

AD-A129 015

ION-SELECTIVE ELECTRODES FOR SOME BETA-ADRENERGIC AND
CALCIUM BLOCKERS(U) ARIZONA UNIV TUCSON DEPT OF
CHEMISTRY L CUNNINGHAM ET AL. MAY 83 TR-6

1/1

UNCLASSIFIED

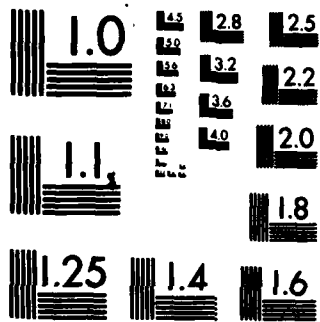
N00014-81-K-0576

F/G 11/9

NL



END
DATA
FOLLOWS
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD A 1 29015

DTIC FILE COPY

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 6	2. GOVT ACCESSION NO. AD A129 015	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) "Ion-Selective Electrodes for Some Beta-Adrenergic and Calcium Blockers"		5. TYPE OF REPORT & PERIOD COVERED Technical -- Interim
7. AUTHOR(s) L. Cunningham and H. Freiser		6. PERFORMING ORG. REPORT NUMBER 13
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Chemistry University of Arizona Tucson, AZ 85721		8. CONTRACT OR GRANT NUMBER(s) N00014-81-K-0576
11. CONTROLLING OFFICE NAME AND ADDRESS Materials Sciences Division Office of Naval Research Arlington, VA 22217		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE May 1983
		13. NUMBER OF PAGES 16
		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) This document has been approved for public release and sale; its distribution is unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) DTIC ELECTRIC JUN 3 1983		
18. SUPPLEMENTARY NOTES submitted to Clinical Chemistry		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) polymer membrane ion selective electrodes, cation selective electrodes, Acebutalol-, Verapamil-, Diltiazem-, Nicardipine-, Lidoflazine-electrode		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Potentiometric sensors based on dinonylnaphthalene sulfonic acid (DNNS) were prepared for several recently developed drugs used in the treatment of cardiovascular disorders. Thus, incorporation of DNNS along with the beta-adrenergic blocker Acebutalol, or calcium-channel blockers Verapamil, Diltiazem, Nicardipine or Lidoflazine into a plasticized polyvinyl chloride membrane resulted in coated wire ion selective electrodes which displayed nearly Nernstian response in the concentration range of 10 ⁻³ to 10 ⁻⁵ M and analytically useful responses down to 10 ⁻⁶ M. Selectivity behavior for each set of electrodes was accurately predicted		

from calculated distribution constants for the respective drugs. Use of these electrodes in analyses of pharmaceutical preparations is discussed, as well as their utility in pharmacokinetic studies.



**Ion-Selective Electrodes for Some Beta-Adrenergic
and Calcium Blockers**

Larry Cunningham and Henry Freiser

**Department of Chemistry
University of Arizona
Tucson, Arizona 85721**

ABSTRACT

Potentiometric sensors based on dinonylnaphthalene sulfonic acid (DNNS) were prepared for several recently developed drugs used in the treatment of cardiovascular disorders. Thus, incorporation of DNNS along with the beta-adrenergic blocker Acebutalol, or calcium-channel blockers Verapamil, Diltiazem, Nicardipine, or Lidoflazine into a plasticized polyvinyl chloride membrane resulted in coated-wire ion selective electrodes which displayed nearly Nernstian response in the concentration range of 10^{-3} to 10^{-5} M and analytically useful responses down to 10^{-6} M. Selectivity behavior for each set of electrodes was accurately predicted from calculated distribution constants for the respective drugs. Use of these electrodes in analyses of pharmaceutical preparations is discussed, as well as their utility in pharmacokinetic studies.

INTRODUCTION

Incorporation of dinonylnaphthalene sulfonic acid as an extractant in PVC membranes plasticized with dioctylphthalate has led to coated-wire electrodes (CWE) having extremely high selectivity for organic cations over common inorganic cationic species.¹ Previous work in this area has focused on the role of analyte structure on the selectivity behavior of these devices² and on development of electrodes selective to many important drugs of abuse³ and other pharmaceuticals⁴⁻⁵.

In each of these studies it was shown that the selectivity of an electrode increases with the analyte extractability, i.e. with its distribution constant. Therefore, the selectivity depends not only on molecular weight, but on the degree of nitrogen substitution and branching of hydrocarbon chains. A more important consideration with pharmaceutical compounds is the influence of hydrophilic substituents, because among a group of drugs similar in size such factors prevail in determining overall membrane selectivity with respect to either metabolites or other potential interferents. A more interesting yet unexplained result from earlier work was the lack of correlation between electrode selectivity and electrode sensitivity.

In this work, CWE's were prepared for several drugs used in treatment of cardiovascular diseases.^{6,7} Among them are drugs awaiting FDA approval for commercial introduction as well as those already available for prescription. The existence of widely varying structures (Fig. 1) among the calcium channel blockers has puzzled researchers in this area in trying to

elucidate a well-defined mechanism of action. The CWE's described here could prove useful in future investigations due to their low cost, small size, durability, as well as their sensitivity and selectivity. All of these compounds are monovalent cations at physiological pH values which make them amenable to analysis via ion-selective electrode methodology. Structural differences will influence drug extractability and hence electrode response characteristics. Response characteristics were critically evaluated in this study in light of their potential application to pharmaceutical assay and in-vivo or in-vitro drug monitoring. In anticipation of routine analysis of pharmaceutical preparations, the effect of high background levels of alpha-lactose, a common packing material, was also determined.

Though previous work did not entail a complete evaluation of electrode pH response, recent studies by other workers^{8,9} have shown that incorporation of a lipophilic base such as tridodecylamine into plasticized PVC results in a pH responsive electrode having little or no interference from inorganic anions provided that sufficient amounts of a lipophilic anion such as tetraphenylborate are also included. Because the electrodes reported here are analogous to these systems, having a lipophilic amine in the form of a high-molecular weight pharmaceutical and a lipophilic anion as DNNS, some kind of pH response might be expected. If so, an optimum pH region for analysis would exist for each electrode.

Materials and Methods

Reagents

The drugs investigated here were Acebutalol (Ives)(I), a beta-adrenergic blocker; and the calcium-channel blockers Lidoflazine (Janssen)(II), Verapamil (Searle,Knoll)(III), Nicardipine (Syntex)(IV), and Diltiazem (Marion)(V). We are grateful to these manufacturers for kindly supplying us with samples. These were dried over CaCl_2 prior to use. Free bases were converted to their hydrochloride form by dissolving in a minimum of conc. HCl; all solutions were prepared at pH 4.0 in 0.01 M acetate buffer. Chromatographic grade polyvinyl chloride (Polysciences, Warrington, Pa.) was used as obtained, as was practical grade dioctyl phthalate (J.T. Baker, Phillipsburg, N.J.). Dinonylnaphthalene sulfonic acid (Henkel Chemical) was purified by the ion-exchange method of Danesi¹⁰. All other chemicals were reagent grade.

Electrode Preparation and Handling

All ISE's were of the coated-wire type constructed as described earlier¹², with the exception that PVC insulated copper wires were substituted for co-axial cables. This facilitated the fabrication of large numbers of electrodes (20 for each drug) with a negligible increase in noise due to absence of shielded wire. After manufacture it was necessary to bathe electrodes in 10^{-3} M to 10^{-4} M solutions of the protonated analyte species for several days before stable responses could be obtained, during which time hydration of the polymer membrane occurred. As was the case in earlier work⁵,

soaking in blank buffer for several minutes prior to calibration resulted in best reproducibility.

Apparatus and EMF Measurements

Previously described micro- and minicomputer systems were used to perform all calibrations within user-specified concentration limits.^{2,11} Except for pH measurements, all potentials reported were measured against a double-junction calomel electrode having 0.1 N NH_4NO_3 in the external junction. Electrode equilibrium was assumed when a maximum of 0.4 mV/min drift was measured.

Sensitivity was obtained from calibration curves in the following manner: The linear segment of the Nernstian response region was extrapolated to meet another taken from the points in the region of no response, typically at concentration levels below 10^{-6}M . The limit of detection is then given by the Log (concentration) value at the intersection of the two linear segments.¹³

At the end of a calibration run, electrodes were situated in a solution of known primary ion concentration. Specified ratios of primary to interfering ion concentration were made by adding standard interferent from a second digital burette, after which responses of each electrode were measured and stored. Selectivity coefficients were then calculated using software based on the two-solution method of Srinivasan and Rechnitz.¹⁴

The pH responses were also evaluated under computer

control. Readings of CWE potentials were made vs. a Ag/AgCl reference contained in a Radiometer/Copenhagen glass combination electrode, which was used to simultaneously monitor pH. Starting at a low initial pH value (< 4.0), small increments (0.2 ml.) of 0.1 N NaOH were added to the blank buffer solution until a minimum specified pH change was realized, at which point electrodes were sampled until each equilibrated. This process was repeated until a desired pH limit was reached. Electrode pH profiles were then plotted as mV vs pH on a digital plotter.

Results and Discussion

All electrodes displayed linear response of nearly Nernstian character in the concentration of analyte above approximately 10^{-5} M as shown in Table I. Because some of these pharmaceuticals have low water solubility, an upper limit of 10^{-3} M was selected for each calibration. Detection limits of at least 10^{-5} M were observed for all electrodes. Somewhat greater sensitivity was obtained with electrodes for III and IV with detection limits down to 10^{-6} M (Fig. 2). Response times ranged from a few seconds for concentrations greater than 10^{-5} M to several minutes for lower concentrations.

It is expected that these electrodes would be exposed to a variety of matrices during analysis of pharmaceutical preparations. The effects of high background levels of neutral compounds were assessed by comparison of calibrations in absence and in presence of 10^3 to 10^4 -fold excess concentrations of alpha-lactose relative to the highest concentration of the drug of interest. It was found that repeated calibrations of various electrodes were virtually identical, demonstrating that no interference is measurable from high levels of this substance in the sample matrix.

Relative selectivity was determined by measuring responses in solutions containing various ratios of tributylammonium to the primary ion. $\log k_{i,j}^{pot}$ values which were calculated from the electrode responses and known activities of each ion are given in Table II along with respective $\log K_D$ values

calculated by the Hansch correlation method¹⁵. The increase in selectivity with drug lipophilicity was consistent with prior investigations of homologous series of alkylated ammonium ion interferents and for electrodes selective to other drugs⁵. Because drug metabolites are usually more hydrophilic than their parent compounds, they would exhibit negligible interference.

Electrode pH response was also a function of the primary ion. A decrease in CWE EMF readings with increasing pH was observed (Fig. 3), though these were not linear over the entire pH range studied and sub-Nernstian in regions where there was a highly pH dependent response (50 mV/pH unit maximum). Because pH can be accurately maintained to ± 0.02 units, an uncertainty of ± 1 mV is expected for the latter region. This corresponds to a concentration error of $\pm 4\%$. However, errors of less than 1% would be expected in regions of less pH dependence, i.e. 10 - 15 mV/pH unit.

The electrodes reported here provide a rapid, inexpensive, and reliable method for analysis through minimum sample preparation, low cost, and high selectivity. Detection limits of 10^{-5} M or lower along with stability in various matrices further enhances their practicality in assays of these and other pharmaceuticals which are protonated amines. Though no commercial preparations were tested here, a suitable procedure would simply involve sample dissolution into an appropriate pH buffer followed by potentiometric measurement. As demonstrated, neutral fillers need not be extracted or accounted for during analysis. It is, of course, recommended that electrode

calibration be confirmed periodically between measurements via a procedure analogous to standardization of a glass pH electrode, i.e. a "two-point" calibration, thereby avoiding errors due to drift.

Perhaps of greater interest is the use of potentiometric sensors for in-vivo monitoring of these drugs during pharmacological or related studies. The way in which each of these drugs inhibit Ca^{+2} uptake by smooth muscle cells, a required step for cellular contraction, is as yet unknown.^{16,17} For this reason a drug monitor would be beneficial in investigations aimed at resolving such problems.

Credit. This research was funded by a grant from the
Office of Naval Research

Literature Cited

1. Martin, C.R.; Freiser, H. Anal. Chem., 1980, 52, 562.
2. Cunningham, L.; Freiser, H. Anal. Chim. Acta., 1981, 132, 43.
3. Martin, C.R.; Freiser, H. Anal. Chem., 1980, 52, 1772.
4. Yamada, T.; Freiser, H. Anal. Chim. Acta., 1981, 125, 179.
5. Cunningham, L.; Freiser, H. Anal. Chim. Acta., 1982, 139, 97.
6. Awan, N.A.; DeMaria, A.N.; and Mason, D.T. Drugs, 1982, 23, 235.
7. Sanders, H.J. Chem. Eng. News, 1982, 60(28), 26
8. Schulthess, P.; Shijo, Y; Pham, H.V.; Pretsch, E.; Ammann, D.; Simon, W. Anal. Chim. Acta, 1981, 131, 111-116.
9. Amman, D.; Lanter, F.; Steiner, R.A.; Schulthess, P.; Shijo, Y.; Simon, W. Anal. Chem., 1981, 53, 2267-2269.
10. Danesi, P.R.; Chirizia, R.; Scibona, G., J. Inorg. Nuc. Chem., 1973, 35, 3926.
11. Martin, C.R.; Freiser, H. Anal. Chem., 1979, 51, 803.
12. Martin, C.R.; Freiser, H. J. Chem Ed., 1980, 57, 512.
13. International Union of Pure and Applied Chemistry, Pure. Appl. Chem., 1976, 48, 129.
14. Srinivasan, K; Rechnitz, G. Anal. Chem., 1969, 41, 1203.
15. Hansch, C.; Leo, A.J. Substituent Constants for Correlation Analysis in Chemistry and Biology, J. Wiley, New York, 1979.
16. Schwartz, A. Am. J. Card., 1982, 49, 497.
17. Millard, R.W.; Lathrop, D.A.; Grupp, G.; Ashraff, M.; Grupp, I.; Schwartz, A. Am. J. Card., 1982, 49, 499.

Table I

<u>Drug</u>	<u>Slope</u>	<u>St. Dev.</u>	<u>Y-intcpt.</u>	<u>St. Dev.</u>
Acebutalol	57.7	+/- 3.0	156	+/- 23
Diltiazem	60.1	+/- 1.7	274	+/- 10
Lidoflazine	60.0	+/- 1.5	311	+/- 8
Verapamil	58.7	+/- 1.7	310	+/- 5
Nicardipine	58.8	+/- 1.6	393	+/- 11

Table II

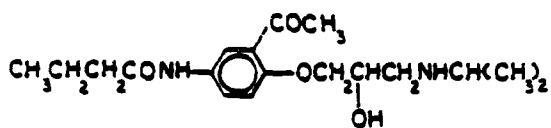
Drug	Log $k_{i,TBA}^{pot}$	+/-	St.Dev.	Log K_D
Acebutalol	2.44	+/-	0.2	-0.2
Diltiazem	0.71	+/-	0.02	2.3
Lidoflazine	0.30	+/-	0.02	5.3
Verapamil	- 0.32	+/-	0.02	5.9
Nicardipine	- 0.89	+/-	0.02	6.3

LEGENDS FOR FIGURES

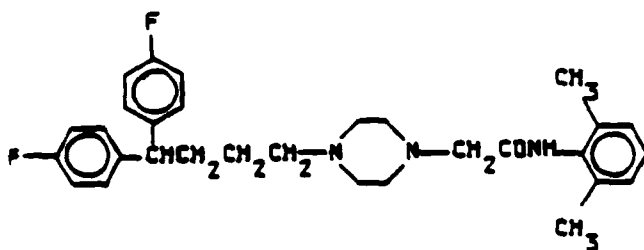
Figure 1. Structures of drugs.

Figure 2. Calibration curves of CWE's for Nicardipine (A), Lidoflazine (B), Verapamil (C), Diltiazem (D), and Acebutalol (E)

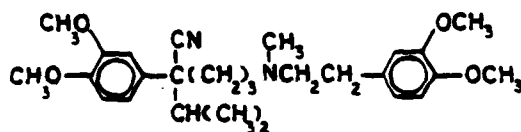
Figure 3. pH responses of CWE's for Nicardipine (A), Acebutalol (B), Verapamil (C), Diltiazem (D), Lidoflazine (E).



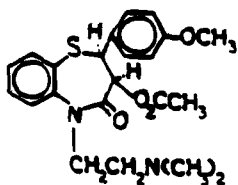
I. Acebutalol



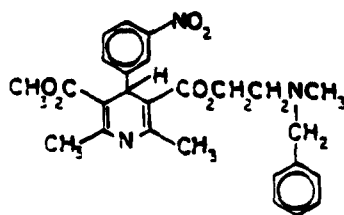
II. Lidoflazine



III. Verapamil

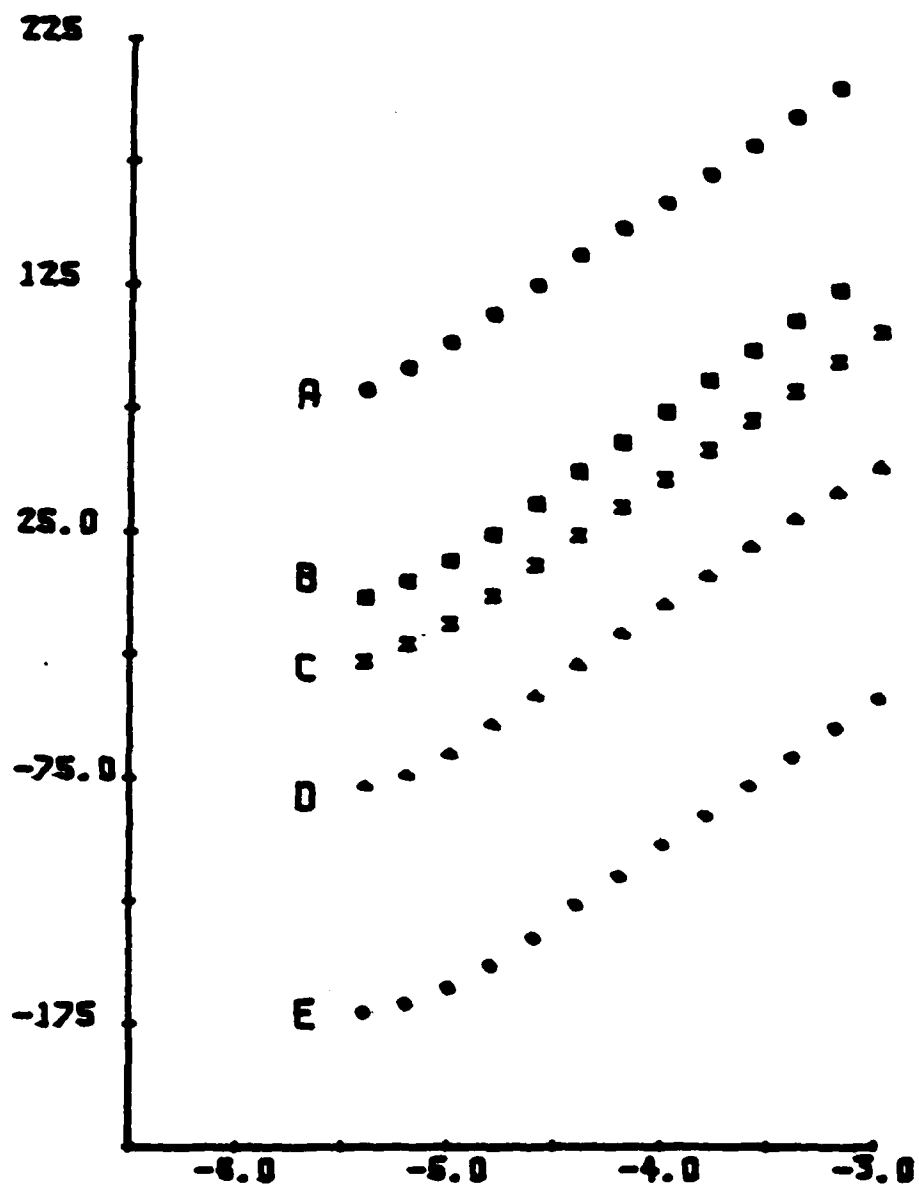


IV. Diltiazem



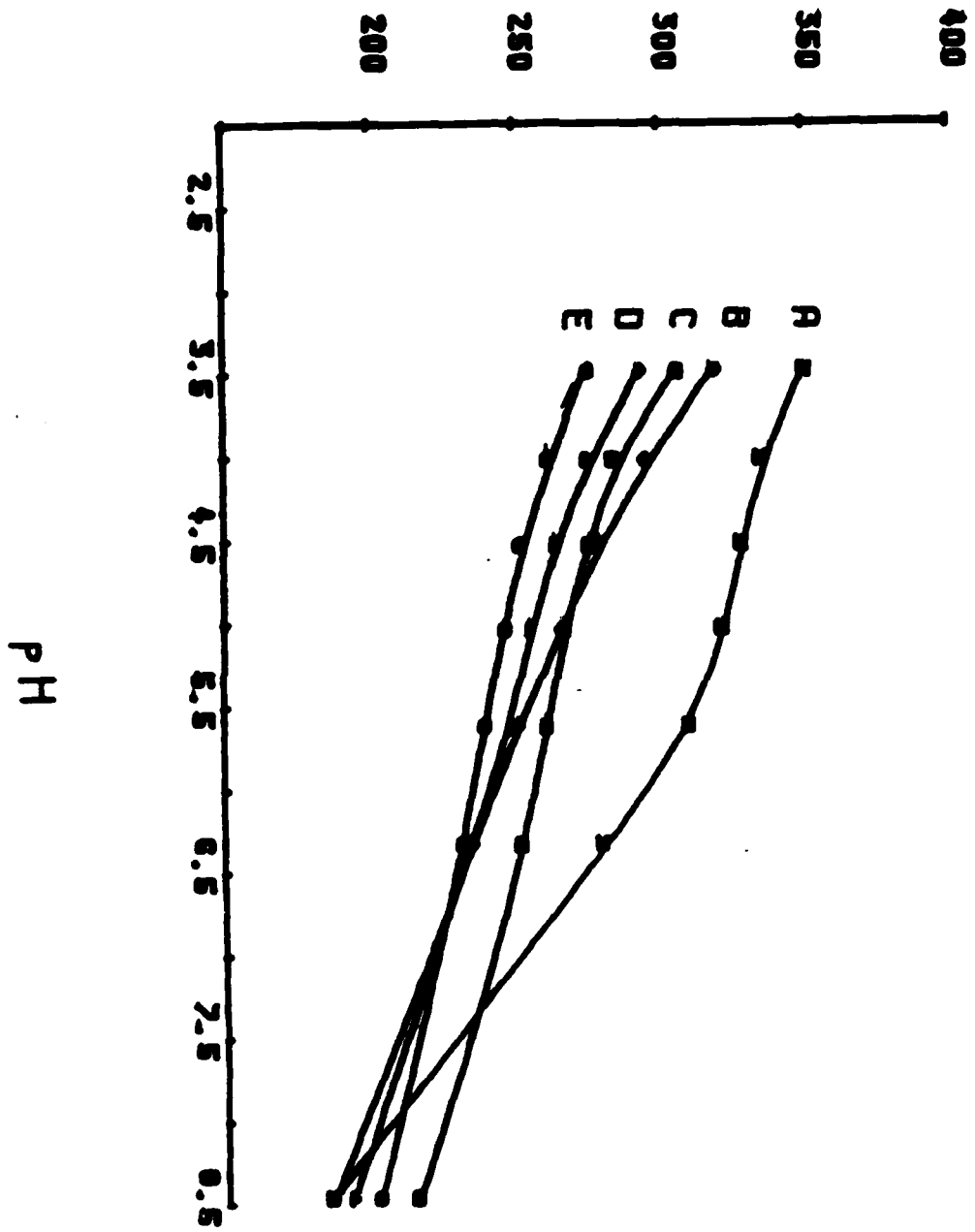
V. Nicardipine

mVs vs CALOMEL REF.



LOG (DRUG)

mVs vs Ag/AgCl



TECHNICAL REPORT DISTRIBUTION LIST, 051C

	<u>No. Copies</u>		<u>No. Copies</u>
Dr. M. B. Denton Department of Chemistry University of Arizona Tucson, Arizona 85721	1	Dr. L. Jarris Code 6100 Naval Research Laboratory Washington, D.C. 20375	1
Dr. R. A. Osteryoung Department of Chemistry State University of New York at Buffalo Buffalo, New York 14214	1	Dr. John Duffin, Code 62 Dn United States Naval Postgraduate School Monterey, California 93940	1
Dr. J. Osteryoung Department of Chemistry State University of New York Buffalo, New York 14214	1	Dr. G. M. Hieftje Department of Chemistry Indiana University Bloomington, IN 47401	1
Dr. B. R. Kowalski Department of Chemistry University of Washington Seattle, Washington 98105	1	Dr. Victor L. Rehn Naval Weapons Center Code 3813 China Lake, California 93555	1
		Dr. Christie G. Enke Michigan State University Department of Chemistry East Lansing, Michigan 48824	1
Dr. D. L. Venezky Naval Research Laboratory Code 6130 Washington, D.C. 20375	1	Dr. Kent Eisentraut, MBT Air Force Materials Laboratory Wright-Patterson AFB, Ohio 45433	1
Dr. H. Freiser Department of Chemistry University of Arizona Tucson, Arizona 85721		Walter G. Cox, Code 3632 Naval Underwater Systems Center Building 148 Newport, Rhode Island 02840	1
Dr. H. Chernoff Department of Mathematics Massachusetts Institute of Technology Cambridge, Massachusetts 02139	1	Professor Isiah M. Warner Department of Chemistry Emory University Atlanta, Georgia 30322	
Dr. A. Zirino Naval Undersea Center San Diego, California 92132	1	Professor George H. Morrison Department of Chemistry Cornell University Ithaca, New York 14853	1

TECHNICAL REPORT DISTRIBUTION LIST, GEN

	<u>No.</u> <u>Copies</u>		<u>No.</u> <u>Copies</u>
Office of Naval Research Attn: Code 413 800 North Quincy Street Arlington, Virginia 22217	2	Naval Ocean Systems Center Attn: Mr. Joe McCartney San Diego, California 92152	1
ONR Pasadena Detachment Attn: Dr. R. J. Marcus 1030 East Green Street Pasadena, California 91106	1	Naval Weapons Center Attn: Dr. A. B. Amster, Chemistry Division China Lake, California 93555	1
Commander, Naval Air Systems Command Attn: Code 310C (H. Rosenwasser) Department of the Navy Washington, D.C. 20360	1	Naval Civil Engineering Laboratory Attn: Dr. R. W. Drisko Port Hueneme, California 93401	1
Defense Technical Information Center Building 5, Cameron Station Alexandria, Virginia 22314	12	Dean William Tolles Naval Postgraduate School Monterey, California 93940	1
Dr. Fred Saalfeld Chemistry Division, Code 6100 Naval Research Laboratory Washington, D.C. 20375	1	Scientific Advisor Commandant of the Marine Corps (Code RD-1) Washington, D.C. 20380	1
U.S. Army Research Office Attn: CRD-AA-IP P. O. Box 12211 Research Triangle Park, N.C. 27709	1	Naval Ship Research and Development Center Attn: Dr. G. Bosmajian, Applied Chemistry Division Annapolis, Maryland 21401	1
Mr. Vincent Schaper DTNSRDC Code 2803 Annapolis, Maryland 21402	1	Mr. John Boyle Materials Branch Naval Ship Engineering Center Philadelphia, Pennsylvania 19112	1
Naval Ocean Systems Center Attn: Dr. S. Yamamoto Marine Sciences Division San Diego, California 91232	1	Mr. A. M. Anzalone Administrative Librarian PLASTEC/ARRADCOM Bldg 3401 Dover, New Jersey 07801	1

TECHNICAL REPORT DISTRIBUTION LIST, 051C

	<u>No.</u> <u>Copies</u>		<u>No.</u> <u>Copies</u>
Dr. M. B. Denton Department of Chemistry University of Arizona Tucson, Arizona 85721	1	Dr. L. Jarris Code 6100 Naval Research Laboratory Washington, D.C. 20375	1
Dr. R. A. Osteryoung Department of Chemistry State University of New York at Buffalo Buffalo, New York 14214	1	Dr. John Duffin, Code 62 Dn United States Naval Postgraduate School Monterey, California 93940	1
Dr. J. Osteryoung Department of Chemistry State University of New York Buffalo, New York 14214	1	Dr. G. M. Hieftje Department of Chemistry Indiana University Bloomington, IN 47401	1
Dr. B. R. Kowalski Department of Chemistry University of Washington Seattle, Washington 98105	1	Dr. Victor L. Rehn Naval Weapons Center Code 3813 China Lake, California 93555	1
		Dr. Christie G. Enke Michigan State University Department of Chemistry East Lansing, Michigan 48824	1
Dr. D. L. Venezky Naval Research Laboratory Code 6130 Washington, D.C. 20375	1	Dr. Kent Eisentraut, MBT Air Force Materials Laboratory Wright-Patterson AFB, Ohio 45433	1
Dr. H. Freiser Department of Chemistry University of Arizona Tucson, Arizona 85721		Walter G. Cox, Code 3632 Naval Underwater Systems Center Building 148 Newport, Rhode Island 02840	1
Dr. H. Chernoff Department of Mathematics Massachusetts Institute of Technology Cambridge, Massachusetts 02139	1	Professor Isiah M. Warner Department of Chemistry Emory University Atlanta, Georgia 30322	
Dr. A. Zirino Naval Undersea Center San Diego, California 92132	1	Professor George H. Morrison Department of Chemistry Cornell University Ithaca, New York 14853	1

END

DATE
FILMED

7-83

DTIC