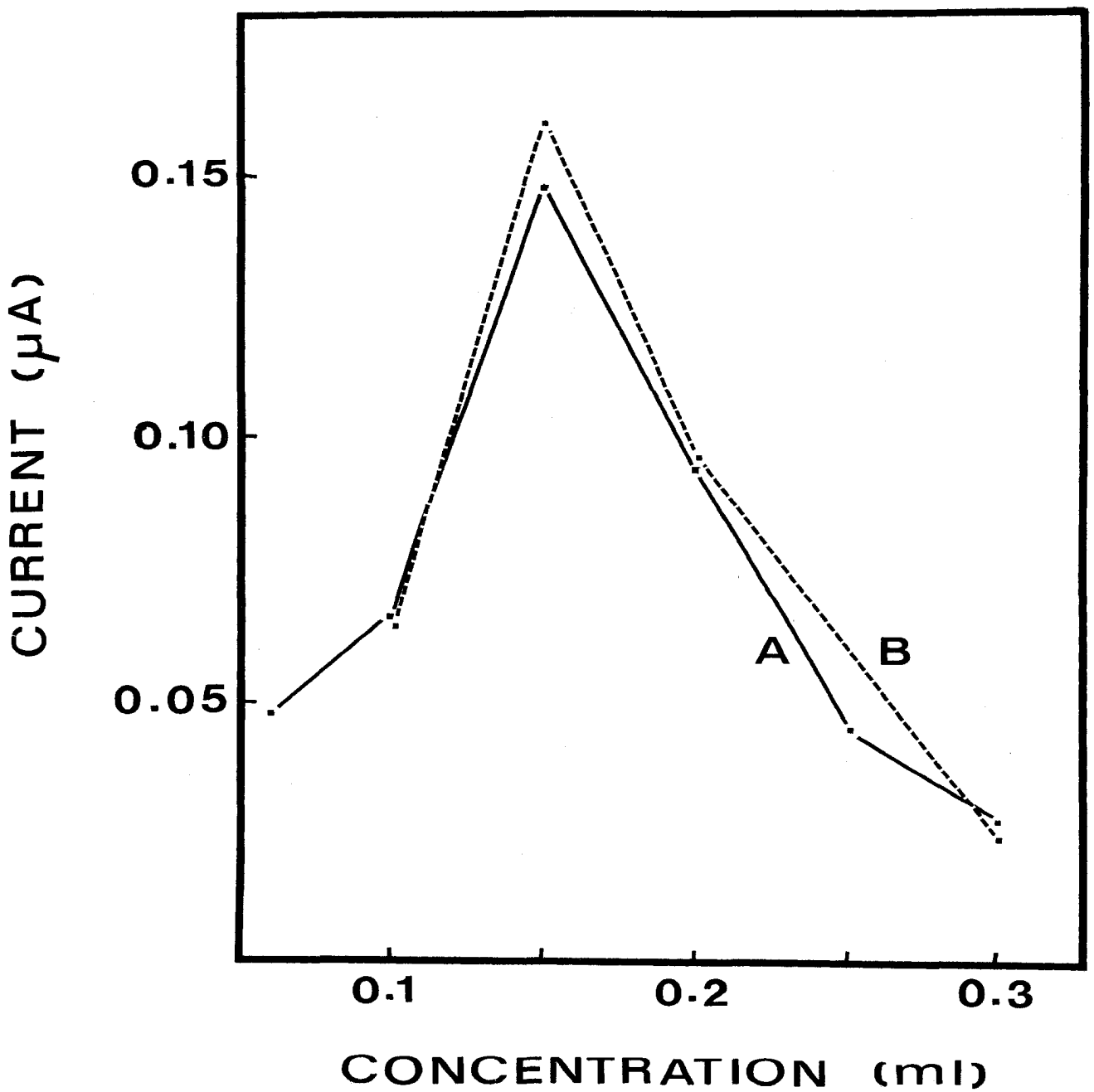


TABLE VIII

EFFECT OF α-METHYL-γ-BUTYROLACTONE ON DIFFUSION CURRENT

α-Methyl-γ-butyrolactone concentration* in ml	Diffusion current** in μA
0.02	0.144
0.04	0.396
0.05	0.480
0.06	0.288
0.07	0.198
0.08	0.252
0.09	0.304
0.10	0.266

* In this preliminary concentration study the lactone was added to 10 ml of 0.2 M $NaC_2H_3O_2$ in methanol.

** Diffusion current values for the first wave of the polarographic reduction of α-methyl-γ-butyrolactone. Refer to text for details.

TABLE IX

EFFECT OF α-METHYL-γ-BUTYROLACTONE ON DIFFUSION CURRENT

α-Methyl-γ-butyrolactone concentration* in ml	Diffusion current** in μA
0.02	0.119
0.04	0.288
0.05	0.339
0.06	0.210
0.07	0.146
0.08	0.128

* In this preliminary concentration study the lactone was added to 10 ml of 0.2 M $NaC_2H_3O_2$ in methanol.

** Diffusion current values for the second wave of the polarographic reduction of α-methyl-γ-butyrolactone. Refer to text for details.

FIGURE 8

EFFECT OF α-METHYL-γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The data for this figure was calculated from the diffusion current of the first reduction wave of α-methyl-γ-butyrolactone. The results of this nonaqueous (CH_3OH-$NaC_2H_3O_2$) study are an average of two analyses for each concentration examined.

FIGURE 8

EFFECT OF α-METHYL-γ-BUTYROLACTONE CONCENTRATION ON DIFFUSION CURRENT

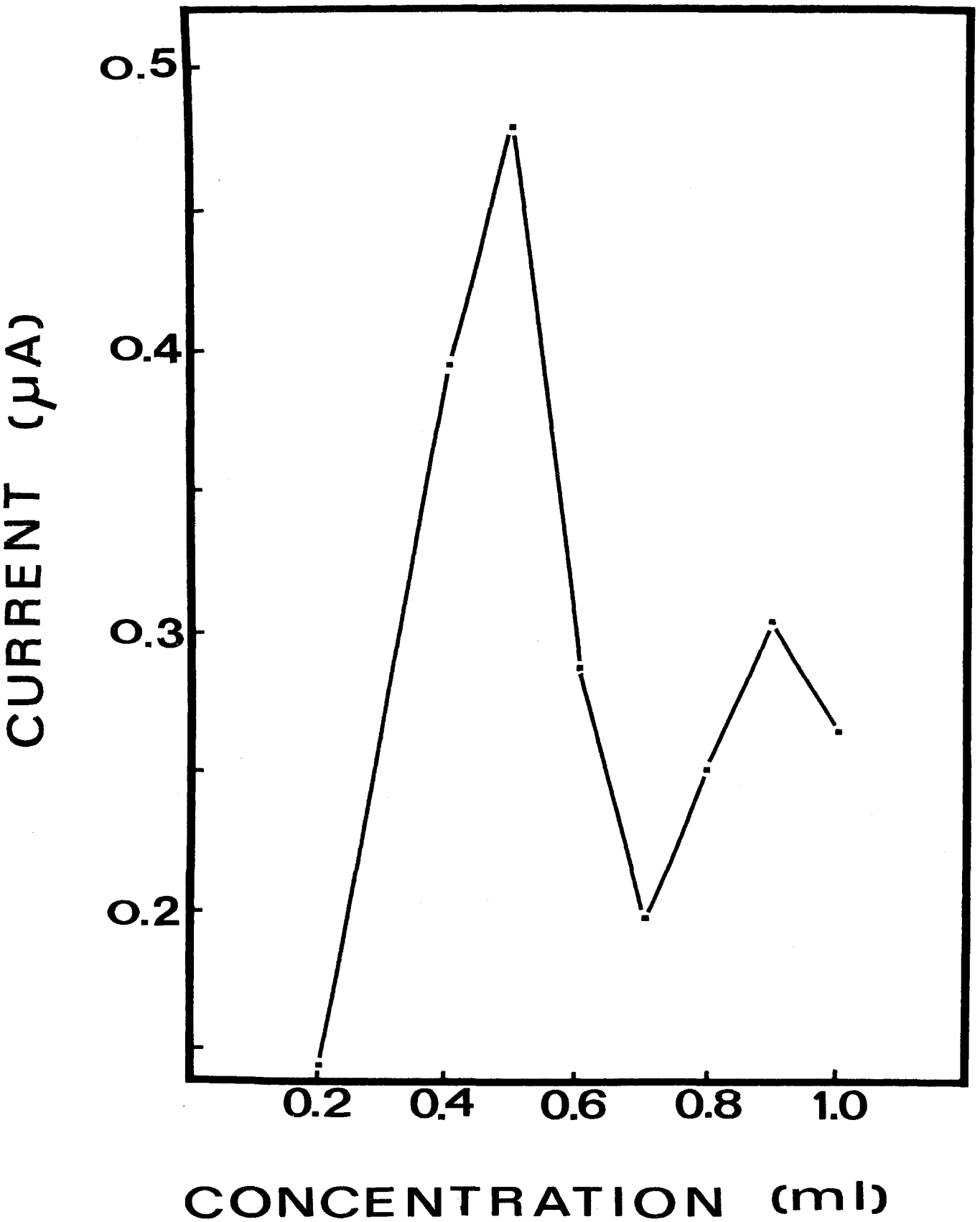


TABLE X

EFFECT OF γ-VALEROLACTONE ON DIFFUSION CURRENT

γ-Valerolactone concentration * in ml of lactone	Diffusion current in μA
0.05**	0.918
0.15	1.536
0.20	1.965
0.30	2.542

* In this preliminary concentration study the lactone was added to 5 ml of aqueous 0.2 M KCl.

** One ml of γ-valerolactone is equivalent to 1.04608 g at 25 °C.

FIGURE 9

EFFECT OF γ-VALEROLACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The data for this aqueous concentration study was calculated from the diffusion current of the composite reduction wave of γ-valerolactone. All analyses were made in duplicate.

FIGURE 9

EFFECT OF γ-VALEROLACTONE CONCENTRATION ON DIFFUSION CURRENT

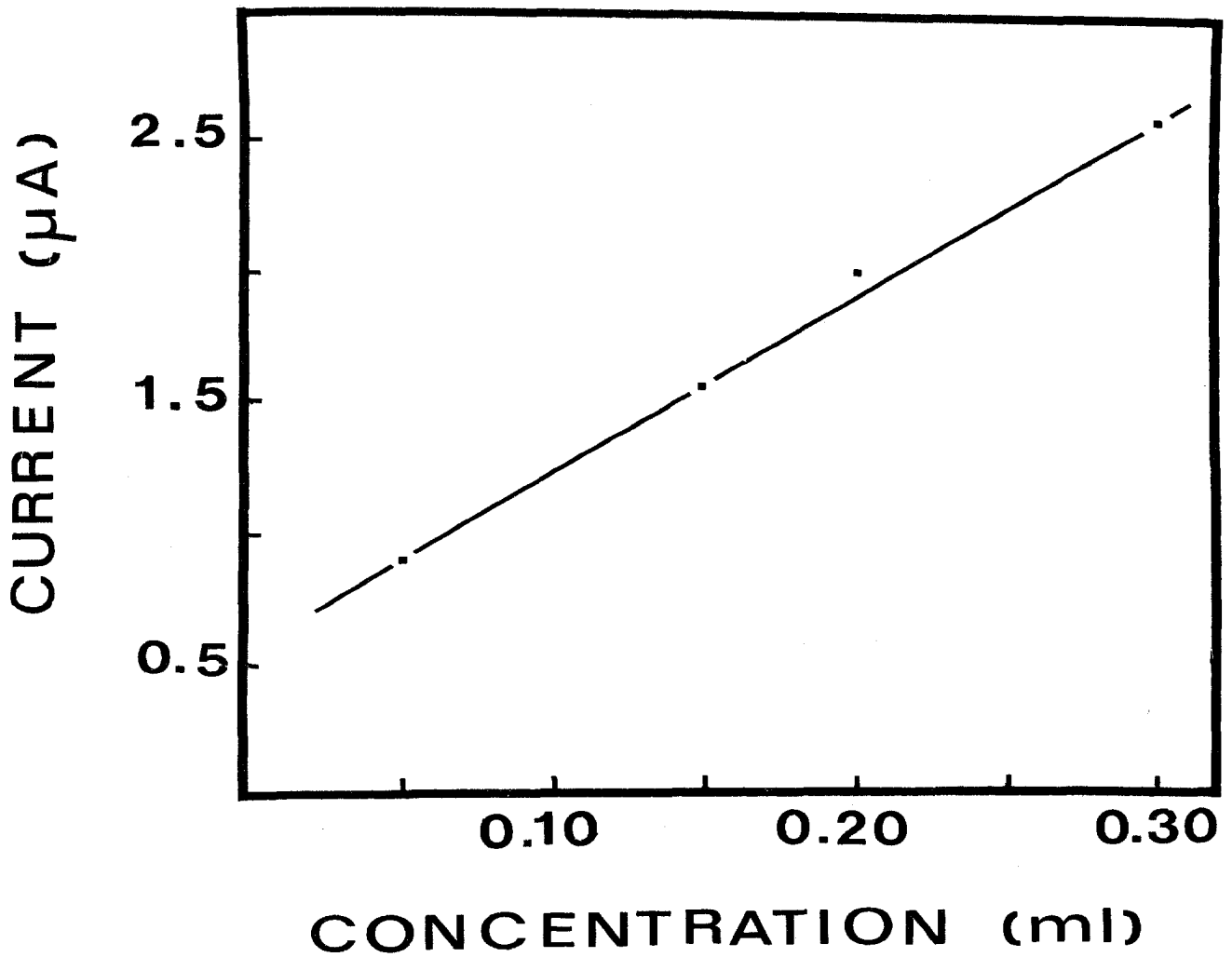


FIGURE 10

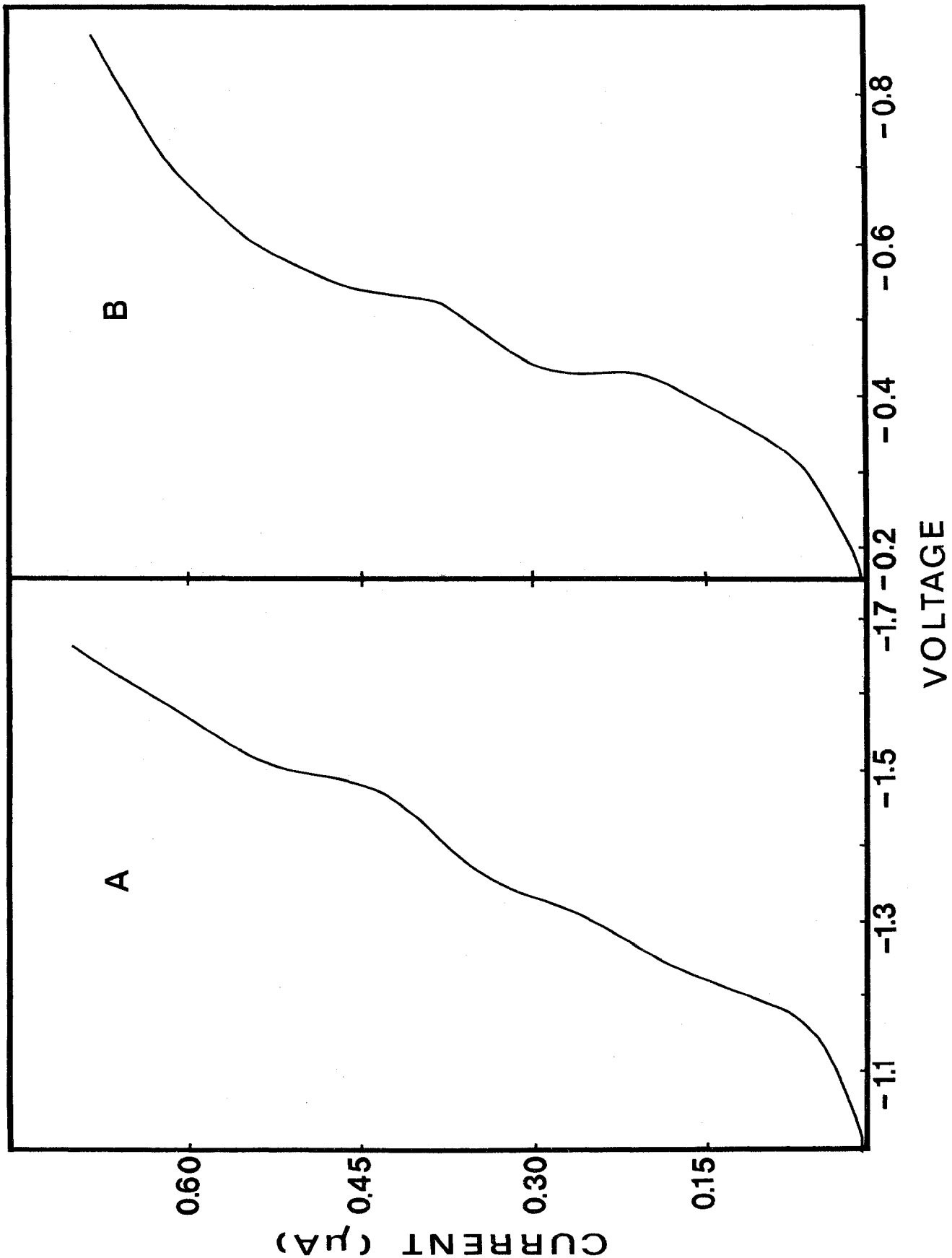
TYPICAL AQUEOUS AND NONAQUEOUS POLAROGRAMS OF γ-VALEROLACTONE

Legend

Part A of this figure shows a polarogram of the aqueous reduction of γ-valerolactone. Part B shows the more pronounced and larger second reduction wave observed for γ-valerolactone in a nonaqueous medium.

FIGURE 10

TYPICAL AQUEOUS AND NONAQUEOUS POLAROGRAMS OF γ-VALEROLACTONE



Model XVI Polarograph.

In Figure 10B is a characteristic polarogram of the reduction of γ-valerolactone in methanol. An apparent $E_{1/2}$ for the combined waves is -0.484 V. A noticeable increase can be seen in the wave at the higher potential in the methanol-sodiumacetate system compared to the aqueous system. In Table XI are the results of a preliminary study of the reduction of γ-valerolactone in methanol using 0.2 M $NaC_2H_3O_2$ as the supporting electrolyte. Figure 11 is a plot of the diffusion current versus concentration for γ-valerolactone in this medium. The aqueous and nonaqueous concentration studies were in essentially the same concentration range, yet a quenching effect on the diffusion current was observed for higher concentrations of the lactone in the methanol system.

δ-Valerolactone

Figure 12 shows that two waves appeared during polarographic reduction of δ-valerolactone in an aqueous 0.2 M KCl supporting electrolyte medium. The apparent $\bar{E}_{1/2}$ of the first wave was -1.41 V. The maximum in this wave could be eliminated by using a final concentration of 0.006% gelatin as the maximum suppressor. No maximum was observed for the second wave whose apparent $\bar{E}_{1/2}$ was -1.63 V.

The first nonaqueous study of the polarographic reduction of δ-valerolactone resulted in three reduction waves, which were not well defined (Figure 13 A). In the second study, only two waves were observed. These were exceptionally well defined (Figure 13 B). A preliminary investigation of the relationship between diffusion current and concentration for the two waves can be seen in Tables XII and XIII. Apparent

TABLE XI

EFFECT OF γ-VALEROLACTONE ON DIFFUSION CURRENT

γ-Valerolactone concentration in ml of lactone added *	Diffusion current in μA
0.025	0.228
0.035	0.308
0.045	0.656
0.065	0.484
0.075	0.432
0.095	0.495
0.145	0.497
0.175	0.286
0.200	0.288

* In this preliminary concentration study the lactone was added to 10 ml of 0.2 M $NaC_2H_3O_2$ in CH_3OH.

FIGURE 11

EFFECT OF γ-VALEROLACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The data for this concentration plot was calculated from the diffusion current of the composite wave, which was obtained in the non-aqueous polarographic reduction of γ-valerolactone. These results are based on a single determination for each concentration tested.

FIGURE 11

EFFECT OF δ-VALEROLACTONE CONCENTRATION ON DIFFUSION CURRENT

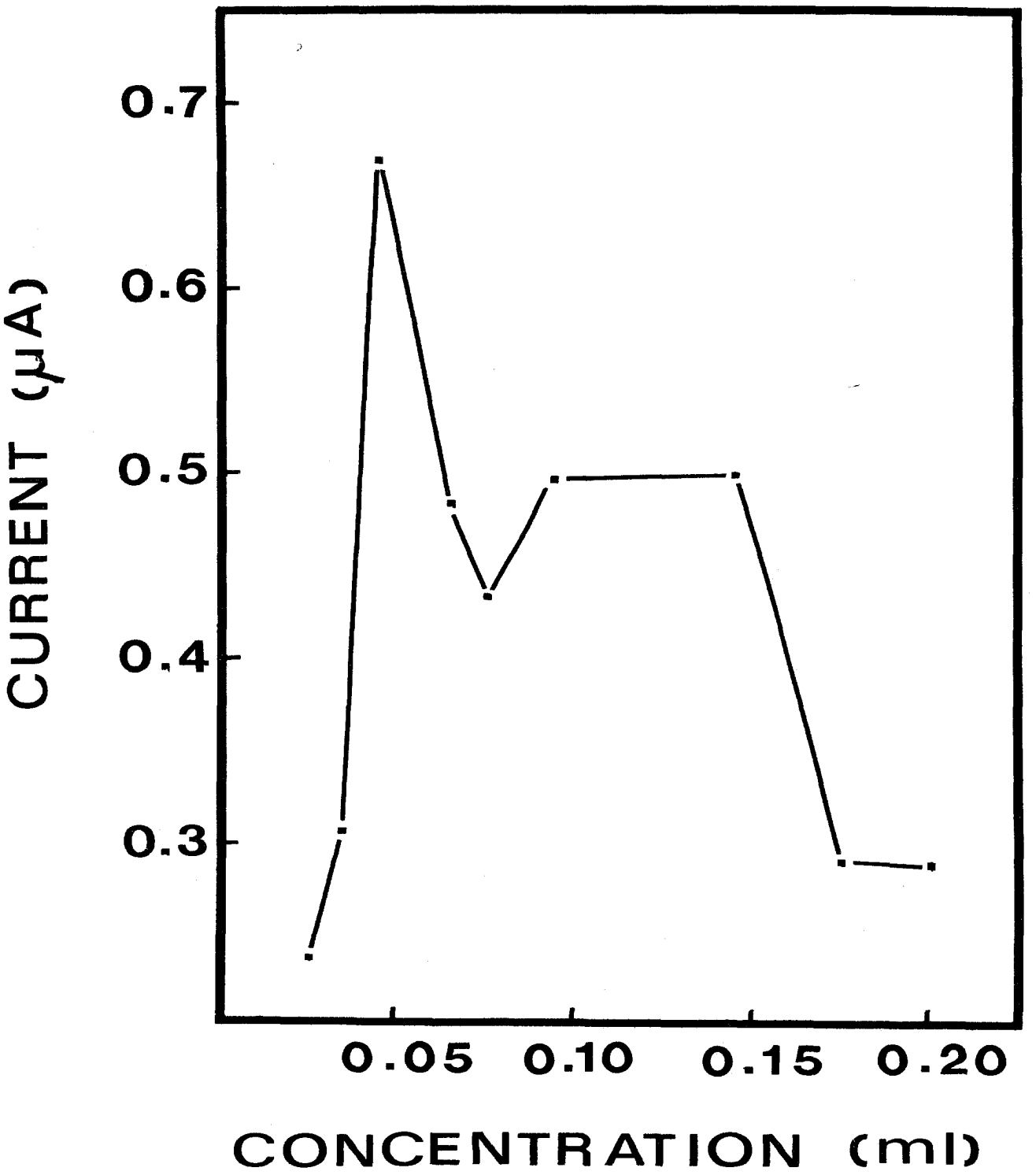


FIGURE 12

TYPICAL AQUEOUS POLAROGRAM OF δ-VALEROLACTONE

Legend

Aqueous polarographic reduction of δ-valerolactone results in the double reduction wave which is seen in this figure. The maximum of the first wave can be eliminated by the addition of a maximum suppressor.

FIGURE 12

TYPICAL AQUEOUS POLAROGRAM OF δ-VALEROLACTONE

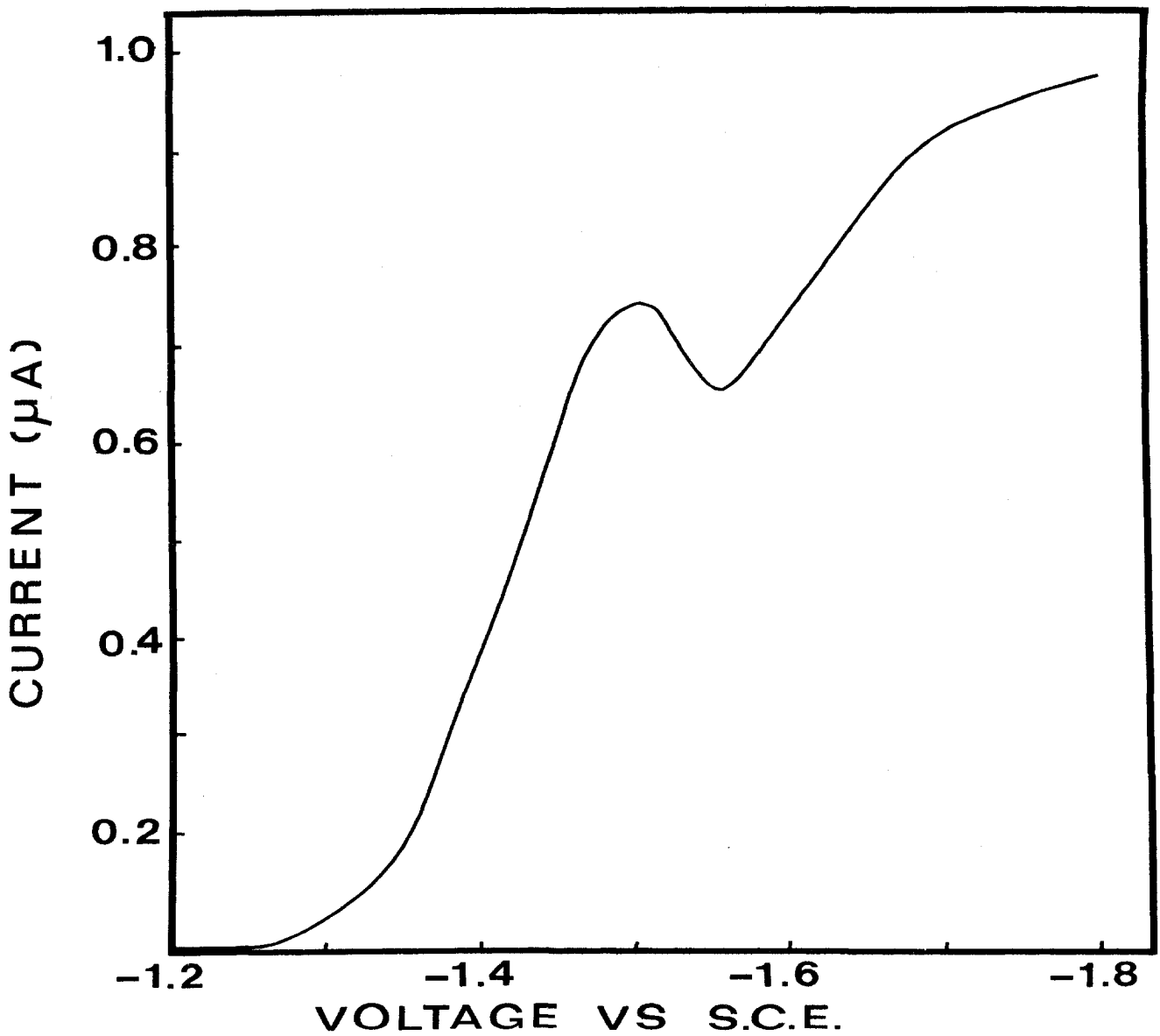


FIGURE 13

TYPICAL NONAQUEOUS POLAROGRAMS OF δ-VALEROLACTONE

Legend

This figure depicts two conflicting studies of the nonaqueous polarographic reduction or δ-valerolactone. Refer to text for detailed information.

FIGURE 13

TYPICAL NONAQUEOUS POLAROGRAMS OF δ-VALEROLACTONE

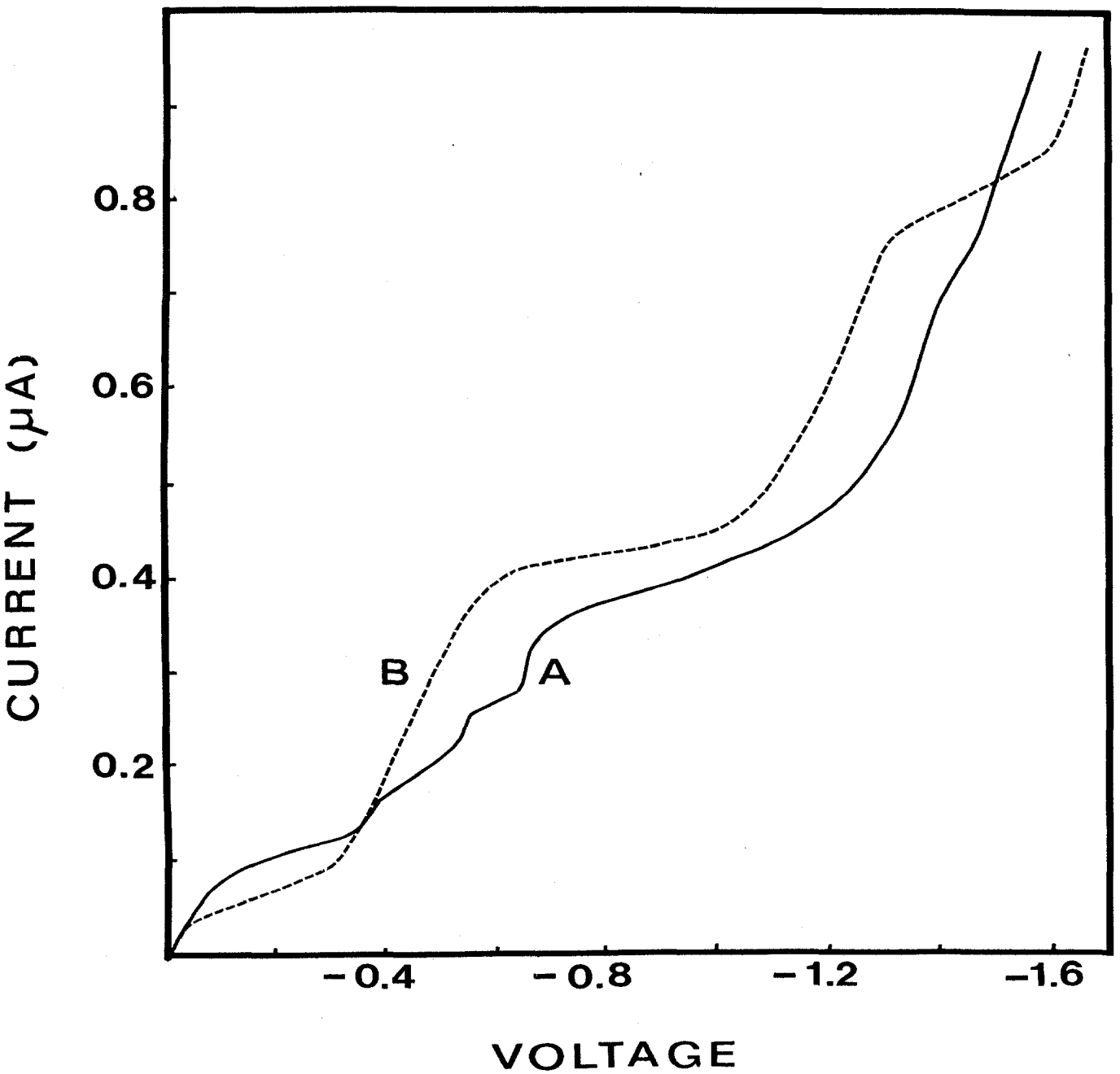


TABLE XII

EFFECT OF δ-VALEROLACTONE ON DIFFUSION CURRENT

δ-Valerolactone concentration in ml of lactone added*	Diffusion current in μA
0.1	0.220
0.12	0.423
0.14	0.534
0.16	0.548
0.18	0.590
0.20	0.600
0.22	0.536
0.24	0.420
0.26	0.377
0.28	0.357
0.32	0.347
0.36	0.321
0.42	0.344
0.50	0.253
0.60	0.249
0.70	0.272
0.80	0.220
0.90	0.211
1.00	0.199

* In this preliminary concentration study the lactone was added to 10 ml of 0.2 M $NaC_2H_3O_2$ in CH_3OH.

TABLE XIII

EFFECT OF δ-VALEROLACTONE ON DIFFUSION CURRENT

δ-Valerolactone concentration in ml of lactone added*	Diffusion current in μA
0.1	0.286
0.12	0.390
0.16	0.512
0.18	0.572
0.20	0.588
0.22	0.488
0.24	0.384
0.26	0.370
0.28	0.336
0.32	0.300
0.36	0.294
0.42	0.363
0.50	0.276
0.60	0.300

* In this preliminary concentration study the lactone was added to 10 ml of 0.2 M $NaC_2H_3O_2$ in CH_3OH.

$\bar{E}_{1/2}$ values for waves one and two are -0.473 V and -1.22 V, respectively. The effect of concentration on the diffusion current of the first and second waves can be seen in Figure 14.

The only difference in the two studies was that the first used anhydrous methanol (prepared as previously described, under EXPERIMENTAL) and the second used Fisher ACS methanol. The possibility of an impurity in the system can be eliminated because blanks run prior to the analyses exhibited no significant impurity waves. Assuming that the Fisher methanol contained water, a possible hydrogen donor would be present, and this might eliminate the third wave which was seen in the anhydrous study.

C. Constant Potential Electrolysis of D-Glucurono-γ-Lactone

The initial current flow for electrolysis E-1 voltage of -2.1 ± 0.1 V was approximately 0.1 amp. After about 8 hr this changed to about 0.2 amp. The initial solution appeared to have a very slight yellow discoloration. This color changed to a pale yellow, and then, after continuously darkening to a brown color, it finally turned green. This final color change may have occurred because deaeration of the solution by nitrogen was discontinued after 5 days. In Figure 14 is a general schematic diagram showing the steps used to attempt separation of the electrolytic solution. Reduction product isolation is still incomplete; however, the acetone extraction (Figure 15) appears to be the best separation step. When an agitated solution of the brown viscous liquid in acetone is allowed to settle, white granular crystals settle first; then, a layer of white flake-like crystals appear, and finally a layer of darker flake-like crystals come to rest. The acetone layer is a

FIGURE 14

EFFECT OF δ-VALEROLACTONE CONCENTRATION ON DIFFUSION CURRENT

Legend

The data for this graph was calculated from the diffusion current of the first and second reduction waves of δ-valerolactone. The results are an average of two analyses for each concentration examined.

A First reduction wave results ——————

B Second reduction wave results - - - - - - - - - - -

FIGURE 14

EFFECT OF δ-VALEROLACTONE CONCENTRATION ON DIFFUSION CURRENT

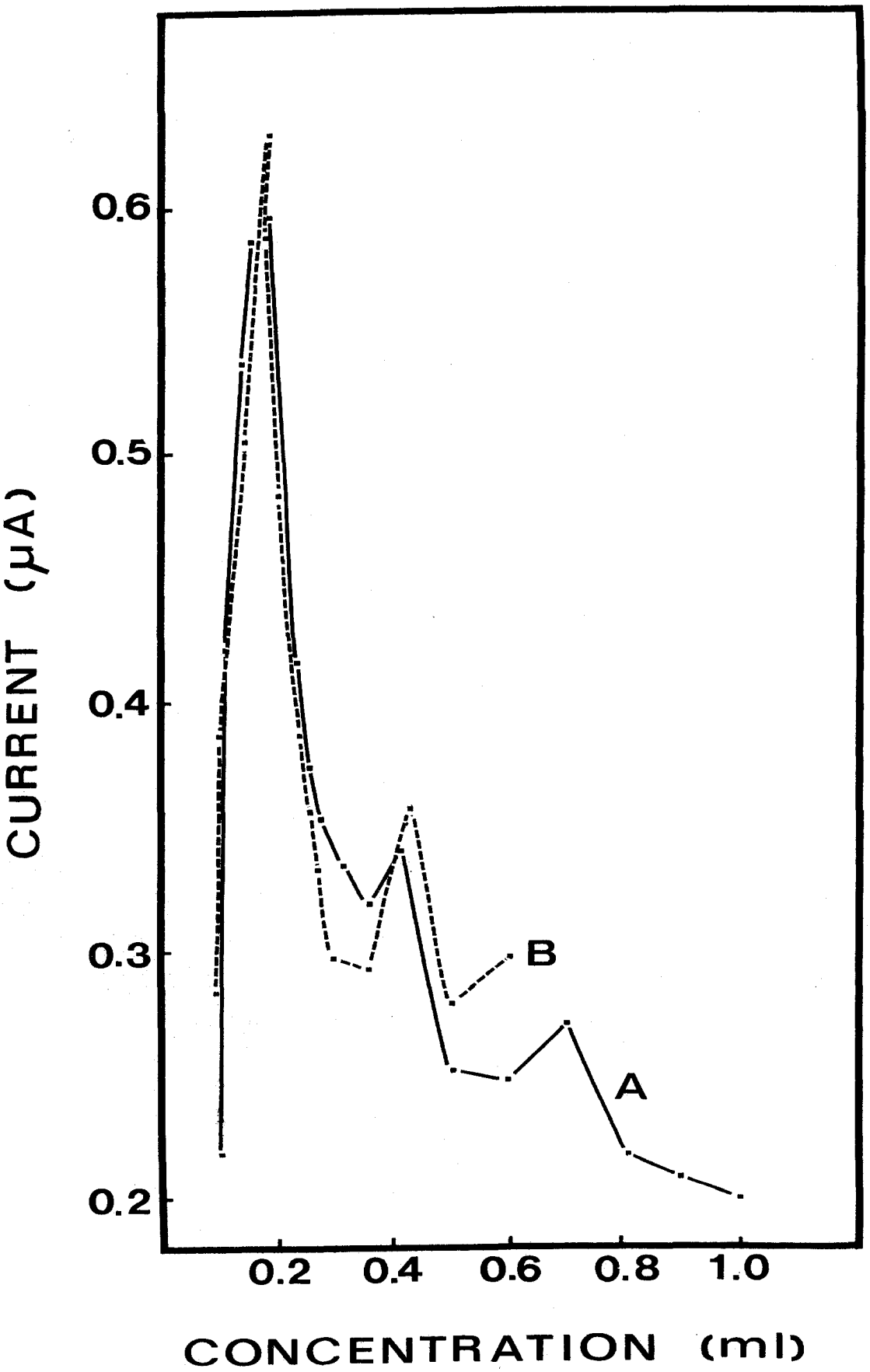


FIGURE 15

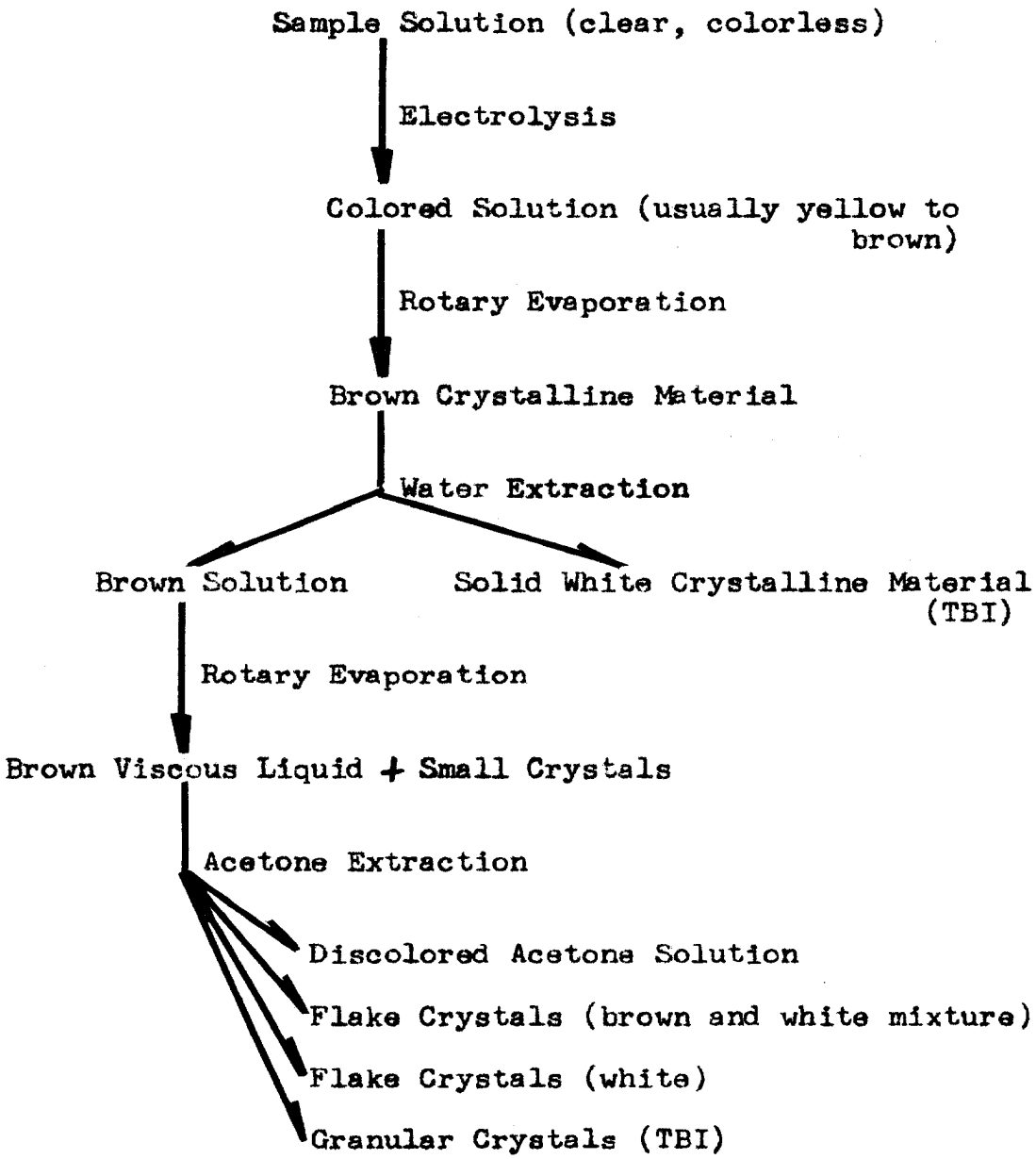
SEPARATION OF ELECTROLYZED SOLUTIONS

Legend

Separation of the components of the electrolyzed solutions E-1 and E-2 was carried out according to the procedure described in this figure.

FIGURE 15

SEPARATION OF ELECTROLYZED SOLUTIONS



dark brown color indicating still another separation. Analyses of these components by i.r. and n.m.r. have shown that the white granular crystals are TBI. The other fractions were not of sufficient purity and quantity to determine accurately; however, DMF, TBI, and D-glucurono-γ-lactone were present.

The semipermeable membrane of electrolysis E-2 prevented most of the anode contamination. The final color of this electrolysis was dark yellow. The brown color that was obtained in electrolysis E-1 was not observed in E-2. A 250-ml fraction of the electrolyzed solution was separated as described for electrolysis E-1. Analysis of i.r. and n.m.r. data was not conclusive.

The constant potential polarographic reduction solution (electrolysis E-3) was analyzed with a Beckman IR-12 spectrophotometer. Blanking difficulties were encountered because of the many strong absorbances of the DMF-TBI medium. The n.m.r. data showed the appearance of a small impurity, possibly due to water, while another stronger peak at -5.62 p.p.m. disappeared. This latter singlet could be removed by adding D_2O to a freshly prepared sample of D-glucurono-γ-lactone.

A summary of all electrolysis blanks is given in Table XIV. Samples appear to discolor more rapidly and to a greater extent in the presence of mercury. Varied constituent blanks (not in the presence of mercury) showed no discoloration after three months when kept at -15 °C.

TABLE XIV

OBSERVATIONS OF TEST SOLUTION DISCOLORATION

Test solution discoloration after 12 hr at room temperature	Observations under the influence of direct current	Observations in the absence of direct current
DMF-TBI	--	no discoloration
DMF-TBI-Hg pool	no discoloration	slight yellow discoloration
DMF-D-Glucurono-γ-lactone	--	no discoloration
DMF-TBI-D-Glucurono-γ-lactone	--	no discoloration
DMF-TBI-D-Glucurono-γ-lactone-Hg pool	yellow	yellow
DMF-D-Glucurono-γ-lactone	--	no discoloration
DMF-D-Glucurono-γ-lactone-Hg pool	--	no discoloration

D. Purification of Tetraalkylammonium Halides

The recrystallization procedures for the purification of TECl and TBI were very efficient and yielded crystalline products of suitable purity to be used as supporting electrolytes for polarographic analyses.

CHAPTER IV

DISCUSSION

A. Polarographic Investigation of D-Glucurono-δ-Lactone

The polarographic determination of D-glucurono-δ-lactone can be carried out most efficiently in an aqueous medium. In this medium, Thibert and Johnston (3) were unable to distinguish between the half-wave potentials of hydrogen ion, D-glucurono-δ-lactone, and D-glucuronic acid. Research involving electrolytic reduction requires a longer period of time, and thus hydrolysis of the lactone must be taken into consideration in an aqueous medium. Upon changing to a nonaqueous medium, one immediately notices that a longer period of deoxygenation is required. When longer deoxygenation times are used, volatile solvents tend to evaporate and this changes the concentration of the solution. To minimize this loss, the gas is scrubbed by passing it through wash bottles filled with the solvent employed. Usually, the last wash bottle prior to the polarographic cell is placed in the same constant temperature bath as the cell. The time and flow rate for the period of deoxygenation is kept constant for all samples.

At the deoxygenation stage various impurities can be introduced as well as removed from the polarographic cell. Hi-pure nitrogen was used in the various studies performed in this laboratory. In past research published from this laboratory (3,8,9) as well as parts of this research, the nitrogen was passed through a set of wash bottles. One set of wash bottles used in the polarographic reduction of beta-substituted phenyl-

cystine derivatives by this laboratory involved a train of six towers (49).

For the carbon dioxide impurity a deoxygenation period of more than one hour is insufficient. When using TBI, aqueous TBOH can be added in order to remove the CO_2 impurity. This was done in the D-glucurono-γ-lactone concentration study, which introduced water, thus bringing about the possibility for hydrolysis of the lactone. This does not appear to be the case. One possible reason for this is that the solutions were prepared fresh and used immediately. The introduction of water would certainly have interfered in the electrolytic reduction of the lactone, because of the duration of the study, but this was avoided simply by not adding the aqueous TBOH in this part of the research. When working with very high concentrations of lactone and low sensitivity on the polarograph CO_2 interference is not normally encountered. In many forms of research a mixed solvent medium is employed. Claver and Murphy (50) used a system consisting of water DMF, and TBI. They found that a maximum of 3 ml of water in 25 ml of DMF could be used before TBI electrolyte decomposition interfered with the polarographic work. The region in which this interference was encountered was 0 to-2 volts versus S.C.E.

In the concentration study, a blank was run on each sample prior to addition of the D-glucurono-γ-lactone. The lactone was then added by pipet and the solution was simultaneously mixed and deoxygenated by the flow of nitrogen gas. During this study one of the samples was allowed to deoxygenate an extra 30 min. The results of this sample were slightly high, indicating the possibility of solvent evaporation resulting in a concentrating of lactone. Although, a 30-min variation of de-

oxygenation time could be tolerated in the concentration study, it is not recommended. The extra-deoxygenated sample was not included in the data and the samples in the study were all deoxygenated equally by the method described in the EXPERIMENTAL section.

A concentration study of D-glucurono-γ-lactone in the range of 50 to 300 μg/ml was obtained in the DMF-H_2O-TBI system. The wave was not well defined compared to the aqueous system. No maximum supressor was required. The best waves were obtained by using a fast chart speed and a slow voltage drive speed in order to stretch the wave along the voltage axis. For ease in seeing the wave, which had a gradual increase in the current axis, only about half of the maximum polarographic sensitivity was used. This allowed about 50 to 75 mm above the limiting current plateau of the lactone for the decomposition of the electrolyte. By using this sensitivity, a sharp contrast in the reduction waves of the electrolyte and lactone occurs. As in the DMF-TBI investigations of Lambert (10), no erratic mercury drops or plugged capillaries were encountered in this concentration study. All values were reproducible. The apparent half-wave potential was calculated and an apparent $\bar{E}_{1/2}$ of a number of polarograms was calculated. An exact $E_{1/2}$ by the manual method described under EXPERIMENTAL could not be obtained, because the current changed with respect to time at a given potential.

The possiblity that O_2 or CO_2 might be diffusing into the system was first suspected, but after the joints were all resealed with high vacuum grease, the current still increased with time. An attempt at deoxygenating for a period of five minutes prior to the determination of each point on the manually obtained polarogram gave results that were

not reproducible. No apparent color change was noticed and polarograms run on the solution were normal, as in the concentration study. The dropping mercury electrode had very few erratic drops as usual.

The same instruments were previously employed in obtaining an accurate $E_{1/2}$ for the beta-substituted phenylcystine derivatives in methanol without encountering any difficulties (49). It can thus be assumed that neither the diffusion of O_2 (less likely CO_2) nor the instrumentation (for measuring $E_{1/2}$) is at fault.

The lactone can also be eliminated because a blank obtained manually could not be run.

Another possible consideration is that the electrolyte could be at fault because a solution of electrolyte in contact with the mercury pool (in the absence of current) turns yellow. This generally takes hours and, as was mentioned previously, the color did not change during the blank polarographic analysis. The system of TBI, TBOH, water, DMF, and mercury must have some presently unexplained interaction which causes the polarogram to shift to increasing current with time. A volatile substance is suspected because of the lowering in current after deoxygenation.

B. Polarographic Reduction of Other Lactones

γ-Butyrolactone

The aqueous γ-butyrolactone study resulted in finding a double wave, but the two waves were not well enough resolved to measure the diffusion current of each. The small second wave at the more negative potential is explained by Hoijtink et al. (23).

In the nonaqueous methanol system with $NaC_2H_3O_2$ as the supporting

electrolyte a larger second wave is observed. This is an indication of slow proton addition.

α-Methyl-γ-Butyrolactone

A well defined double wave was observed in the methanol-sodium acetate medium. The aqueous investigation was not undertaken; however, one would expect that a double wave similar to the γ-butyrolactone aqueous reduction would result.

D-Mannurono-δ-Lactone

A double reduction wave was observed in DMF employing TECl supporting electrolyte. In the aqueous system examined by Johnston and Thibert only one reduction wave was observed (8). This follows a pattern which is similar to the other lactones previously examined.

α-Valerolactone

In the results shown in Figure 10B a noticeable increase can be seen in the diffusion current of the second wave compared to the same wave observed in the aqueous analysis shown in Figure 10A.

The nonaqueous reduction of γ-valerolactone yields polarographic waves that are not as well defined as some of the lactones previously examined.

δ-Valerolactone

The polarographic analysis of δ-valerolactone in an aqueous medium resulted in a double reduction wave. The first wave had a maximum. This was not found for the other lactones examined in this section.

The second wave was also more pronounced than would be expected from the previous lactone investigations.

The first nonaqueous polarographic reduction resulted in finding a triple wave. Studies of this unusual triple wave have not been repeated.

C. Constant Potential Electrolysis of D-Glucurono-γ-Lactone

Three constant potential reductions of D-glucurono-γ-lactone were carried out. A cell modeled after Lingane's (18) was prepared. The anode and cathode was separated by an extremely coarse sintered glass disk. The cell was prepared on a larger scale to increase the mercury surface at the anode and cathode. When tested, a negligible amount of current was obtained, which means a longer period of reduction must take place. This was not feasible and so another cell was designed. The new cell attempted to reduce the resistance by bringing the anode and cathode compartments as close as possible. The most efficient way was to place the anode directly above the cathode. The cathode was a large mercury pool in a 4-liter beaker and the platinum anode was suspended in a large diameter hollow tube with a coarse sintered glass disk on one end. The tube with the sintered glass disk was lowered within 1 cm of the mercury pool. Inside the tube, resting on the sintered glass disk, were four connected platinum electrodes. A small current of about 0.05 amp was obtained. It should also be noted that in both of these attempts stirring was very important, and was employed in both the anode and cathode compartments.

In order to obtain a larger current, the reduction had to be carried out in one vessel with the platinum anode suspended above the mer-

cury pool cathode. This resulted in an increase in current to about 0.3 amp and the first reduction (electrolysis experiment E-1) of the lactone was carried out.

Still wishing to separate the cell into two compartments an attempt was made by using a semiperméable cellulose membrane to contain the Pt anode electrodes. This was successful in eliminating part of the anode impurities of about 0.2 amp. In order to slow down diffusion of the dark-brown anode solution which passed through the membrane, an attempt was made at changing the anode solution a number of times throughout the electrolysis. This procedure removed part of the contamination by anode products while at the same time giving current values (0.1 amp) which were high enough for constant potential reduction of the lactone.

The third attempt was on a much smaller scale and employed the Sargent Model XVI Polarograph as a potentiostat. This reduction was carried out over a period of two weeks. Polarograms of the solution were obtained throughout the electrolysis period. It was hoped that a minimum reduction of about 25% of the diffusion current would be obtained. Sample calculations indicated that a one electron reduction would reduce 50% of the sample. These calculations were very conservative estimates using a diffusion current of 100 μa while the true value was 150 μa. Similarly, they were based on 10 days while the run actually lasted 14 days. The foregoing estimates were also to make up for the fact that the current is not continuous at the DME. Over the two-week period, the solution changed from colorless to a pale yellow, which gradually changed to a dirty yellow. Samples were taken every three days and kept at -15 °C. These samples had a gradual color gra-

dient when placed side by side in order of collection. A blank solution was run similarly, but no color change was observed. The blank remained clear and colorless throughout the electrolysis.

The series of polarographic sample reductions were analyzed by i.r. and n.m.r. spectroscopy. The i.r. results were inconclusive possibly due to the fact that low concentrations of the lactone were used and blanking difficulties were encountered for the strong absorbing solvent. The n.m.r. revealed the disappearance of a peak. This occurred rather abruptly in the concentration study. It was also too early to have been due to the removal of the lactone by polarographic reduction. The peak was due to a hydroxyl hydrogen and its disappearance could be brought about by adding D_2O to a freshly prepared DMF-TBI-lactone solution. One would suspect that traces of water are entering the electrolytic system and thus quenching of the peak due to the hydroxy group would occur, but one does not observe a noticeable water peak in the n.m.r. This remains a very interesting but unexplained phenomenon.

The constant potential reduction samples were placed in a rotary evaporator. The removal of the DMF was very difficult, and a rotary evaporator was the only way to remove it. This was followed by water and acetone extractions after which n.m.r. analysis revealed that DMF was still present. The reduction product was not isolated in the present study, and only a very small amount of the starting lactone was recovered. The DMF solvent was probably not the best choice for the constant potential electrolysis.

Blank solutions were prepared in 25-ml volumetric flasks. These were deoxygenated for 45 minutes and then stoppered. They were stored

in the dark. The solutions contained similar volumes and concentrations as those employed in electrolysis E-3. After 12 hours the solution containing DMF, TBI, D-glucurono-γ-lactone, and mercury pool on the bottom, had turned yellow. Solutions which did not have a mercury pool did not turn yellow. Other similar blanks in which the constituents were varied revealed that mercury speeds up discoloration. The yellow discoloration of TBI solutions in DMF generally takes weeks at room temperature. Solutions kept at -15 °C showed no discoloration after as long as three months. All solutions that were colored retained their degree of discoloration if stored at -15 °C. This discoloration gradient was noticed in the constant potential reduction study of D-glururono-γ-lactone.

What is believed to be the Lobry de Bruyn-van Ekenstein transformation of carbohydrates was also observed in a number of studies. The appearance of a greenish-yellow color was noticed after 5 hr at room temperature in a solution of DMF containing TBI, and D-glucurono-γ-lactone. A green color was also observed in the constant potential reduction of the lactone (electrolysis E-1). In general, it can be said that a solution containing DMF, TBI, D-glucurono-γ-lactone, and a mercury pool will first turn from a clear colorless solution to a pale yellow. This solution then turns continuously darker at room temperature. After a month, the solution is a dark redish-brown. Without mercury, the solution turns a very pale-yellow and this discoloration does not progress any further. This pale-yellow color was also noticed immediately upon dissolving TBI salts which were not recrystallyzed.

D. Purification of Tetraalkylammonium Halides

The largest problem in the polarographic investigation of D-glucurono-γ-lactone was the purification of the quaternary ammonium salts. The TEB may decompose upon recrystallization. It loses Br_2 upon exposure to air. The chloride salt is hygroscopic and loses Cl_2 if placed in a desiccator over CaO. The tetrabutylammonium salts have similar traits (51). Numerous difficulties were encountered in the present investigation during the purification of the tetraethyl- and tetrabutylammonium chloride and bromide salts. The yields upon recrystallization are generally very poor, and many times the third or fourth recrystallizations turned out to be more highly colored and their use in polarographic blanks also showed this increase in impurity. One minor success was the formation of a large crystal of TECl weighing approximately one gram. Although it was only a first recrystallization, material of this purity would have been satisfactory as a supporting electrolyte for polarographic investigations. Any check in the literature on the use of the quaternary ammonium salts in polarography reveals that they are widely employed, but their use at more negative potentials, although exclusive, is difficult to find for the chloride and bromide salts. The iodide form is widely used because of its ease in purification. The TBI is insoluble in water and this allows for its recrystallization from a 3:1 acetone-methanol solution to which water is added. The salt after being recrystallized three times is placed in a vacuum desiccator. The clear crystals turn to a white powder as the methanol, acetone, and/or water are removed *in vacuo*.

CHAPTER V

SUMMARY AND CONCLUSIONS

A polarographic wave for the reduction of D-glurucono-γ-lactone was observed in a medium containing DMF, water, TBI, and TBOH. The reduction wave has an apparent $\bar{E}_{1/2} = -1.94$ V. An exact manual measurement of $E_{1/2}$ was not possible because the current increased with time. A five-minute deoxygenation after each manually determined point on the polarogram also failed to give reproducible results. The diffusion current has been shown to be proportional in a concentration range of 50-300 μg/ml. No maximum suppressors were employed as no maxima or other disturbance were observed. The water and TBOH were added as 0.1 ml of 0.02 M aqueous TBOH in order to remove a CO_2 impurity. A more concentrated solution or a larger sample of TBOH discolored the solution to a faint pink. No hydrolysis of the lactone was observed and the diffusion current on all the samples was extremely reproducible.

Three constant potential electrolyses of the lactone were attempted. The results with respect to discoloration of the samples were similar. All changed from a clear colorless solution to a faint yellow which gradually darkened to a light brown. The solvent was removed by rotary evaporation and water and acetone extractions were used to attempt separation from a dark-brown viscous liquid. The product of the electrolytic reduction has not been identified.

A summary of the lactones examined in this study is given in Table III. These lactones gave well defined two step reduction waves when a nonaqueous medium was employed. In the aqueous medium the wave at the more negative potential would be eliminated or greatly reduced while the less negative wave would increase proportionally, because of the presence of a good proton donor (H_2O).

BIBLIOGRAPHY

1. M. ISHIDATE and T. SHIMOZAWA. J. Pharm. Soc. Jap. 64, 53 (1944).

2. R. J. THIBERT and A. J. BOYLE. Talanta, 7, 175(1961).

3. R. J. THIBERT and J. R. JOHNSTON. Can. J. Chem. 47, 265(1969).

4. I. M. KOLTHOFF and J. J. LINGANE. Polarography. Vol. II. Interscience Publishers, Inc., New-York. 1952. p. 720.

5. H. MATHESON, H. S. ISBELL, and E. R. SMITH. J. Res. Natl. Bur. Stand. 28, 95(1942).

6. R. M. DOWBEN. Biochim. Biophys. Acta, 31, 454(1959).

7. Corn Products Sales Company. Tech. Bull. JN-2.

8. J. R. JOHNSTON and R. J. THIBERT. Can. J. Chem. 47, 1433(1969).

9. R. J. THIBERT and C. PATEL. Can. J. Chem. 48, 2000(1970).

10. F. L. LAMBERT. Anal. Chem. 30, 1018(1958).

11. F. L. LAMBERT and K. KOBAYASHI. J. Org. Chem. 23, 773(1958).

12. B. E. CONWAY, R. E. VERRALL, and J. E. DESNOYERS. Trans. Faraday Soc. 62, 2740(1966).

13. L. SILVERMAN and W. BRADSHAW. U. S. Atomic Energy Comm. NAA-SR-1966, 6(1956).

14. H. A. LAITINEN and S. WAWZONEK. J. Amer. Chem. Soc. 64, 1765 (1942).

15. P. Van RYSSELBERGHE and J. M. McGEE. J. Amer. Chem. Soc. 67, 1039(1945).

16. S. WAWZONEK, E. W. BLAHA, R. BERKLEY and M. E. RUNNER. J. Electrochem. Soc. 102, 235(1955).

17. P. L. PICKARD and W. E. NEPTUNE. Anal. Chem. 27, 1358(1955).

18. J. J. LINGANE, C. G. SWAIN, and M. FIELDS. J. Electrochem. Soc. 65, 1348(1943).

19. S. WAWZONEK and A. GUNDERSEN. J. Electrochem. Soc. 107(6)537 (1960).

20. P. H. GIVEN, M. E. PEOVER, and J. SCHOEN. J. Chem. Soc. 2674 (1958).

21. S. WAWZONEK and D. WEARRING. J. Amer. Chem. Soc. 81, 2067 (1959).

22. S. WAWZONEK, R. BERKEY, E. W. BLAHA, and M. E. RUNNER. J. Electrochem. Soc. 103, 456(1956).

23. G. J. HOIJTINK, J. van SCHOOTEN, E. de BOER, and W. Ij AALBERSBERG. Rec. trav. chim. 73, 355(1954).

24. J. GIVEN. J. Chem. Soc. 2684(1958).

25. M. D. RAUSCH, W. E. McEWEN, and J. KLEINBERG. J. Amer. Chem. Soc. 76, 3622(1954).

26. C. P. ANDRIEUX and J. M. SAVANT. Bull. Soc. Chim. Fr. 11, 4671(1968).

27. K. TAKAOKA. Rev. Polarog. 14(2),63(1966).

28. L. Y. KHEIFETS and V. D. BEZUGLYI. Elektrokhimiya, 2(7), 800 (1966).

29. J. PASCIAK. Zeszyty Nank. Politech. Slask. 32, 3(1966).

30. V. G. MAIRANOVSKII and G. I. SAMOKHVALOV. Elektrokhimiya, 2(6), 711(1966).

31. J. N. GAUR and N. K. GOSWAMI. Electochim. Acta, 11(7), 939 (1966).

32. J. R. JEZOREK and H. B. MARK. Anal. Lett. 3(10),515(1970).

33. P. FIORDIPONTI, F. RALLO, and L. RAMPAZZO. Ann. Chim. 60(7), 497(1970).

34. M. UEHARA and J. NAKAYA. Bull. Chem. Soc. Jap. 43(10), 3136 (1970).

35. D. Kh. RASULEVA, A. A. VOLOD'KIN, V. V. ERSHOV, A. I. PROKOF'EV, and S. P. SOLODOVNIKOV. Ser. Khim. 7, 1659(1970).

36. M. K. KALINOWSKI. Chem. Phys. Lett. 7(1),55(1970).

37. Y. M. KARGIN and V. Kh. IVANOVA. Reakts. Sposobnost Org. Soedin. 7(2),255(1970).

38. G. TAKEYA, H. ITOH, K. HATANAKA, and M. NAGAYAMA. Mem. Fac. Eng. Hokkaido Univ. 12(4),439(1970).

39. Y. MATSUI and Y. DATE. Bull. Chem. Soc. Jap. 43(9),2828(1970).

40. G. PALIANI. Z. Naturforsch. B, 25(8),786(1970).

41. B. A. KISELEV, Y. N. KOZLOV, and V. B. EVSTIGNEEV. Biofizika,

15(4), 594(1970).

42. R. SCHMID and V. GUTMANN. Chem. Zvesti, 23(10), 746(1969).

43. Y. MATSUI, Y. KUROSAKI, Y. DATE. Bull. Chem. Soc. Jap. 43(7), 2046(1970).

44. R. ANDRUZZI, M. E. CARDINALI, and I. CARELLI. J. Electroanal. Chem. Interfacial Electrochem. 26(2-3), 211(1970).

45. L. FIESER. Experiments in organic chemistry. Ed. 3, D. C. Heath and Co., Boston, 1955. p. 286.

46. E. V. KRYUKAVA and A. P. TOMILOV. Zh. Analit. Khim. 20(2), 265(1965).

47. H. H. WILLARD, L. L. MERRITT, and J. A. DEAN. Instrumental methods of analysis. Ed. 3, D. Van Nostrand Co. (Canada).

48. J. K. TAYLOR and S. W. SMITH. J. Res. Natl. Bur. Stand. 56, 143(1956).

49. C. PATEL. Masters Thesis, University of Windsor. (1969).

50. G. C. CLAVER and M. E. MURPHY. Anal. Chem. 31, 1682(1959).

51. Beilstein's Handbuch der Organischen Chemie. Vol. IV. Suppl. II. Julius Springer Verlag, Berlin, 1943. p. 596.

VITA AUCTORIS

1945: Born in Mariazell, Austria, February 5, 1945.

1963: Graduated with a Silver Dollar Scholastic Award from Hazel Park High School, Hazel Park, Michigan.

1967: Assistant Fencing Coach for Wayne State University, Detroit, Michigan.

1968: Graduated with a B. A. Degree from Wayne State University, Detroit, Michigan.

1969: Accepted into the Faculty of Graduate Studies at the University of Windsor, Windsor, Ontario.

Professional Societies:

Chemical Institute of Canada.