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An Analytical Study of Berberine

W. Riley McGaughran

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ANALYTICAL
STUDY OF
BERBERIN

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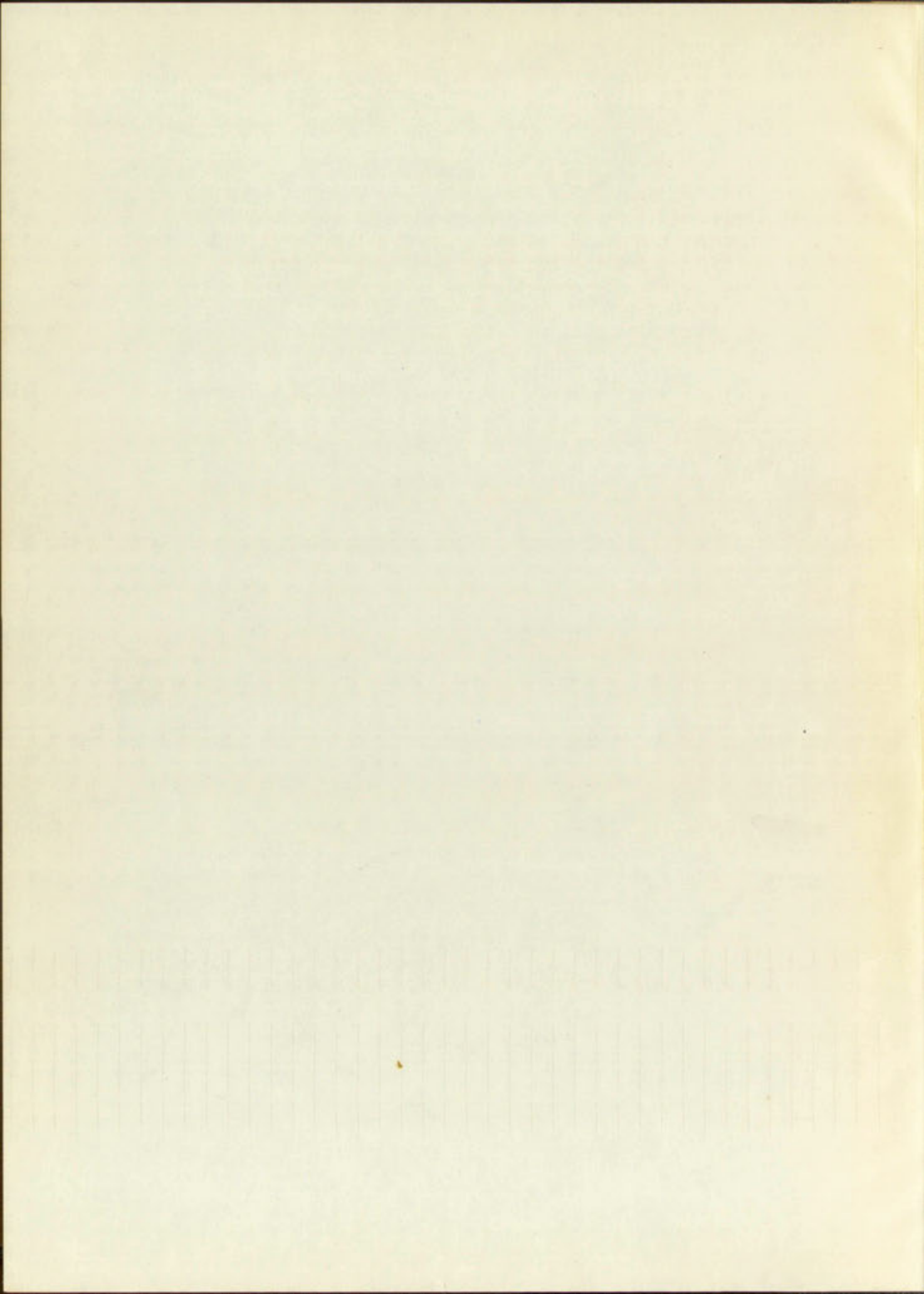
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AN ANALYTICAL STUDY OF
BERBERINE

By

W. Riley McGaughran

A Thesis

In partial fulfillment of the
Requirements for the Degree of
Master of Science in Chemistry

The University of New Mexico
1950

AN INVESTIGATION OF THE

EFFICIENCY OF

18

W. R. RILEY, M.D.

EFFICIENCY OF EZERASE BOND IN THE REMOVAL OF PAC CONTENT

In partial fulfillment of the
Requirements for the Degree of
Master of Science in Chemistry

The University of New Mexico
1955

This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

E. H. Castetter
DEAN

August 7, 1950
DATE

AN ANALYTICAL STUDY
OF BERBERINE

Thesis committee

E. L. Martin
CHAIRMAN

Raymond N. Castle

William J. Koster

J. L. Kiebsomer

This thesis directed and approved by the candidate's committee has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

E. J. Cantler

Accepted for the degree of

AN ANALYTICAL STUDY
OF STEREOTYPE

Thesis Committee

W. J. Cantler
Chairman

W. J. Cantler
W. J. Cantler

W. J. Cantler

W. J. Cantler

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FIGURE

1. Polarographic cell's construction and polarographic measurements
2. Electrolytic apparatus

CHAPTER I

THE PROBLEM

Berberine, an alkaloid, has unique pharmacological properties as well as unusual chemical and physical characteristics. This compound has been used for many years as a remedy for eye diseases. In contrast to most alkaloids, berberine hydroxide is more soluble than its salts. Its chemical structure has been established, but a correlation of many of its properties and its structure has not been made.

The purpose of this work was to undertake an analytical study of berberine, hoping to collect data which would make possible the correlation of certain properties of the compound with its structure. The study was planned to include reactions involving electro-reducible groups present in the molecule and was to be executed primarily by polarographic and spectrophotometric methods.

CHAPTER I

THE SUBJECT

Berberine, an alkaloid, has unique pharmacological properties as well as unusual chemical and physical characteristics. This compound has been used for many years as a remedy for eye diseases. In contrast to most alkaloids, berberine hydroxide is more soluble than the latter. Its chemical structure has been established, but a correlation of many of its properties and its structure has not been made.

The purpose of this work was to undertake a systematic study of berberine, hoping to obtain data which would make possible the correlation of certain properties of the compound with its structure. The work included to include reactions involving electron-reducing agents present in the molecule and was to be executed primarily by polarographic and spectrophotometric methods.

CHAPTER II

REVIEW OF THE LITERATURE

A. LITERATURE ON CHEMISTRY OF BERBERINE

Scientists began the study of berberine in 1826. The research covering its chemistry, especially that involved in the proof of structure, is well summarized by T. A. Henry¹ in his monograph on plant alkaloids. The original chemical research including the synthesis was done chiefly by W. H. Perkin, Jr. between 1889-1925.

Berberine, $C_{20}H_{19}O_5N$, which belongs to the isoquinoline group, is widely distributed in nature, occurring unreduced in Ranunculaceae, Berberidaceae, and other botanical families.

Structural formulas for berberine and related compounds of significance in this work are listed on the following pages.

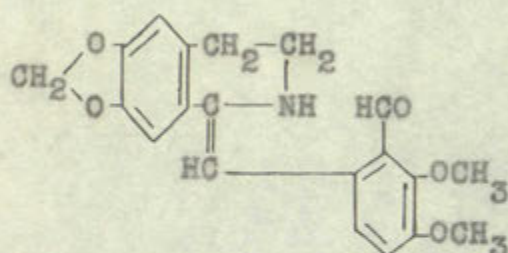
¹Henry, The Plant Alkaloids, (fourth edition Blakiston, 1949), p. 328-336.

CHAPTER II

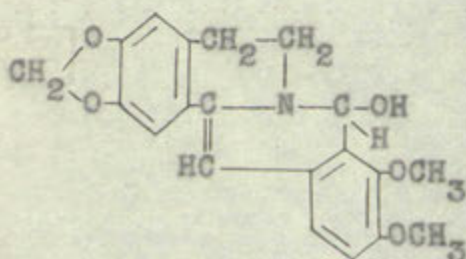
REVIEW OF THE LITERATURE

A. LITERATURE ON THE CHEMISTRY OF BROMINE

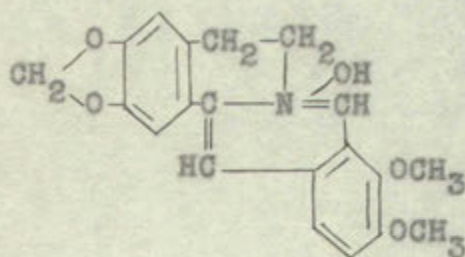
Scientific work on the chemistry of bromine in 1930. The
research covering the chemistry, especially that involving the
the group of elements, is well represented by J. A. Henry
in his monograph on element bromine. The early work on
research including the elements was done mainly by J. A.
Parker, Jr. between 1900-1930.
Bromine, Br_2 , which belongs to the halogen-
oid group, is widely distributed in nature, occurring as
reduced in bromides, bromates, and other bromine
compounds.
Structural formulas for bromine and related com-
pounds of significance in this work are listed on the fol-
lowing pages.



Berberine: Imino-aldehyde form²



Berberine: Carbinol form²



Berberine: Ammonium or quaternary base form²

²Henry, op. cit., p. 333



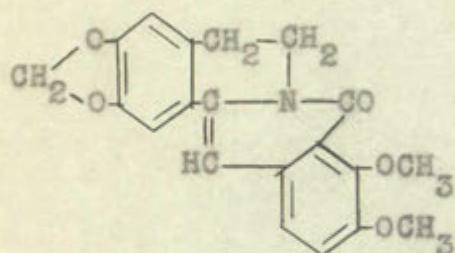
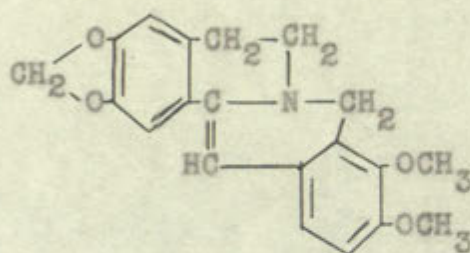
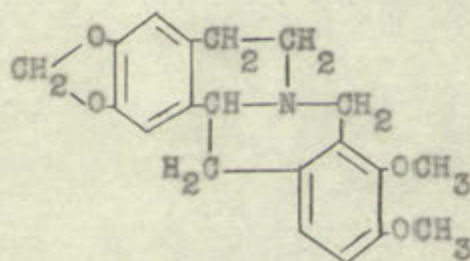
Berberine: 1,2,3,4-tetrahydro-5,6-dimethoxy-1,2,3,4-tetrahydroquinoline



Berberine: 1,2,3,4-tetrahydro-5,6-dimethoxy-1,2,3,4-tetrahydroquinoline



Berberine: 1,2,3,4-tetrahydro-5,6-dimethoxy-1,2,3,4-tetrahydroquinoline

Oxyberberine³Dihydroberberine³Tetrahydroberberine^{4, 5}

Epiberberine⁶: Same as berberine, with methylenedioxy and ortho-dimethoxy groups reversed.

³Tinkler, J. Chem. Soc., 99, 1346-7 (1911)

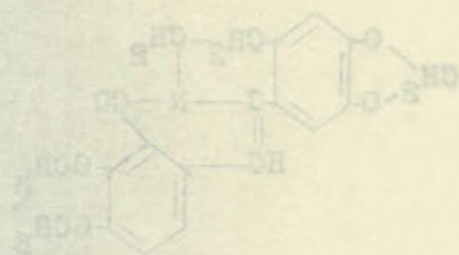
⁴Perkin, Jr., ibid., 113, 764 (1918)

⁵Perkin, Ray and Robinson, ibid., 127, 740 f. (1925)

⁶Perkin, Jr., ibid., 113, 503 (1918)



Epigallocatechin gallate²



Epigallocatechin²



Epigallocatechin gallate²

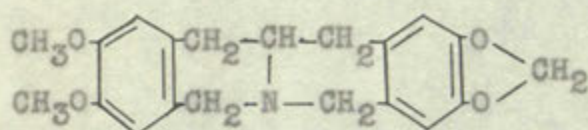
Epigallocatechin²: Same as epigallocatechin, with structure
 given and other chemical groups
 removed.

Epigallocatechin²: Same as epigallocatechin, with structure
 given and other chemical groups
 removed.

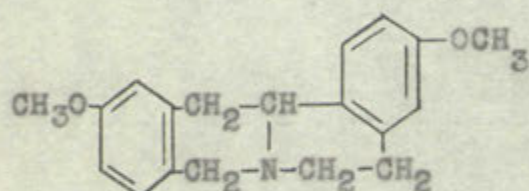
Epigallocatechin²: Same as epigallocatechin, with structure
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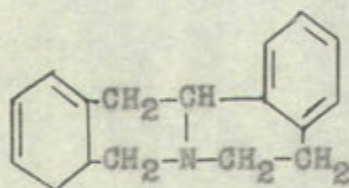
Epigallocatechin²: Same as epigallocatechin, with structure
 given and other chemical groups
 removed.



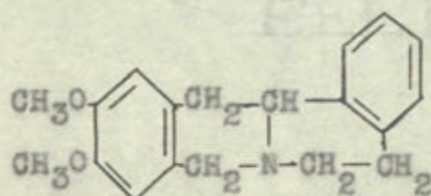
2,4-Methylene-dioxy-11,12-dimethoxy-
6,15,16,17-tetrahydro-paraberberine⁷



3,11-Dimethoxy-tetrahydroprotoberberine⁷



Tetrahydroprotoberberine⁷



Tetrahydro-psi-berberine⁷

⁷Chakravarti, Haworth & Perkin, Jr., ibid., 1927, 2266 f. and 2275.



2,4-dihydroxy-6-methyl-3,5-bis(methoxycarbonyl)benzoic acid



2,4-dihydroxy-6-methyl-3,5-bis(methoxycarbonyl)benzoic acid



2,4-dihydroxy-6-methyl-3,5-bis(methoxycarbonyl)benzoic acid

2,4-dihydroxy-6-methyl-3,5-bis(methoxycarbonyl)benzoic acid



2,4-dihydroxy-6-methyl-3,5-bis(methoxycarbonyl)benzoic acid

The three forms of berberine given above are not entirely confirmed. No evidence has been obtained by experimenters to confirm the imino-aldehyde form, but the other two forms have been suggested by reactions. Reaction of berberine with large excess of alkali yields a mixture of oxyberberine and dihydroberberine⁸. Tetrahydroberberine, a colorless derivative, is obtained by electrolytic reduction. Epiberberine and berberine have similar properties, indicating the similarity of the reversed groups⁹.

The last four compounds given above lack the intense colors characteristic of the berberine structure and do not react as readily. This shows the significance of the rearranged geometrical configurations and of the absence of the catecholic groups¹⁰.

B. LITERATURE ON POLAROGRAPHY

1. Polarography

The word polarograph is a trade name in this country used for instruments made to be utilized in the polarographic

⁸Tinkler, loc. cit.

⁹Perkin, Jr., loc. cit.

¹⁰Chakravarti, Haworth, and Perkin, Jr., loc. cit.

The three forms of berberine given above are not
entirely identical. No evidence has been obtained by
experimenters to confirm the false-aldehyde form, but the
other two forms have been suggested by Tschirner.² The
form of berberine with large excess of alkali yields a
mixture of oxyberberine and dihydroberberine. Dihydrober-
berine, a colorless derivative, is obtained by electro-
lytic reduction. Dihydroberberine and berberine have similar
properties, indicating the similarity of the reversed
groups.³

The last four compounds given above lack the intense
color characteristic of the berberine structure and do not
react as readily. This shows the slight change of the re-
versed structural configuration and of the absence of the
catechol groups.⁴

2. LITERATURE ON POLYMERIZATION OF BERBERINE
The word oligomer is a Greek name for a small number.
used for substances that do not polymerize in the ordinary way.

²Tschirner, *Ann.*, 1901, 311.
³Reichstein, *Ann.*, 1901, 311.
⁴Reichstein, *Ann.*, 1901, 311.

method of analysis as outlined by Heyrovsky. This process of analysis has become so widespread that the term polarograph and related words, polarographic, polarogram, polarography, have become commonly used words associated in current literature with Heyrovsky's method¹¹.

The method consists in the application of an increasing potential through a dropping mercury electrode to an electro-oxidizable or electro-reducible substance in dilute solution until decomposition results. The decomposition potential is characteristic and is known as the half wave potential of the substance; under the proper conditions the magnitude of diffusion current obtained is proportional to concentration of substance oxidized or reduced^{12,13,14}.

The theoretical equation for the diffusion current, first derived by Ilkovic, is¹³:

$$i_d = 605nD^{1/2}C_m^{2/3}t^{1/6}.$$

¹¹Kolthoff and Lingane, Polarography, (N.Y., Interscience Publishers, Inc., 1941), pp. 1f., and 55.

¹²Muller, The Polarographic Method of Analysis, (Easton, Pa., Jr. Chem. Ed., 1941), pp. 42-49.

¹³Kolthoff and Lingane, Chem. Revs., 24, 3-4 (1939).

¹⁴Weissberger, Physical Methods of Organic Chemistry, Vol. 2 (N.Y., Interscience Publishers, Inc., 1946), p. 1115.

method of analysis as outlined by Hays. This process of analysis has been so widespread that the term "analysis" and related words, chromatography, colorimetry, etc., have become commonly used words associated in current literature with Hays' work.

The method outlined in this report is an adaptation of the method through a growing medium, electrolysis, or oxidation of electro-oxidizable substances in which the initial decomposition results. The decomposition potential is characteristic and is known as the half-wave potential of the substance; under the proper conditions the magnitude of the current output depends on the concentration of the substance oxidized or reduced.

The theoretical equation for the diffusion current first derived by Ilkovic is:

$$i_d = 607 n D^{1/2} C \nu^{1/2}$$

Ilkovic and Lingane, Electrochemistry, 2nd ed., Interscience Publishers, Inc., New York, 1957, p. 11.

Ilkovic, The Polarographic Determination of Analytes, (London, Pa., J. Chem. Soc., 1959, p. 21).

Ilkovic and Lingane, Electrochemistry, 2nd ed., Interscience Publishers, Inc., New York, 1957, p. 11.

Ilkovic, Physical Methods of Electrochemistry, Vol. 2 (N.Y., Interscience Publishers, Inc., 1959, p. 11).

Recently this equation has been modified to take into account the curvature of the dropping mercury electrode surface, as follows¹⁵:

$$i_d = 607nD^{1/2}C_m^{2/3}t^{1/6} \cdot \left[1 + 39 \frac{D^{1/2}t^{1/6}}{m^{1/3}} \right],$$

where

i_d = average current in microamperes during life of drop;

n = number of faradays of electricity required per mole of electrode reaction;

D = diffusion coefficient of the oxidizable or reducible substance, in the units, square centimeters per second;

C = concentration, in the units, millimoles per liter;

m = rate of flow of mercury from the dropping electrode capillary, in milligrams per second; and

t = drop time, in seconds.

The value of the term in parenthesis is about 1.1 with capillaries of the usual characteristics.

Limiting potentials of the dropping mercury electrode are about +0.4 volt, the oxidation potential of mercury itself, and about -2.0 volts up to -2.9 volts, depending on the decomposition potential of the supporting electrolyte cation and other factors. Potassium, lithium, and tetramethyl ammonium cations have the highest negative decomposition

¹⁵Lingane and Loveridge, J. Am. Chem. Soc., 72, 438, (1950).

Recently this equation has been modified to take into account the curvature of the dropping mercury electrode surface, as follows:

$$i_d = 607 n D^{1/2} C \left[1 + \frac{0.41 V}{r} \right]^{1/2}$$

where

i_d = average current in microamperes during life of drop;

n = number of faradays of electricity required per mole

of electrode reaction;

D = diffusion coefficient of the substance or substance

substance, in the units, square centimeters per

second;

C = concentration, in the units, millimoles per liter;

m = rate of flow of mercury from the dropping electrode

capillary, in milligrams per second; and

t = drop time, in seconds.

The value of the rate in parentheses is about 1.1.

capillaries of the usual characteristics.

Limiting potentials of the dropping mercury electrode

are about +0.4 volt, the oxidation potential of mer-

cury itself, and about -2.0 volt up to -2.5 volt, depending

on the decomposition potential of the supporting electrolyte

and other factors. Potassium, lithium, and cesium

ammonium cations have the highest negative decomposition

potentials and overvoltage, J. A. Harrison, *Anal. Chem.*, 32, 177, 1960.

(1960).

potentials^{16,17}.

Organic compounds, usually reduced irreversibly,^{18,19} must be in buffered solutions for polarographic measurements. Some organic compounds, such as quinine and iso-quinoline type compounds, produce catalytic hydrogen waves²⁰. These are the result of a lowering of the overvoltage of hydrogen on mercury and are proportional to the concentration of the catalytic substance but of a larger order than reduction waves of the same substance.

By means of the controlled electro-oxidation or electro-reduction of a compound, at potentials determined with the polarograph, it is possible not only to measure coulometrically the quantity of electricity involved in the reaction but also to obtain the oxidized or reduced derivatives in quantities large enough for analytical studies²¹.

¹⁶Rysselberghe and McGee, ibid., 67, 1039 (1945).

¹⁷Perley, Trans. Electrochem. Soc., 76, 98 (1939).

¹⁸Kolthoff and Lingane, op. cit., Chem., Revs., p. 79.

¹⁹Müller, Chem. Revs., 24, 105 (1939).

²⁰Kolthoff and Lingane, Polarography, loc. cit.

²¹Lingane, J. Am. Chem. Soc., 67, 1916 (1945).

Organic compounds, mainly reduced hydrocarbons, must be in buffered solutions for electrochemical analysis. Some organic compounds, such as alcohols and aldehydes, are the result of a reaction of the electrode with hydrogen on mercury and are objectionable to the determination of the catalytic substance due to a large over-potential wave of the same substance.

By means of the controlled electro-oxidation or reduction of a compound, its potential is determined with the polarograph, it is possible not only to measure the quantity of the substance involved in the reaction but also to obtain the nature of the product. Quantities large enough for analytical studies.

-
18. Wassermann and Weiss, Z. 1934 (1935).
 19. Conley, J. and Weiss, Z. 1934 (1935).
 20. Kolthoff and Weiss, Z. 1934 (1935).
 21. Witt, G. and Weiss, Z. 1934 (1935).
 22. Kolthoff and Weiss, Z. 1934 (1935).
 23. Witt, G. and Weiss, Z. 1934 (1935).

2. Polarography of berberine

In the literature three authors have reported polarographic studies on berberine.

H. F. W. Kirkpatrick²² found reduction waves for berberine solutions in the range of $1 \times 10^{-4}M$ berberine, in acetate buffers, as shown in Table I.

TABLE I
REDUCTION WAVES OF BERBERINE SOLUTIONS

pH:		4	5	6	7	8
Reduction wave, half-wave potential versus S.C.E., volts	1st.	-1.07	-1.05	-1.06	-1.03	-1.02
	2nd.	-1.19	-1.21	-1.25	-1.25	-1.21
	3rd.	-	-	-	-1.50	-
Catalytic wave decomposition potential (estimated from curves) volts		-	-1.7	-1.75	-1.7	-1.65

Kirkpatrick found²³ that the reduction waves were subject to considerable variation with concentration, "...presumably due to tautomeric changes. . .", and that the half wave potentials also varied with concentration. At a pH of 3, in the range of $0.75-1.5 \times 10^{-4}M$ berberine, he found a linear relationship between the diffusion current

²²Kirkpatrick, Quart. Jr. Pharm. Pharmacol. (London), 19, 11 (1946).

²³Ibid., p. 10.

2. Volatility of hydrogen
 In the literature there is no reported polarographic studies on hydrogen.
 H. F. W. Kirkpatrick²² found reduction waves for hydrogen in the range of 1.0V to 1.2V in acetate buffer, as shown in Table I.

TABLE I

REDUCTION WAVES OF HYDROGEN IN ACETATE BUFFER

Conc.	E _{1/2}	E _{1/2}	E _{1/2}	E _{1/2}	E _{1/2}
Reduction wave	1.15	1.07	1.05	1.03	1.01
Half-wave potential	1.12	1.10	1.08	1.06	1.04
versus S.C.E., volts	2.0	2.0	2.0	2.0	2.0
Catalytic wave					
decomposition potential					
(estimated from curves)					
volts	1.7	1.7	1.7	1.7	1.7

Kirkpatrick found²² that the reduction waves were subject to considerable variation with concentration. . . . presumably due to adsorption effects. . . . and that the half wave potential also varied with concentration. At a pH of 5, in the range of 0.05-0.20M hydrogen, he found a linear relationship between the diffusion current

²²Kirkpatrick, Trans. Faraday Soc., **47**, 100 (1951).
 12. 11 (1951).
 Table I, p. 10.

and concentration of berberine, the value of $i_d/C_m^{2/3}t^{1/6}$ being 3.37. From the Ilkovich relationship and the value of the diffusion coefficient of berberine computed from its molar volume, he deduced that two electrons were required for the reduction of one molecule of the alkaloid, so that the wave corresponded to the formation of dihydroberberine.

O. F. Uffellie²⁴ stated that berberine had a conjugated double bond with nitrogen, which bond was reducible to give tetrahydroberberine. He found in an acid medium a reduction wave at -0.97 volts, with the curve rising gradually, "... likely on account of a secondary reaction taking place. ...". In a neutral medium he obtained two reduction waves, less clear, at -1.08 volts and -1.65 volts. In an alkaline medium he obtained the same waves but at a more negative potential, -1.14 volts, -1.75 volts.

F. Santavy²⁵ obtained a series of polarograms on berberine in a pH range of 2 to 12, using Britton and Robinson buffers²⁶. Half wave reduction potentials estimated from his curves are shown in Table II.

²⁴Uffellie, Polarografisch Onderzoek van Enige Alkaloiden, (Utrecht, Keminkten Zoon, 1945), p. 101-2.

²⁵Santavy, Coll. Czechoslov. Chem. Commun. XIV 377-87 (1949).

²⁶Britton, Hydrogen Ions (2nd. Ed., N. Y., Van Nostrand Co., Inc., 1932) p. 225.

TABLE II
REDUCTION POTENTIALS OF BERBERINE SOLUTIONS

Approximately $1 \times 10^{-3} M$ Berberine; half wave potentials, volts

pH:	<u>2-6</u>	<u>7-8</u>	<u>9-10</u>	<u>10.5-12</u>
1st.	-0.7*	-0.68*	-0.64*	-1.0*
2nd.	-	-1.3 to -1.4	-1.3 to -1.4	-1.3**
3rd.	-	-1.7 to -1.8	-1.75 to -1.8	-1.8**

Approximately $2 \times 10^{-4} M$ Berberine; half wave potentials, volts

pH:	<u>2-6</u>	<u>7-8</u>	<u>9-10</u>	<u>10.5-12</u>
1st.	-0.8*	-0.8*	-0.8*	-0.9*
2nd.	-1.0	-1.2	-0.9*	-1.3*
3rd.	-	-1.4; -1.6	-1.3	-1.7*

* waves small.

** waves, at pH of 2-12, proportional to concentration of berberine.

TABLE II

REDUCTION POTENTIALS IN AQUEOUS SOLUTION

Approximately 1x10⁻⁴ M; 25°C; pH 7.0; all values are standard potentials

	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
1st	-0.76	-0.52	-0.34	-0.16	0.00	0.16	0.34	0.52	0.76	0.92	1.08	1.24
2nd	-	-	-1.32 to -1.4	-1.4 to -1.5	-1.5 to -1.6	-1.6 to -1.7	-1.7 to -1.8	-1.8 to -1.9	-1.9 to -2.0	-2.0 to -2.1	-2.1 to -2.2	-2.2 to -2.3
3rd	-	-	-1.7 to -1.8	-1.8 to -1.9	-1.9 to -2.0	-2.0 to -2.1	-2.1 to -2.2	-2.2 to -2.3	-2.3 to -2.4	-2.4 to -2.5	-2.5 to -2.6	-2.6 to -2.7

Approximately 1x10⁻⁴ M; 25°C; pH 7.0; all values are standard potentials

	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
1st	-0.76	-0.52	-0.34	-0.16	0.00	0.16	0.34	0.52	0.76	0.92	1.08	1.24
2nd	-	-	-1.32 to -1.4	-1.4 to -1.5	-1.5 to -1.6	-1.6 to -1.7	-1.7 to -1.8	-1.8 to -1.9	-1.9 to -2.0	-2.0 to -2.1	-2.1 to -2.2	-2.2 to -2.3
3rd	-	-	-1.7 to -1.8	-1.8 to -1.9	-1.9 to -2.0	-2.0 to -2.1	-2.1 to -2.2	-2.2 to -2.3	-2.3 to -2.4	-2.4 to -2.5	-2.5 to -2.6	-2.6 to -2.7

* waves small.

** waves, at pH of 2-12, reversed to concentration of pyridine.

As these different reduction potentials indicate, considerable variation is possible and more information on the factors causing the variation is needed. Santavy's curves show that the effect of pH can be divided into three categories. At a pH in the acid range up to about 5 there is little change in the reduction potential of berberine with variation of pH; in the neutral range, at a pH of 6 to 7, there is a zone of transition; in the alkaline range, 8 and above, reduction occurs in additional waves of different reduction potentials as compared with the acid range.

The nature of the reduced compounds of berberine has been deduced but not proved.

In addition to berberine, Kirkpatrick²⁷⁻³² made studies of other alkaloids in the isoquinoline group, which in some respects are comparable to berberine. These are summarized partially in Table III. Structures are shown in Table IV.

²⁷Kirkpatrick, Quart. Jr. Pharm. Pharmacol. (London) 18, 245 (1945).

²⁸Ibid., 18, 338 (1945).

²⁹Ibid., 19, 8 (1946).

³⁰Ibid., 19, 127.

³¹Ibid., 19, 526.

³²Ibid., 20, 87 (1947).

As these different reactions are observed, considerable variation in results may be expected in the factors causing the variation in results. The curves show that the effect of α can be divided into two categories. At a α in the first range, as α increases, is little change in the reduction potential of the system with variation of α ; in the second range, as α increases, there is a large variation in the reduction potential. In the second range, reduction occurs in addition to the first reduction potential, as compared with the first range.

The nature of the reduced compounds of the various elements has been deduced but not verified.

In addition to the various reactions, some of other elements in the periodic table, such as some reactions are comparable to the reactions of the elements mentioned partially in Table IV. Reactions are shown in

Table IV.

27	Aluminum, 19, 1945.
(London)	19, 1945.
28	Ibid., 19, 1945.
29	Ibid., 19, 1945.
30	Ibid., 19, 1945.
31	Ibid., 19, 1945.
32	Ibid., 19, 1945.

TABLE III

POLAROGRAPHIC DATA ON SOME ALKALOIDS

Alkaloid	Concentration	Wave Type	Reduction Potentials ^a Catalytic Potentials ^b versus S.C.E., volts	Reduction Factor e/m ²	Reduction $i_d/Cm^{2/3}t^{1/6}$ Ilkovic equation factor
Cotarnine	1×10^{-3} to 1×10^{-4}	pH: reduction	$\frac{3-4}{-1.1}$ $\frac{5-8}{-1.0}$; -1.15	1 + 1	3.21
Narceine	1×10^{-3}	pH: reduction	$\frac{3-7}{-1.2 \text{ to } -1.45}$	2	3.26
Papaverine	1×10^{-4} 1×10^{-4}	pH: reduction catalytic	$\frac{3-10}{-1.45}$ $\frac{6}{-}$ $\frac{8}{-}$ -1.6 -1.6	2	3.5
Narootine	1×10^{-4} to 1×10^{-6}	pH: catalytic	$\frac{6}{-1.5}$	-	-
	1×10^{-3} to 1×10^{-4}	catalytic	-	-1.5	-

TABLE III (continued)

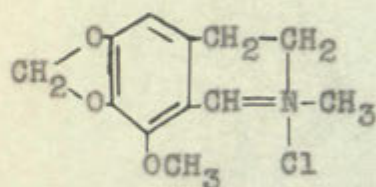
POLAROGRAPHIC DATA ON SOME ALKALOIDS

Alkaloid	Concentration	Wave Type	Reduction Potentials ^a Catalytic Potentials ^b versus S.C.E., volts	Reduction Factor ^a e/m	$i_d/Cm^{2/3}t^{1/6}$ Ilkovic equation factor
Hydrastine: smaller catalytic waves than narcotine, occurring in similar manner					
Hydrastinine	1×10^{-3}	pH:	3-6	7-10	
		reduction	-1.1 to -1.2	-1.1 to -1.0; -1.3	2 3.29
Colchicine	1×10^{-3}	pH:	3-4	6 10	
		reduction	-1.3	-1.35; -1.65	1 + 1 2 acid:1.42 alk.:2.63
Strychnine and Brucine		pH:	6	8	
		catalytic	-1.65 approximate	-1.55	

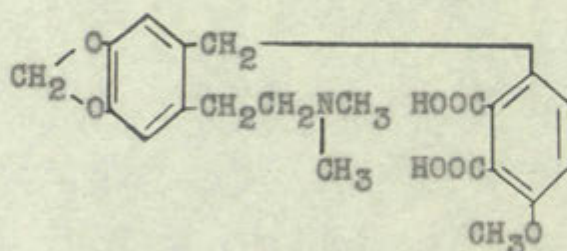
^aReduction Potentials, half-wave potentials; ^bCatalytic Potentials, self explanatory; 0.9.C.E., saturated calomel electrode.

* e/m, electrons per molecule.

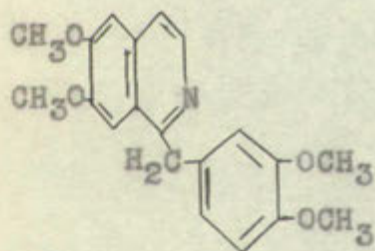
TABLE IV
STRUCTURES OF ALKALOIDS IN TABLE III



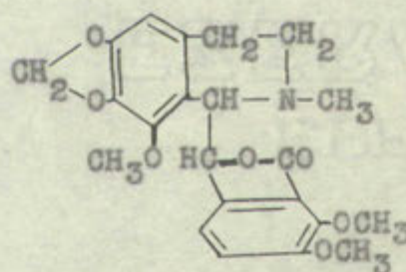
Cotarnine chloride³³
(pale yellow color)



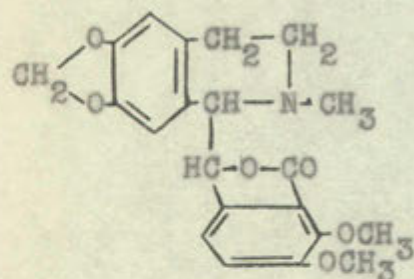
Narceine (inactive)³⁴
(colorless)



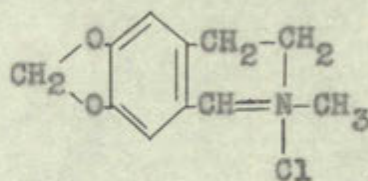
Papaverine³³
(colorless)



Narcotine³³
(colorless)



Hydrastine³³
(colorless)



Hydrastinine chloride³³
(yellow color)

Tetrahydropapaverine: same as papaverine with pyridine ring saturated.

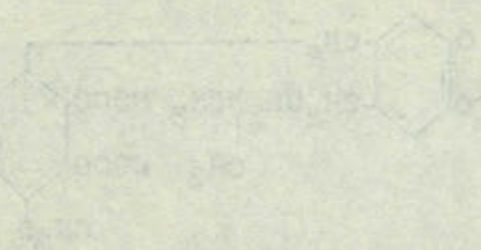
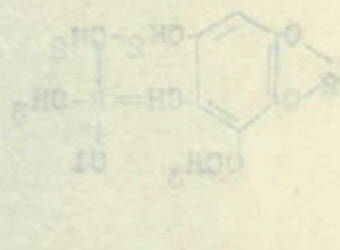
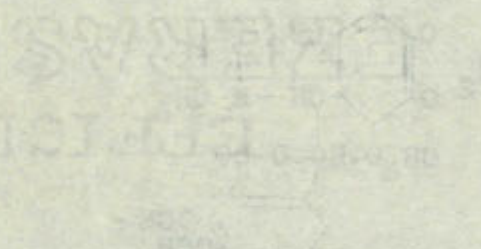


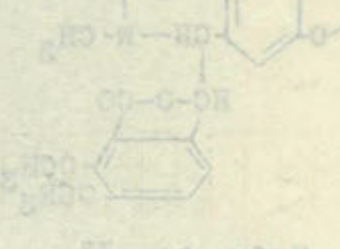
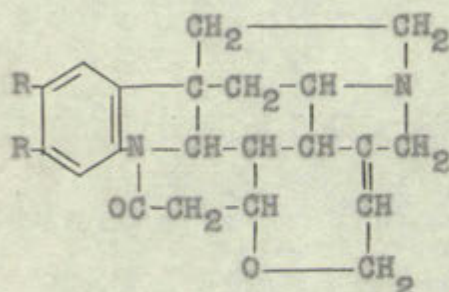
TABLE IV	STRUCTURES OF ALKALOIDS IN T. B. 117
	
<p>Gutaric acid chloride (pale yellow color)</p>	<p>Gutaric acid chloride (pale yellow color)</p>
	
<p>Papaverine (colorless)</p>	<p>Papaverine (colorless)</p>
	
<p>Hydrastine (pale yellow)</p>	<p>Hydrastine (pale yellow)</p>
<p>Tetrahydropapaverine: same as papaverine with two methoxy groups.</p>	<p>Tetrahydropapaverine: same as papaverine with two methoxy groups.</p>

TABLE IV (continued)
STRUCTURE OF ALKALOIDS IN TABLE III

Colchicine, $C_{22}H_{25}O_6N$ ^{35,36}: structure uncertain
(yellow color)



R = H-

Strychnine³³

R = CH_3O-

Brucine³³

(both colorless)

Laudanosine: same as tetrahydropapaverine with methyl group on nitrogen atom.

³³Gilman, Organic Chemistry, Vol. II, (Second Edition, N. Y. C., J. Wiley and Sons, Inc. 1943), pp. 1211-17, 1220, 1236.

³⁴Kirkpatrick, op. cit., 20, p. 88.

³⁵Ibid., 19, p. 533.

³⁶Lange, Handbook of Chemistry, (Fourth Edition, Sandusky, Ohio, Handbook Publishers, Inc., 1941), p. 506.

TABLE IV (continued)

STRUCTURE OF ANALOGS OF 1,2,3,4,5-PENTAZOLONE

Colophane, $C_{15}H_{12}O_2$, $M_p 155-156^\circ$, $d_4^{25} 1.075$, $n_D^{25} 1.515$

(yellow solid)



1,2,3,4,5-Pentazalone

1,2,3,4,5-Pentazalone

(both enantiomers)

1,2,3,4,5-Pentazalone: same as 1,2,3,4,5-Pentazalone with methyl group on nitrogen atom.

1,2,3,4,5-Pentazalone, $C_{15}H_{12}O_2$, $M_p 155-156^\circ$, $d_4^{25} 1.075$, $n_D^{25} 1.515$, $IR 1720, 1620, 1520, 1420, 1320, 1220, 1120, 1020, 920, 820, 720, 620, 520, 420, 320, 220, 120, 20$

1,2,3,4,5-Pentazalone, $C_{15}H_{12}O_2$, $M_p 155-156^\circ$, $d_4^{25} 1.075$, $n_D^{25} 1.515$

1,2,3,4,5-Pentazalone, $C_{15}H_{12}O_2$, $M_p 155-156^\circ$, $d_4^{25} 1.075$, $n_D^{25} 1.515$

1,2,3,4,5-Pentazalone, $C_{15}H_{12}O_2$, $M_p 155-156^\circ$, $d_4^{25} 1.075$, $n_D^{25} 1.515$, $IR 1720, 1620, 1520, 1420, 1320, 1220, 1120, 1020, 920, 820, 720, 620, 520, 420, 320, 220, 120, 20$

Kirkpatrick³⁷ stated that the catalytic waves were probably due to the reduction of hydrogen ions to atomic hydrogen. Since the height of the wave was normally a function of the concentration of the alkaloid, the course of the reaction could be a preliminary transfer of electrons from the drop of mercury to the alkaloid molecules, and the subsequent transfer to hydrogen ions. The catalytic power, in such a mechanism, ". . . would be dependent upon the electron-accepting capacity of the alkaloid molecule, which is determined by electron displacements and electrical effects inherent to the molecule . . ."

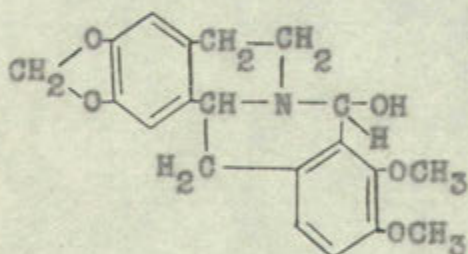
This author found³⁸ in the irreducibility of strychnine and brucine and the reducibility of colchicine further support for the conclusions of other authors³⁹ that the carbonyl group in aromatic compounds was not polarographically reducible unless a double bond was in conjugation with the carbonyl. The waves produced by strychnine and brucine in acid solution, as found by Kirkpatrick, were of catalytic origin and could not be associated with the carbonyl group in their structure, especially since it was shown that colchicine was catalytically inactive.

³⁷Kirkpatrick, op. cit., 20, p. 89.

³⁸Ibid., p. 92.

³⁹Eisenbrand and Picher, Z. Physiol. Chem., 260, 83 (1939), cited by Kirkpatrick, loc. cit.

According to Kirkpatrick's results⁴⁰, the reduction of berberine involved two electrons, and the reduced product was dihydroberberine. However, berberine is also electro-reducible to tetrahydroberberine⁴¹. If these reductions occur separately, berberine to dihydroberberine and dihydroberberine to tetrahydroberberine, then the question arises where each reduction takes place. Structures for the three possible theoretical structural forms of berberine, and also for oxyberberine, dihydroberberine, and tetrahydroberberine have been given (page 3). The other possible reduced product involving only two electrons is ethylene dihydroberberine:



⁴⁰Kirkpatrick, op. cit., 19, p. 10.

⁴¹Perkin, Jr., op. cit., 113, p. 764.

According to Kipke's results⁴⁰, the reduction of berberine involved two electrons, and the reduced product was dihydroberberine. However, berberine is also electro-reducible to tetrahydroberberine⁴¹. In these reductions occur separately, berberine to dihydroberberine and dihydroberberine to tetrahydroberberine, there was question arises where each reduction takes place. Answers for the three possible theoretical structural forms of berberine, and also for oxyberberine, dihydroberberine, and tetrahydroberberine have been given (page 7). The other possible reduced products involving only two electrons is ethylene dihydroberberine:



⁴⁰Kipke, J., *Ann. Chem.*, 19, 20.
⁴¹Kipke, J., *Ann. Chem.*, 19, 20.

Several authors^{42,43,44} have reported that berberine heated in strongly alkaline solutions undergoes a mutual oxidation-reduction reaction to give equal parts of oxyberberine and dihydroberberine. In electro-reduction at a controlled potential in which only two electrons are involved, ethylene dihydroberberine may be the reduced product instead of dihydroberberine as given on page 4; this would be the case if the ethylene double bond in berberine was more easily reducible than the aldehyde group or carbinol-nitrogen group or the carbon-ammonium-nitrogen group.

3. Reduction potentials of some carbonyl compounds

Adkins and Cox⁴⁵ reported reduction potentials for several aldehydes and ketones. According to their observations enolizable compounds might show a different reduction potential in alkaline solution than in acid solution; for example vanillin gave a reduction potential 0.3 volts lower in acid than in alkaline medium. They found that the

⁴²Tinkler, loc. cit.

⁴³Henry, op. cit., p. 333.

⁴⁴Gilman, op. cit., p. 1215.

⁴⁵Adkins and Cox, J. Am. Chem. Soc., 60, 1151-52 (1938).

Several authors^{42, 43} have reported that reactions
 heated in strongly alkaline solutions undergo a radical
 oxidation-reduction reaction to give equal parts of oxy-
 peroxide and dihydroperoxide. In electro-reduction a
 controlled potential in which only two electrons are
 involved, ethylene dihydroperoxide may be the reduced
 product instead of dihydroperoxide as given on page 4. This
 would be the case if the ethylene dihydroperoxide
 was more easily reduced than the dihydroperoxide group in some
 diol-alcohol group or the diol-alcohol-alcohol group.

3. Potential difference of some organic compounds
 Adams and Cox⁴⁴ reported reduction potentials for
 several aldehydes and ketones. According to their obser-
 vations enolizable compounds might show a different reduc-
 tion potential in alkaline solution than in acid solution.
 For example vanillin gave a reduction potential of 0.3 volts
 lower in acid than in alkaline solution. They found that the

⁴² Tinkler, loc. cit.

⁴³ Henry, loc. cit., p. 383.

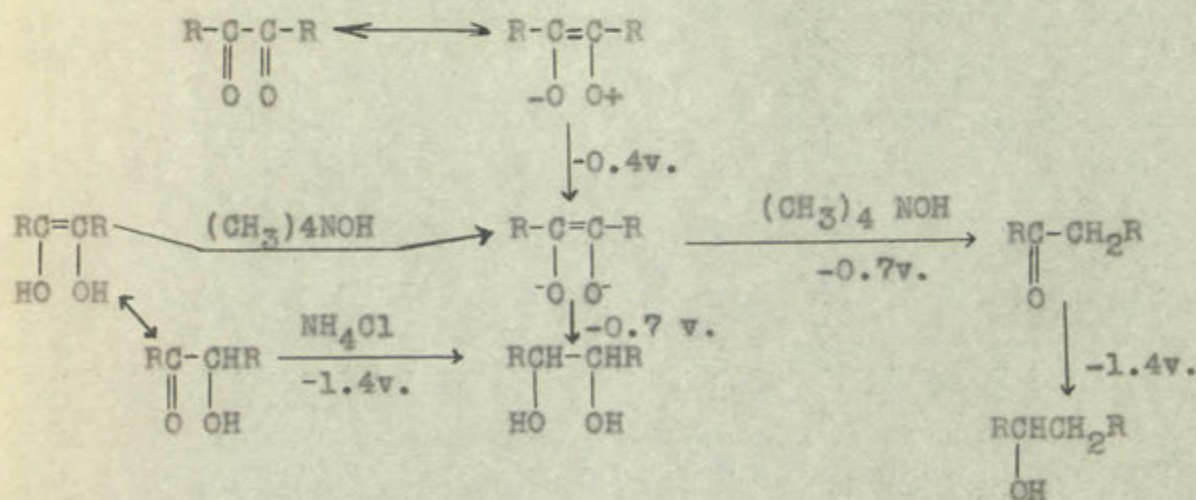
⁴⁴ Adams, loc. cit., p. 1116.

⁴⁵ Adams and Cox, J. Am. Chem. Soc., 61, 111-12.

reduction potentials of ketones as measured polarographically were somewhat arbitrary; averages of widely varying values were usually as much as 0.2 volt higher than the lowest potential at which reduction would take place as determined by other means. They found that aliphatic aldehydes showed reduction potentials in the vicinity of -1.9 volts; phenyl alkyl ketones were lower, -1.5 to -1.6 volts; benzophenone, benzaldehyde still lower, -1.3 volts. Reduction potentials of certain methoxy and hydroxy benzaldehydes were higher than that of the parent compound. Benzalacetone and similar compounds and pyruvic acid had reduction potentials of -1.1 to -1.2 volts. In general, they found that: an increase in unsaturation decreased the reduction potential of the carbonyl group; aromatic aldehydes had much lower reduction potentials than saturated aldehydes; alkyl aryl ketones and mesityl oxide, were lower than saturated ketones; quinones and alpha-diketones had the lowest of all reduction potentials. The effectiveness of the alkene linkage in lowering the reduction potential of the RCO group was only a little less than that of an aryl group. For benzil and benzoin in alkaline solution they found half wave potentials of -0.7 and -1.4 volts. For benzil in acid, -0.4 and -0.7 volts, and for benzoin in ammonium chloride solution, -1.4 volts. The probable

reduction potentials of ketones as measured potentiometrically were somewhat arbitrary; average of slightly varying values were usually as much as 0.2 volt higher than the lowest potential at which reduction would take place as determined by other means. They found that aliphatic aldehydes showed reduction potentials in the vicinity of -1.0 volt; phenyl alkyl ketones were lower, -1.2 to -1.5 volt; benzophenone, benzaldehyde, still lower, -1.5 volt. Reduction potentials of certain aldehydes and hydroxy ketones were higher than that of the parent compound. Benzalacetone and aliphatic hydroxy ketones were found to have reduction potentials of -1.1 to -1.2 volt. In general, they found that an increase in conjugation lowered the reduction potential of the carbonyl group; aromatic aldehydes had much lower reduction potentials than saturated aldehydes; alkyl alkyl ketones and aryl alkyl ketones were lower than saturated ketones; quinoxaline and naphthoquinone had the lowest of all reduction potentials. The reduction potentials of the alkene linkage in favoring the reduction potential of the RCO group was only a factor less than that of an aryl group. For benzil and benzil in alkaline solution they found half wave potentials of -0.5 V and -1.2 V. For benzil in acid, -0.4 and -0.5 V, and for benzil in ammonium chloride solution, -1.4 volt. The probable

reaction is:



Some compounds taken from their paper are listed in Table V. There are other compounds included which have been taken from a list compiled by E. H. Sargent and Co.^{46,47}

⁴⁶ Adkins and Cox, op. cit., p. 1153.

⁴⁷ Sargent and Co., Scientific Apparatus and Methods Vol. 2, Fall, 1949, p. 118.

TABLE V
HALF WAVE POTENTIALS OF SOME COMPOUNDS

Compound	Half Wave Potential Volts	Reference
1. Benzaldehyde	-1.34	46
2. Anisaldehyde	-1.46	46
3. 2,3-Methylenedioxy-benzaldehyde	-1.39	46
4. Vanillin	-1.73	46
5. Acetophenone	-1.52	46
6. Benzoyl acetone	-1.80	46
7. Dibenzal acetone	-1.22	46
8. Benzal acetone	-1.24	46
9. Benzal acetone	-1.12;-1.36	46
10. Cinnamal acetophenone	-0.93;-1.11	46
11. Crotonaldehyde	-1.37;-1.80	46
12. Benzoin	-0.7 ; -1.4	46
13. n-Heptaldehyde	-1.90	46
14. Methyl-ethyl-ketone	none	47
15. Methyl-vinyl-ketone	-1.42	47
16. 2-Benzoyl-benzoic acid	-1.11;-1.60	47
17. 2-Benzoyl-benzoic-acid-anilide	-1.48;-2.20	47
18. N-Methyl-2-benzoyl-benzanilide	-1.46;-1.97	47
19. 2-Benzoyl-benzoic-acid-amide	-1.52;-1.94; -2.26	47

TABLE V
HALF WAVE POTENTIALS OF SOME COMPOUNDS

Compound	Half Wave Potential Volts	Reference
1. Benzaldehyde	-1.54	48
2. Anisaldehyde	-1.48	48
3. 2,3-Methylenedioxy-benzaldehyde	-1.38	48
4. Vanillin	-1.75	48
5. Acetophenone	-1.52	48
6. Benzyl acetate	-1.80	48
7. Ethyl acetate	-1.52	48
8. Benzal acetate	-1.54	48
9. Benzal acetate	-1.12; -1.55	48
10. Cinnamyl acetophenone	-0.85; -1.11	48
11. Crotonaldehyde	-1.37; -1.80	48
12. Benzoin	-0.7; -1.4	48
13. n-Heptaldehyde	-1.80	48
14. Methyl-ethyl-ketone	none	47
15. Methyl-vinyl-ketone	-1.42	47
16. 2-Benzoyl-benzoic acid	-1.11; -1.80	47
17. 2-Benzoyl-benzoic acid-ethylide	-1.48; -2.20	47
18. 4-Methyl-2-benzoyl-benzoic acid	-1.46; -1.97	47
19. 2-Benzoyl-benzoic acid-ethylide	-1.52; -1.94; -2.58	47

TABLE V (continued)
HALF WAVE POTENTIALS OF SOME COMPOUNDS

Compound	Half Wave Potential Volts	Reference
20. ortho-Methoxy-acetophenone	-1.67	47
21. meta-Methoxy-acetophenone	-1.71	47
22. para-Methoxy-acetophenone	-1.80	47

TABLE V - SUPPLEMENT

Compound	pH	Half Wave Potential vs. S.C.E. volts	Current micro- amperes	Reference
Benzaldehyde	1.3	-0.98	2.3*	48
	7.2	-1.36	4.2	48
Acetophenone		-1.7	7, est.**	49
Benzalacetophenone		-1.2;-1.7	7;7, est.**	49

* equivalent to one electron per molecule
** estimated from curves

^{46,47} See page 22.

⁴⁸ Pasternak, Helv. Chim. Acta, 31, 755 (1948).

⁴⁹ Pasternak and Halban, ibid., 29, 195-96 (1946).

TABLE V (continued)

HALF-WAVE POTENTIALS OF SOME COMPOUNDS

Compound	Half-Wave Potential Volts	Reference
20. ortho-Methoxy-acetophenone	-1.87	47
21. meta-Methoxy-acetophenone	-1.77	47
22. para-Methoxy-acetophenone	-1.80	47

TABLE V - SUPPLEMENT

Compound	Half-Wave Potential Volts	Current micro-ampere	Reference
Benzaldehyde	-1.2	0.30	48
	-1.25	1.1	48
Acetophenone	-1.7	0.01-0.40	48
Benzoinacetophenone	-1.6; -1.7	1.7, 0.01-0.40	48

* equivalent to one electron per molecule
 ** estimated from curve

48, 47 See page 23.

49 Preterman, *Anal. Chem.*, **34**, 700 (1962).

48 Preterman and Kellom, *Anal. Chem.*, **34**, 105-106 (1962).

A comparison of various reduction potentials listed in Table V indicates a number of things. A methoxy or methylene-dioxy substitution increases the reduction potential of benzaldehyde, as shown by anisaldehyde and 2,3-methylenedioxy-benzaldehyde. According to the compounds acetophenone and ortho-, meta-, and para-substituted methoxy acetophenones, ortho, meta and para methoxy substitutions on the benzene ring increase the reduction potential of a ketone group attached to the ring. An amine nitrogen atom attached to a carbonyl apparently increases the reduction potential of the latter, as in the compounds 2-benzoyl-benzoic-acid-anilide, N-methyl-2-benzoyl-benzanilide, and 2-benzoyl-benzoic-acid-amide compared to 2-benzoyl-benzoic acid.

A compound such as crotonaldehyde, number 11, with two reduction potentials, has two groups that might be reduced. Either might be reduced in two steps, with one electron per molecule at each step; a dimer would be formed at the first step. Or both groups might be reduced, involving a total of four electrons. The question of what kind of reduction takes place requires data showing which group is reducible at the lower potential and how many electrons per molecule are involved, for an answer. Other compounds, presenting the question as to whether a carbonyl group in

A comparison of various reduction potentials listed in Table V indicates a number of things. A methoxy or methylene-dioxy substitution increases the reduction potential of benzaldehyde, as shown by benzaldehyde and 2,5-methylenedioxy-benzaldehyde. According to the compounds acetophenone and ortho-, meta-, and para-substituted methoxy acetophenones, ortho, meta and para methoxy substituents on the benzene ring increase the reduction potential of a ketone group attached to the ring. An amine nitrogen atom attached to a carbonyl apparently increases the reduction potential of the latter, as in the compounds 2-benzyloxy-benzaldehyde, 2-benzyloxy-benzaldehyde, 2-benzyloxy-benzaldehyde, and 2-benzyloxy-benzaldehyde. 2-benzyloxy-benzaldehyde is a solid.

A compound such as acetophenone, number 11, with two reduction potentials, has two groups that might be reduced. Either might be reduced in two steps, with one electron per molecule at each step; a dimer would be formed at the first step. Or both groups might be reduced, involving a total of four electrons. The question of what kind of reduction takes place requires data showing which group is reducible at the lower potential and how many electrons per molecule are involved, for an answer. Other compounds, presenting the question as to whether a carbonyl group in

conjugation with an ethylene group or the ethylene group was reduced at the lower potential, are methyl-vinyl-ketone compared to methyl-ethyl-ketone, benzal acetone compared to benzoyl acetone, and cinnamal acetophenone compared to dibenzal acetone.

The previous compounds supposedly are reduced at the carbonyl group. Definite evidence that this group, when in conjugation with an ethylene group, is not necessarily reduced at a lower potential than the ethylene group was shown by Pasternak. Some of his data are included in Table V-Supplement, page 24.

As an example of unsaturated carbonyl compounds, Pasternak⁵⁰ found that benzalacetophenone was reduced in two steps, each involving two electrons per molecule; the second reduction potential had the same half wave potential as acetophenone when it was reduced in one step involving two electrons per molecule. This indicated that the ethylene group was reduced in the first step at a lower potential than the carbonyl group in conjugation with it.

Pasternak⁵¹ measured the half wave potentials and the quantity of electricity involved in the reductions at

⁵⁰Pasternak and Halban, loc. cit.

⁵¹Pasternak, op. cit., pp. 755, 761-2, 765, 769, 776.

conjugation with an ethylene group or the ethylene group was reduced at the lower potential, the methyl-vinyl-ketone was reduced to methyl-ethyl-ketone, benzyl acetone compared to benzoyl acetone, and dimethyl acetophenone compared to diphenyl acetone.

The previous compounds supposedly are reduced at the carbonyl group. Belinfante advanced that this group, when in conjugation with an ethylene group, is not necessarily reduced at a lower potential than the ethylene group was shown by Pasternak. Some of his data are included in Table V-Supplement, page 24.

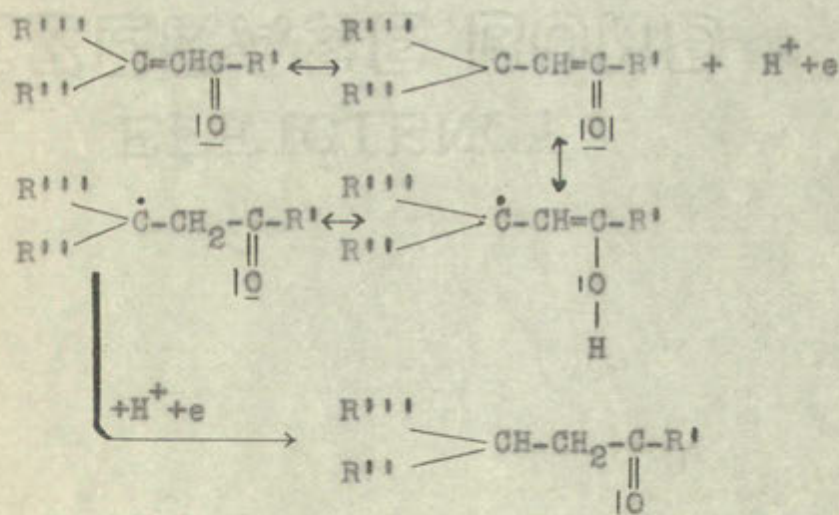
As an example of unsaturated carbonyl compounds, Pasternak⁵⁰ found that benzalacetophenone was reduced in two steps, each involving two electrons per molecule; the second reduction potential had the same half wave potential as acetophenone when it was reduced in one step involving two electrons per molecule. This indicated that the ethylene group was reduced in the first step at a lower potential than the carbonyl group in conjugation with it.

Pasternak⁵¹ measured the half wave potentials and the quantity of electricity involved in the reductions of

⁵⁰Pasternak and Nelson, *loc. cit.*

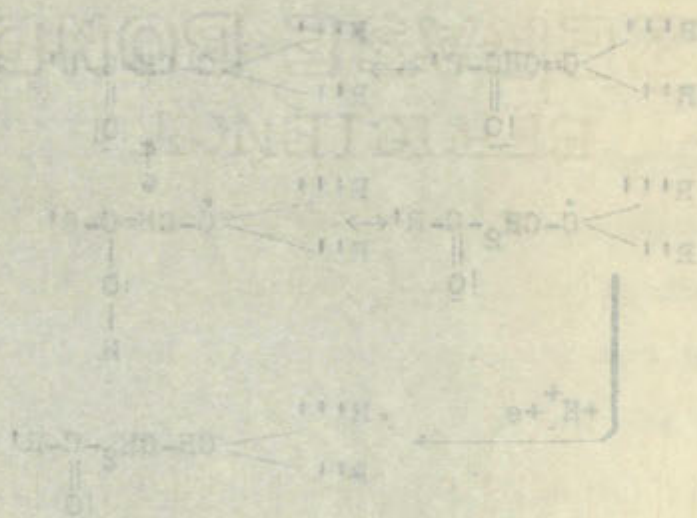
⁵¹Pasternak, *op. cit.*, pp. 785, 786, 787, 788, 789, 790.

these potentials for a number of organic compounds. He found that at a pH of 1.3 the reduction of benzaldehyde involved one electron per molecule, which suggests that a pinacone must have been formed. At a higher pH the reduction required between one and two electrons per molecule; the product formed was a mixture of a pinacone and an alcohol. In fact, the electro-reduction of carbonyl groups and of ethylene bonds in many compounds took place in two one-electron steps. A dimer or a radical would be the intermediate product. Pasternak postulated the following mechanism for the reaction:



The taking up of the first electron is reversible and rapid; the second one is irreversible and slow.

these potentials for a number of organic compounds. It
 found that at a pH of 1.5 the reduction of benzaldehyde
 involved one electron per molecule, which suggests that
 a pinacol must have been formed. At a higher pH the
 reduction required between one and two electrons per
 molecule; the product formed was a mixture of a pinacol and
 an alcohol. In fact, the electro-reduction of carbonyl groups
 and of ethylene bonds in many compounds took place in two
 one-electron steps. A clear-cut radical would be the inter-
 mediate product. However, we prepared the following mech-
 anism for the reaction:



The taking up of the first electron is reversible and rapid;
 the second one is irreversible and slow.

C. LITERATURE ON SPECTROPHOTOMETRIC ABSORPTION

1. Berberine and Related Alkaloids

Tinkler⁵² (1911) found berberinium hydroxide and berberine salts to have the same absorption spectra and therefore similar constitution. Different absorption spectra were observed for berberine treated with an excess potassium hydroxide. Since these were also different from the absorption spectra of tetrahydroberberine, Tinkler deduced that berberine in the carbinol form reacted with the alkali to give equal parts of oxyberberine and dihydroberberine. Tinkler considered the various salts in aqueous (or alcoholic) solutions to have the ammonium form. He found no evidence for the existence of berberine in the imino-aldehyde form. These conclusions paralleled those of other authors on other alkaloids with structures similar in some respects to berberine.

In their report on cotarnine, Dobbie, Lauder and Tinkler⁵³ stated that dilute aqueous or alcohol solutions of cotarnine had spectra practically identical to the

⁵²Tinkler, op. cit., pp. 1342-47.

⁵³Dobbie, Lauder and Tinkler, J. Chem. Soc., 83, 599-602; (1903).

C. LITERATURE ON SPOTROSCOPIC ASSORTION

1. Barbitone and Related Alkaloids

Tinkler⁵² (1911) found barbitone hydrazide and barbitone salts to have the same absorption spectra and therefore similar constitution. Different absorption spectra were observed for barbitone treated with an excess potassium hydroxide. Since these were also different from the absorption spectra of tetrahydrobarbitone, Tinkler deduced that barbitone in the solid state is heated with the alkali to give equal parts of oxobarbitone and dihydrobarbitone. Tinkler considered the various salts in question for which he found no evidence for the existence of barbitone in the false-aldehyde form. These conclusions established those of other authors on other alkaloids with structures similar to those respects to barbitone.

In their report on cotarnine, Langer, Langer and Tinkler⁵³ stated that alkali exposure of alkaloid solutions of cotarnine had spectra practically identical to the

⁵²Tinkler, UK. J. Chem. Soc., 1911, 1545-47.

⁵³Dobbin, Langer and Tinkler, J. Chem. Soc., 1913, 1545-47.
1913, (1913).

hydrochloride salt with two absorption bands; on the other hand, the ether or chloroform solutions of cotarnine and hydrocotarnine had only one absorption band, which was different from that of the salts. They considered the former to have an ammonium form and the latter a carbinol form of structure. These authors⁵⁴ found that 1-Normal alkali converted 98% of the ammonium form into the carbinol form.

Dobbie and Lauder⁵⁵ found papaverine to have different absorption spectra from that of tetrahydropapaverine and hydrastinine different spectra from that of oxyhydrastinine. Corydaline and tetrahydroberberine had similar spectra.

Dobbie and Tinkler⁵⁶ found that aqueous or alcoholic solutions of the various salts of hydrastinine had spectra similar to those of the free base; but an ether solution of hydrastinine and the compound obtained by reaction of the hydrochloride salt with excess sodium hydroxide had different spectra. They considered the former to have an ammonium form structure and the latter a carbinol form.

Absorption bands for these compounds are shown in Table VI.

⁵⁴Ibid., 85, 121-28 (1904).

⁵⁵Dobbie and Lauder, ibid., 83, 606, 616 (1903).

⁵⁶Dobbie and Tinkler, ibid., 85, 1007 (1904).

hydrochloride salt with two absorption bands; on the other hand, the ether or chloroform solutions of cocaine and hydrococaine had only one absorption band, which was different from that of the salts. They considered the former to have an ammonium form and the latter a carbinol form of structure. These authors⁵⁴ found that 1-Normal alcohol converted 88% of the ammonium form into the carbinol form. Dobbie and Langer⁵⁵ found cocaine to have different absorption spectra from that of tetrahydrocannabinol and hydrocannabinol different spectra from that of oxyhydrocannabinol. Cocaine and tetrahydrocannabinol had similar spectra. Dobbie and Tinkler⁵⁶ found that aqueous or alcoholic solutions of the various salts of hydrocannabinol had spectra similar to those of the free base; but an ether solution of hydrocannabinol and the compound obtained by reaction of the hydrochloride salt with excess sodium hydroxide had different spectra. They considered the former to have an ammonium form structure and the latter a carbinol form. Absorption bands for these compounds are shown in

Table VI.

⁵⁴ Ibid., 88, 151-52 (1934).
⁵⁵ Dobbie and Langer, <i>ibid.</i> , 88, 606, 608 (1935).
⁵⁶ Dobbie and Tinkler, <i>ibid.</i> , 88, 1007 (1934).

TABLE VI

ABSORPTION BANDS OF BERBERINE AND RELATED COMPOUNDS

Compound	Absorption Bands millimicrons	Refer- ence
Berberine nitrate (page 3)	318-60; 405-58	57
Berberine salts (page 3)	277; 347-48; 438-42	58
Berberine reacted with potassium hydroxide (pp. 4, 6)	342-89	58
Methyl-dihydroberberine	364-400	58
Tetrahydroberberine (p. 4)	277-301 (in EtOH)	57
Corydaline (p. 31)	278-294 (in EtOH)	57
Cotarnine (salts) (p. 16)	250; 328	59
Hydrocotarnine (p. 31)	285.5	59
Hydrastine (p. 16)	286-312 (in EtOH)	57
Hydrastinine (p. 16)	250-56; 284-303; 347-92	57
Oxyhydrastinine	287-316	57
Hydrohydrastinine	285-97	60
Narceine (p. 16)	none	57
Narcotine (p. 16)	290-317 (in EtOH)	57
Papaverine (p. 16)	220-53; 304-33 "	57
Tetrahydropapaverine (p. 16)	267-90 "	57
Tetrahydropapaverine, Tetrahydroberberine, Corydaline, & Laudanosine (p. 17).	all 282	61
6,7-Dimethoxy-2-methyl-3,4-di- hydroisoquinolinium-chloride (p. 31)	232-58; 286-316; 341-84	62
6,7-Dimethoxy-2-methyl-tetra- hydro-isoquinoline (p. 31)	286-94	62

⁵⁷Dobbie & Lauder, op. cit., pp. 606-09, 616-17.

⁵⁸Tinkler, op. cit., 99, pp. 1342-44

⁵⁹Dobbie, Lauder, & Tinkler, op. cit., 83, p. 600.

⁶⁰Dobbie & Tinkler, op. cit., p. 1007.

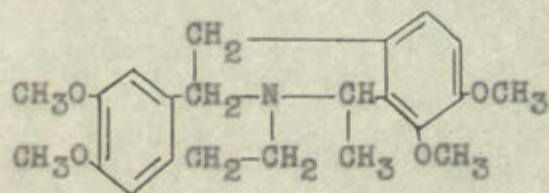
⁶¹Tinkler, op. cit., 101, pp. 1252-53.

⁶²Dobbie & Fox, J. of Chem. Soc., 105, 1639 (1914).

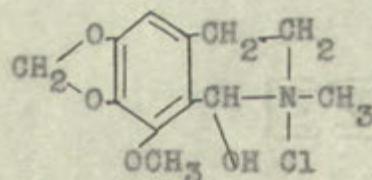
TABLE VI (continued)

ABSORPTION BANDS OF BERBERINE AND RELATED COMPOUNDS

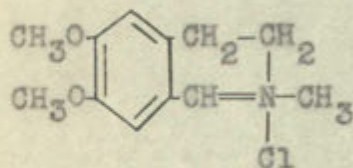
Structural formulas of compounds listed above not given previously:



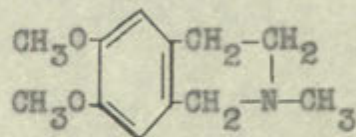
Corydalline



Hydrocotarnine



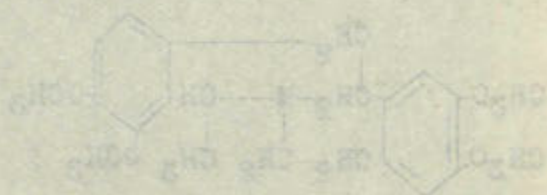
6,7-Dimethoxy-2-methyl-
3,4-dihydroisoquinolinium-
chloride



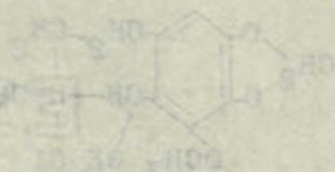
6,7-Dimethoxy-2-methyl-
tetrahydroisoquinoline

ABSORPTION BANDS OF RADIATION AND RELATED COMPOUNDS

Structural formulae of compounds listed above are given previously:



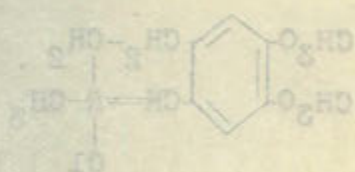
Compound 1



Compound 2



Compound 3



Compound 4

2. Absorption Spectra Correlated with Structure

A great deal of literature in recent years has been concerned with attempts to correlate absorption spectra with molecular structure. Absorption spectra are produced by electrons being forced from normal or ground levels to higher levels. The return of electrons to lower levels can result in emission spectra such as fluorescence⁶³.

In absorbing systems certain parts of the molecules are usually considered to be responsible for the absorption. In the visible and ultraviolet regions of the spectrum these parts or groups have been considered as unsaturated groups and designated as chromophores. Such groups as the ethylene, carbonyl, isocyano, and the benzene ring come under this classification. Other groups which increase or decrease the resonance of the so called chromophores and result in a change of the absorption band are known as auxochromes. If the absorption band is changed to longer wavelengths the change is called a bathochromic effect. Some auxochromes, listed in order of increasing bathochromic effects, are⁶⁴: (a) Ortho and para-directing, or electron donating:

⁶³Brode, Chemical Spectroscopy (Second Edition, N. Y., J. Wiley and Sons, Inc., 1943), pp. 213-15, 286.

⁶⁴Ferguson, Chem. Revs., 43, 395 (1948).

CH₃, Cl, Br, OH, OCH₃, NH₂, O⁻; (b) Meta-directing, or electron attracting: NH₃, COOH, COCH₃, CHO.

When there is a large electric moment connected with the electronic transition in the molecule with light absorption, strong absorption occurs⁶⁵. The energy producing electron displacements is $-\frac{1}{2}kx^2$, where x = displacement from equilibrium or ground state, and k = restoring force constant. The relation of wavelength, λ , to k is shown by the following⁶⁶:

$$\lambda = \frac{c}{\nu_0} = \frac{2\pi c}{\sqrt{k/m}}$$

λ = wavelength

c = speed of light

ν_0 = incident light frequency

or, the wavelength is
inversely proportional

m = effective reduced mass of vibrating electrons

to the square root of k .

k = restoring force

The effect on the light absorption of any given chromophoric group by various factors can be summarized as follows⁶⁷:

⁶⁵Ferguson, op. cit., p. 394.

⁶⁶Ibid., p. 391.

⁶⁷Ibid., p. 439.

CH₃, Cl, Br, OH, OCH₃, NH₂, C₆H₅, (p) Meta-director, or

electron attracting: NO₂, COOH, C=O, CHO.

When there is a large electric moment associated with

the electronic transition in the molecule with light absorp-

tion, strong association occurs.⁶⁵ The energy producing

electron displacement is $\sim \frac{1}{2}kx^2$, where x = displacement from

equilibrium or ground state, and k = restoring force con-

stant. The relation of wavelength, λ , to k is given by the

following⁶⁶:

$$\lambda = \frac{h}{\nu} = \frac{h}{2\pi c \sqrt{\frac{k}{m}}} \quad \lambda = \text{wavelength}$$

ν = speed of light

ν_0 = natural frequency

m = reduced mass

k = effective reduced

mass of vibrating

electrons

k = restoring force

or, the wavelength is

inversely proportional

to the square root of k .

The effect on the light absorption of any given

chromophore group by various factors can be summarized as

follows⁶⁷:

66. Peterson, *op. cit.*, p. 104.

67. *Ibid.*, p. 107.

68. *Ibid.*, p. 108.

1. (a) Resonance in the excited state lowers the potential energy of the excited state;

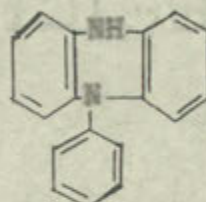
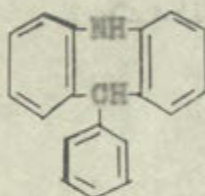
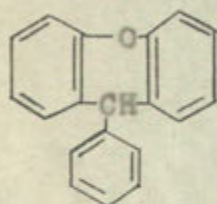
(b) Steric hindrance in the ground state raises the potential energy of the ground state; these effects produce a bathochromic shift or longer wavelength absorption band.

2. (a) Resonance in the ground state decreases the potential energy of the ground state;

(b) Steric strain in the excited state increases the potential energy of the excited state; these effects produce a hypsochromic shift or shorter wavelength absorption band.

3. Fluorescence

Fluorescence is characteristic of a number of organic compounds but is marked in xanthene, acridine, and azine groups⁶⁸:



Substituents such as hydroxyl or alkyl-amine groups in either side ring do not change fluorescence, but if the

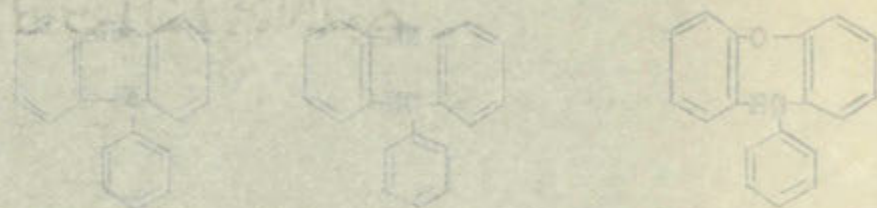
⁶⁸Lewis and Calvin, Chem. Revs., 25, 322 (1939).

1. (a) Resonance in the excited state lowers the potential energy of the excited state.
 (b) Resonance in the ground state lowers the potential energy of the ground state. These effects produce a bathochromic shift of longer wavelength absorbing band.

2. (a) Resonance in the ground state decreases the potential energy of the ground state.
 (b) Resonance in the excited state increases the potential energy of the excited state. These effects produce a hypsochromic shift of shorter wavelength absorbing band.

3. Fluorescence

Fluorescence is characteristic of a group of organic compounds but is absent in inorganic compounds and salts.



Substituents such as hydroxyl or alkyl groups on the other side ring do not cause fluorescence, but if the

substituent is a phenyl-amine group fluorescence is destroyed. Any substituent may be placed in the lower group without changing fluorescence.

Hydrocarbons with two benzene rings are usually fluorescent and fluoresce more towards the visible region as the molecule becomes heavier⁶⁹.

4. Absorption Bands of Compounds

The next two tables list some chromophores and auxochromes, and a number of compounds, with corresponding absorption bands, that pertain to the present problem.

⁶⁹Radley and Grant, Fluorescence Analysis in Ultraviolet Light (Third Edition, N. Y., D. Van Nostrand, 1949), p. 289.

substituent is a phenyl-oxide group fluorescence is destroyed. Any substituent not so placed in the lower group without changing fluorescence.

Hydrocarbons with two benzene rings are usually fluorescent and fluorescence is observed in the visible region as the molecule becomes heavier.⁶⁸

4. Absorption Bands of Compounds

The next two tables list some compounds and their absorption bands, and a number of compounds, with corresponding absorption bands, that contain the π -conjugated system.

TABLE VII

ABSORPTION BANDS OF SOME CHROMOPHORES AND AUXOCHROMES

Chromophore Group	Auxochrome Substituent	Absorption Band millimicrons	Molecular Extinction Coefficient	Absorption Band Change millimicrons	Reference
C = C		195	10,000		70
C = O		275	100		70
C = N		230			70
C=C-C=O	More polar solvent	220-260;	10,000;	+	70;71
	Alkyl group;	310-330.	100	-	
-C=C-C=O	R = H.	215		+10, each;	71-74
-C=C-C=N-	Alkyl group	267-7.5	10,000 to 35,000	-5, none	73
CH ₂ =CHCH=CH ₂	Alkyl group	217		+5, each	70;74
CH=CH-OH	Saturated alkyl or allyl group;	268		Both the same + ;	70
CH=CH-CH	-CH=CH-CH ₃ .			20 to 30 additional	

⁷⁰Ferguson, op. cit., pp. 392-93, 397, 402, 404, 406, 411.

⁷¹Woodward, J. Am. Chem. Soc., 63, 1123, 1125 (1941).

⁷²Evans & Gillam, J. Chem. Soc., 1945, 432-434.

⁷³Ibid., 1943, 565-568.

⁷⁴Woodward, J. Am. Chem. Soc., 64, 73, 76 (1942).

TABLE VIII
ABSORPTION BANDS OF VARIOUS COMPOUNDS

Compound	Absorption Band millimicrons	Molecular Extinction Coefficient	Refer- ence
1. Ethylene	200		75
2. Trimethyl- ethylene	234		76
3. Isopropyl- idine meth- ylamine	230		75
4a. Benzene	203.5; 254.	7400; 204.	77
b. "	234; 239; 243; 249; 254.5; 261; 268.	34; 54; 96; 153; 208; 143; 9.	78; 79
5. Benzyl alcohol	248; 252.5; 258.5; 264; 268.	117; 155; 185; 138; 90.	78
6. Piperonyl alcohol	220-238**; 285.5	--; strong	80

⁷⁵Ferguson, op. cit., p. 392.

⁷⁶Buroway, J. Chem. Soc., 1939, 1178.

⁷⁷Doub and Vandenbelt, J. Am. Chem. Soc. 69, 2716
(1947).

⁷⁸Campbell, et.al., J. Am. Chem. Soc., 69, 880-83
(1947).

⁷⁹Orndorff, et.al., ibid., 50, 835 (1928).

⁸⁰Purvis, J. Chem. Soc., 125, 410 (1924).

TABLE VIII
ABSORPTION BANDS OF TROPIC COMPOUNDS

Compound	Absorption Band Wavelengths	Molar Extinction Coefficient	Notes
1. Ethylene	200	70	
2. Trimethyl- ethylene	204	70	
3. Isopropyl- idene meth- ylamine	200	70	
4a. Benzene	202.5-204.5	70	
b.	204.5-206.5	70	
5. Benzyl alcohol	202.5-204.5	70	
6. Piperonyl alcohol	200-202	70	

75. Targuon, *et al.*, 1952.

76. Barovsky, *et al.*, 1952, 1953.

77. Bond and Vandenbrouk, *et al.*, 1952.

(1952).

78. Caspelli, *et al.*, 1952, 1953.

(1952).

79. Bond, *et al.*, 1952, 1953.

80. Barovsky, *et al.*, 1952, 1953.

TABLE VIII (continued)
ABSORPTION BANDS OF VARIOUS COMPOUNDS

Compound	Absorption Band millimicrons	Molecular Extinction Coefficient	Refer- ence
7. 4,4'-Dihydroxy-diphenyl-ethane	227;279	17,600;3,700	81
8. Anisole	217;269	6,400;1,480	82
9. o-Veratrole	227;278	5,000;2,000	83
10. Piperic acid	326;broad	- -	84
11. Pyridine	253		85
12. Benzaldehyde	199;240; 278;320	20,000;13,000; 1,040;50	86
13. Vanillin	310;353	710;1,980	87

⁸¹Hunter, Morton and Carpenter, ibid., 1950, 441.

⁸²Doub and Vandenbelt, loc. cit.

⁸³International Critical Tables, V., p. 362.

⁸⁴Dobbie and Fox, J. Chem. Soc., 103, 1194 (1913).

⁸⁵Baker and Baly, ibid., 91, 1122 (1907).

⁸⁶Morton and Stubbs, ibid., 1940, 1349-50.

⁸⁷Lemon, J. Am. Chem. Soc., 69, 2998 (1947).

TABLE VIII (continued)
ABSORPTION BANDS OF VARIOUS COMPOUNDS

Compound	Absorption Band millimicrons	Molecular Extinction Coefficient	Refer- ence
14. o-Methoxy- benzaldehyde	253;319	11,700;4,160	88
15a. Piperonal	275;315	6,300;10,000	88
b. "	234;272;312		89
16. Benzal-meth- ylamine	247	17,200	90
17. Styrene	240	10,000***	90
18. l-Phenyl- propene	249;284;293	14,700;1,030 640	91
19. l-para-methoxy- phenylpropene	258	20,350	91
20a. Iso-eugenol	258-59		92
b. "	249-75;313**		93

⁸⁸Leonard, et al, ibid., 71, 2997-3005 (1949).

⁸⁹Purvis, loc. cit., footnote 80.

⁹⁰Ferguson, op. cit., pp. 398, 419-20, 401.

⁹¹Campbell, et al, loc. cit., footnote 78.

⁹²Purvis, op. cit., p. 408.

⁹³Crymble, et al., J. Chem. Soc., 99, 453-55 (1911).

TABLE VIII (continued)
ABSORPTION BANDS OF VARIOUS COMPOUNDS

Compound	Absorption Band millimicrons	Molecular Extinction Coefficient	Refer- ence
21a. Eugenol	280-81	- -	94
b. "	278-86	- -	95
22. Iso-safrole	249-76** 281-312**	- -	95
23. Safrole	278-94	- -	95
24a. Transstilbene	295	23,400	96;97
b. "	222;288; 322	13,000;20,000*** 20,000***	98
25. o-Hydroxystilbene	248	34,900	99
26. Bibenzyl	262	- -	97

⁹⁴Purvis, loc. cit., footnote 92.

⁹⁵Crymble, et al, loc. cit., footnote 93.

⁹⁶Gillam and Hey, J. Chem. Soc., 1939, 1173.

⁹⁷Ferguson, op. cit., pp. 420, 397.

⁹⁸Morton, Hassan, and Calloway, J. Chem. Soc., 1934,
898.

⁹⁹Booker, Evans, and Gillam, ibid., 1940, 1456.

TABLE VII (continued)
ASSOCIATION RATES OF VARIOUS COMPOUNDS

Compound	Association Rate millimoles per mole of compound	Association Rate per mole of compound	Reference
91a. Benzene	100-150	-	91
91b. Benzene	100-150	-	92
92. 1,2-dichloro	100-150	-	93
93. Benzene	100-150	-	94
94a. Trans-2-butene	100-150	100-150	95
94b. Trans-2-butene	100-150	100-150	96
95. o-Dichlorobenzene	100-150	100-150	97
96. o-Dichlorobenzene	100-150	100-150	98

99. Propane, 1,2-dichloro, 1,3-dichloro, 1,4-dichloro
 100. Cyclohexane, 1,2-dichloro, 1,3-dichloro, 1,4-dichloro
 101. Styrene and 1,2-dichloro, 1,3-dichloro, 1,4-dichloro
 102. Phenylacetylene, 1,2-dichloro, 1,3-dichloro, 1,4-dichloro
 103. Acetylene, 1,2-dichloro, 1,3-dichloro, 1,4-dichloro
 104. Propyne, 1,2-dichloro, 1,3-dichloro, 1,4-dichloro
 105. Butyne, 1,2-dichloro, 1,3-dichloro, 1,4-dichloro
 106. Pentynes, 1,2-dichloro, 1,3-dichloro, 1,4-dichloro

TABLE VIII (continued)
ABSORPTION BANDS OF VARIOUS COMPOUNDS

Compound	Absorption Band millimicrons	Molecular Extinction Coefficient	Refer- ence
27. P,P'-dihydroxy- bibenzyl	227;279	17,600;3,700	100
28. Benzil (yellow)	259;370	20,400;78	101
29. Piperonil (yellow)	235;281; 324	19,000;9,540; 13,150	101
30. 3,3'-dimeth- oxybenzil (yellow)	262;322; 380**	11,450;15,100; 470	101
31. 2,2'-dimeth- oxybenzil (colorless)	254;318	19,500;8,300	101

** Absorption Band an inflection point

*** E approximate only

¹⁰⁰Hunter, Morton and Carpenter, loc. cit., footnote
81.

¹⁰¹Leonard, et al, loc. cit., footnote 88.

TABLE VIII (continued)
ABSORPTION BANDS OF VARIOUS COMPOUNDS

Compound	Absorption Band Wavelength Wavenumber	Reference	Notes
27. 2,2'-dihydroxy- diphenyl	288; 289	17,400; 1,700	100
28. Benzil (yellow)	288; 289	17,400; 1,700	101
29. Phthalonitrile (yellow)	288; 289	17,400; 1,700	101
30. 2,2'-dihydroxy- diphenyl (yellow)	288; 289	17,400; 1,700	101
31. 2,2'-dihydroxy- diphenyl (colorless)	288; 289	17,400; 1,700	101

** Absorption band in infrared region

see E approximately only

100 Hunter, Johnson and Carpenter, *loc. cit.*, footnote 10

31.

101 Leonard, *et al.*, *loc. cit.*, footnote 10

5. Alkyl Substitution Versus Ionization Potentials on Some Chromophores

In a paper published by W. C. Price¹⁰² the effect of alkyl substitution on spectra and ionization potentials of some fundamental chromophores is analyzed. The author showed that ". . .the effect of alkyl substitution on the absorption spectra and ionization potentials of many classes of molecules . . ." was ". . .due mainly to preferential stabilization of the ionic or excited state . . ." He believed that this stabilization was probably due largely to a result of hyperconjugation and charge transfer. Some values taken from tables in Price's paper are listed in Tables IX, X, XI.

According to Price¹⁰³ the magnitude of the changes in ionization potential were of much greater magnitude ". . . than any changes in ground-state stabilization (resonance energy) which have been shown by heats of hydrogenation or formation to accompany substitution. The conclusion to be drawn from this is that the main cause of the reduction is to be sought for in an increase in the stability of the molecular ion resulting from the substitution." The

¹⁰²Price, Chem. Revs., 41, 257, 271 (1947).

¹⁰³Ibid., p. 269-70.

5. Alkyl Substitution Effects on Ionization Potentials

Introduction and Summary

In a paper published by W. H. Laidler and co-workers in 1957, alkyl substitution on aromatic and heterocyclic systems was investigated. The authors showed that the effect of alkyl substitution on the ionization potential and electron affinity of many classes of molecules . . . was . . . the ability to stabilize the ionization of the ion or excited state . . . It is believed that this stabilization can be due to the effect of hyperconjugation and other factors. Some values taken from Laidler's paper are listed in Table IX, X, XI.

According to Table IX the magnitude of the change in ionization potential with respect to alkyl groups is . . . than any changes in ground-state electron affinity (resonance energy) which have been shown to result from substitution on formation to aromatic substitution. The conclusion to be drawn from this is that the main cause of the variation in ionization potential is the stabilization of the molecular ion resulting from the substitution.

¹⁰² Laidler, *Proc. Roy. Soc. (London)*, **241**, 1957.

¹⁰³ Ibid., p. 150-151.

electrons concerned are "...non-bonding electrons on the appropriate atoms. . . ."

TABLE IX¹⁰⁴

EFFECT OF SUBSTITUTION ON IONIZATION POTENTIALS

Substance	Ionization Potential	Substance	Ionization Potential
C_6H_6	9.24	NH_3	10.8
$C_6H_5-CH_3$	8.92	CH_3NH_2	9.8
$C_6H_5-C_2H_5$	8.75*	$(CH_3)_2NH$	9.6
$C_6H_4(CH_3)_2$ o,m, or p	8.3*	$(CH_3)_3N$	9.4

* approximately

TABLE X¹⁰⁵

STABILIZATION ENERGY AND REDUCTION OF IONIZATION POTENTIALS

Compound	Heat of Stabilization	Reduction of Ionization Potential
Alkyl aromatics relative to benzene		
Toluene	0.30	9.7
n-Propyl-benzene	1.17	14.7, approximately
ortho-Xylene	-	18.0, "
values in kilocalories per mole		

electrons centered on the two bonding electrons on the appropriate atom.

TABLE IV
EFFECT OF SUBSTITUTION ON FIRST IONIZATION POTENTIALS

Substance	Ionization Potential (eV)	Substance	Ionization Potential (eV)
C_6H_6	8.54	$\text{C}_6\text{H}_5\text{CH}_3$	8.54
$\text{C}_6\text{H}_5\text{CH}_3$	8.54	$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_3$	8.54
$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_3$	8.54	$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_3$	8.54
$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_3$	8.54	$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	8.54

* Experimentally determined.

DISCUSSION

TABLE V

STABILIZATION ENERGY AND HEAT OF FORMATION OF BENZENE

Compound	Heat of Formation (kcal/mole)	Stabilization Energy (kcal/mole)
Benzene	19.8	36.0
1,3-Cyclohexadiene	16.7	36.0
1,4-Cyclohexadiene	16.7	36.0
1,5-Cyclohexadiene	16.7	36.0

TABLE XI¹⁰⁶
 HEATS OF COMBUSTION, CH₂-INCREMENTS, AND
 DERIVED STABILIZATION ENERGIES

Substance	Heat of Combustion Increase*	Stabilization Energy with Substitution*	Energy Change of Ion**
NH ₃	-----	-----	-----
CH ₃ NH ₂	166.1	-8.7	+14.3
C ₂ H ₅ NH ₂	156.2	+1.2	-
(CH ₃) ₂ NH	161.3	-3.9	+0.7
(C ₂ H ₅) ₂ NH	156.2	+1.2	-
(CH ₃) ₃ N	161.3	-3.9	+0.7

* in kilocalories per mole at 25°C.

** change in Stabilization Energy of ION with Substitution.

¹⁰⁴Ibid., p. 258-59.

¹⁰⁵Ibid., p. 263.

¹⁰⁶Price, loc. cit., see footnote 103.

TABLE XI
HEATS OF COMBUSTION, ΔH_c° , AND
DERIVED STABILIZATION ENERGIES

Substance	Heat of Combustion Increase ^a	Stabilization Energy with Substitution ^b	Energy Change of Ion ^c
NH_3	100.1	-0.7	+1.2
CH_3NH_2	100.2	+1.2	-
$\text{C}_2\text{H}_5\text{NH}_2$	101.3	-0.8	+0.7
$(\text{CH}_3)_2\text{NH}$	100.2	-0.7	-
$(\text{C}_2\text{H}_5)_2\text{NH}$	101.2	-0.8	+0.7

* in kilocalories per mole at 25°C.
** change in stabilization energy of ion with substitution.

^a Ibid., p. 222-23.

^b Ibid., p. 222.

^c Ibid., loc. cit., see footnote 102.

CHAPTER III

SOURCE AND ANALYSIS OF THE BERBERINE USED IN STUDY

A. SOURCE

The berberine used in the present study was one-eighth ounce of berberine hydrochloride Merck, Merck and Co., number 1462, lot 41129. This lot was used for all analyses and experimental work.

B. ANALYSIS

The theoretical empirical formula and composition of Berberine Hydrochloride are as follows:

Formula: $C_{20}H_{17}O_4N \cdot HCl \cdot 2H_2O$ Molecular weight: 407.85

Composition: C--58.89% H--5.43%

N--3.435% Cl--8.694% CH_3O --15.21%

Micro-analysis by standard methods gave the following results^{107,108}:

C--58.92% H--5.55%

N--3.38%; 3.44% Cl--8.65% CH_3O --14.7%

¹⁰⁷Clark, Semimicro Quant. Org. Anal., (N.Y. Academic Press, 1943), p. 25, 37, 53.

¹⁰⁸Niederl and Niederl, Micromethods of Quantitative Organic Analysis (N.Y., J. Wiley and Sons, Inc., 1942), p. 239-43.

SOURCE AND ANALYSIS OF THE SUBSTANCE USED IN THE

A. SOURCE

The substance used in the present study was one-eighth ounce of barbitone hydrochloride, lot 100, number 100, lot 100, and lot 100, all analyzed and experimentally tested.

B. ANALYSIS

The theoretical molecular formula and weight of barbitone hydrochloride are as follows:

Formula: $C_4H_5N_2O_3 \cdot HCl$ Molecular weight: 191.5

Composition: C-42.35% H-3.15% N-14.50% O-24.00%

N-3.45% Cl-12.55% (theoretical)

Micro-analytic of sample: found: C-42.35% H-3.15% N-14.50%

Results: 100.00%

C-42.35% H-3.15% N-14.50% O-24.00%

N-3.45% Cl-12.55% (theoretical)

100.00% (theoretical)
Found: 100.00% (theoretical)

100.00% (theoretical)
Found: 100.00% (theoretical)
p. 232-233

CHAPTER IV

APPARATUS AND EXPERIMENTAL TECHNIQUE

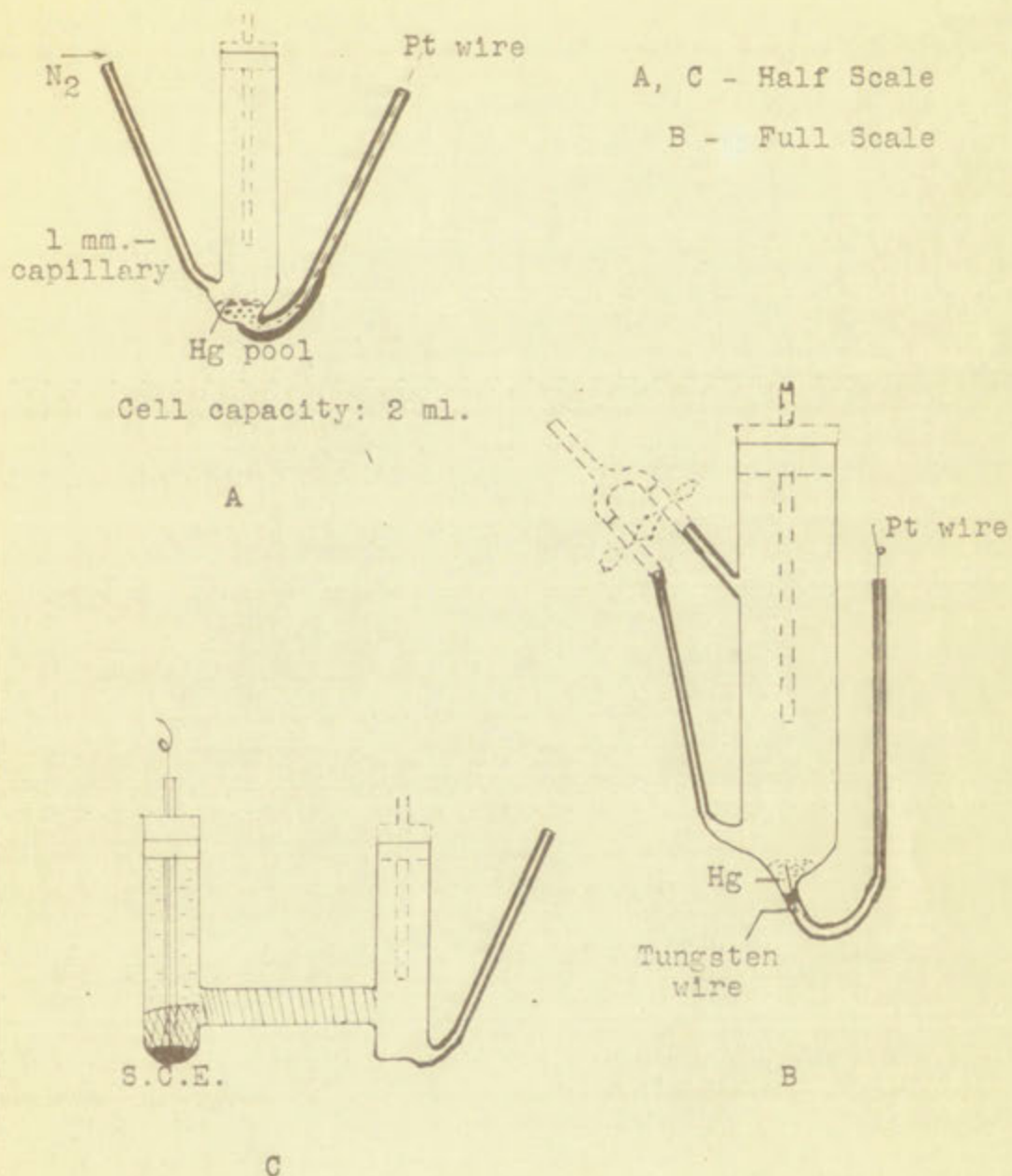
A. APPARATUS USED

Considerable apparatus had to be constructed for some of the necessary experimental work.

The Fisher Electropode was used for making most of the polarograms, but the cell furnished by the manufacturer for the instrument was inadequate. The Heyrovsky cell obtained for use with the Sargent Polarograph was better. It provided for the protection of the sample being polarographed against exposure to air containing oxygen. However, it required too much mercury for its mercury-pool, per sample analyzed. The cells constructed for use with the Electropode are shown in Figure 1.

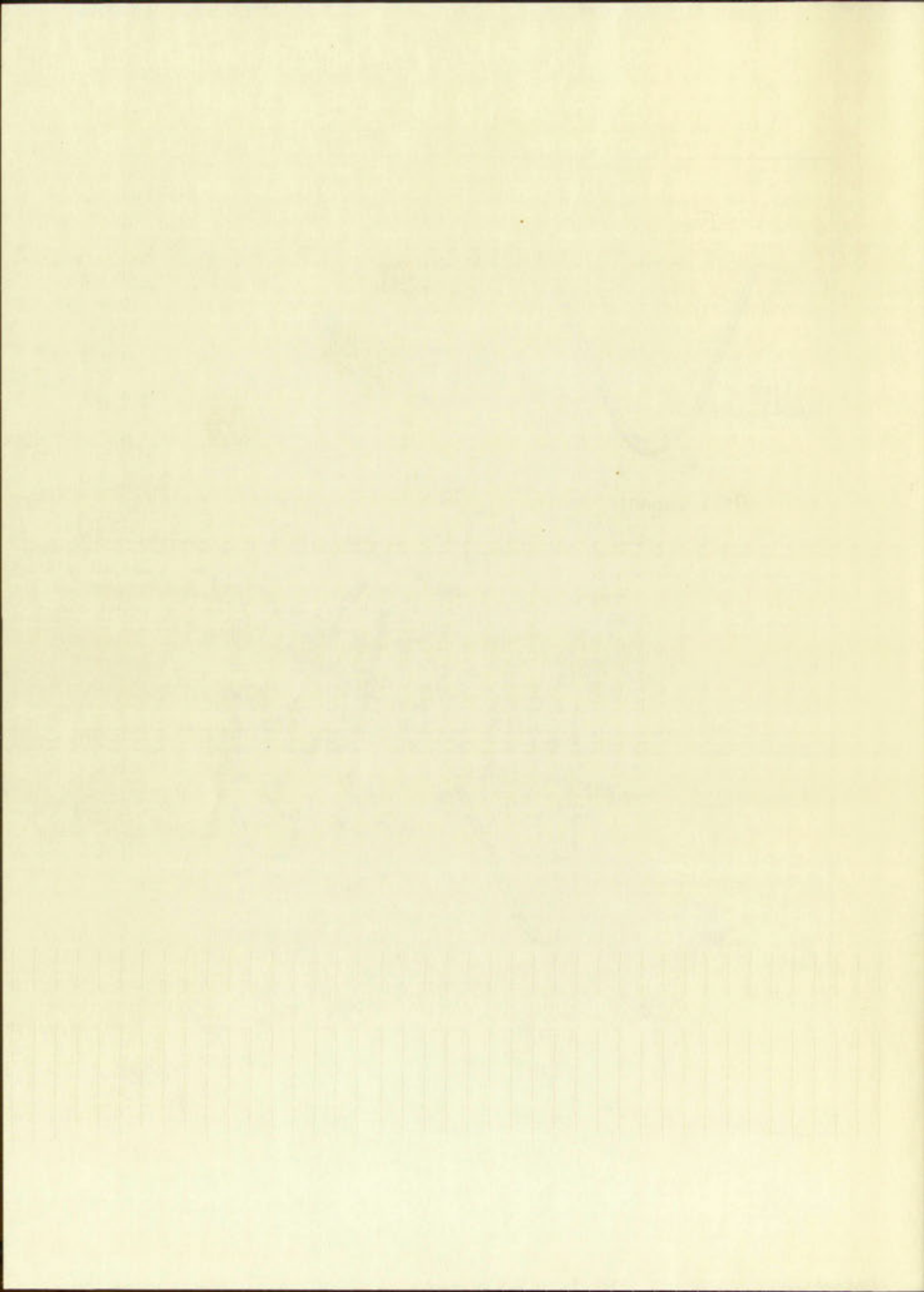
Cell (B) similar to the Heyrovsky cell provided the greatest protection of sample from air. Cell (C)¹⁰⁹ provided for the measurement of the reduction potential directly against the saturated calomel electrode half cell, (S.C.E.), when the latter was connected to the mercury-pool in the polarograph. This cell could also be used like cell (A).

¹⁰⁹cf. Kolthoff and Lingane, Polarography, (N. Y. Interscience Pub. Inc., 1941), p. 242.



POLAROGRAPHIC CELLS CONSTRUCTED FOR POLAROGRAPHIC MEASUREMENTS

Figure 1



EFFICIENCY
ERASE BOARD
RAC CONTENT

An electrolytic cell was required for proposed controlled reduction experiments. Also a coulometer to be used in conjunction with this electrolytic cell was necessary. The electrolytic cell used was a type similar to the one described by Lingane¹¹⁰ and modified by Pasternak¹¹¹. It was constructed as shown in Diagram A of Figure 2. The coulometer described by Lingane¹¹² was constructed for use with the electrolytic cell. It is shown in Diagram B of Figure 2.

The electrolytic cell and coulometer were set up as illustrated in Diagram C of Figure 2. The Sargent Polarograph galvanometer was used in these experiments to control the reduction potential¹¹³.

¹¹⁰Lingane, J. Am. Chem. Soc., 67, 1917, (1945).

¹¹¹Pasternak, Helv. Chim. Acta., 31, 772, (1948).

¹¹²Lingane, op. cit., p. 1918.

¹¹³Lingane, I. and E. C. Anal. Ed., 16, 150, (1944).

An electrolytic cell was required for proposed controlled reduction experiments. Also a voltmeter to be used in conjunction with this electrolytic cell was necessary. The electrolytic cell used was a type similar to the one described by Lingane¹¹⁰ and modified by Westerman¹¹¹. It was constructed as shown in Figure 4 of Figure 2. The voltmeter described by Lingane¹¹² was constructed for use with the electrolytic cell. It is shown in Figure 5 of Figure 2.

The electrolytic cell and voltmeter were set up as illustrated in Figure 6 of Figure 2. The control potentiograph galvanometer was used in these experiments to control the reduction potential.¹¹³

-
- ¹¹⁰Lingane, J. Am. Chem. Soc., 67, 1917, (1945).
- ¹¹¹Westerman, Helv. Chim. Acta, 31, 779, (1948).
- ¹¹²Lingane, op. cit., p. 1918.
- ¹¹³Lingane, J. and E. G. Appel, Ed., 16, 100, (1944).

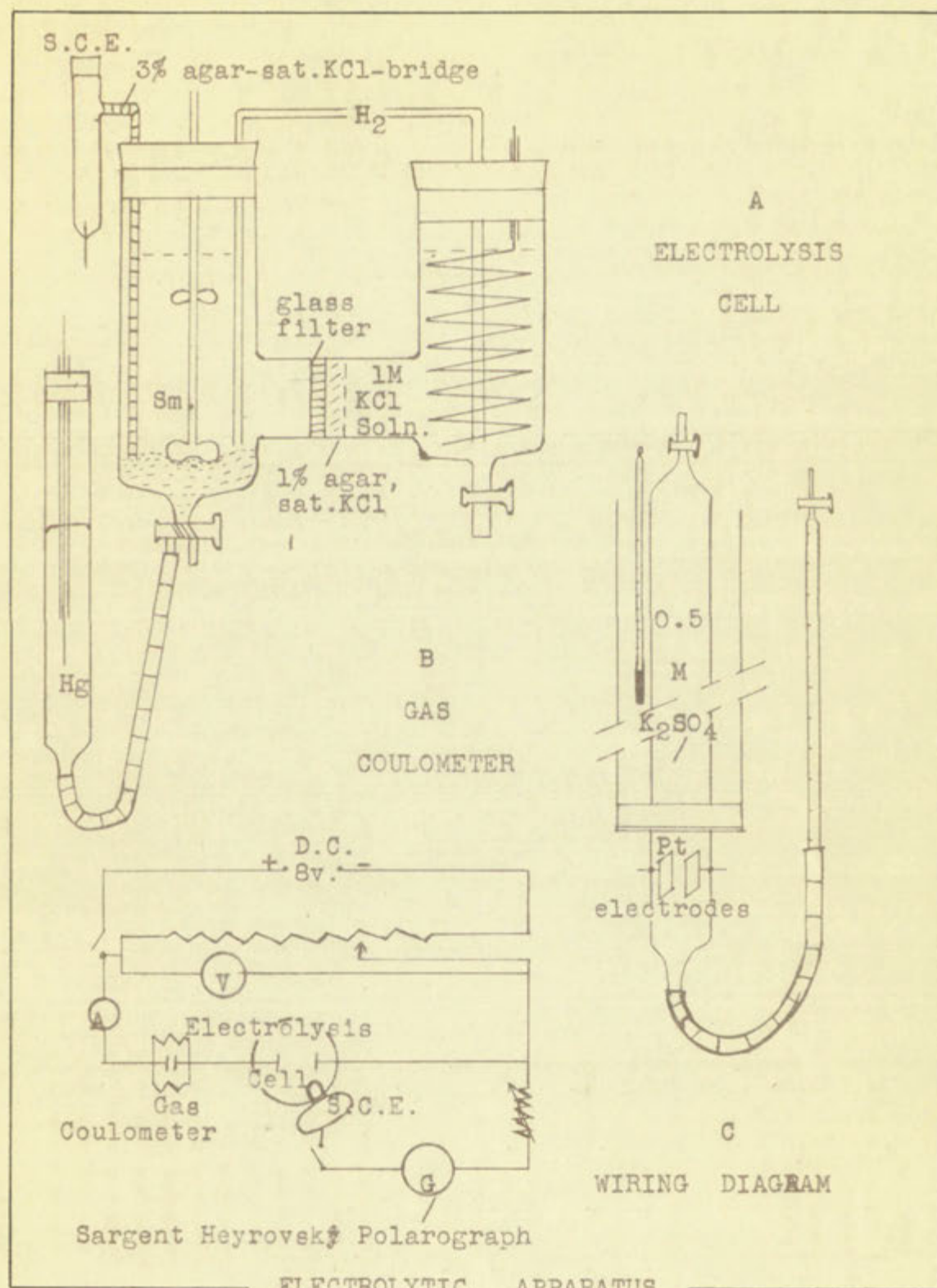
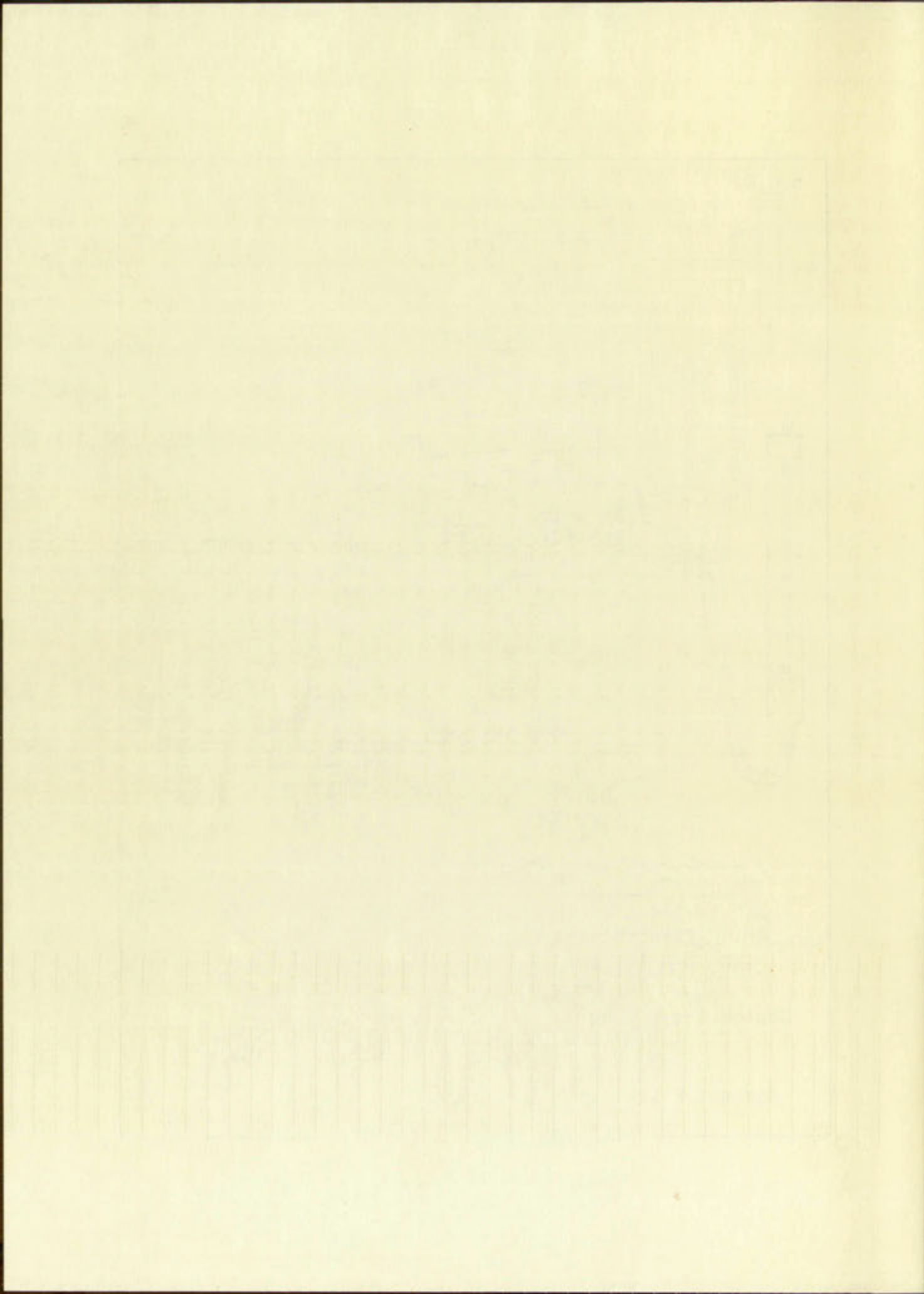


Figure 2



BY
BOND

12

B. EXPERIMENTAL TECHNIQUE

1. Polarography

The sample to be examined polarographically contained the test material, a supporting electrolyte, and necessary buffers. Nitrogen or hydrogen gas, previously washed with water, was bubbled through the sample to remove dissolved oxygen. In the case of cell (B), Figure I the nitrogen or hydrogen gas was kept flowing above the sample during the experiments in order to assure the absence of oxygen. The residual current was measured by polarographic observation of a blank containing everything except test material. No means of constant temperature control were available so that the prevailing room temperature, which varied considerably, was necessarily the temperature used in the studies.

2. Electrolysis

In the construction of the apparatus (Fig. 2, p. 49) a few details are noted. The glass filter in the electrolysis cell was made by pulverizing broken pyrex glass and sieving out the 60 mesh particles which were fused in the bottom of an asbestos padded porcelain crucible to form the filter (at about 750-800°C.). The filter was then sealed in the cross arm of the electrolytic cell. The saturated

2. EXPERIMENTAL METHOD

2.1. Electrolysis

The samples to be examined polycrystalline contained the test material, a supporting electrolyte, and a buffer. Nitrogen or hydrogen gas, previously passed through water, was bubbled through the sample to remove dissolved oxygen. In the case of cell (2), Figure 1, the nitrogen or hydrogen gas was kept flowing above the sample during the experiments in order to remove the traces of oxygen. The residual current was measured by reference to the observation of a blank containing everything except the sample. As means of constant temperature control were provided so that the prevailing room temperature, which varied only slightly, was necessarily the temperature used in the analysis.

2.2. Electrode

In the construction of the electrodes (Fig. 1) a few details are noted. The glass fitted in the electrolytic cell was made by withdrawing a glass tube and leaving out the 60 mesh particles which were fused in the bottom of an asbestos gasket potometer provided to the filter (at about 750-800°C.). The filter was then sealed in the crown end of the electrolytic cell. The electrode

potassium chloride, agar-agar gel was made by heating agar-shreds in water until the agar dispersed and later adding enough potassium chloride to obtain a saturated solution; the agar-agar would not disperse readily if potassium chloride was added first. Rubber tubing for both cell and coulometer was boiled in concentrated sodium hydroxide solution for an hour or more and then thoroughly washed. The electrodes were prepared and sealed in the coulometer tube as follows: the tungsten wire used for sealing leads into the pyrex glass walls was first broken or cut into desired lengths. This was done because tungsten is brittle and will shatter at a fused joint (made with another metal) at the instant it is broken anywhere along its length. A length previously covered with molten borax to minimize oxidation was sealed to a platinum bead. Back of the platinum bead a glass sleeve was fused on to the wire. The wire was then touched to a platinum foil electrode, about 1.7 square centimeters in area, at the platinum bead end in an oxygen flame to seal the electrode to the bead. Two electrodes thus prepared were then sealed into the walls of the coulometer tube at the glass sleeves, and the coulometer tube was annealed. The silver wire anode was made by winding 10 gauge silver wire into a closely packed helix about 33 millimeters in diameter and 55 millimeters long, with a total area of

potassium chloride, agar-agar, gel was made by heating water.

shreds in water until the agar dissolved and later adding

enough potassium chloride to obtain a saturated solution; the

agar-agar would not dissolve readily if potassium chloride was

added first. Rubber tubing for both cell and counter

was boiled in concentrated sodium hydroxide solution for an

hour or more and then thoroughly washed. The electrodes

were prepared and sealed in the counter tube as follows:

The tungsten wire used for sealing leads into the cell

glass walls was first broken or cut into desired lengths.

This was done because tungsten is brittle and will fracture

at a fused joint (made with another metal) at the instant

it is broken anywhere along its length. A length previously

covered with molten borax to minimize oxidation was sealed

to a platinum lead. Back of the platinum lead a glass

sleeve was fused on to the wire. The wire was then sealed

to a platinum foil electrode, about 1.5 square centimeters

in area, at the platinum lead and in an oxygen flame to seal

the electrode to the lead. Two electrodes thus constructed

were then sealed into the walls of the counter tube at

the glass sleeves, and the counter tube was mounted.

The silver wire anode was made by winding 10 gauge silver

wire into a closely packed helix about 50 millimeters in

diameter and 50 millimeters long, with a total area of

approximately 80 square centimeters. Analytical reagent grade potassium sulfate was used to make the 0.5 Molar electrolyzing solution used in the coulometer¹¹⁴.

The electrolytic apparatus was set up according to the wiring diagram shown in Fig. 2, (p. 49). The controlling reduction potential was measured by means of a high resistance voltmeter, obtained by connecting an external three megohm resistance to the Sargent Heyrovsky polarograph¹¹⁴ and using it as such; with the sensitivity of the Sargent instrument set at 5, the external resistance was adjusted until 50 millimeters on the scale corresponded to exactly 1 volt. Total scale was 150 millimeters.

In general the recommendations given by Lingane¹¹⁵ were followed. A current of 50-100 milliamperes was passed through the coulometer solution in the vicinity of the electrodes for 5 to 10 minutes with coulometer stopcock open to saturate the potassium sulfate solution with hydrogen and oxygen before starting the electrolysis. The supporting solution, minus the compound to be reduced, was electrolyzed at a potential 0.1 to 0.2 volt more negative than any reduction potential to be employed, before the test

¹¹⁴Lingane, I. and E. C. Anal. Ed., loc. cit.

¹¹⁵Lingane, J. Am. Chem. Soc., loc. cit.

approximately 50 square centimeters. Analytical reagent grade potassium sulfate was used to make the 0.2 molar electrolytic solution used in the experiments.¹¹⁴

The electrolytic apparatus was set up according to the wiring diagram shown in Fig. 2, (p. 48). The controlling reduction potential was measured by means of a high resistance voltmeter, obtained by connecting an external three megohm resistance to the Sargent-Waterson potentiometer¹¹⁵ and using it as such; with the sensitivity of the Sargent instrument set at 5, the external resistance was adjusted until 55 millivolts on the scale corresponded to exactly 1 volt. Total resistance was 100 millivolts.

In general the procedures given by Lingane¹¹⁶ were followed. A current of 50-100 milliamperes was passed through the constant solution in the vicinity of the electrode for 5 to 10 minutes with constant electrode open to exhaust the potassium sulfate solution with hydrogen and oxygen before starting the electrolysis. The supporting solution, when the compound to be reduced, was electrolyzed at a potential 0.1 to 0.2 volt more negative than any reduction potential to be employed, before the test

¹¹⁴Lingane, J. and E. G. Allen, *Anal. Chem.*, 30, 104 (1958).

¹¹⁵Lingane, J. and Allen, *Anal. Chem.*, 30, 104 (1958).

compound was introduced, to remove any reducible impurities which might be present. The test compound was added from a stock solution about one hundred fold more concentrated than the final dilution. The electrolysis was carried out at a potential 0.1 to 0.2 volt more negative than the half-wave reduction potential as determined by the polarograph. The electrolysis was continued at any given potential setting until the current ceased to flow. When the silver chloride coating on the silver anode became thick, it was periodically removed by reversed electrolysis in dilute sulfuric acid solution. After the first two or three experiments were carried out, it was found that whenever a neutral or alkaline solution was electrolyzed it was necessary to remove all of the mercury from the mercury-cathode reservoir and clean it by repeated shaking in distilled water¹¹⁶ before carrying out the next electrolysis; unless this was done some of the alkaline solution invariably seeped into the reservoir mercury. The seepage did not occur in acid solutions.

The first experiments were carried out by preparing a separate smaller sample for use in polarographic

¹¹⁶Weissberger, Physical Methods of Organic Chemistry, Vol. 2, (N.Y.C., Interscience Publishers, Inc., 1946), pp. 1191-92.

compound was introduced, to remove any reducible impurities which might be present. The test compound was added from a stock solution about one hundred fold more concentrated than the final dilution. The electrolysis was carried out at a potential 0.1 to 0.2 volt more negative than the half-wave reduction potential as determined by the polarograph. The electrolysis was continued at any given potential setting until the current ceased to flow. When the silver chloride coating on the silver anode became thick, it was periodically removed by repeated electrolysis in dilute sulfuric acid solution. After the first two or three experiments were carried out, it was found that whenever a neutral or alkaline solution was electrolyzed it was necessary to remove all of the mercury from the mercury-cathode reservoir and clean it by repeated shaking in distilled water before carrying out the next electrolysis; unless this was done some of the alkaline solution invariably seeped into the reservoir mercury. The seepage did not occur in acid solutions.

The first experiments were carried out by preparing a separate analysis sample for use in polarographic

measurements while the supporting solvent was pre-electrolyzed. The smaller sample was polarographed to determine reduction potential to be used. The smaller sample was 10 milliliters; the larger one to be electro-reduced was 100 milliliters altogether. After the test solution was electrolyzed, a ten milliliter portion was set aside for tests. In case more than one reduction potential was used on same test solution a ten milliliter portion was withdrawn for tests after each reduction step. Polarograms, pH measurements, and spectral curves were run on the test samples removed and set aside.

For the last experiments carried out, the electrolytic apparatus was modified by combining the polarograph and electrolytic apparatus. This was done by placing the dropping mercury capillary of the Fisher Electropode through another hole bored in the stopper covering the cathode compartment so that it became partly immersed in the test solution to be electrolyzed. The electrical lead from the dropping mercury capillary was connected by an extension to its regular jack in the Fisher Electropode. The mercury pool jack was connected to a wire which could be connected either to the cathode cell mercury pool lead or to the calomel half-cell lead, depending on whether it was desirable to polarograph the solution against the mercury pool or the

measurements while the supporting solvent was stirred. The smaller sample was polarographed to determine reduction potential to be used. The smaller sample was 10 milliliters; the larger one to be electro-reduced was 100 milliliters altogether. After the first reduction was also, analyzed, a ten milliliter portion was set aside for use. In cases where more than one reduction potential was used on the first reduction a ten milliliter portion was set aside for each after each reduction step. Polarograms, or measurements, and potential curves were run on the test samples removed and set aside.

For the last experiments carried out, the electro-lytic apparatus was modified by combining the cathode and anodic compartments. This was done by placing the hydrogen-evolving mercury cathode of the Fisher Electrode Company in another hole bored in the stopper covering the cathode compartment so that it became partly immersed in the test solution to be electrolyzed. The electrical lead from the dropping mercury cathode was connected by an extension to its regular lead in the Fisher Electrode. The mercury pool tank was connected to a wire which could be connected either to the cathode cell mercury pool lead or to the anode half-cell lead, depending on whether it was desired to polarograph the solution against the mercury pool or the

saturated calomel electrode. The calomel half-cell originally made with the side arm that extended into cathode compartment constructed from 4 millimeter glass tubing was replaced by a larger saturated calomel electrode side arm of 6 millimeter tubing so that a larger conducting path would be provided. The latter was constructed with a sealed in tungsten wire in the bottom of the tube to provide a better electrical path to the mercury contact in the saturated calomel electrode.

For each electrolysis carried out with the combined apparatus, $(110-x)$ milliliters of supporting solution, where x milliliters of $1 \times 10^{-2} M$ berberine hydrochloride solution were later added to get the concentration of berberine hydrochloride desired in solution to be electro-reduced, were placed in cathode compartment and pre-electrolyzed. Ten milliliters were removed and set aside as a blank for later tests. The solution was polarographed after the usual removal of dissolved oxygen. Enough of the stock berberine hydrochloride solution was added to make 100 milliliters of solution. After mixing well, this solution was polarographed. Ten milliliters were removed and set aside for later tests, leaving 90 milliliters (90%) to be electro-reduced. The electro-reduction was carried out at a potential of 0.1 to 0.2 volt more negative than the half-wave

exhausted calomel electrode. The calomel half-cell origin-
ally made with the side arm that extended into cathode com-
partment was connected from 4 millimeter glass tubing was
replaced by a larger saturated calomel electrode which was
of 8 millimeter tubing so that a larger conducting path would
be provided. The latter was connected with a sealed in
tungsten wire in the bottom of the tube to provide a better
electrical path to the mercury contained in the saturated
calomel electrode.

For each electrolysis carried out with the combined
apparatus, (10-2) milliliters of supporting solution,
where a milliliters of $10^{-2}M$ barbituric acid solution
solution were later added to get the concentration of bar-
bituric acid desired in solution to be electro-
reduced, were placed in cathode compartment and pre-electro-
lyzed. Ten milliliters were removed and set aside as a
blank for later tests. The solution was electrolyzed after the
usual removal of dissolved oxygen. Enough of the stock bar-
bituric acid solution was added to make 100 milli-
liters of solution. After mixing well, this solution was
polarographed. Ten milliliters were removed and set aside
for later tests, leaving 90 milliliters (90%) to be electro-
reduced. The electro-reduction was carried out at a potential
of 0.1 to 0.2 volt more negative than the half-wave

decomposition potential indicated by polarogram. A ten milliliter sample was removed and set aside for later tests, leaving eighty milliliters (80%) to be further electro-reduced, if the polarogram showed more than one half-wave potential. After the final reduction another polarogram was made on the remaining solution, and then ten milliliters were removed for later tests. When a polarogram was made, the electrolytic circuit had to be disconnected so that polarograms were only possible on the initial and final solutions. Otherwise, reverse electrolysis could occur.

The solutions set aside for tests were measured for pH values with a Beckman pH-meter and then used for spectral curve measurements. The ten milliliter sample set aside was large enough for polarographic tests also, when such tests were necessary, such as on intermediate samples not polarographed in electrolytic cell.

3. Spectrophotometry

Spectral curves of the various solutions measured were made with the Beckman Model DU Quartz Spectrophotometer with UV attachment. Two matched silica cells were used for all measurements. The samples were diluted one-tenth to approximately $1 \times 10^{-5} M$ concentration before measurement in the spectrophotometer. The spectral band width was kept as

deposition potential indicated by potentiometer. A can
 millimeter sample was removed and set aside for later
 tests, leaving slightly millimeter (50) to be tested
 electro-thermally. If the potentiometer showed more than one
 half-wave potential. After the final reduction process
 potentiometer was made on the remaining solution, and then the
 millimeter was removed for later tests. When a millimeter
 was made, the electro-thermally tested and to be tested
 needed so that potentiometer was only possible on the
 initial and final solution. Otherwise, various electro-
 lytes could occur.

The solutions and millimeter were removed for
 pH values with a Beckman pH meter and then used for electro-
 lyte curve measurements. The two millimeter sample and
 acid was large enough for potentiometric tests and, when
 such tests were necessary, such as to measure the amount
 not polarized in electrolytic cell.

5. The Electrode

General source of the electrode solution was
 were made with the Beckman Model 55 glass electrode
 with UV attachment. The electrode glass was a 1/2 inch
 all measurements. The angles were divided into 1/2 inch
 approximately 1/2 inch. A concentration of 0.1 molar was used
 the spectrograph. The electrode was used with a 1/2 inch

low as possible in order to provide maximum resolution of absorption bands; this was done by using as small slit width openings as possible. However, the range was still not under 1.5 millimicrons, varying from 1.5 to about 2.8. With some Beckman Spectrophotometers it is possible to get a 1.0 millimicron range in the entire ultra violet region. As far as could be told, the 1.5 to 2.8 millimicron range was adequate in the present case.

In addition to spectral curve measurements, fluorescent tests were made on the various berberine test solutions. A drop of the 10^{-4} molar test solution, dried on a piece of filter paper, was examined in ultraviolet light by means of a Mineralight 2537 millimicron wavelength lamp. One sample was also examined in the Beckman instrument over its range, with maximum slit width opening.

low as possible in order to provide maximum resolution of absorption bands; this was done by using an slit width opening as possible. However, the range was still not as low as 1.5 millimicrons, varying from 1.5 to about 2.5. With some Beckman Spectrophotometers it is possible to get a 1.0 millimicron range in the entire ultra violet region. As far as could be told, the 1.5 to 2.5 millimicron range was adequate in the present case.

In addition to spectral curve measurements, fluorescence tests were made on the various polymers and solvents. A drop of the 10% polymer test solution, dried on a piece of filter paper, was examined in ultraviolet light by means of a Beckman DU-2 millimicron wavelength lamp. One sample was also examined in the Beckman fluorescence ray the range, with various slit widths opening.

CHAPTER V

PRELIMINARY POLAROGRAPHIC WORK

The Fisher Electropode showed a sensitivity of 0.0225 microamperes per scale division.

A test solution was prepared containing $1 \times 10^{-4} M$ berberine hydrochloride, 0.1M tetramethyl ammonium chloride (E. K. Co., P-3592), 0.01% gelatin, and acetate buffer to establish a pH of 6¹¹⁷. This solution was analyzed polarographically and was found to have one half-wave for berberine at approximately -1.15 volts. A second half-wave was reached at about -1.7 volts, but the blank also showed this half-wave; this indicated that impurities were present in one or more of the reagents.

In order to assure the absence of oxygen Cell B shown in Fig. 1, p. 47, was used. The gelatin had impurities in it so its use as a suppressor was discontinued. Unless the acetate buffer solution was prepared immediately before use, extraneous waves were obtained. It was found necessary to recrystallize the tetramethylammonium chloride twice from ethanol to obtain a solution which would give satisfactory results. Unbuffered solutions prepared from this twice crystallized compound did not decompose until a voltage of

¹¹⁷ Kirkpatrick, Quart. J. Pharm. Pharmacol. (London) 19, 10--11 (1946).

CHAPTER V

PRELIMINARY POLAROGRAPHIC WORK

The Polar Electrode showed a sensitivity of 0.0250 microamperes per scale division. A test solution was prepared containing 10^{-3} M barbitone hydrochloride, 0.1M tetramethylammonium chloride (E. K. Co., P-3562), 0.01% gelatin, and acetate buffer to establish a pH of 8.17. This solution was analyzed polarographically and was found to have one half-wave for barbitone at approximately -1.15 volts. A second half-wave was reached at about -1.7 volts, but the blank also showed this half-wave; this indicated that oxidation was present in one or more of the reagents. In order to ensure the absence of oxygen Cell A shown in Fig. 1, p. 47, was used. The gelatin had been treated as usual and a supporting electrolyte was discontinued. Unless the acetate buffer solution was prepared immediately before use, extensive waves were obtained. It was found necessary to reprecipitate the tetramethylammonium chloride twice from ethanol to obtain a solution which would give satisfactory results. Undisturbed solutions prepared from this twice recrystallized compound did not decrease until a voltage of

17
 Kikunori, Chem. J. Japan, (Tokyo), (1946).
 18, 10-11 (1946).

-2.3 to -2.4 volts was reached. This decomposition voltage was somewhat less than that indicated previously¹¹⁸.

In order to determine the limits of various electrolytes and buffers, a series of polarograms was made on solutions containing the cations, sodium, potassium, lithium, ammonium, and tetramethylammonium and the anions, iodide, bromide, chloride, fluoride, hydroxyl, acetate, carbonate, borate, biphthalate, and tartrate. Some of the solutions were buffered, others were not.

Solutions of potassium ion and of the tetramethylammonium ion had relatively high decomposition potentials and it was ascertained that they were the only cations which could be used successfully in these studies.

Of the halides the chloride and fluoride were about equal and exceeded the others in their negative decomposition potentials. The weak organic acid anions had more negative decomposition potentials than the chloride ion. The remaining anions examined were unsatisfactory.

Accordingly further experimental tests were confined to supporting electrolytes and buffers containing the cations potassium and tetramethylammonium and the anions chloride, hydroxyl, carbonate, acetate, tartrate, and phosphate.

¹¹⁸Rysselberghe and McGee, J. Am. Chem. Soc., 67, 1039 (1945).

-2.3 to -2.4 volts was reached. This decomposition voltage was somewhat less than that indicated previously.¹¹⁸

In order to determine the limits of various electro-

lytes and buffers, a series of polarograms was made on solutions containing the cations, sodium, potassium, lithium, ammonium, and tetramethylammonium and the anions, iodide, bromide, chloride, fluoride, hydroxyl, acetate, carbonate, borate, phosphate, and tartrate. Some of the solutions were buffered, others were not.

Solutions of potassium ion and of the tetramethylammonium ion had relatively high decomposition potentials and it was ascertained that they were the only cations which could be used successfully in these studies.

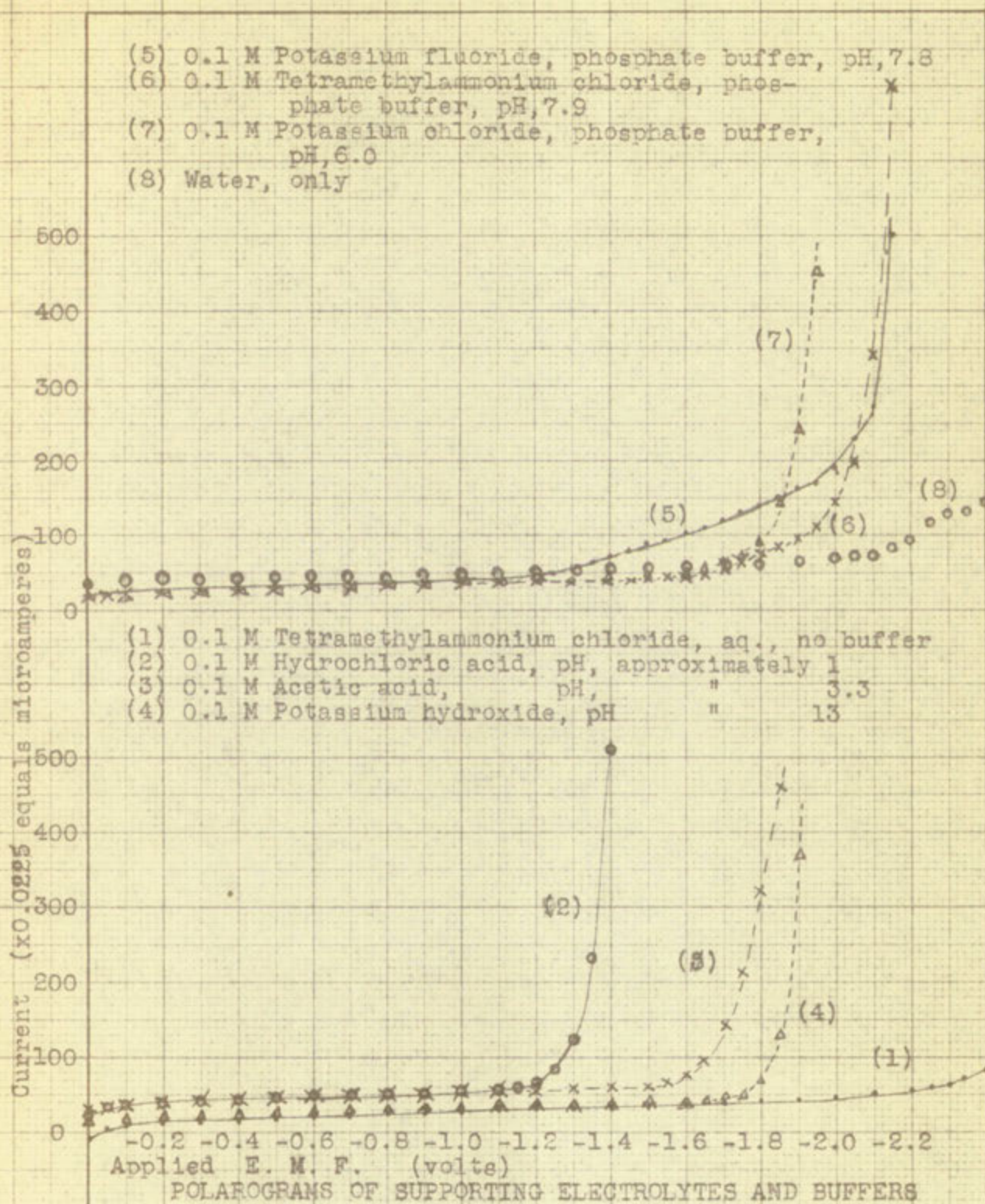
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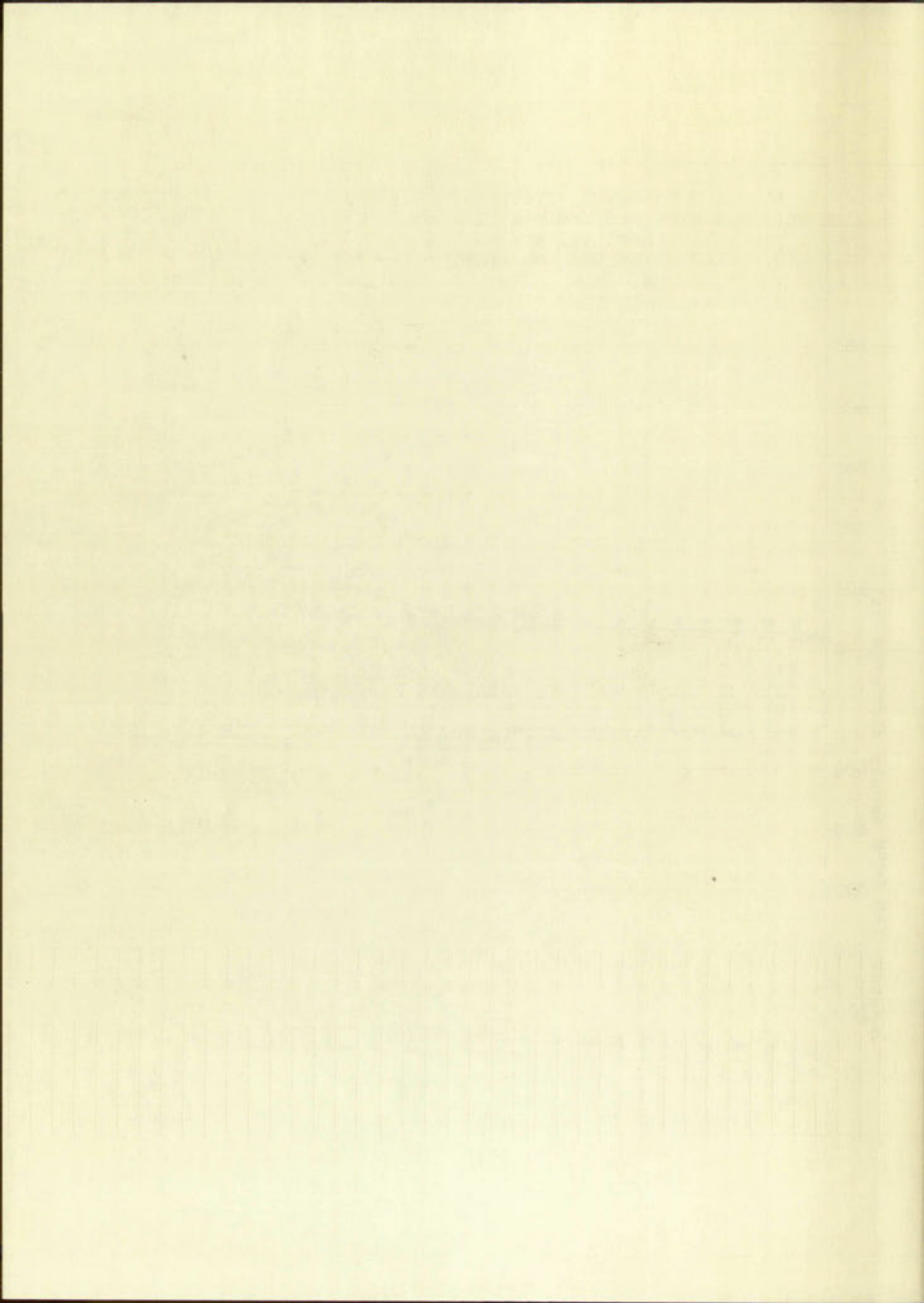
¹¹⁸ Wassilberghs and McGee, J. Am. Chem. Soc., 67, 1026 (1945).

In all instances the decomposition potentials were lower in acid solutions and became more negative with increasing pH. Mercury dropping times were 19-21 drops per minute and the temperature was 25-31°C. Some typical polarograms obtained are shown in Graph I.

In all instances the decomposition potentials were lower in cold solutions and became more negative with increasing pH. Mercury dropping times were 15-20 drops per minute and the temperature was 25-30°C. Some typical polarograms obtained are shown in Graph 1.



GRAPH 1



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FAB CONTENT

CHAPTER VI

POLAROGRAPHIC MEASUREMENTS OF BERBERINE

For the first series of measurements on berberine a stock solution was prepared containing 40.81 milligrams of berberine hydrochloride in 100 milliliters of solution, equivalent to 1.00×10^{-3} molar. Appropriate volumes of this solution plus the necessary supporting electrolytes and buffers were used to make the test solutions.

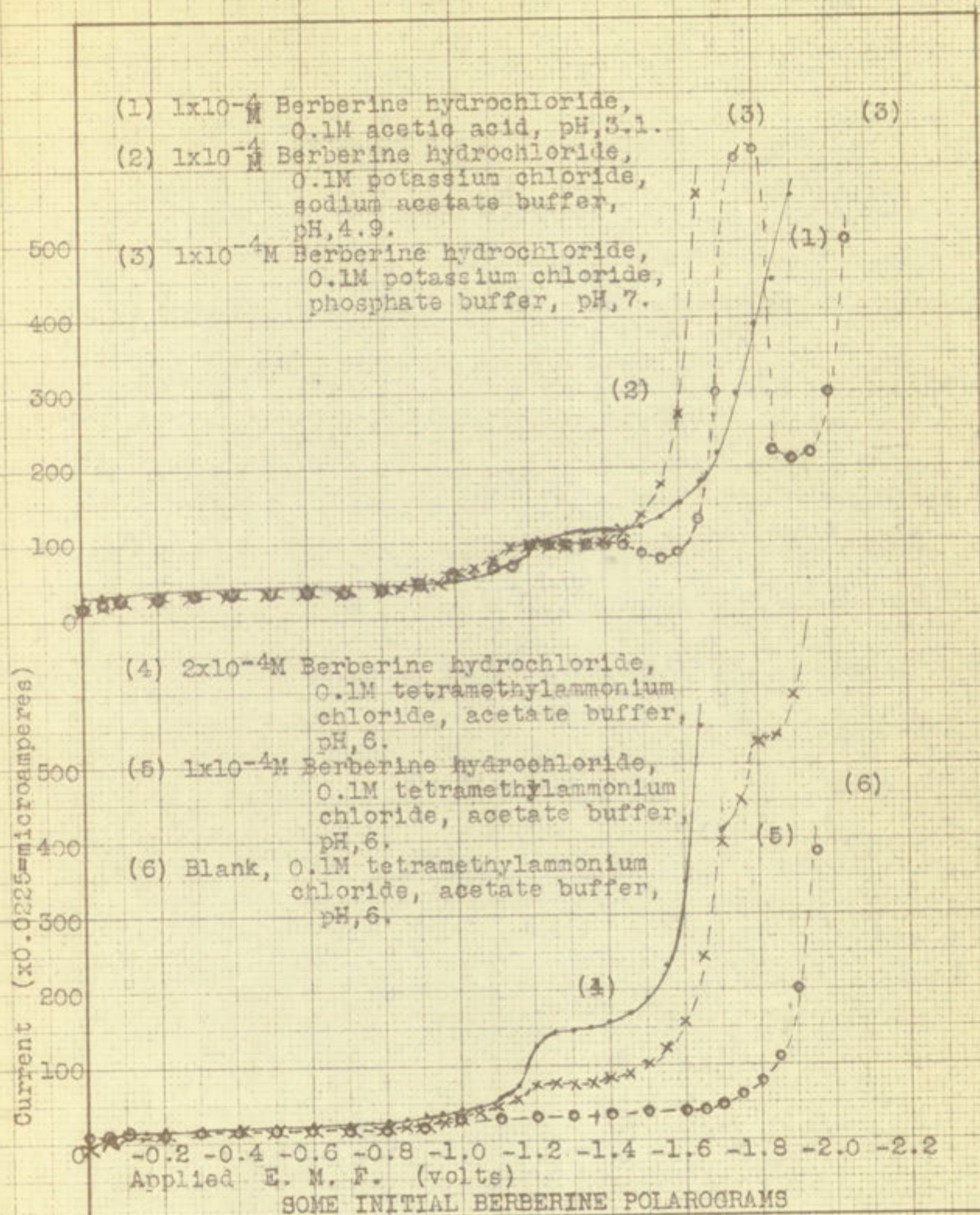
Some of the initial polarograms are shown in Graphs 2, 3, and 4. All these curves were obtained without a suppressor. The polarographic waves obtained in the range of -1.6 to -1.7 volts are probably catalytic waves due to hydrogen discharge rather than an indication of the reduction of berberine. The results tabulated from graphs are given in Table XII. These results are summarized in Table XIII.

CHAPTER VI

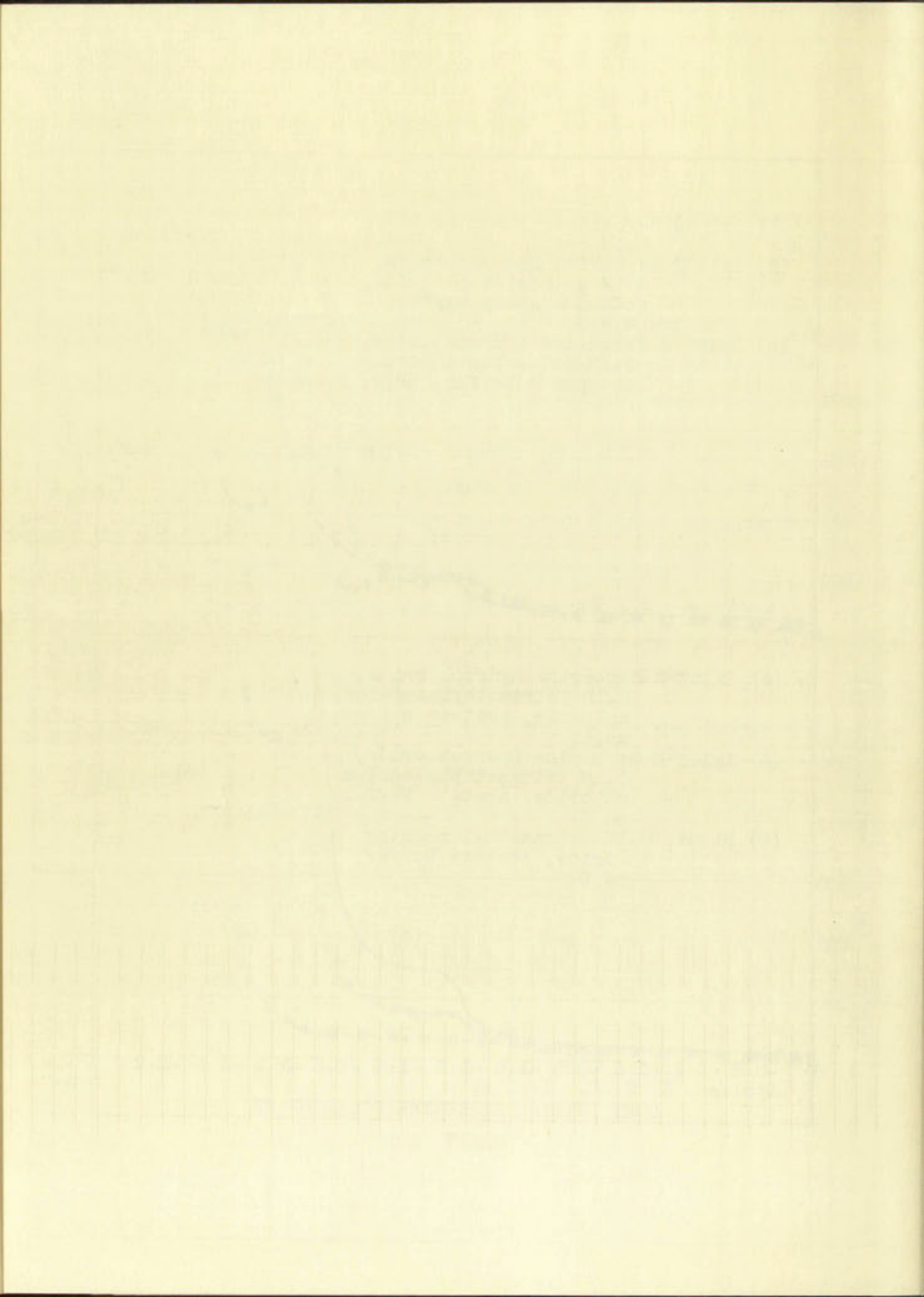
POLAROGRAPHIC MEASUREMENTS OF BERBERINE

For the first series of measurements on berberine a stock solution was prepared containing 40.81 milligrams of berberine hydrochloride in 100 milliliters of solution, equivalent to 1.00×10^{-5} molar. Appropriate volumes of this solution plus the necessary supporting electrolytes and buffers were used to make the test solutions.

Some of the initial polarograms are shown in Figure 3, 3, and 4. All these curves were obtained without a suspension. The polarographic waves obtained in the range of -1.8 to -1.7 volts are probably catalytic waves due to hydrogen discharge rather than an indication of the reduction of berberine. The results tabulated from graphs are given in Table XII. These results are summarized in Table XII.

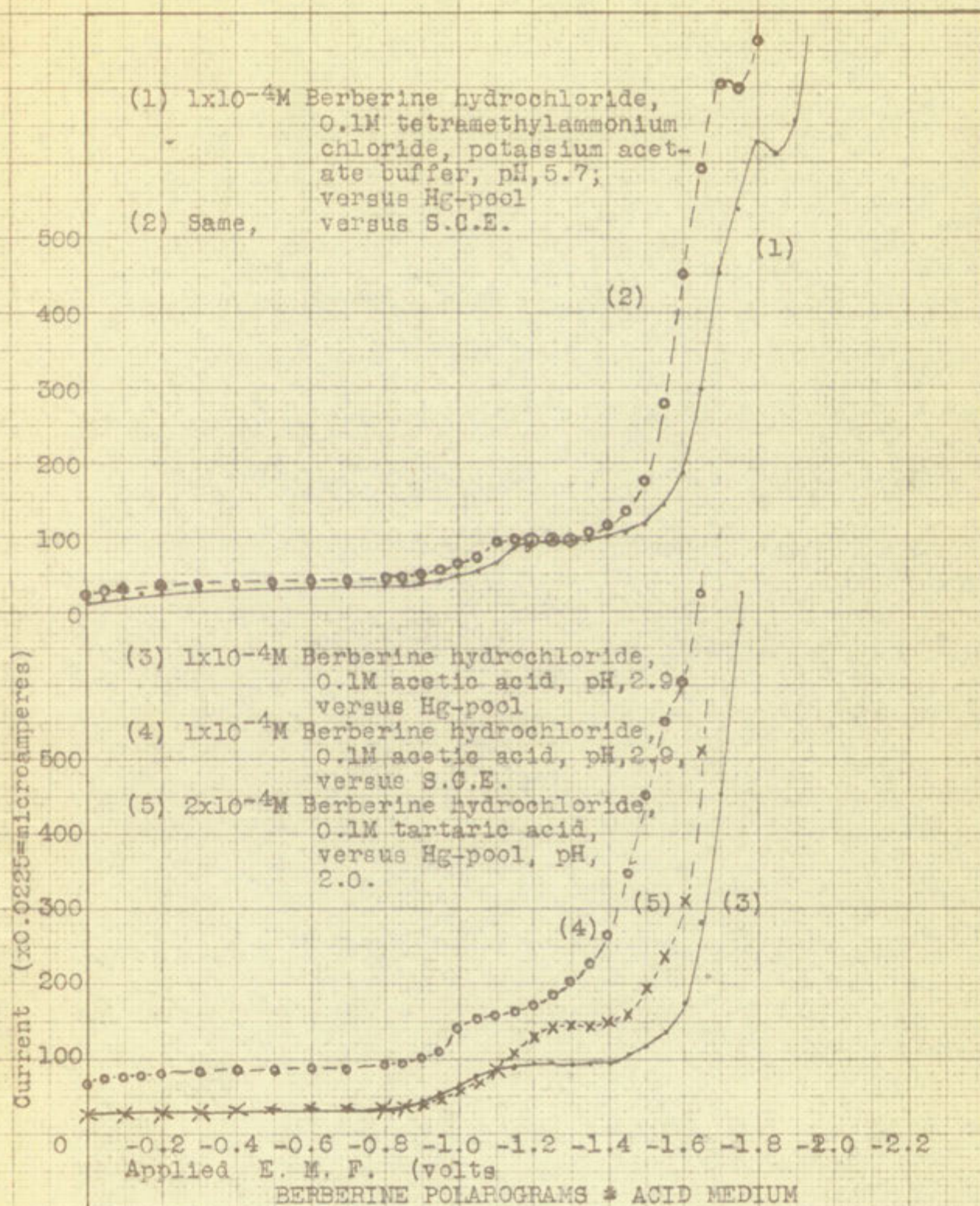


GRAPH 2

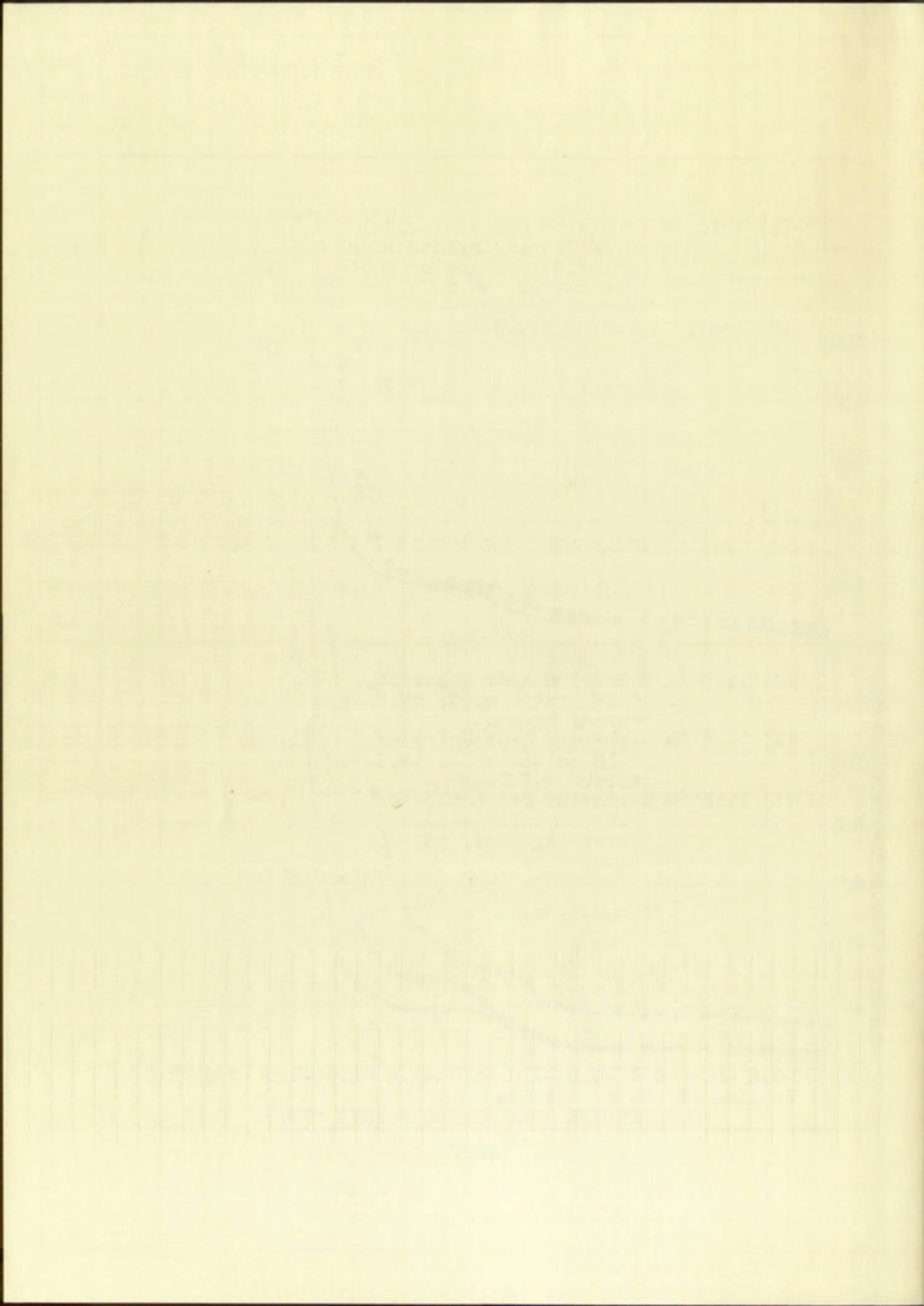


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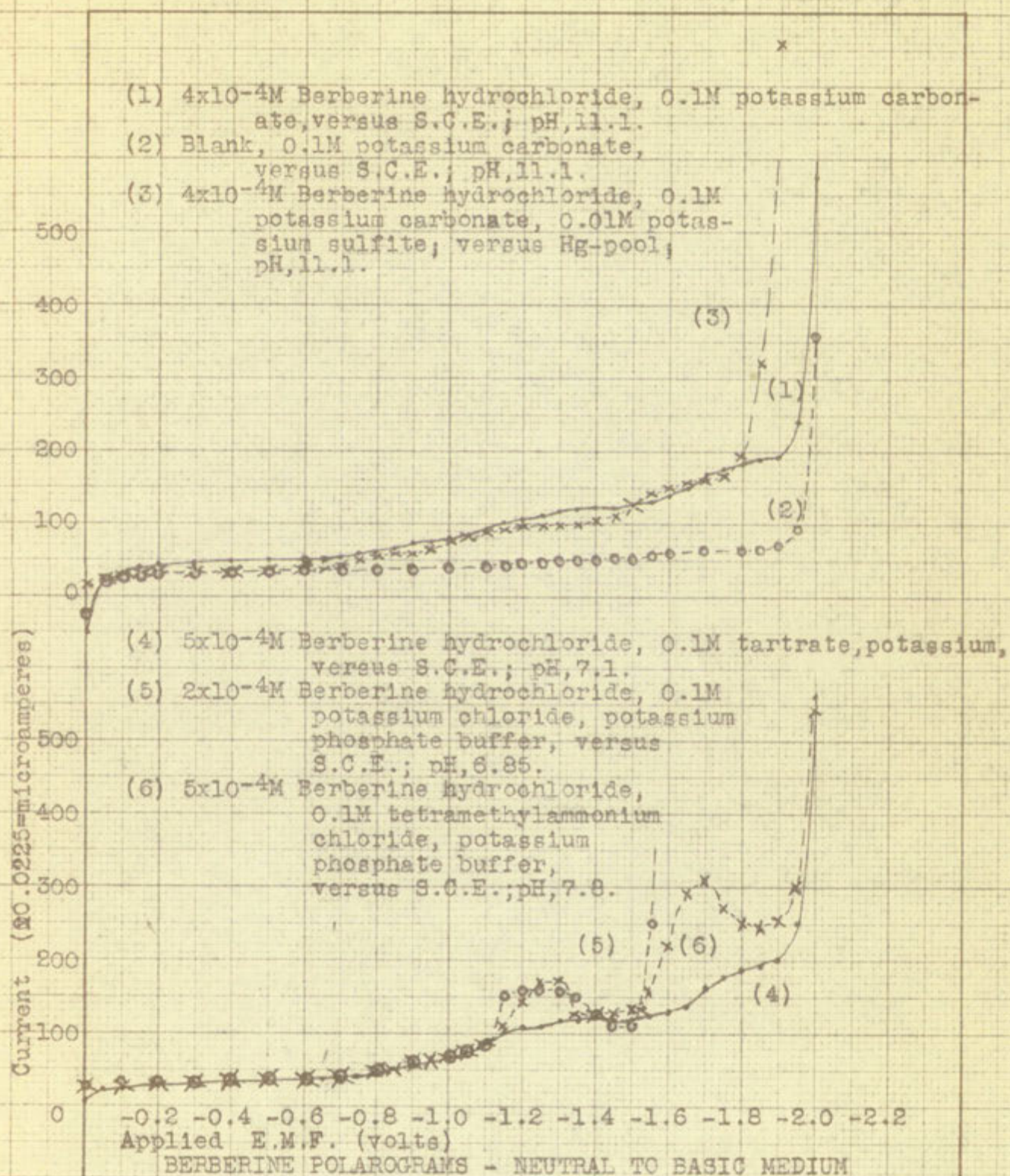


GRAPH 3

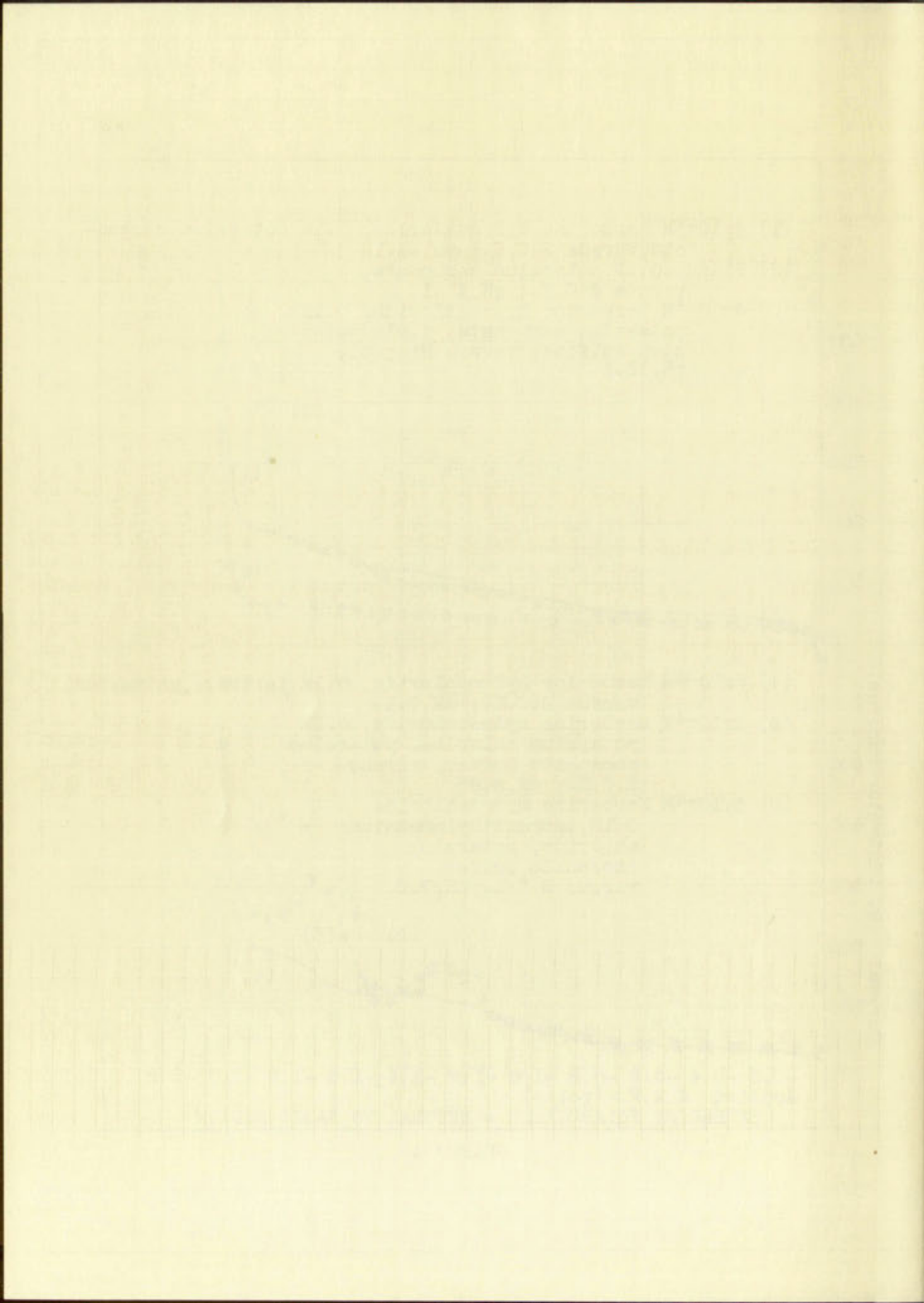


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GRAPH 4



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UNIT

TABLE XII
POLAROGRAPHIC RESULTS OF BERBERINE SOLUTIONS

Concen- tration, Berberine hydro- chloride	Supporting Electrolyte and Buffer	pH	Half-wave Potentials A* B**	Current Rise microamperes per millimole
$2 \times 10^{-4} M$	0.2M tartar- ic acid	2.0	-1.10	12.3
$1 \times 10^{-4} M$	0.1M acetic acid	2.9	-0.98 -1.00	13.5
$1 \times 10^{-4} M$	0.1M $(CH_3)_4$ NCl & potas- sium acetate	5.7	-1.10 -1.04; -1.56.	13.5 135.
$2 \times 10^{-4} M$	0.1M KCl, & KH_2PO_4	6.85	-1.15; -1.60.	7.9 (13.5max.*); 168.
$5 \times 10^{-4} M$	0.1M potas- sium tartrate	7.1	-0.85; -1.10; -1.68.	1.5; 2.25; 2.7.
$5 \times 10^{-4} M$	0.1M $(CH_3)_4$ NCl & KH_2PO_4	7.8	-0.85; -1.15; -1.60.	1.13; 2.9 (4.9max.*); 3.4 (8.1max.*).
$1 \times 10^{-4} M$	0.1M KCl, & KH_2PO_4	8.5	-0.88; -1.28; -1.62.	3.4; 3.4; 6.8.
$4 \times 10^{-4} M$	0.1M potas- sium carbon- ate	11.1	-0.747; -0.88; -1.15; -1.62.	0.56; 0.8; 2.25; 3.4
$4 \times 10^{-4} M$	0.1M K_2CO_3 0.01M K_2SO_3	11.1	-0.80; -1.00; -1.52.	1.1; 2.0; 3.4.
$(1 \times 10^{-3} M$	Benzaldehyde, 0.1M KCl	7	-1.35	4.7)

Mercury dropping time, 20 drops per minute; temperature range, 27-30°C.

* A, versus mercury pool; **B, versus S.C.E.; * maximum.

TABLE XII

POLAROGRAPHIC RESULTS OF BISMUTHINE SOLUTIONS

Concentration, Bismuthine Hydro- chloride	Supporting Electrolyte and Buffer	pH	Half-wave Potential, V vs SCE	Current Rise after 10 minutes
$1 \times 10^{-4} M$	0.2M tartaric acid	3.0	-1.15	11.5
$1 \times 10^{-4} M$	0.1M acetic acid	3.9	-0.98 -1.20	15.5
$1 \times 10^{-4} M$	0.1M (CH ₃) ₂ NH HCl + 0.05M sodium acetate	5.7	-1.10 -1.24; -1.20	13.8 12.8
$2 \times 10^{-4} M$	0.1M KCl, 0.1M KH ₂ PO ₄	8.85	-1.15; -1.20; -0.98	7.5 (15.5 max. 2); 10.8; 1.5
$3 \times 10^{-4} M$	0.1M tartaric acid	7.1	-1.10; -1.20	8.25; 5.7
$5 \times 10^{-4} M$	0.1M (CH ₃) ₂ NH HCl + 0.05M KH ₂ PO ₄	7.6	-1.15; -1.20	11.5; 3.9 (15.5 max. 2); 2.8 (10.8 max. 2)
$1 \times 10^{-4} M$	0.1M KCl, 0.1M KH ₂ PO ₄	8.5	-0.98; -1.18; -1.20	5.4; 5.4; 5.8
$4 \times 10^{-4} M$	0.1M tartaric acid	11.1	-0.74; -0.98; -1.15; -1.20	0.65; 0.5; 1.20; 1.4
$4 \times 10^{-4} M$	0.1M K ₂ CO ₃ , 0.01M VCl ₃	11.1	-0.60; -1.00; -1.20	1.1; 0.5; 1.4
($1 \times 10^{-5} M$) Bismuthine 0.1M KCl		8	-1.15	4.7

Mercury dropping time, 50 drops per minute; temperature range, 25-30°C.

* A, versus activity pool; ** B, versus 0.1M VCl₃ solution.

TABLE XIII
DECOMPOSITION POTENTIALS OBTAINED ON BERBERINE SOLUTIONS

Concentration	Conditions	pH	Decomposition Potentials*		
			First Wave	Second Wave	Third Wave
1 to $2 \times 10^{-4}M$	acid range	2-5	-1.0 to -1.3	-1.56?	
1 to $5 \times 10^{-4}M$	neutral range	6-7	-0.85 to -0.9	-1.1 to -1.2	-1.6 to -1.7
1 to $5 \times 10^{-4}M$	alkaline range	8-11	-0.85 to -0.9	-1.1 to -1.3	-1.5 to -1.65

* in volts

TABLE VIII

DECOMPOSITION POTENTIALS OBTAINED IN HYDROLYTIC SOLUTIONS

Concentration	Condition	First Wave	Second Wave	Decomposition Potential, Volts
1 to 5% Fe^{+2}	acid range	0.5 to 0.8	-1.5 to -1.8	
1 to 5% Fe^{+3}	acid range	0.5 to 0.8	-1.5 to -1.8	
1 to 5% Fe^{+2}	alkaline range	0.5 to 0.8	-1.5 to -1.8	
1 to 5% Fe^{+3}	alkaline range	0.5 to 0.8	-1.5 to -1.8	

all in volts

CHAPTER VII

THE CONTROLLED REDUCTION AND COULOMETRIC
MEASUREMENTS OF BERBERINE

For the remaining experiments with berberine a stock solution was prepared containing 407.05 milligrams of berberine hydrochloride in 100 milliliters aqueous solution at 25° C., equivalent to 0.998×10^{-2} molar berberine hydrochloride. One to 5.00 milliliters of this solution diluted to 100 milliliters, including supporting electrolytes and buffers, served as the test solution in each experiment. The electrolytic apparatus and essential procedure have been described (pp. 49, 52). Hydrogen gas was used to remove and keep out oxygen.

The first series of experiments were made on the basis of results recorded in the last chapter. Each experiment consisted of electrolyzing a known concentration of berberine hydrochloride in an aqueous solution containing a supporting electrolyte and in most instances a buffer. Solutions were electrolyzed in both acid and alkaline media. The extent of reduction varied with conditions. The essential data are given in Tables XIV and XV. Data are arranged in order of increasing pH in Table XIV, and in

CHAPTER VII

THE CONTROLLED REDUCTION AND OXIDATION

MEASUREMENTS OF REDUCTION

For the remaining experiments with butyric acid, a solution was prepared containing 10.00 millimoles of butyric acid in 100 milliliters of 0.1N sodium hydroxide. This solution was then diluted to 100 milliliters, including supporting electrolyte and solvent, served as the test solution in each experiment. The electrolytic apparatus and essential procedure have been described (pp. 42, 43). Hydrogen gas was used to remove and keep out oxygen.

The first series of experiments were made on the basis of results recorded in the last chapter. Each experiment consisted of electrolyzing a known concentration of butyric acid in an aqueous solution containing a supporting electrolyte and at various potentials. Solutions were electrolyzed in both acid and alkaline media. The extent of reduction varied with conditions. The results are given in Tables XIV and XV. These are arranged in order of increasing E in Table XIV, and in

order carried out in Table XV. Coulometric data are arranged by letters of respective solutions. Corrections for the partial pressure of water vapor in the coulometer were taken from published data¹¹⁹.

Polarograms on some of these solutions are shown in Graphs 5, 6, and 7 following Table XV.

The second and last series of electrolytic experiments were made with only hydrochloric acid, potassium hydroxide, potassium chloride, tetramethyl ammonium chloride, and potassium hydrogen phosphate, used as supporting electrolytes or buffers or both. The essential data are shown in Tables XVI and XVII in a similar manner as in the previous tables. Polarograms of some of the solutions are shown in Graphs 8 and 9.

Spectral curves, covered in the next chapter were determined for these solutions.

Qualitative tests of several of the berberine solutions for the aldehyde group, such as the test with Tollen's reagent were negative. Neither berberine hydrochloride nor reduced berberine derivatives showed a test any different from a blank.

¹¹⁹Lingane, J. Am. Chem. Soc., 67, 1918 (1945).

order carried out in Table XV. Conductivity data are arranged by factors of respective solutions. Corrections for the partial pressure of water vapor in the condenser were taken from published data.¹⁹

Polarograms on some of these solutions are shown in

Graphs 5, 6, and 7 following Table XV.

The second and last series of electrolytic experi-

ments were made with only hydrochloric acid, potassium hydroxide, potassium chloride, tetramethyl ammonium chloride, and potassium hydrogen phosphate, used as supporting electrolytes or buffers or both. The essential data are shown in Tables XVI and XVII in a similar manner as in the previous tables. Polarograms of some of the solutions are shown in Graphs 8 and 9.

Boiling curves, covered in the next chapter were

determined for these solutions.

Qualitative tests of several of the barbituric acid-

ions for the aldehyde group, such as the test with Fehling's reagent were negative. Neither barbituric hydrochloride nor reduced barbituric derivatives showed a test any different from a blank.

TABLE XIV

ELECTROLYTIC DATA, POLAROGRAPHIC, FIRST SERIES

Solution	Berberine Concentration Molar	Electrolyte	pH	Half-wave Potentials versus Hg-pool volts	Current Rise microamperes per millimole
A	2×10^{-4}	0.1M (CHOHCOOH) ₂	2.0 2.1	-1.12 none	12.3 none
E	1×10^{-4}	0.05M (CH ₃) ₄ NCl, CH ₃ COOK buffer	5.8 5.8 6.9 9.6	-1.1; -1.7 -1.1; -1.7? -1.7? none	13.5; 140. 6.7; 124? 11.2? none
B	5×10^{-4}	0.1M (CHOHCOOH) ₂	7.0	-1.2; -1.8	3.4 (4.3max.); 2.0 (2.9max.);
H	1×10^{-4}	0.5M KCl, KH ₂ PO ₄ buffer	8.0	-1.1; -1.55	4.5; 6.7.
			8.4	-1.05; -1.55	4.5; 5.6.
			9.1	-1.15; -1.75	4.5; 24.7.
G	2×10^{-4}	0.1M KCl, KH ₂ PO ₄ buffer	8.1	-0.8; -1.2; -1.7	3.3; 2.2 (6.7max)
			8.4	-1.3	0.55 (5.1max.);
			9.2	-1.35	2.2 (3.3max.);
D	5×10^{-4}	0.1M K ₂ CO ₃	11.2 11.2 11.2	-0.65; -1.0; -1.65 -0.7; -1.0; -1.65 -0.7; -1.15; -1.6	1.1; 2.9; 2.7 1.7; 1.7; 2.26. 1.7; 1.1; 1.1.

TABLE XIV (continued)
ELECTROLYTIC DATA, POLAROGRAPHIC, FIRST SERIES

Solution	Berberine Concentra- tion Molar	Electrolyte	pH	Half-wave Potentials versus Hg-pool volts	Current Rise microamperes per milli- mole
C	5×10^{-4}	0.1M K_2CO_3	11.1	-0.7; -1.0; -1.25; -1.55	1.1; 1.6; 1.35; 1.8.
C-reduced at -1.25 volts*		0.01M K_2SO_3	11.15	-0.7; -1.15; -1.55	1.1; 0.7; 1.6.
C-reduced at -1.65 volts*			11.15	-0.7; -1.15; -1.55	0.9; 0.5; 1.6.
C-reduced at -1.9 volts*			11.15	-0.75; -1.25; -1.5	0.9; 0.4; 1.35.

Mercury dropping time, 20 drops per minute; temperatures, 25-30°C.

* versus S. C. E. (saturated calomel electrode).

(Boulevard) via street

Station 1000, 1000-1000, 1000-1000

Station	1000-1000	1000-1000	1000-1000
Station 1000	1000-1000	1000-1000	1000-1000
Station 1000	1000-1000	1000-1000	1000-1000
Station 1000	1000-1000	1000-1000	1000-1000

Station	1000-1000	1000-1000	1000-1000
Station 1000	1000-1000	1000-1000	1000-1000
Station 1000	1000-1000	1000-1000	1000-1000
Station 1000	1000-1000	1000-1000	1000-1000

Station 1000, 1000-1000, 1000-1000

(Station 1000, 1000-1000, 1000-1000)

TABLE XV

ELECTROLYTIC DATA, COULOMETRIC, FIRST SERIES

Solu- tion	A Berb. concn.	B Bar. Pr. in.	C Coul.T. C	R Redn. EMF.	D Vol. H ₂ -O ₂	E P.Pr.H ₂ O Coul., mm.	Changes in Solution	Electrons per Molecule*
A	2x10 ⁻⁴ M	24.920	29.0-28.9	-1.25	1.74	29.4	colorless	3.72
A, ck.	2x10 ⁻⁴	25.080	30.4-28.9	-1.25	1.75	30.7	colorless	3.76+5%
B	5x10 ⁻⁴	25.076	27.2-26.8	-8.2	0.12	26.0	none	0.10
C	5x10 ⁻⁴	24.953 24.963	34.2-34.1 33.8-32.0	-1.25 -1.65	0.545 0.91	42, est. 35,	cloudy "	0.45 0.85 1.3, tot.
D	2x10 ⁻⁴	24.963	30.6-30.3 30.3-30.0	-1.65 -1.95	0.19 0.455	32 31	cloudy "	0.40 0.97 1.4, tot.
E	1x10 ⁻⁴	24.896	31.8-31.9	-1.25 -1.6	0.45 5.30	35, est.	lighter color colorless	1.9 24.7
G	2x10 ⁻⁴	24.951 to 24.876 to 24.915	32.1-32.2 32.2-30.0	-1.25 -1.8	0.79 1.11	35, est. 31	cloudy "	2.6 2.9 5.5, tot.
H	1x10 ⁻⁴	24.785 to 24.813	33.0-30.1	-1.8	2.04	31	cloudy	8.6

See next page for footnotes on table

TABLE XV (continued)

ELECTROLYTIC DATA, COULOMETRIC, FIRST SERIES

Column A, Berberine concentration, molar;
" B, Barometric pressure in inches of mercury;
" C, Temperature of coulometer, °Centigrade;
" R, Reduction potential applied, in volts;
" D, Volume of hydrogen-oxygen gas collected in coulometer;
" E, Partial pressure of water vapor in coulometer, over 0.5M potassium sulfate solution.

* Electrons per molecule calculated as follows: equal to:

$$D \times \frac{273}{273 + C} \times \frac{(B \text{ in millimeters} - E)}{760} \times \frac{1}{22,400 \times 3/4 \times A/10}$$

when corresponding values in columns A, B, C, D, and E, are substituted as indicated; the symbol, x, is used to signify multiplication.

Abbreviations: ck. equals check
in. " inches
mm. " millimeters (of mercury)
est. " estimated

1. The first of these is the fact that the
 2. Government has been unable to obtain
 3. the necessary information from the
 4. various sources which it has
 5. been using for this purpose.

6. The second of these is the fact that
 7. the Government has been unable to
 8. obtain the necessary information from
 9. the various sources which it has
 10. been using for this purpose.

11. The third of these is the fact that
 12. the Government has been unable to
 13. obtain the necessary information from
 14. the various sources which it has
 15. been using for this purpose.

16. The fourth of these is the fact that
 17. the Government has been unable to
 18. obtain the necessary information from
 19. the various sources which it has
 20. been using for this purpose.

21. The fifth of these is the fact that
 22. the Government has been unable to
 23. obtain the necessary information from
 24. the various sources which it has
 25. been using for this purpose.

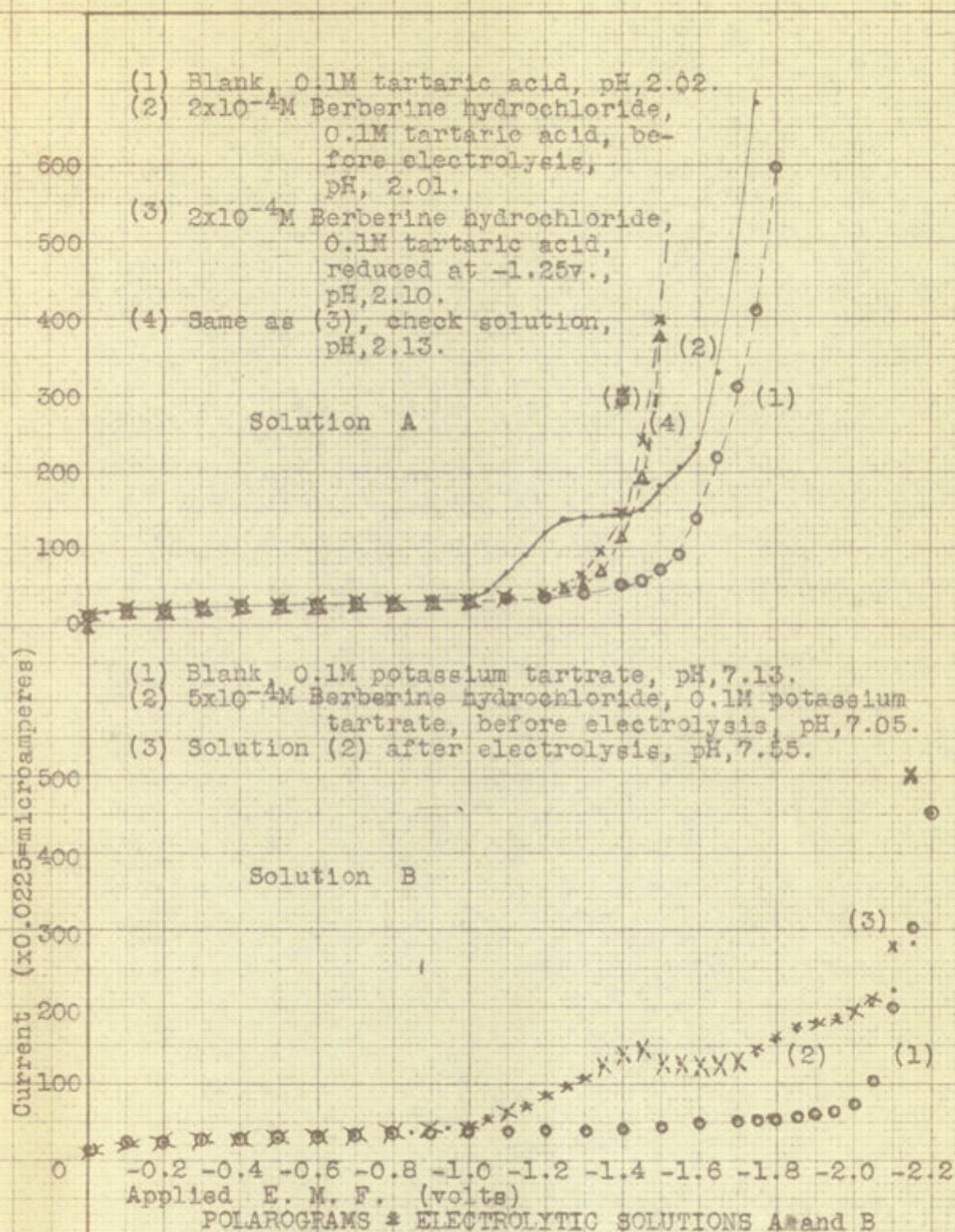
26. The sixth of these is the fact that
 27. the Government has been unable to
 28. obtain the necessary information from
 29. the various sources which it has
 30. been using for this purpose.

31. The seventh of these is the fact that
 32. the Government has been unable to
 33. obtain the necessary information from
 34. the various sources which it has
 35. been using for this purpose.

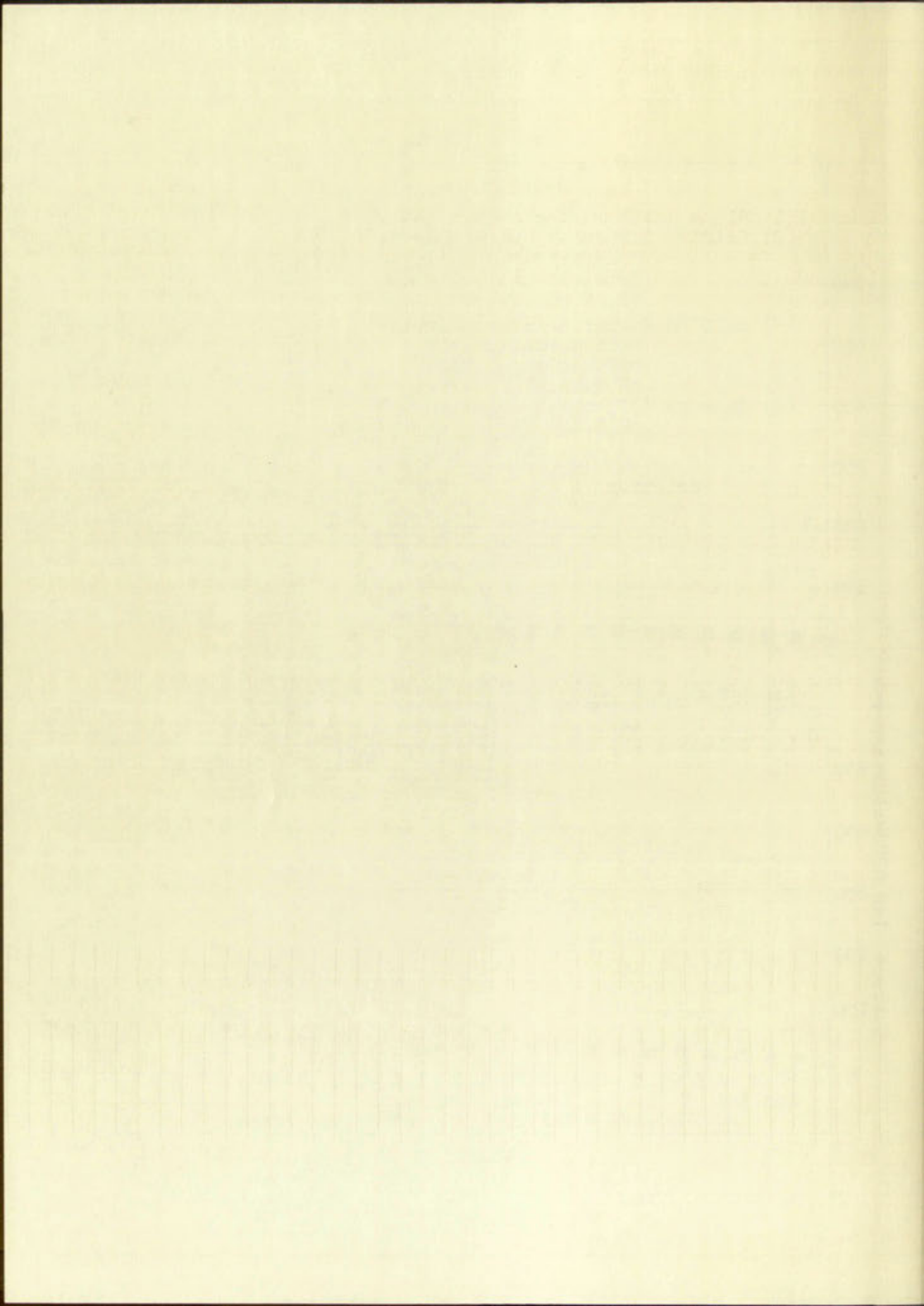
36. The eighth of these is the fact that
 37. the Government has been unable to
 38. obtain the necessary information from
 39. the various sources which it has
 40. been using for this purpose.

41. The ninth of these is the fact that
 42. the Government has been unable to
 43. obtain the necessary information from
 44. the various sources which it has
 45. been using for this purpose.

46. The tenth of these is the fact that
 47. the Government has been unable to
 48. obtain the necessary information from
 49. the various sources which it has
 50. been using for this purpose.

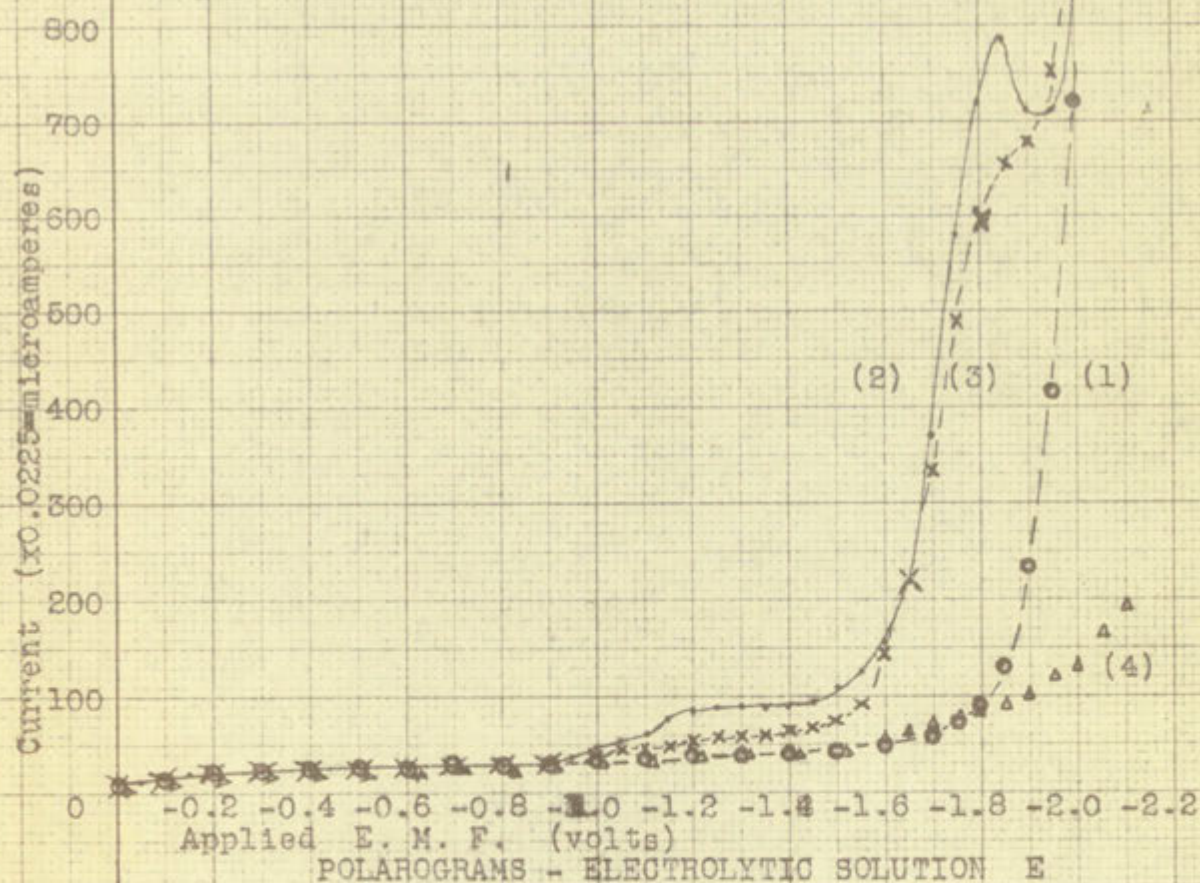


GRAPH 5

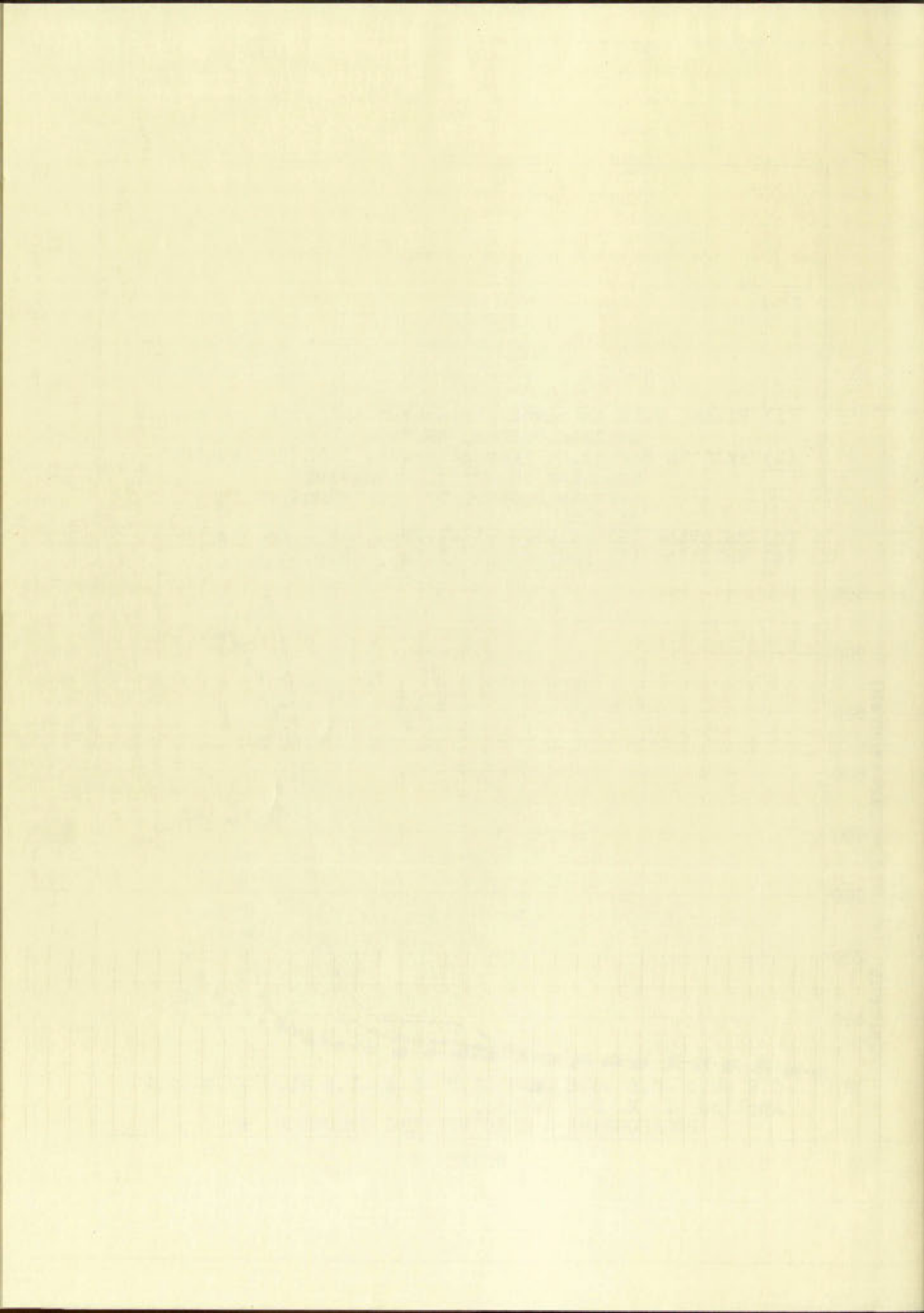


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PAGE

- (1) Blank, 0.1M tetramethylammonium chloride, potassium acetate buffer, pH, 5.7.
 (2) 1×10^{-4} M Berberine hydrochloride, 0.1M tetramethylammonium chloride, potassium acetate buffer, before reduction, pH, 5.8.
 (3) Solution (2) reduced at -1.25v., pH, 5.85.
 (4) Solution (3) reduced at -1.6v., pH, 6.95.

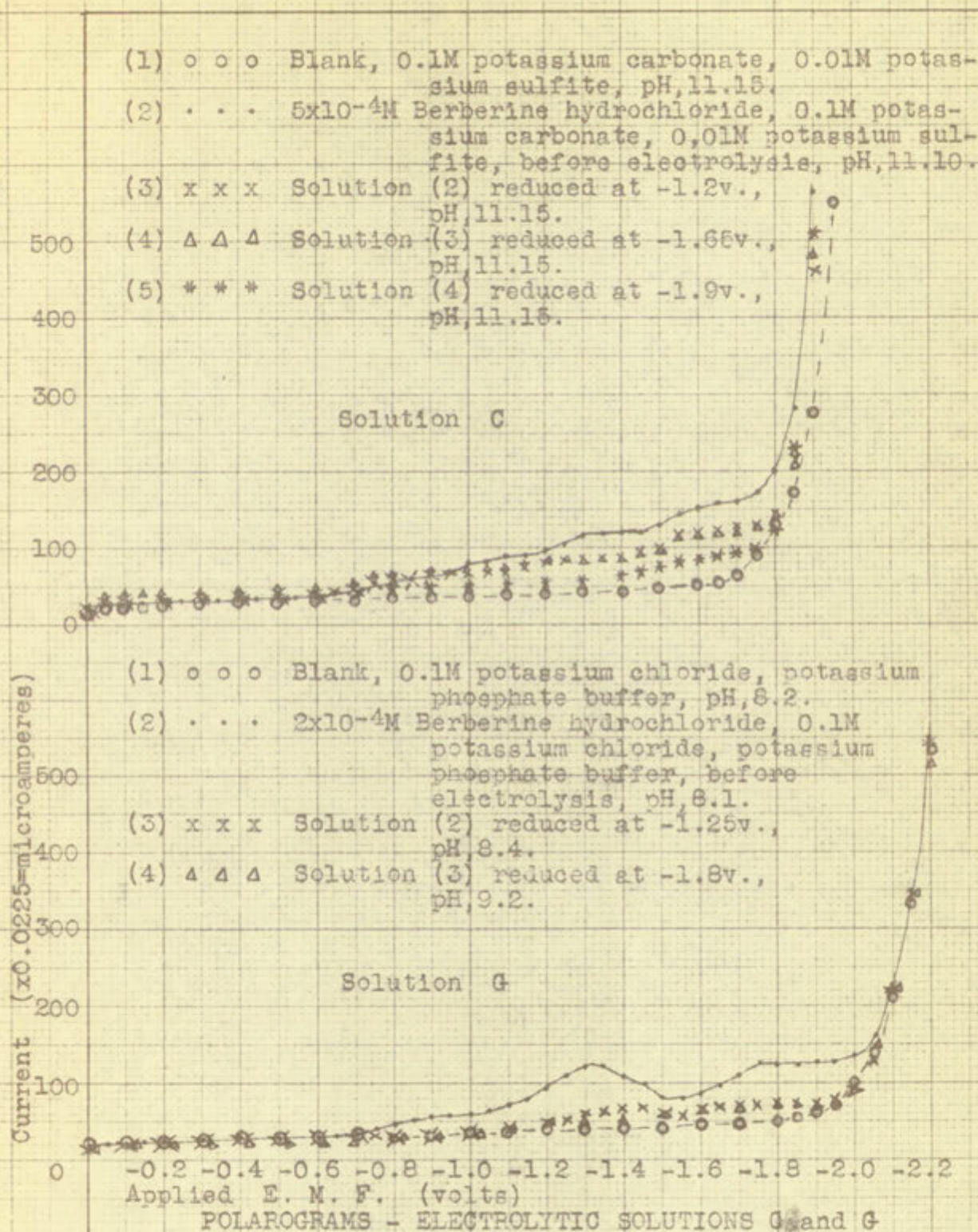


GRAPH 6

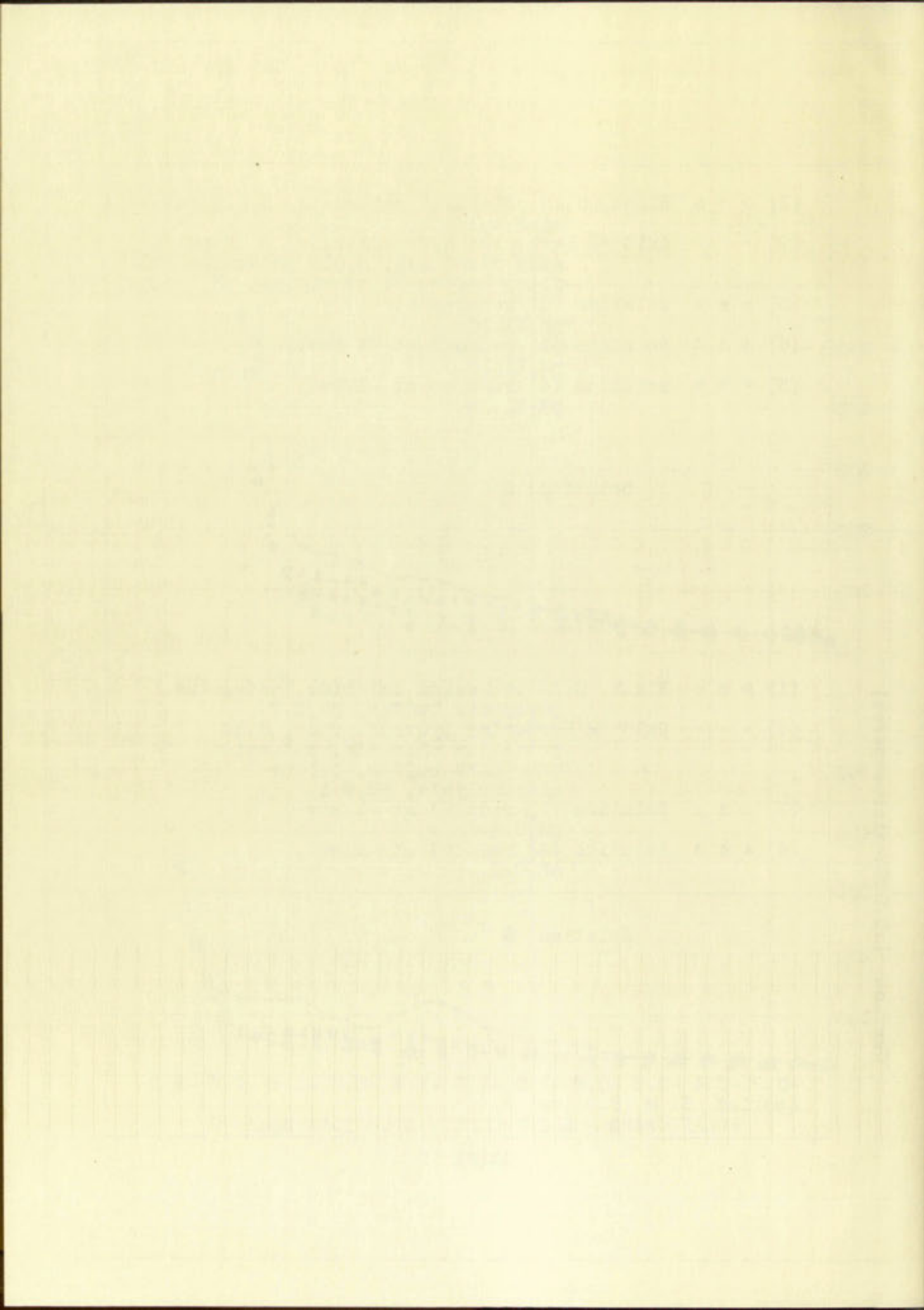


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GRAPH 7



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CONTENT

TABLE XVI

ELECTROLYTIC DATA, POLAROGRAPHIC, SECOND SERIES

Solution	Berberine Concentration Molar	Electrolyte	pH	Half-wave Potentials versus S. C. E. volts	Current Rise microamperes per millimole
I	2×10^{-4}	0.1M HCl	1.3	-0.88**	12.3
I-reduced at -0.94±0.05 volts*			1.3	none	none
V	2×10^{-4}	0.5M KCl, KH_2PO_4 buffer	6.75	-1.15; -1.55; -1.76.	7.3 (11.2max.); 13.3 (17.4max.); 6.7 (7.8max.).
V-reduced at -1.6 volts*			6.85	-1.15; -1.55.	2.8; 5.1, (8.4max.).
IV	2×10^{-4}	0.1M $(\text{CH}_3)_4\text{NCl}$	6.8	-1.1; -1.55; -2.1.	5.6; 4.5 (5.6max.); 45.
IV-reduced at -1.95 volts*			10.3	none, but berberine partly precipitated out of solution	
II	2×10^{-4}	0.5M KCl	8.85	-1.0; -1.35; -1.8. -1.057	2.25; 2.8 (7.3max.); 3.3 (4.5max.); 21.3
II-reduced at -1.15 volts*			9.2		
III	2×10^{-4}	0.1M KOH	12.64	-0.84; -1.05; -1.65.	2.25; 2.25; 6.75
III-reduced, not polarographed.					

TABLE XVII

ELECTROLYTIC DATA, COULOMETRIC, SECOND SERIES

Solu- tion	A Berb. concn.	B Bar. Pr. in.	C Coul. T. cc	R Redn. EMF, mm.	D Vol. H ₂ -O ₂	E P.Pr.H ₂ O Coul., mm.	Changes in solution	Electrons per Molecule*
I	2x10 ⁻⁴ M	24.896 to 24.790	24.8-28.8	-0.9	0.84	29	colorless	2.0**
II	2x10 ⁻⁴	24.897	24.0-22.8	-1.15	2.08	20	cloudy	4.6
III	2x10 ⁻⁴	24.830	20.0-27.0	-1.8*	6.0+		"	-
IV	2x10 ⁻⁴	24.996	24.1-23.8	-1.95	0.49	22	"	1.2
V	2x10 ⁻⁴	25.033	24.1-25.6	-1.6	4.50	24	some berber- ine precip- itated cut from super- saturation.	11.0***

Columns A, B, C, R, D, E - same as in Table XVI

* Electrons per molecule calculated as shown in Table XVI footnotes

* Reduction potential for solution III oscillated between -1.8 & -5.2 v.

** Temperature fluctuation on coulometer was found to cause a volume fluctuation of 0.03 to 0.07 milliliters per degree temp. change; the figure 2.0 was more correctly (1.5 to 2.0). *** 11.0 evidently due to the catalytic discharge of hydrogen, not to reduction of berberine.

1. The first of the three series of experiments was conducted in the summer of 1934. The results of these experiments are given in Table I. The second series of experiments was conducted in the autumn of 1934. The results of these experiments are given in Table II. The third series of experiments was conducted in the spring of 1935. The results of these experiments are given in Table III.

2. The results of the three series of experiments are summarized in Table IV. It will be seen from this table that the results of the three series of experiments are in good agreement. This indicates that the method of determining the rate of reaction is reliable.

Time	Temp.	Conc.	Rate	Calc.	Obs.	Error	Remarks
1.0	25.0	0.10	0.001	0.001	0.001	0.000	
1.5	25.0	0.10	0.001	0.001	0.001	0.000	
2.0	25.0	0.10	0.001	0.001	0.001	0.000	
2.5	25.0	0.10	0.001	0.001	0.001	0.000	
3.0	25.0	0.10	0.001	0.001	0.001	0.000	
3.5	25.0	0.10	0.001	0.001	0.001	0.000	
4.0	25.0	0.10	0.001	0.001	0.001	0.000	
4.5	25.0	0.10	0.001	0.001	0.001	0.000	
5.0	25.0	0.10	0.001	0.001	0.001	0.000	
5.5	25.0	0.10	0.001	0.001	0.001	0.000	
6.0	25.0	0.10	0.001	0.001	0.001	0.000	
6.5	25.0	0.10	0.001	0.001	0.001	0.000	
7.0	25.0	0.10	0.001	0.001	0.001	0.000	
7.5	25.0	0.10	0.001	0.001	0.001	0.000	
8.0	25.0	0.10	0.001	0.001	0.001	0.000	
8.5	25.0	0.10	0.001	0.001	0.001	0.000	
9.0	25.0	0.10	0.001	0.001	0.001	0.000	
9.5	25.0	0.10	0.001	0.001	0.001	0.000	
10.0	25.0	0.10	0.001	0.001	0.001	0.000	

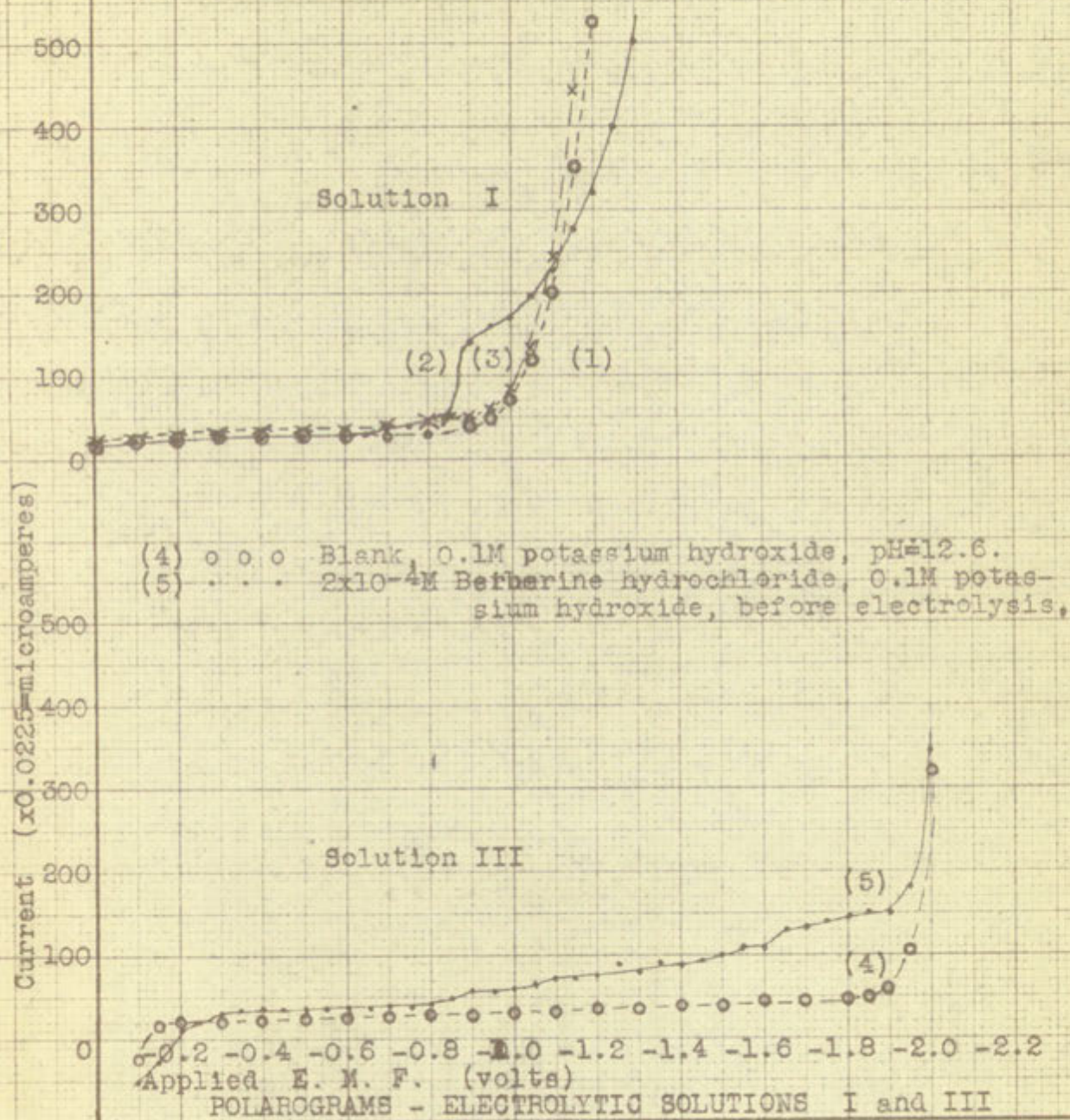
Time	Temp.	Conc.	Rate	Calc.	Obs.	Error	Remarks
1.0	25.0	0.10	0.001	0.001	0.001	0.000	
1.5	25.0	0.10	0.001	0.001	0.001	0.000	
2.0	25.0	0.10	0.001	0.001	0.001	0.000	
2.5	25.0	0.10	0.001	0.001	0.001	0.000	
3.0	25.0	0.10	0.001	0.001	0.001	0.000	
3.5	25.0	0.10	0.001	0.001	0.001	0.000	
4.0	25.0	0.10	0.001	0.001	0.001	0.000	
4.5	25.0	0.10	0.001	0.001	0.001	0.000	
5.0	25.0	0.10	0.001	0.001	0.001	0.000	
5.5	25.0	0.10	0.001	0.001	0.001	0.000	
6.0	25.0	0.10	0.001	0.001	0.001	0.000	
6.5	25.0	0.10	0.001	0.001	0.001	0.000	
7.0	25.0	0.10	0.001	0.001	0.001	0.000	
7.5	25.0	0.10	0.001	0.001	0.001	0.000	
8.0	25.0	0.10	0.001	0.001	0.001	0.000	
8.5	25.0	0.10	0.001	0.001	0.001	0.000	
9.0	25.0	0.10	0.001	0.001	0.001	0.000	
9.5	25.0	0.10	0.001	0.001	0.001	0.000	
10.0	25.0	0.10	0.001	0.001	0.001	0.000	

Time	Temp.	Conc.	Rate	Calc.	Obs.	Error	Remarks
1.0	25.0	0.10	0.001	0.001	0.001	0.000	
1.5	25.0	0.10	0.001	0.001	0.001	0.000	
2.0	25.0	0.10	0.001	0.001	0.001	0.000	
2.5	25.0	0.10	0.001	0.001	0.001	0.000	
3.0	25.0	0.10	0.001	0.001	0.001	0.000	
3.5	25.0	0.10	0.001	0.001	0.001	0.000	
4.0	25.0	0.10	0.001	0.001	0.001	0.000	
4.5	25.0	0.10	0.001	0.001	0.001	0.000	
5.0	25.0	0.10	0.001	0.001	0.001	0.000	
5.5	25.0	0.10	0.001	0.001	0.001	0.000	
6.0	25.0	0.10	0.001	0.001	0.001	0.000	
6.5	25.0	0.10	0.001	0.001	0.001	0.000	
7.0	25.0	0.10	0.001	0.001	0.001	0.000	
7.5	25.0	0.10	0.001	0.001	0.001	0.000	
8.0	25.0	0.10	0.001	0.001	0.001	0.000	
8.5	25.0	0.10	0.001	0.001	0.001	0.000	
9.0	25.0	0.10	0.001	0.001	0.001	0.000	
9.5	25.0	0.10	0.001	0.001	0.001	0.000	
10.0	25.0	0.10	0.001	0.001	0.001	0.000	

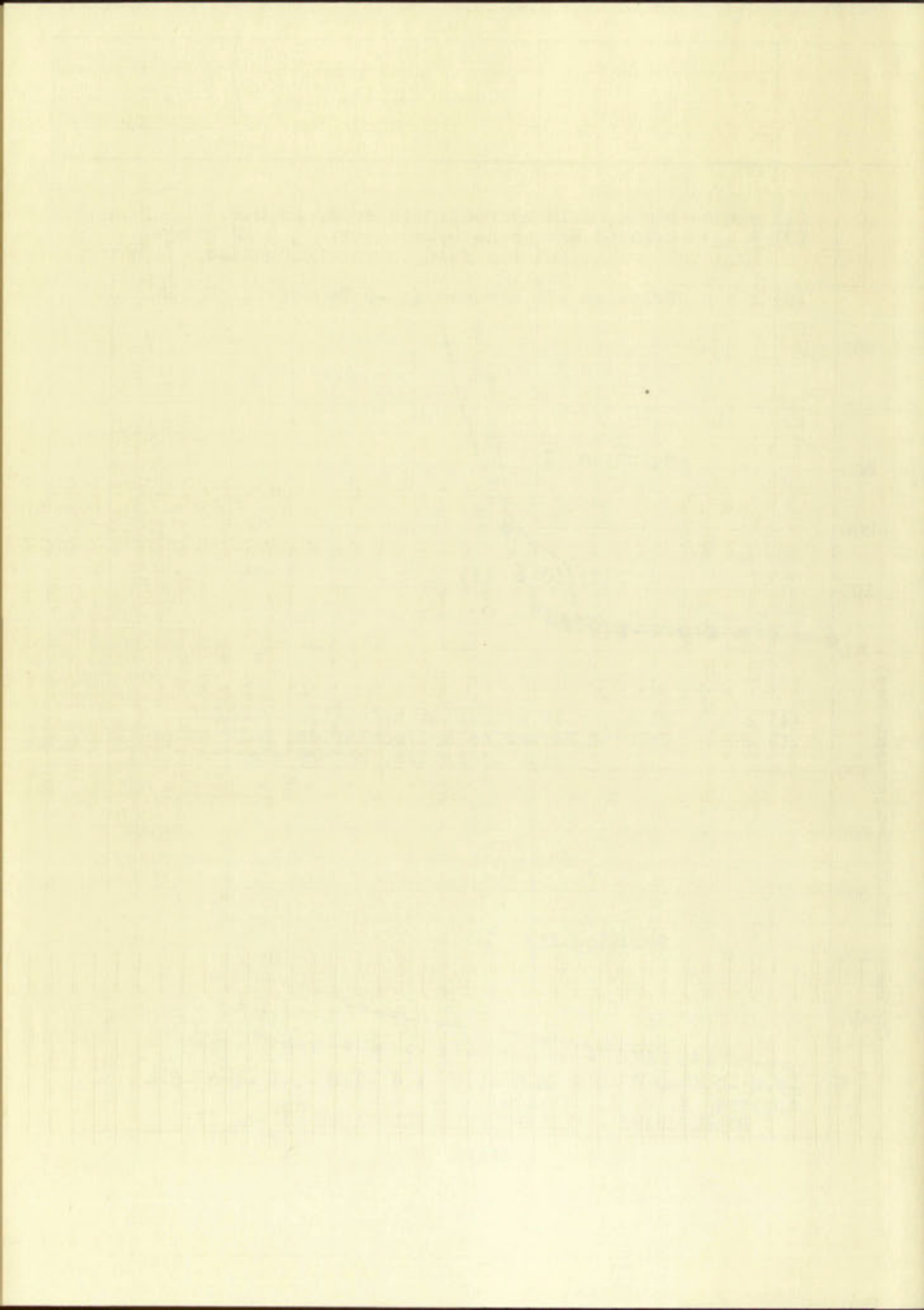
THEORY OF THE EXPERIMENT

APPENDIX

- (1) o o o Blank, 0.1M hydrochloric acid, pH, 0.8.
 (2) . . . 2×10^{-4} M Berberine hydrochloride, 0.1M hydrochloric acid, before reduction, pH, 1.3.
 (3) x x x Solution (2) reduced at -0.9 ± 0.05 v., pH, 1.3.

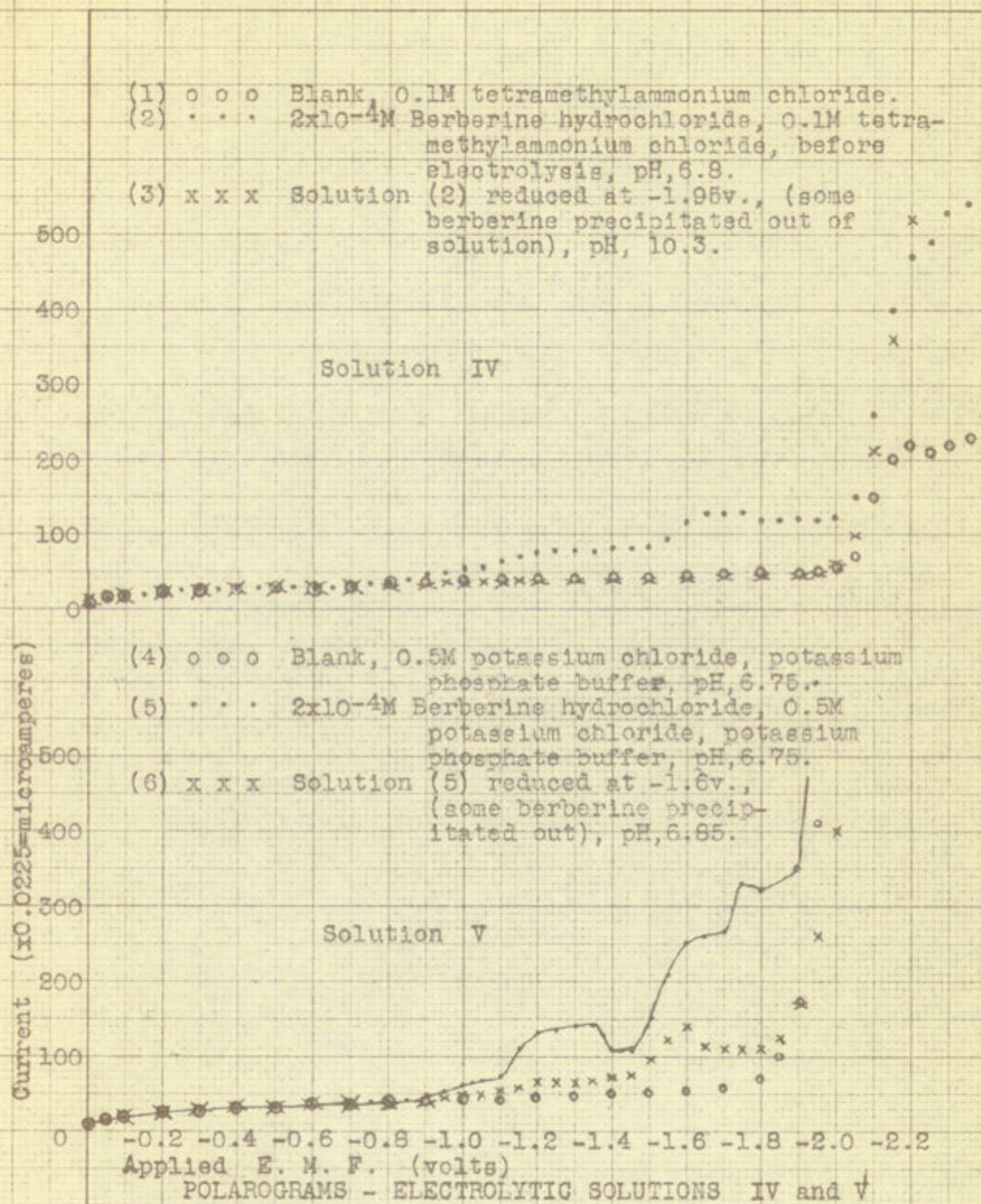


GRAPH 8

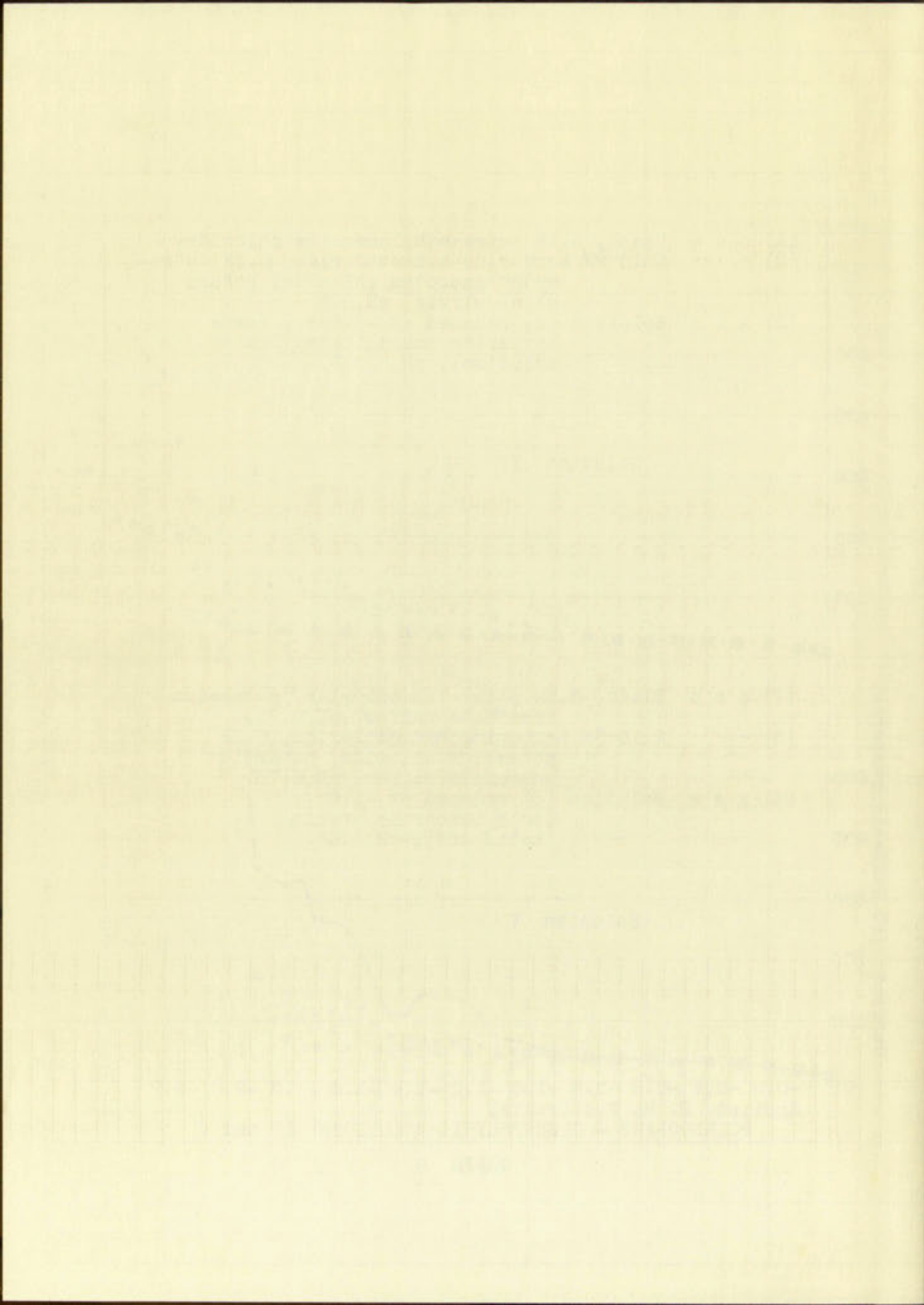


EVERETT BOND
F. F. BOND
H. C. BOND

EFFICIENCY
EVERASE BOND
RAG CONTENT



GRAPH 9



NEW YORK
CROSS ST
CENTRAL

JENNY
THE BOND
CONTENT

CHAPTER VIII

SPECTROPHOTOMETRIC MEASUREMENTS OF BERBERINE SOLUTIONS

A. ABSORPTION SPECTRA

Spectrophotometric measurements, made as previously described (p. 56), were recorded for all of the solutions electrolyzed. A few measurements showed that the absorption band in visible range was the same for all the yellow solutions. Therefore, only a few of the solutions were checked in the visible range. Table XVIII lists the maximum absorption bands and molecular extinction coefficients obtained for the various solutions before electrolysis. The berberine solutions measured in both series of experiments are listed together in the order of increasing pH.

Table XIX gives the spectrophotometric data for the same solutions after reduction. Besides the absorption bands and molecular extinction coefficients, the reduction currents and the color changes are tabulated.

Spectral curves for the various solutions are shown in graphs following the tables. Graphs 10, 11, 12, and 13 show the first series; graphs 14, 15, and 16 show the second series.

It was found in the first series that organic acids

CHAPTER VIII

SPECTROPHOTOMETRIC MEASUREMENTS OF BENZENE SOLUTIONS

A. ABSORPTION SPECTRA

Spectrophotometric measurements, made as previously described (p. 80), were recorded for all of the solutions electrolyzed. A few measurements showed that the absorption band in visible range was the same for all the yellow solutions. Therefore, only a few of the solutions were checked in the visible range. Table XVII lists the maximum absorption bands and molar extinction coefficients obtained for the various solutions before electrolysis. The molar extinction coefficients measured in both series of experiments are listed together in the order of increasing λ . Table XIX gives the spectrophotometric data for the same solutions after reduction. Besides the absorption bands and molar extinction coefficients, the reduction currents and the color changes are tabulated. Spectral curves for the various solutions are shown in graphs following the tables. Graphs 10, 11, 12, and 13 show the first series; graphs 14, 15, and 16 show the second series. It was found in the first series that organic acids

TABLE XVIII

SPECTROPHOTOMETRIC DATA ON BERBERINE SOLUTIONS BEFORE ELECTROLYSIS
Absorption Bands (A) and Molecular Extinction Coefficients (E)

Berberine solution as	pH ^a aqueous soln.	A mu**	E	A mu**	E	A mu**	E
Sulfate		228	28,400	263	27,300	344-45	24,950
Hydrochloride: With CH ₃ COOH							5850
Soln. A	2.9	229	27,000	263	28,300	344	26,500
Soln. A, EtOH	2.0	229	29,000	263	28,300	344	26,000
Soln. I	1.3	231	28,400	265	28,000	350	26,600
Soln. E	5.8	228	29,100	264	28,600	345	26,500
Soln. IV	6.8	228	28,600	262	28,000	344	26,000
Soln. V	6.75	228	29,400	262	28,600	343	26,200
Soln. B	7.0	228	30,000	263	29,200	343	26,400
Soln. G	8.1	228	27,800	263	29,600	344	26,500
Soln. H	8.0	228	28,600	263	27,300	342	25,300
With K ₂ CO ₃							6000
Soln. C	11.1	235 ^a	27,000 ^b	263	28,600	344	26,700
Soln. III	11.1	233 ^c	28,000 ^d	263	29,500	344	27,000
	12.6 ^f	227	30,250	262	20,300	336	18,000
					28,750	343	25,850

* pH values measured before one-tenth dilutions for spectral measurements

** mu equals millimicrons, the wavelength of absorption band

^a band less than 235 mu.

^b extinction more than 27,000

^c band an inflection point

^d extinction approximate, only

See Tables XV and XVII for further information on solutions.

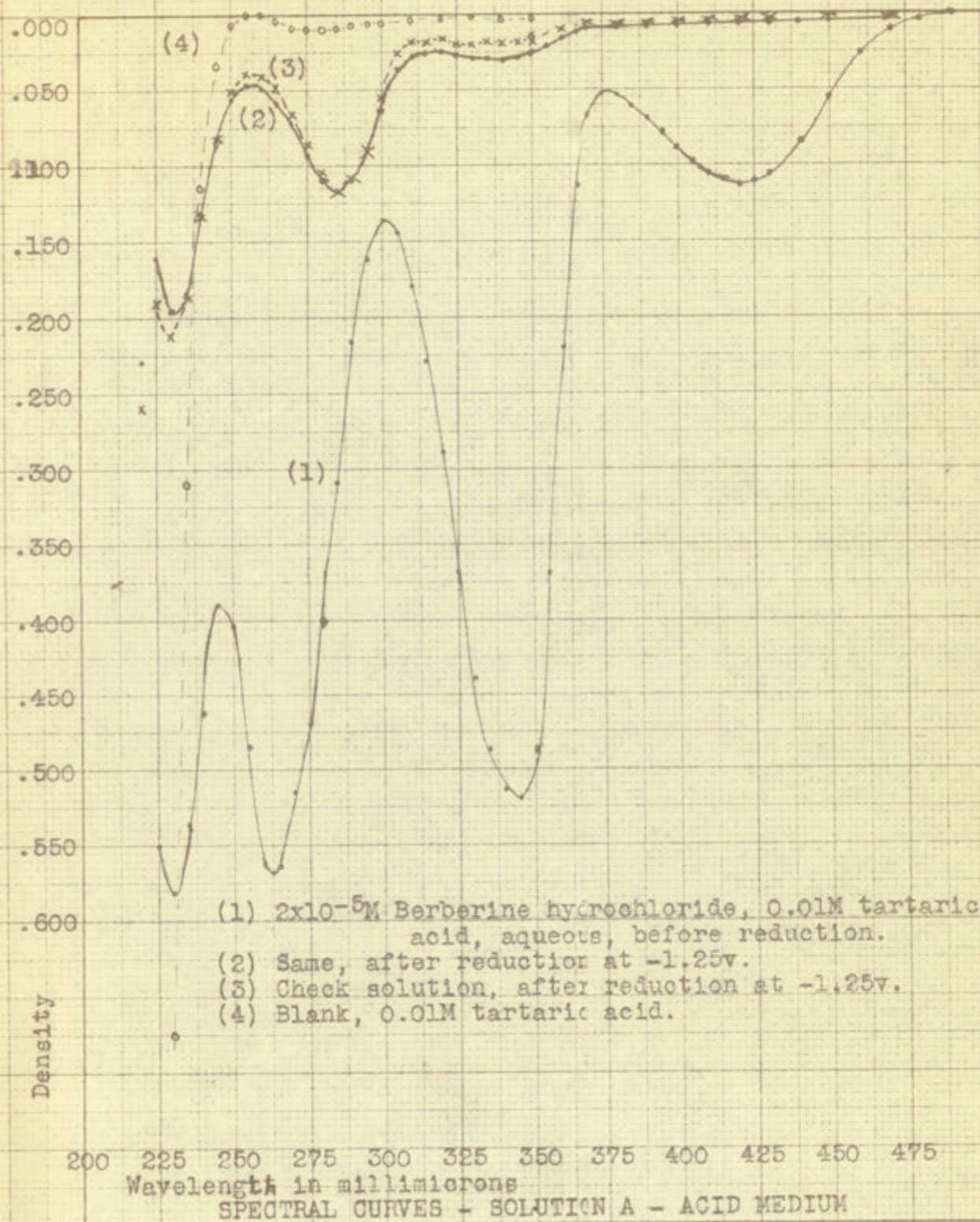
TABLE XIX

SPECTROPHOTOMETRIC DATA ON REDUCED BERBERINE
Absorption Bands (A) and Molecular Extinction Coefficients (E)

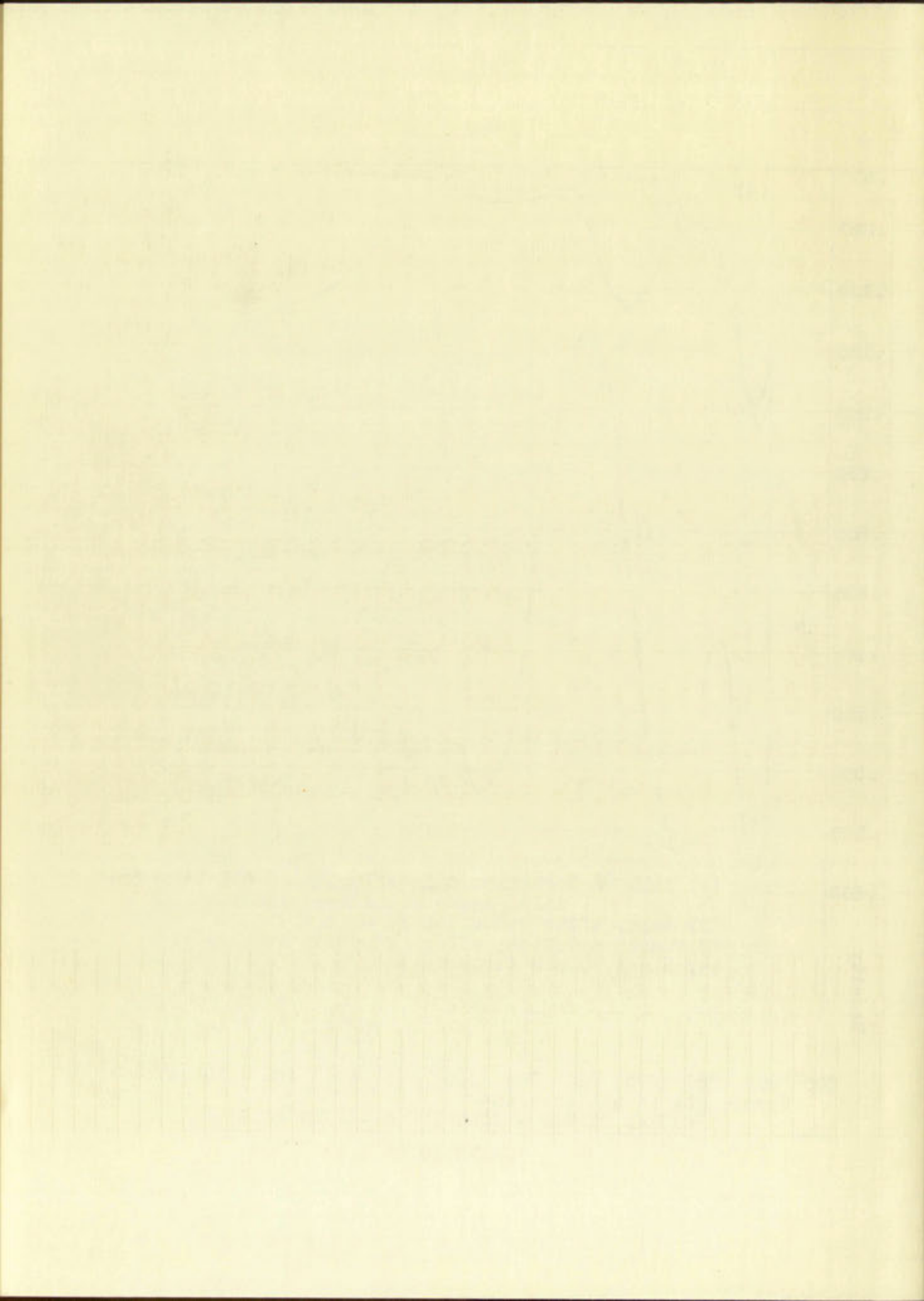
Solution	Reduction Potential volts	A mu*	E	A mu*	E	A mu*	E	A mu*	E
A	-1.25	228	9800	285	5900	340	1500	none	
A-check	-1.25	228	10,500	285	5900	340	1000	none	
I	-0.9	228 ^d	11,600 ^d	285	6300	345	1000	none	
E	-1.25	228	18,000	265	13,000	344	11,100	423	2600
E	-1.6	227	4700	285 [†]	2500 [†]	345	1200	none	
E	-2.0	228	3400	285 [†]	1700 [†]	345	1100	none	
IV	-1.95	228	12,500	263	11,100	344	11,000	omitted	
V	-1.6	225	9200	263	7100	343	6100	omitted	
B	-8.2**	-	-	-	-	-	-	-	
G	-1.25	228	5250	264	4350	342	4000	omitted	
G	-1.8	228	5300	265	3500	342	3000	omitted	
II	-1.15	225	7500	263	6300	342	5500	omitted	
H	-1.8	230	8300	263	7800	342	7500	omitted	
H	-1.95	230	8800	263	8200	342	8000	omitted	
C	-1.25	-	-	262	14,000	342	12,800	415	2600
C	-1.65	-	-	263	15,600	343	14,000	417	2600
C	-1.9	-	-	263	12,800	343	10,800	417	1820
D	-1.7	229	21,800	263	20,700	344	18,800	omitted	
D	-1.9	229	21,000	263	19,400	344	17,700	omitted	
III	-1.8?	227	5200	262	4500	343	4000	omitted	

* mu, millimicrons;
c inflection point;

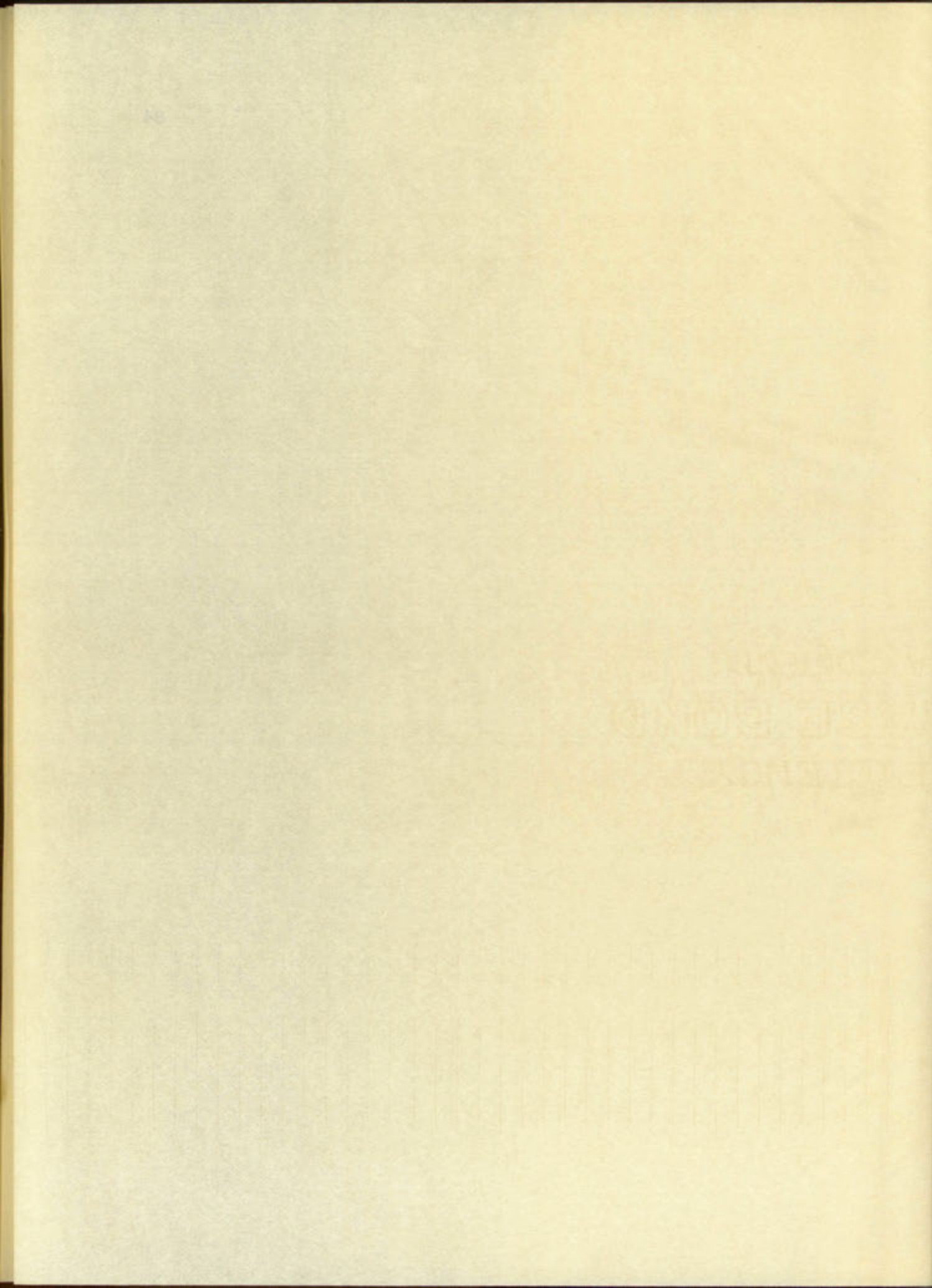
** B sample, no reduction
d approximate

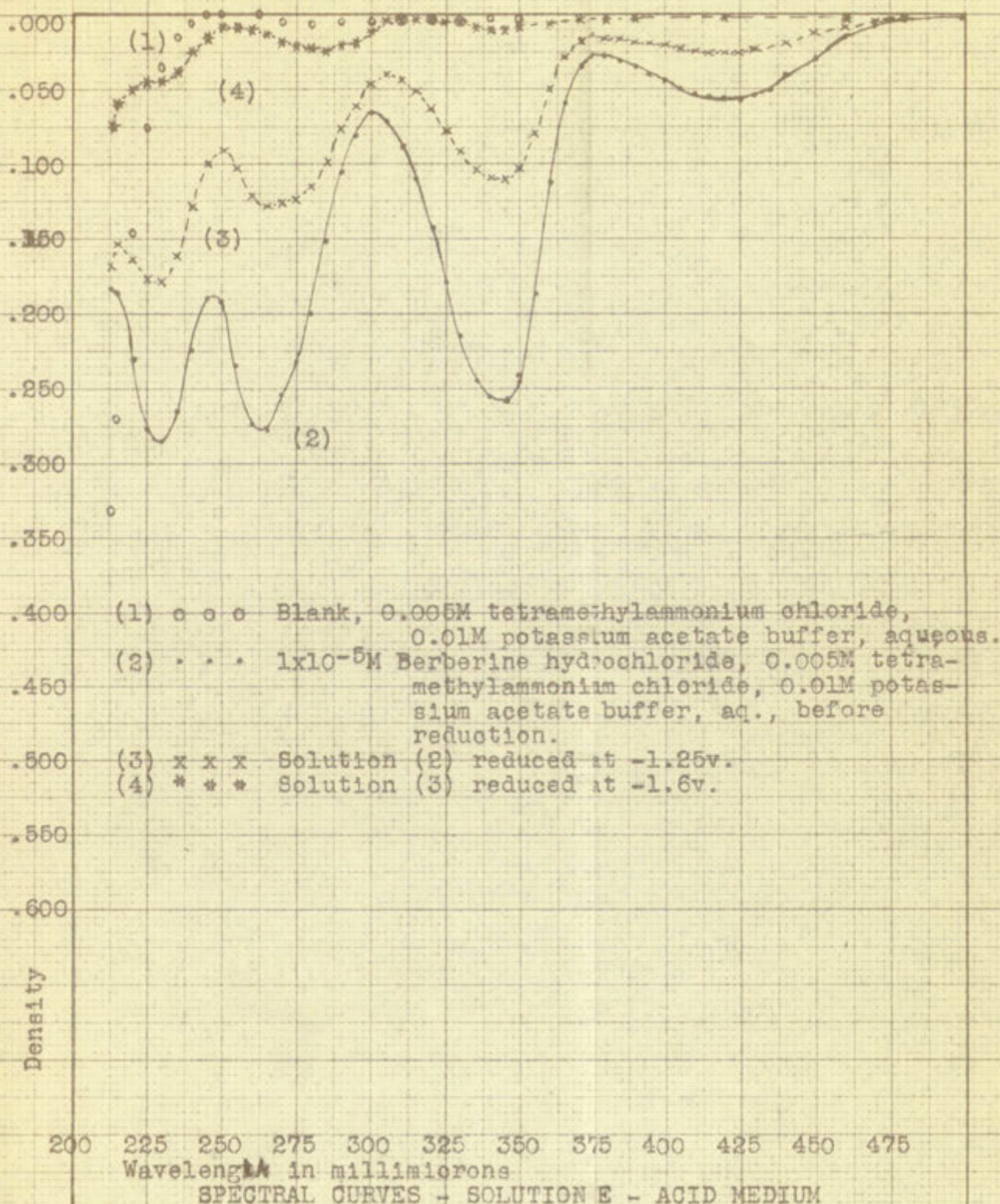


GRAPH 10

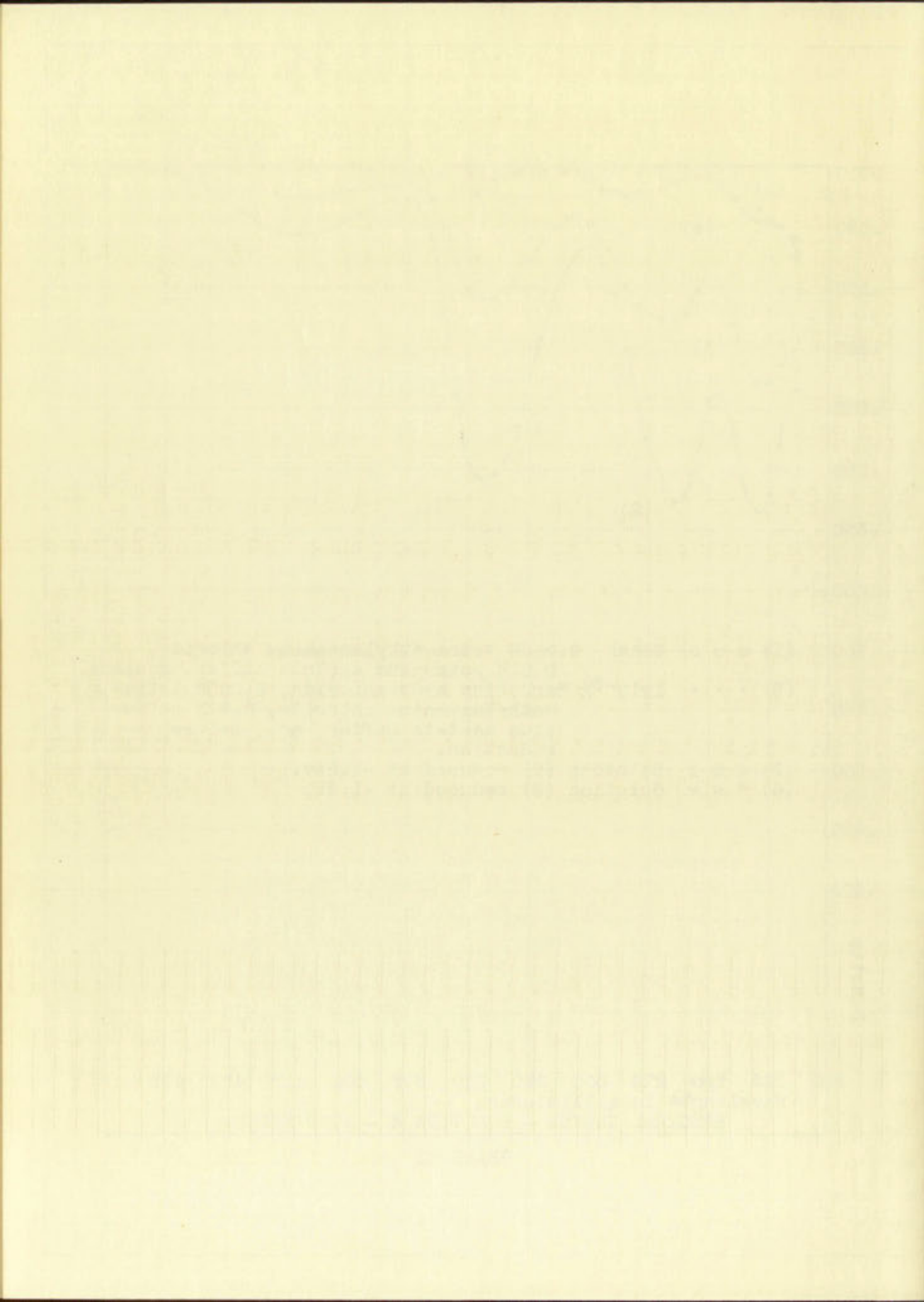


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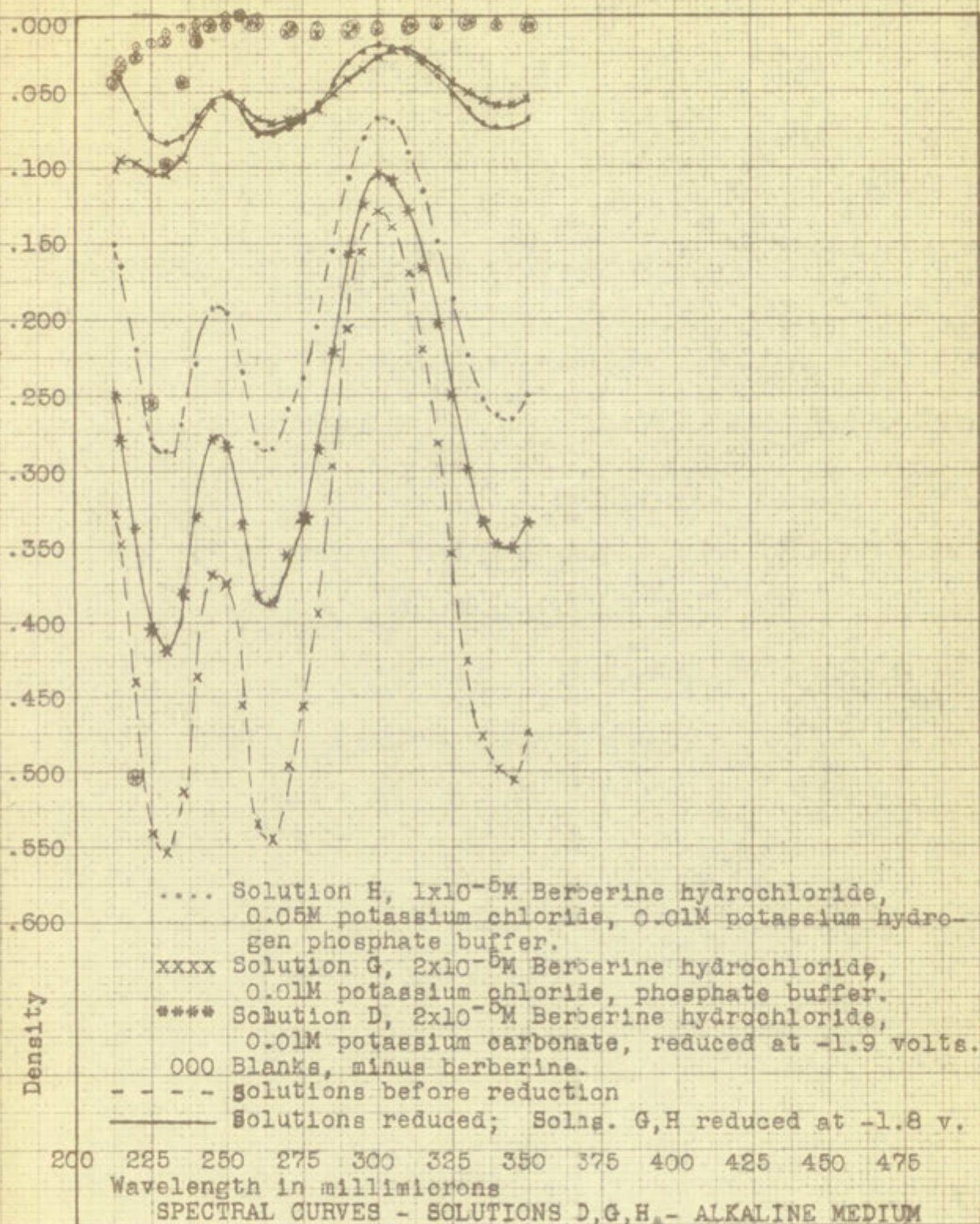




GRAPH 11



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ESEB/2
ELEE



GRAPH 12



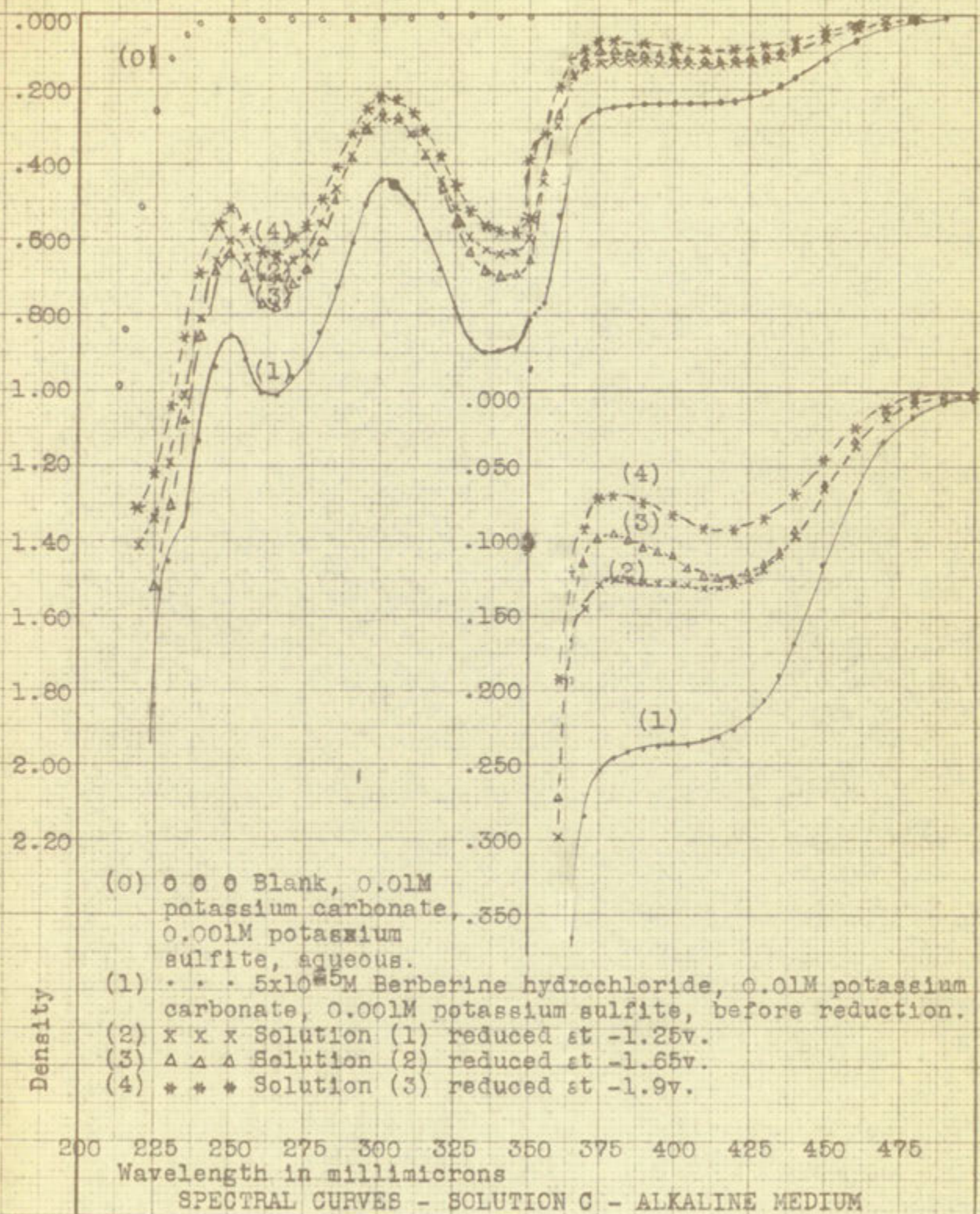
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SUGGESTION
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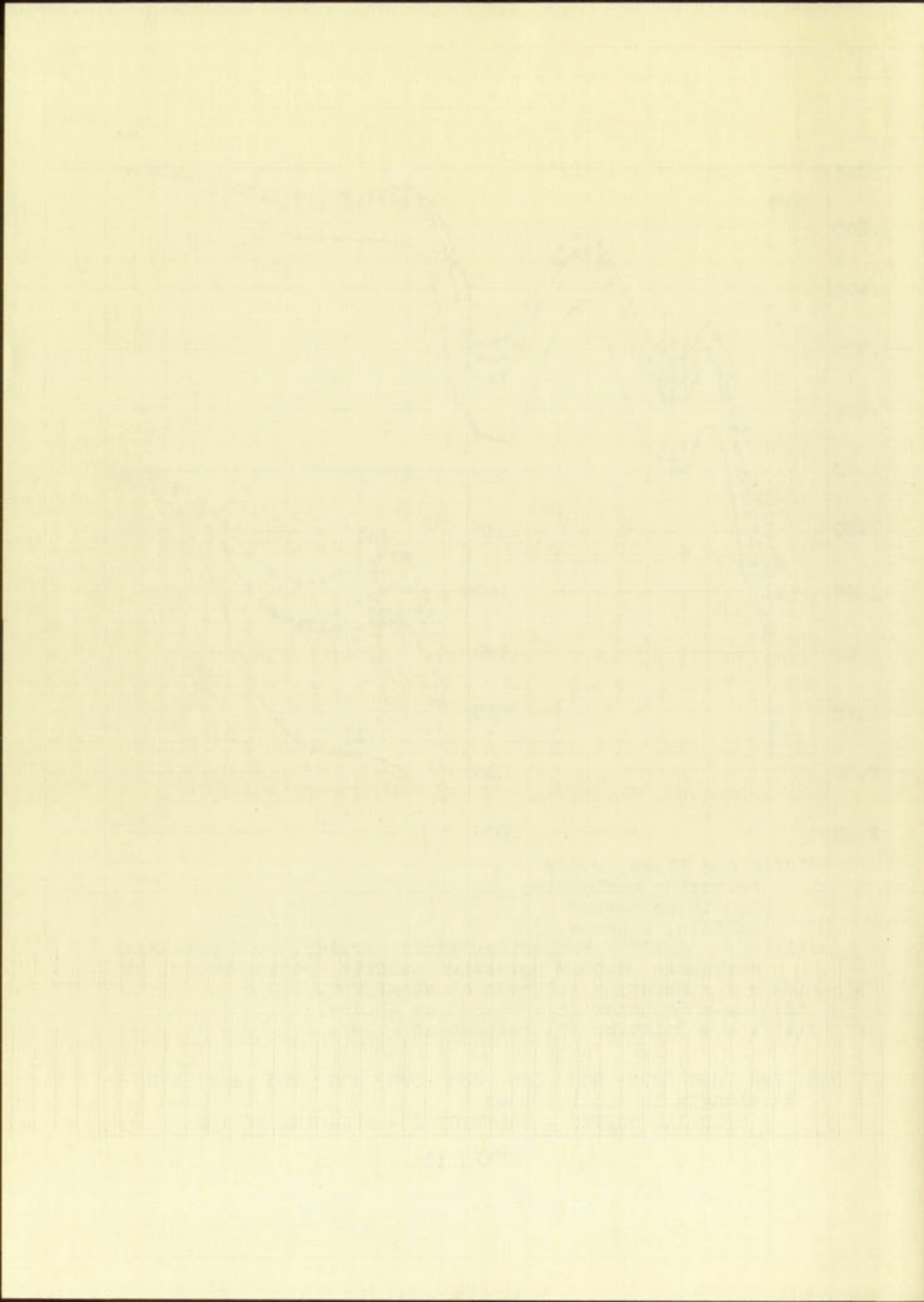
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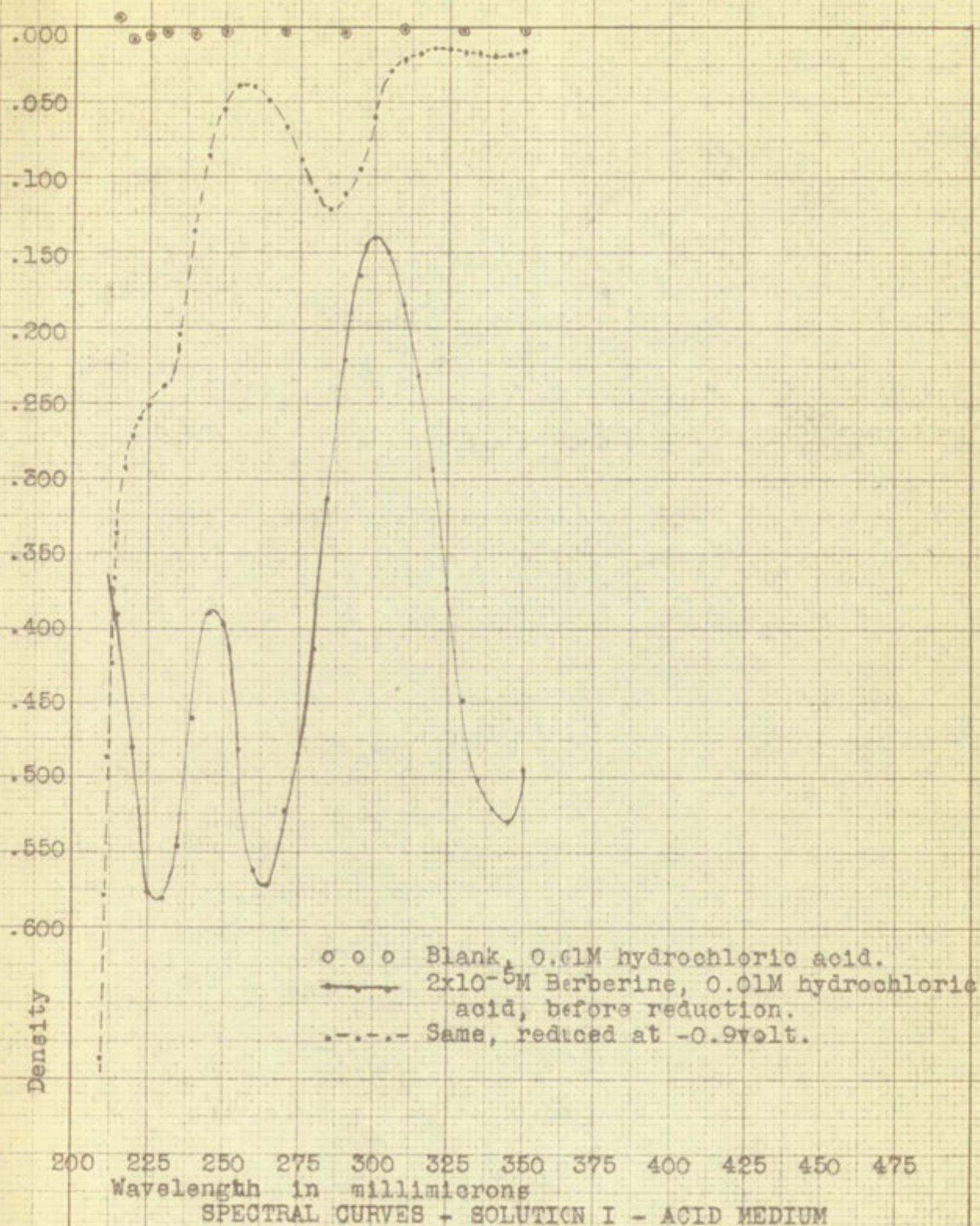


GRAPH 13

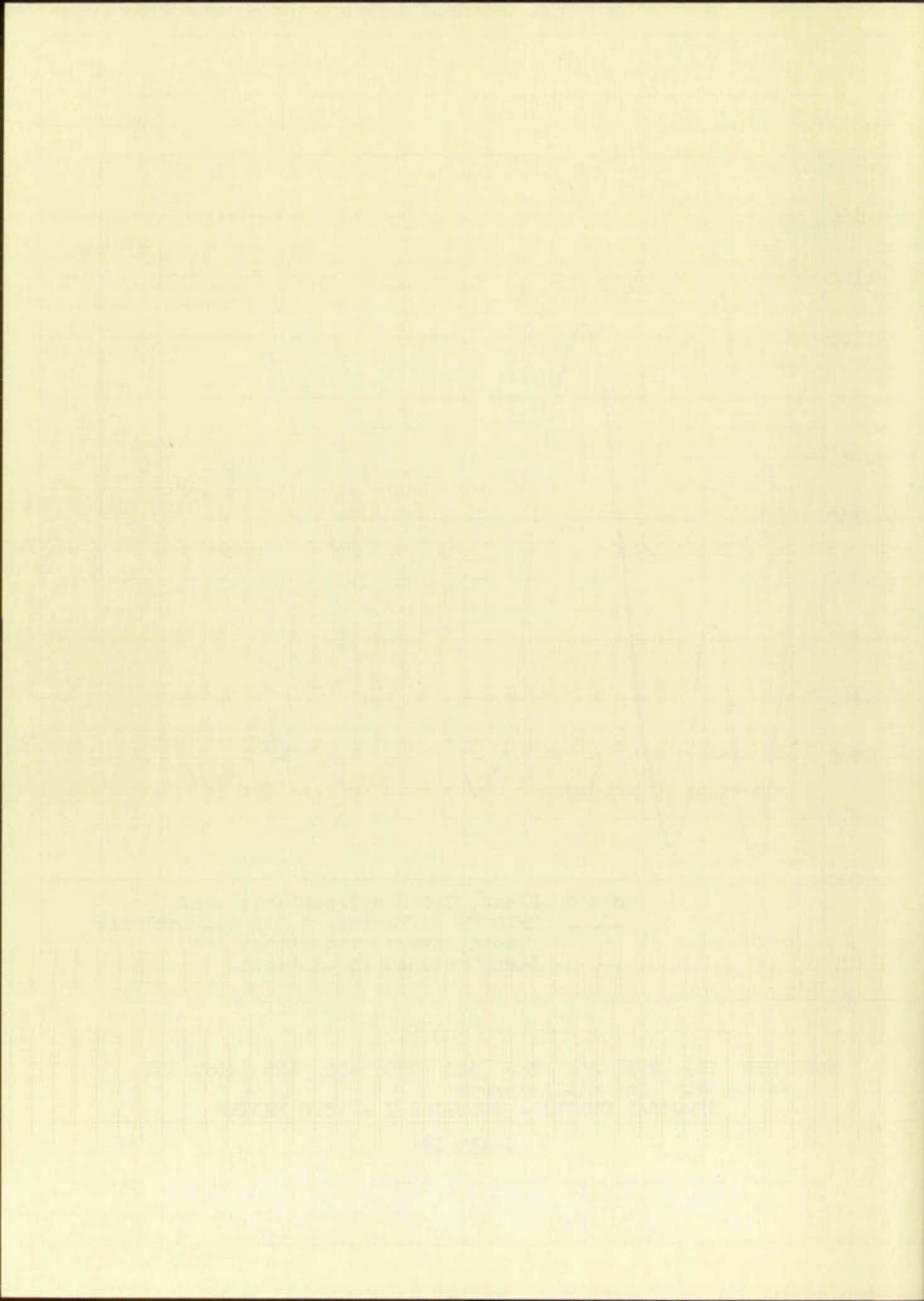


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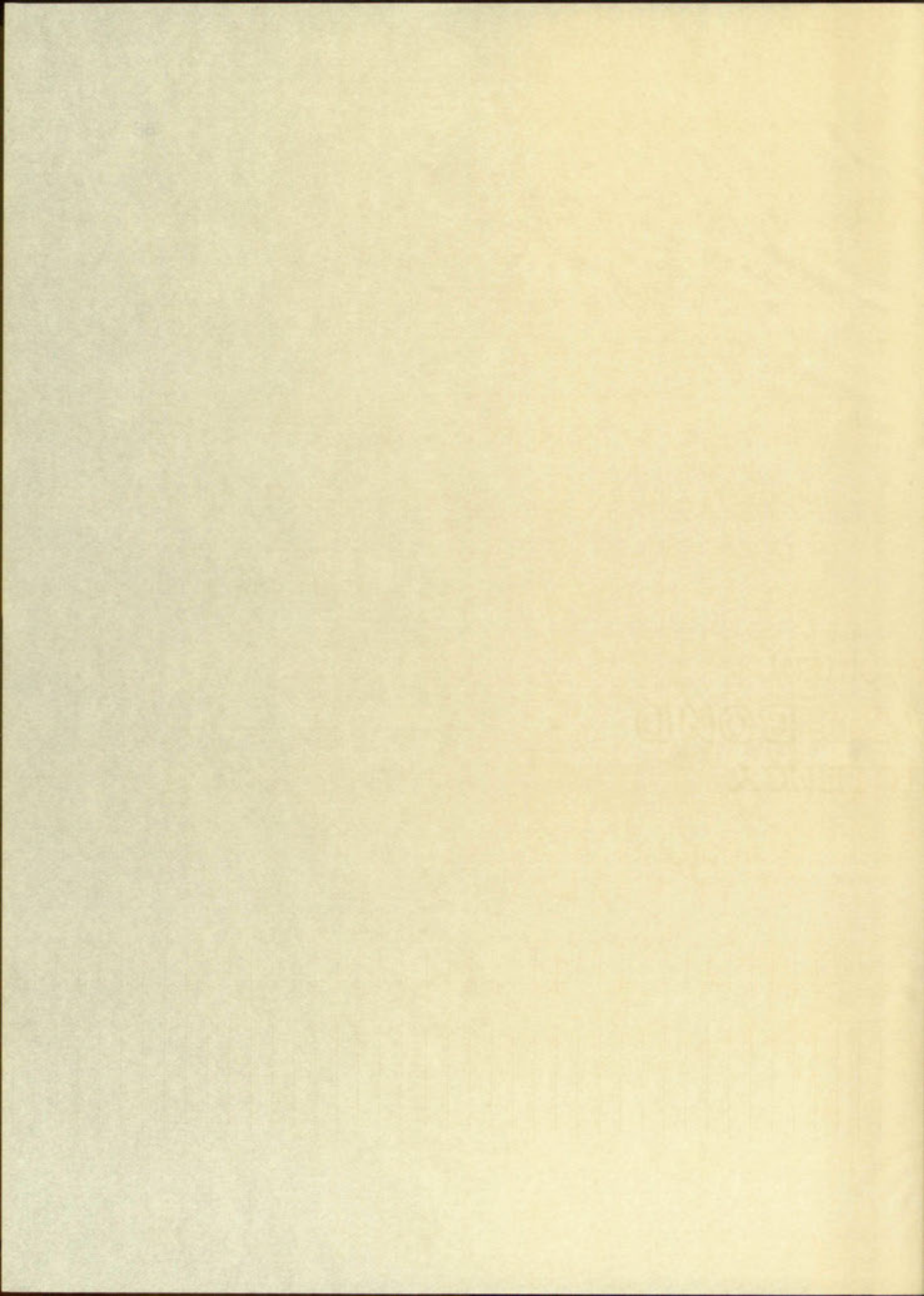
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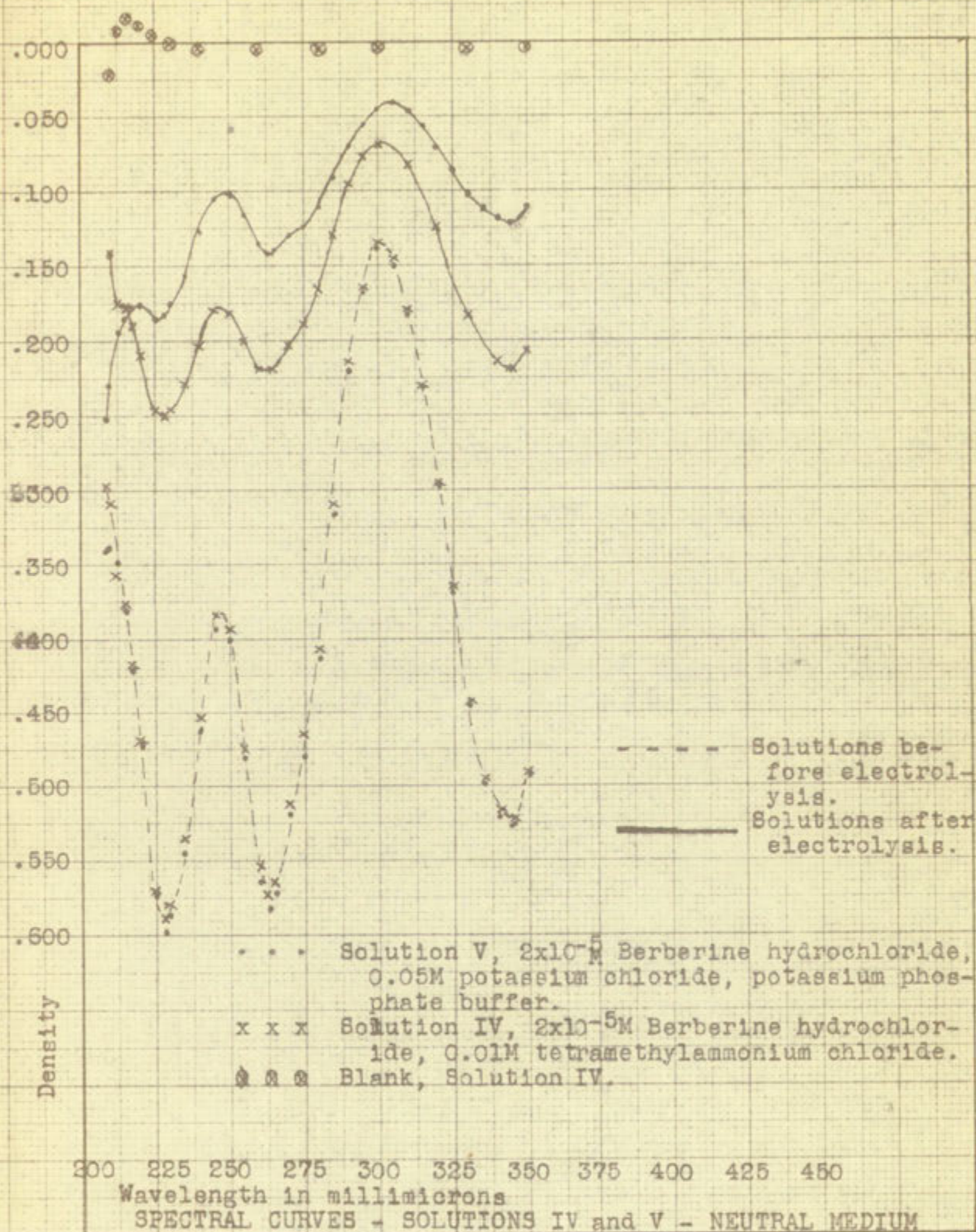


GRAPH 14

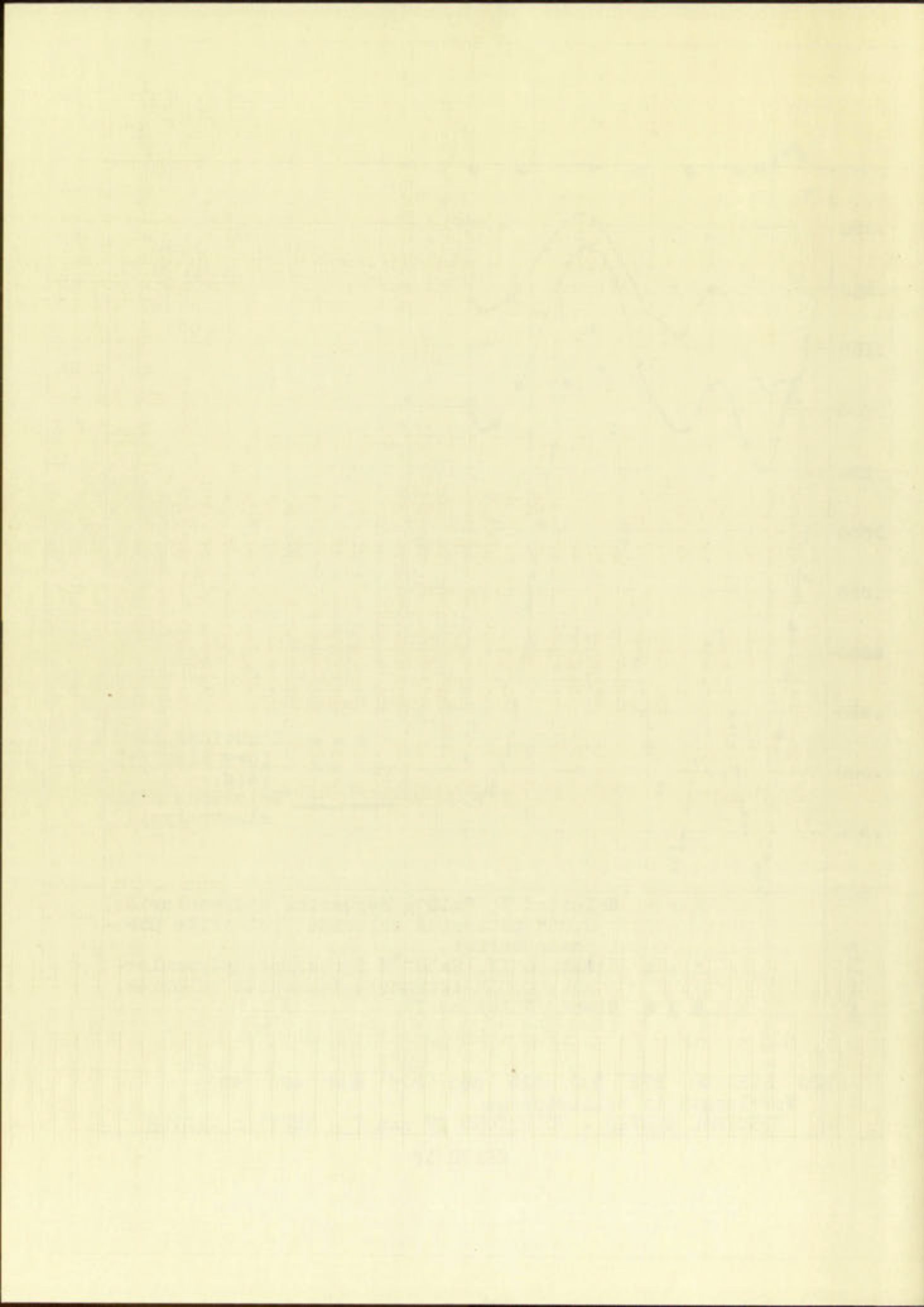


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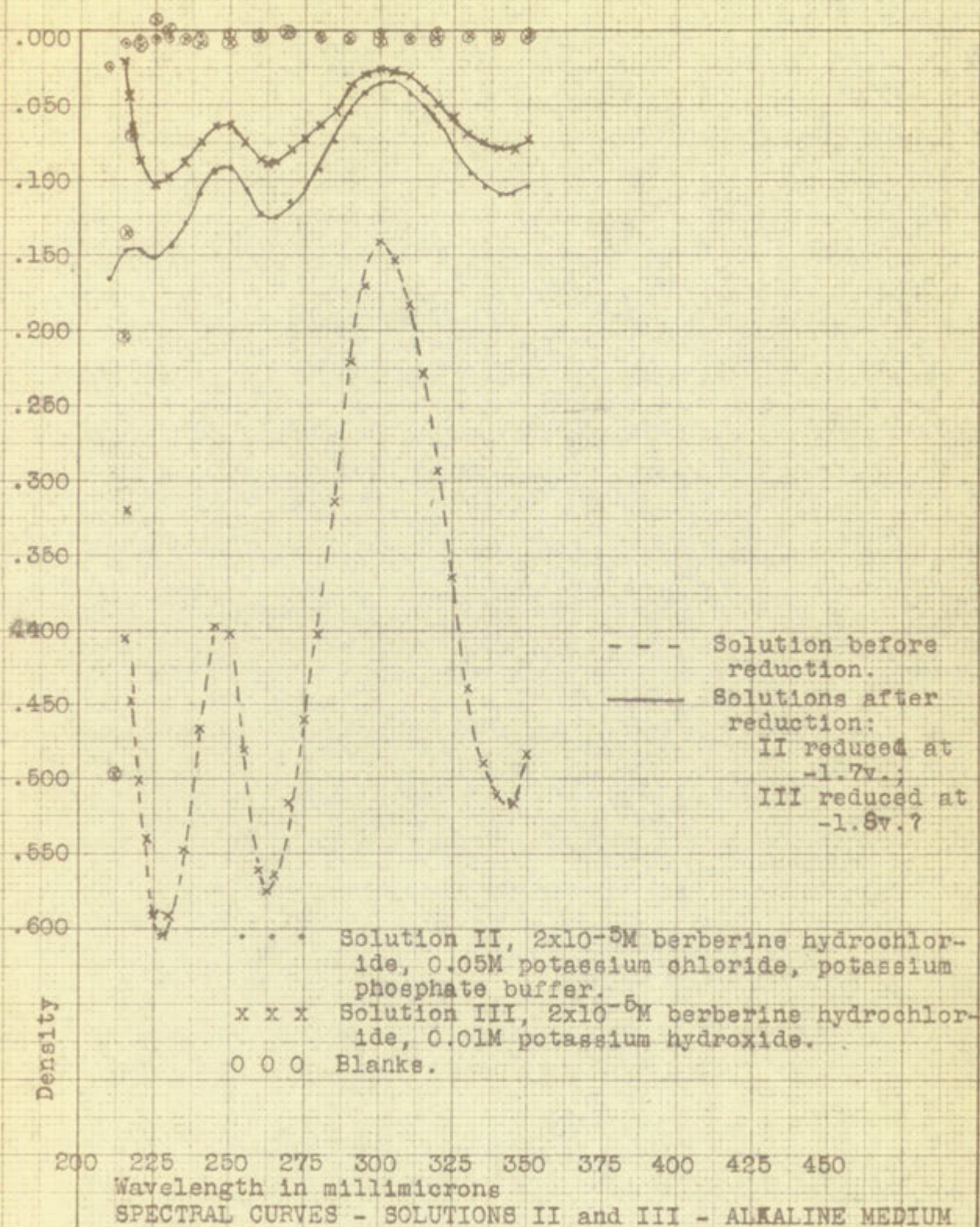


GRAPH 16

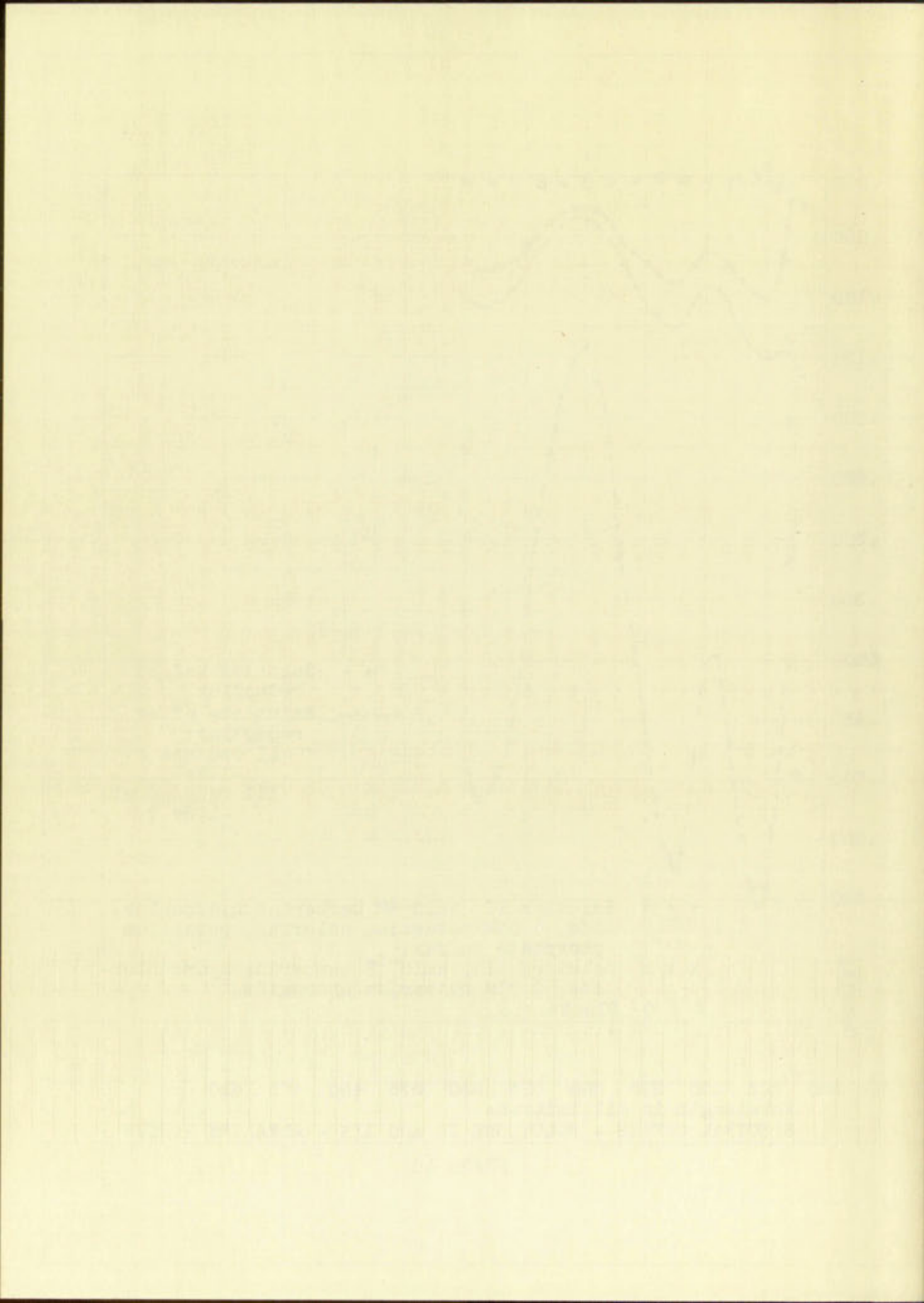


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GRAPH 16



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absorbed light in the far ultraviolet region, overlapping the shortest wavelength band of berberine solutions. Therefore, only inorganic compounds were used as supporting electrolytes in the second series.

In order to determine the effect of varying the pH of berberine solutions, spectral curves of berberine hydrochloride dissolved in strongly acid, neutral, and strongly alkaline aqueous solutions were determined. These are shown in Graph 17. Since it has been reported¹²⁰ that berberine changed into dihydroberberine and oxyberberine (Cannizzaro type reaction) in strong alkali, some berberine was heated in potassium hydroxide for one hour on a steam bath (1.00 milliliters of 1×10^{-3} molar berberine hydrochloride plus 1.00 milliliters of 1 molar potassium hydroxide) then diluted to a solution containing 1×10^{-5} molar berberine hydrochloride and 0.101 molar potassium hydroxide. A similar but unheated solution was prepared to run in parallel. The spectral curves for these are shown in Graph 18.

Other alkaloids of the isoquinoline group have structures somewhat similar to berberine. Three of these were available, namely cotarnine chloride, papaverine hydrochloride, and hydrastine. Maximum absorption bands and molecular

¹²⁰Tinkler, J. Chem. Soc. 99, 1346-47 (1911).

absorbed light in the far ultraviolet region, overlapping the shortest wavelength band of barbituric solutions. Therefore, only inorganic compounds were used as supporting electrolytes in the present study.

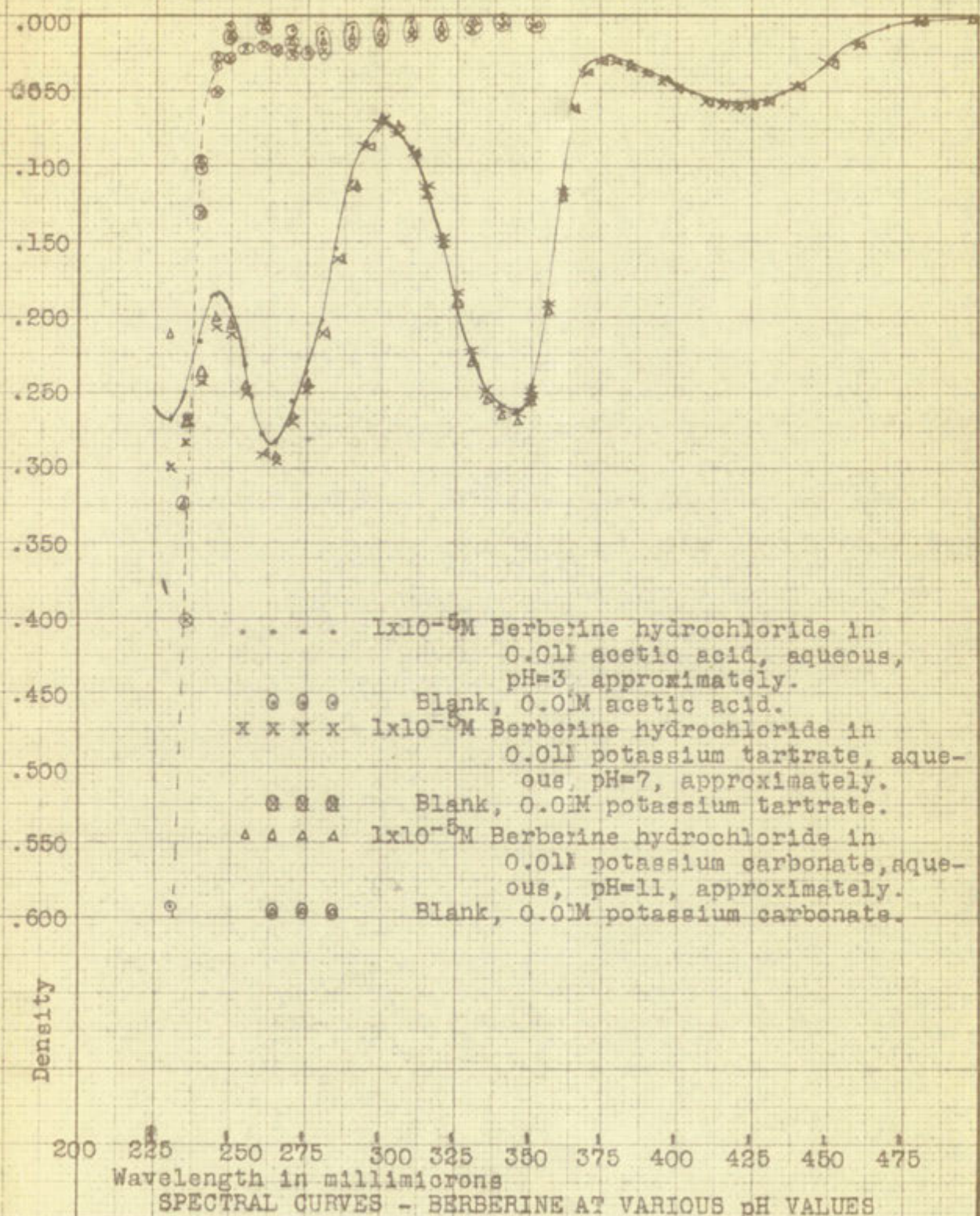
In order to determine the effect of varying the pH of barbituric solutions, spectral curves of barbituric hydrochloride dissolved in strongly acidic, neutral, and strongly

alkaline aqueous solutions were determined. These are shown in Graph 17. Since it has been reported¹²⁰ that barbituric acid changes into dihydrobarbituric acid and oxybarbituric acid (type reaction) in strong alkali, some barbituric acid was heated in potassium hydroxide for one hour on a steam bath (1.00 milliliters of 1×10^{-2} molar barbituric hydrochloride plus 1.00 milliliters of 1 molar potassium hydroxide) then diluted to a solution containing 1×10^{-2} molar barbituric hydrochloride and 0.101 molar potassium hydroxide. A similar but unheated solution was prepared to run in parallel.

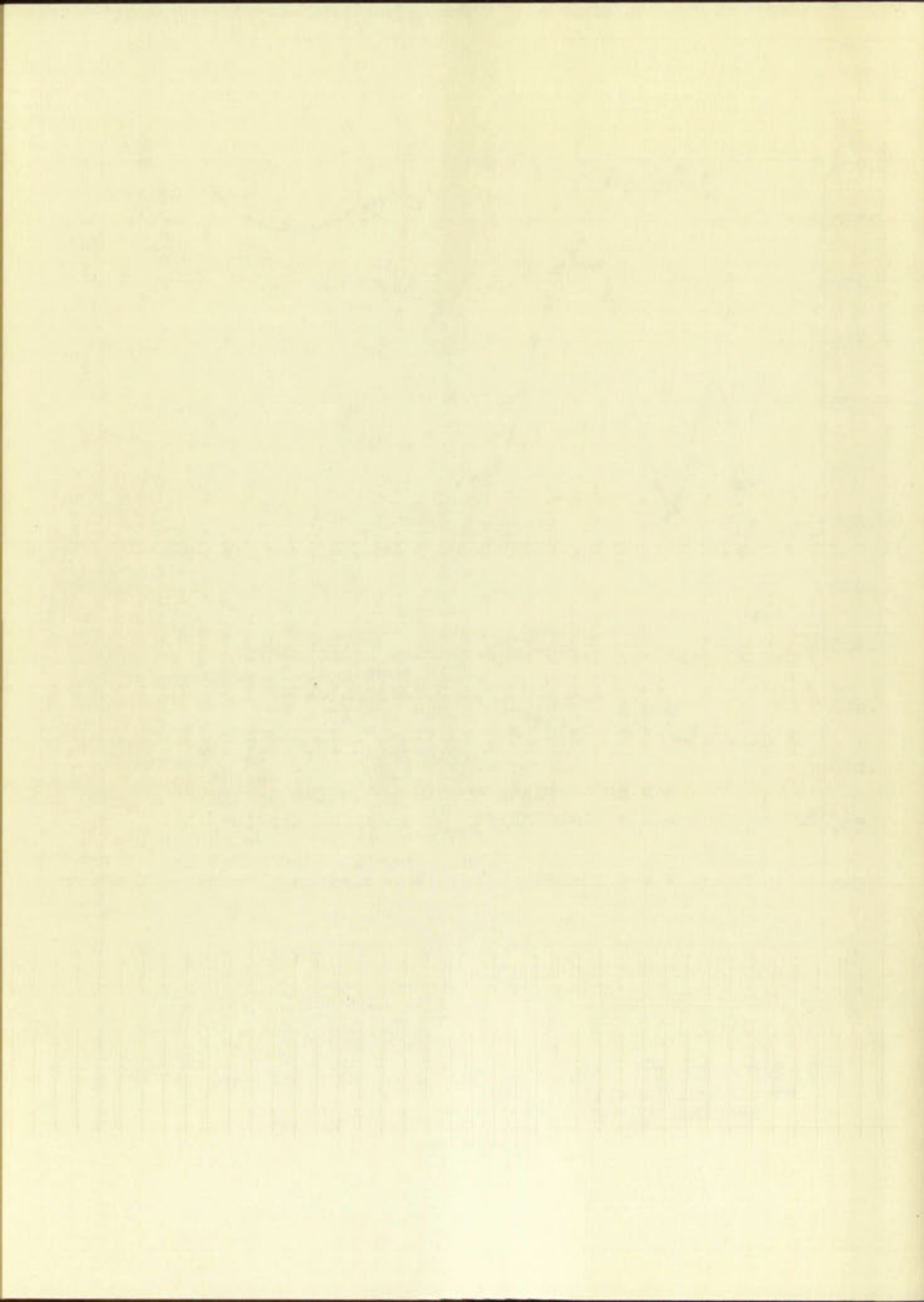
The spectral curves for these are shown in Graph 18. Other alkalis of the isocyanide group have given

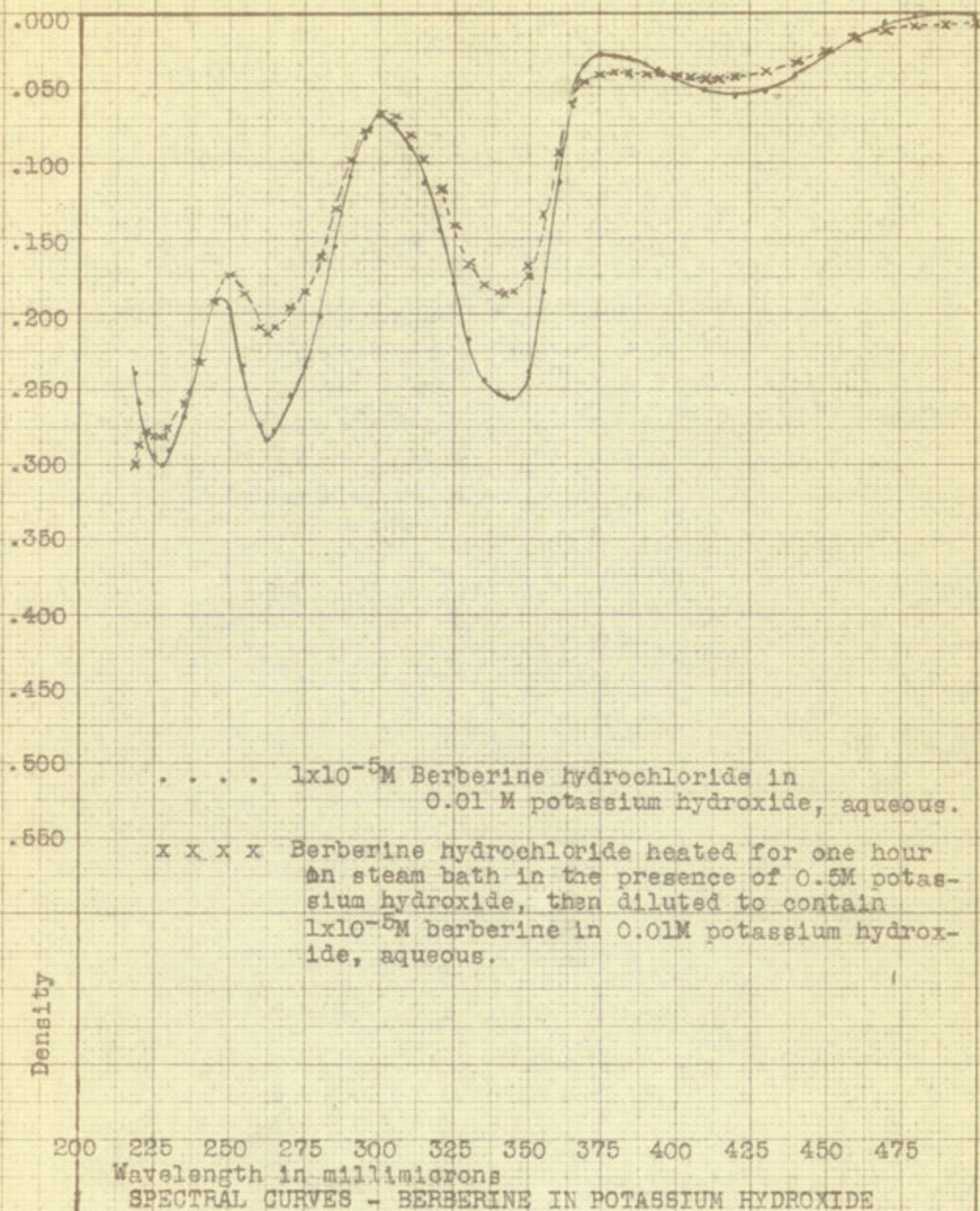
curves somewhat similar to barbituric. Three of these were available, namely sodium chloride, potassium hydrochloride, and pyridine. Maximum absorption bands and molecular

¹²⁰ Tinkler, J. Chem. Soc., 1242-47 (1911).

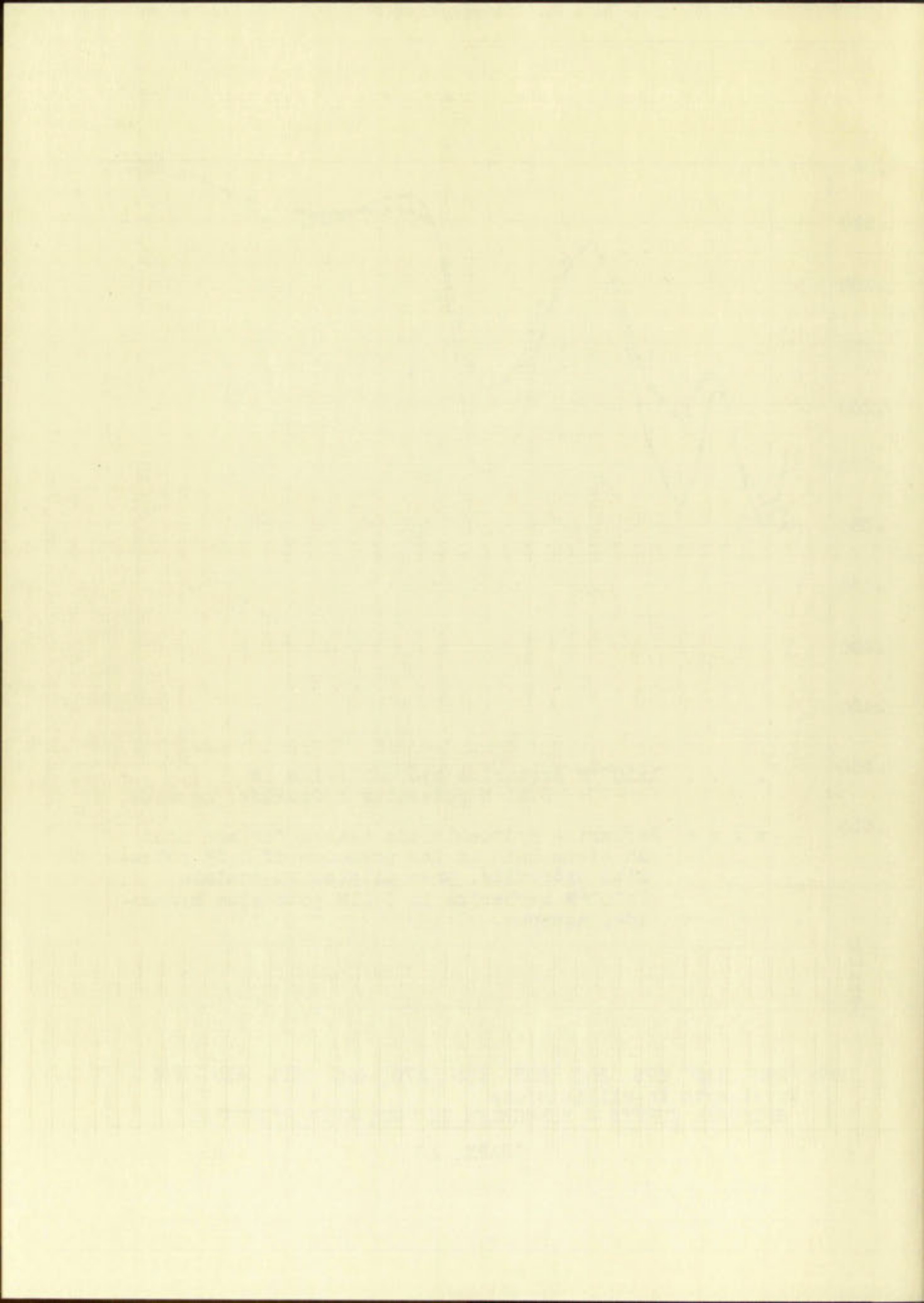


GRAPH 17





GRAPH 18



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FREE

extinction coefficients obtained for them are shown in Table XX. Spectral curves obtained from solutions of these compounds are shown in Graph 19.

B. FLUORESCENCE

Berberine salts are intensely fluorescent. In order to determine how the fluorescence varies with changing wavelength a drop of 1×10^{-4} molar berberine hydrochloride solution was placed on a piece of filter paper and let dry. The spot was examined in the path of the light beam of the Beckman Spectrophotometer, as it entered the sample cell compartment. With maximum slit width opening, it was possible, in a dark room, to observe the fluorescence of spot over the entire range of the instrument. In going from visible to ultraviolet wavelengths, the berberine showed a slight yellowish fluorescence at 420 millimicrons, and the fluorescence increased to its brightest around 360 to 350 millimicrons, thereafter diminishing. Below about 265 millimicrons the fluorescence decreased more rapidly and apparently disappeared below 206 millimicrons. The maximum fluorescent regions were approximately the same as the maximum absorption bands, but they were not in the same order of intensity.

extinction coefficients obtained for these are shown in Table XX. Spectral curves obtained from solutions of these compounds are shown in Graph 19.

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TABLE XX

ABSORPTION BANDS OF COTARNINE, PAPAVERINE, AND HYDRASTINE

Compound	Color	Absorption Band Millimicrons	Molecular Extinction Coefficient
Cotarnine chloride	yellow;	217	27,700
Aqueous	light yellow-	252	27,500
solution	blue fluores- cence	333	34,100
Papaverine	colorless;	250	25,400
hydrochloride	light bluish	284	2970
Aqueous	fluorescence	309	3700
Solution		325*	3100
Hydrastine	colorless;	210-**	37,000+**
Alcoholic	no fluorescence	296	7050
solution			

* Inflection point

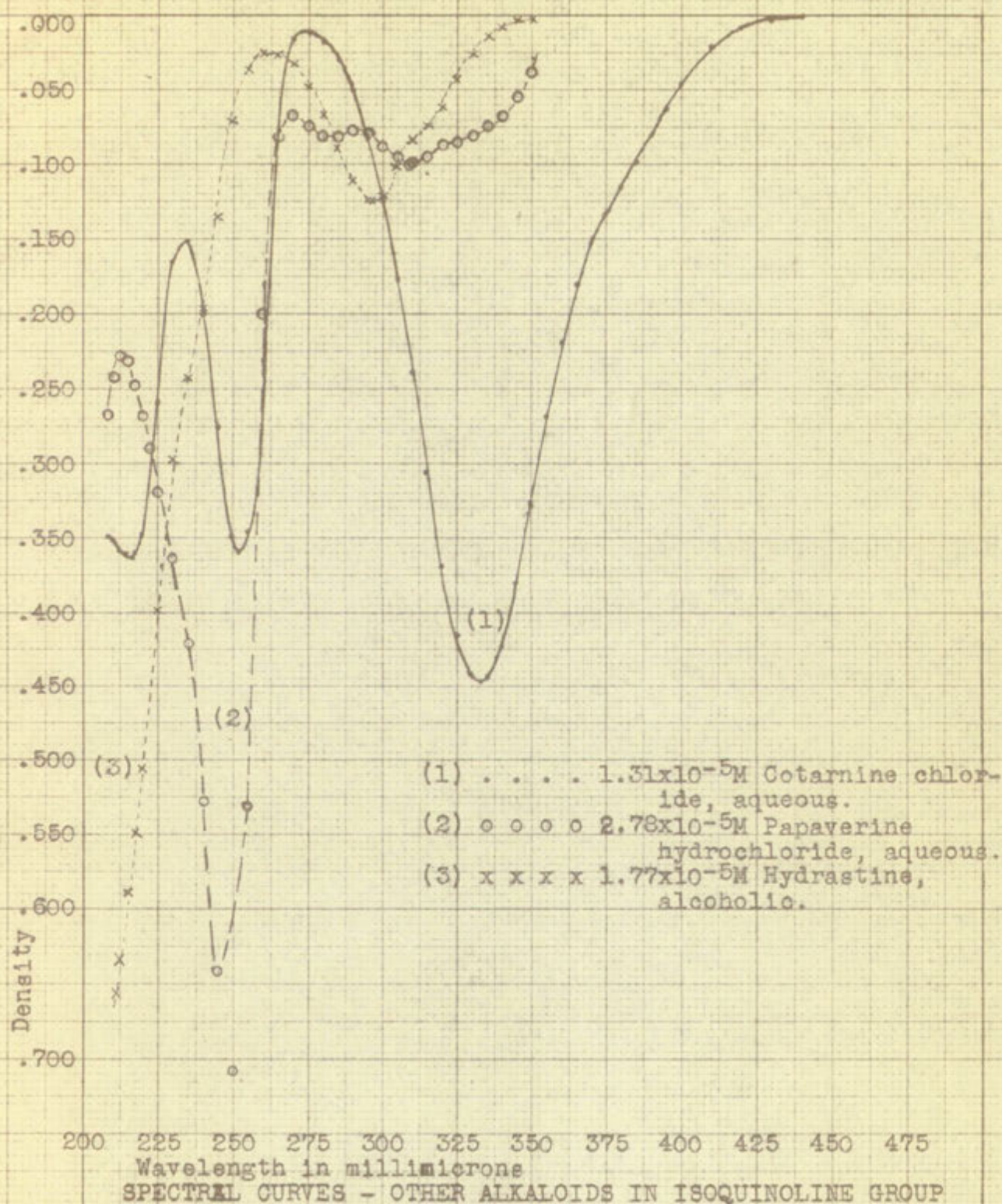
** - less than; + greater than (band below range of Beckman Spectrophotometer).

TABLE XX

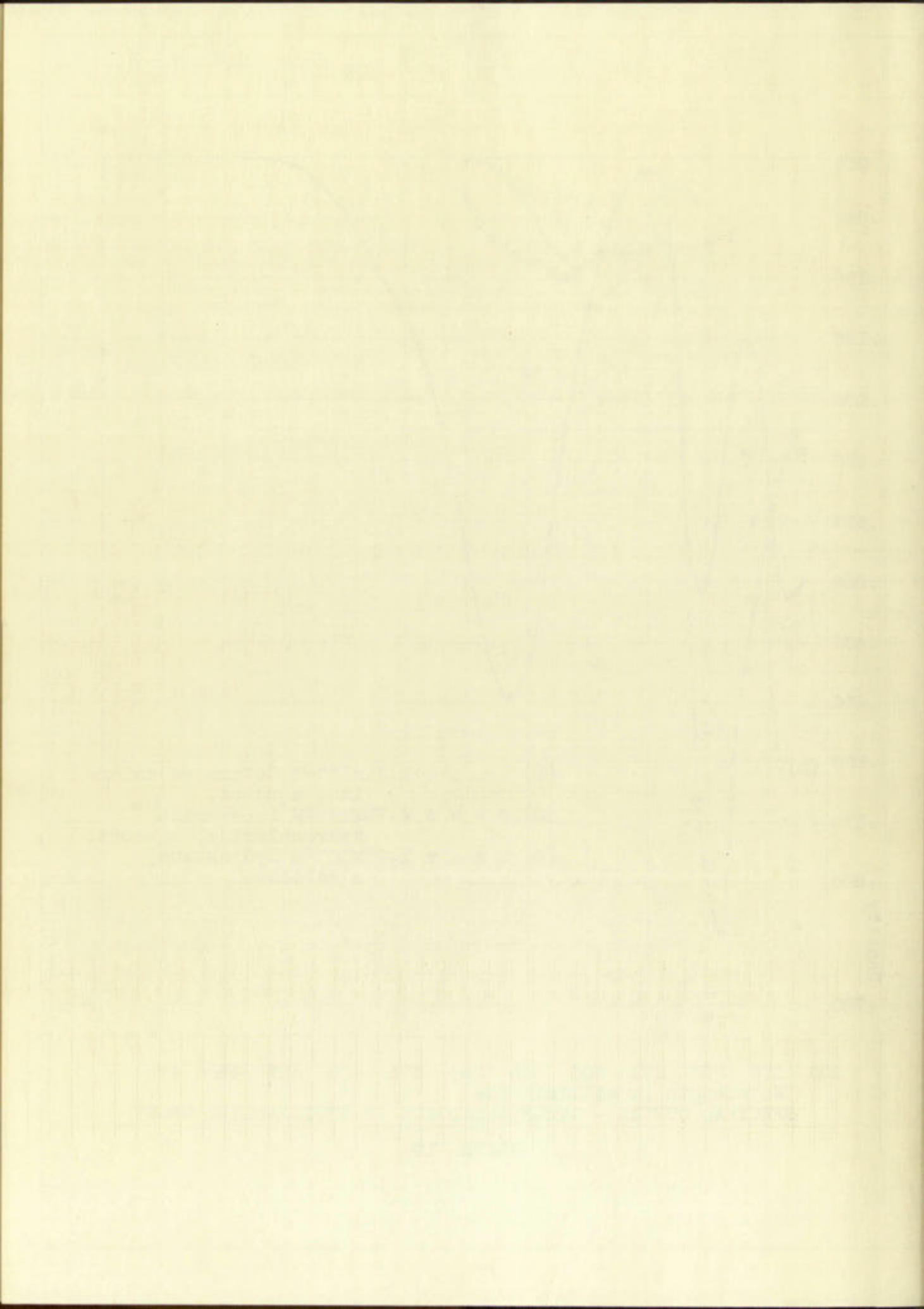
ABSORPTION BANDS OF COTHRINE, CATHARTIC, AND NITRATES

Compound	Color	Absorption Band (mμ)	Molar Extinction Coefficient
Cocaine chloride	yellow	217	27,700
Agonist	light yellow	228	27,700
Solution	dark yellow	235	24,100
Prochloride	colorless	250	22,400
Agonist	light yellow	254	27,700
Solution	colorless	261	27,700
Hydroxide	colorless	270	27,700
Agonist	no fluorescence	280	27,700

* Inflection point
 ** - less than; + greater than (band below range of sodium spectrograph).



GRAPH 19



CHAPTER IX

ANALYSIS OF DATA

A. POLAROGRAPHY OF BERBERINE

As indicated by the data in Table XII, the maximum over-all current rise for the electro-reduction of berberine was 12.5 to 13.5 microamperes. When these observations are compared with the data previously found by Pasternak (Table V, Supplement), it can be assumed that a current of the above magnitude involved four electrons per molecule. Therefore, a current of approximately 3.4 microamperes per millimole was involved for each electron per molecule entering into the reduction.

On this basis four electrons per molecule caused the complete electro-reduction of berberine in acid solution. With neutral solutions results were essentially the same as those found for acid solutions, though less consistent. With slightly alkaline solutions the reduction currents indicated that reduction occurred in three steps involving one electron per molecule for each of the first two waves and two electrons per molecule for the third wave. In strongly alkaline solutions the total reduction involved only two or less electrons per molecule, with only one

CHAPTER IX ANALYSIS OF DATA

A. POLAROGRAPHY OF BISMUTH

As indicated by the data in Table XII, the cathodic over-all current rise for the electro-reduction of bismuth was 12.5 to 13.5 microamperes. When these observations are compared with the data previously found by Rastbach (Table V, Supplement), it can be assumed that a current of the above magnitude involved four electrons per molecule. Therefore, a current of approximately 2.5 microamperes per millimole was involved for each electron per molecule entering into the reduction.

On this basis four electrons per molecule seemed the complete electro-reduction of bismuth in acid solution. With neutral solutions results were essentially the same as those found for acid solutions, though less consistent. With slightly alkaline solutions the reduction current indicated that reduction occurred in three stages involving one electron per molecule for each of the first two waves and two electrons per molecule for the third wave. In strongly alkaline solutions the total reduction involved only two or less electrons per molecule, with only one

electron per molecule at any step. On the basis of this experiment, when only two electrons per molecule are involved, it is difficult to ascertain whether reduction took place at the carbinol-nitrogen-atom group or at the ethylene linkage.

B. CONTROLLED REDUCTION OF BERBERINE

Controlled coulometric reduction of berberine solutions (Tables XV and XVII) gave data which more definitely confirms several facts; (a) complete electro-reduction in acid solution to a colorless compound involves four electrons per molecule; (b) reduction in alkaline solution to a lighter colored product involves two electrons per molecule; (c) reduction can take place so that a lighter colored product reduced with two electrons per molecule is obtained, which product can be further reduced with two electrons per molecule to a colorless compound; and (d) under certain conditions reduction can take place to a colorless compound involving only two electrons per molecule, which compound does not reduce further electrolytically. With the disappearance of color, fluorescence also disappears.

This indicates the formation of tetrahydroberberine (colorless) when four electrons per molecule are involved, and dihydroberberine (light yellow color) when two electrons per molecule are involved. In the case of reduction

electron per molecule at any stage. On the basis of this experiment, when only two electrons per molecule are involved, it is difficult to ascertain whether reduction took place at the carbonyl-nitrogen-atom group or at the ethylene linkage.

B. CONTROLLED REDUCTION OF BENZOPHENONE

Controlled coulometric reduction of benzophenone solutions (Tables IV and VII) gave data which were definitely confirmatory of the following facts: (a) complete electro-reduction in acid solution to a colorless compound involves four electrons per molecule; (b) reduction in alkaline solution to a lighter colored product involves two electrons per molecule; (c) reduction can take place so that a lighter colored product reduced with two electrons per molecule is obtained, which product can be further reduced with two electrons per molecule to a colorless compound; and (d) under certain conditions reduction can take place to a colorless compound involving only two electrons per molecule, which compound does not reduce further electrolytically. With the disappearance of color, fluorescence also disappears.

This indicates the formation of tetrahydrobenzophenone (colorless) when four electrons per molecule are involved, and dihydrobenzophenone (light yellow color) when two electrons per molecule are involved. In the case of reduction

to a colorless compound involving two electrons per molecule, reduction must take place at the ethylene group to give ethylene-dihydroberberine, since the carbinol group is reduced when dihydroberberine is formed (pp. 4, 6).

According to experimental results electrolytic reduction does not always take place according to polarographic data, particularly in alkaline solutions. It is thought that an internal electrolytic cell back - E.M.F. is a factor in such differences.

C. SPECTROPHOTOMETRIC MEASUREMENTS

As shown in Table XVIII aqueous berberine hydrochloride solutions showed four maximum absorption bands, three intense ones in the ultraviolet region, and one considerably less intense in the visible region near the ultra violet. These bands are 228-229 millimicron, 262-264 millimicron, 342-345 millimicron and 420-422 millimicron, with corresponding molecular extinctions at 27,000-30,000; 27,000-30,000; 25,000-27,000; and 5000-6000. With the exception of the solution of berberine hydrochloride in potassium carbonate-potassium sulfite solution, all solutions measured, at all pH values, fell within the above ranges. Anomalous values were obtained in the exceptional case, but they were not due to the high pH, because a solution of still higher

to a colorless compound involving two electrons per molecule, reduction must take place at the ethylene group to give ethylene-dihydroberberine, since the carbonyl group is reduced when dihydroberberine is formed (pp. 4, 5).

According to experimental results electrolytic reduction does not always take place according to polarographic data, particularly in alkaline solutions. It is thought that an internal electrolytic cell back - E.M.F. is a factor in such differences.

C. SPECTROPHOTOMETRIC MEASUREMENTS

As shown in Table XVII various berberine hydrochloride solutions showed four maximum absorption bands, three intense ones in the ultraviolet region, and one considerably less intense in the visible region near the ultra violet. These bands are 238-239 millimicrons, 262-264 millimicrons, 268-269 millimicrons and 430-432 millimicrons, with corresponding molar extinction coefficients at 27,000-30,000; 25,000; 25,000-27,000; and 5000-6000. With the exception of the solution of berberine hydrochloride in potassium carbonate-potassium sulfate solution, all solutions measured at pH values, fell within the above ranges. Anomalous values were obtained in the exceptional case, but they were not due to the high pH, because a solution of still higher

pH was similar to all other solutions measured. The sulfite ion probably caused the difficulty. An alcoholic solution of berberine hydrochloride measured for comparison gave absorption bands with small bathochromic shifts, varying from 2 millimicrons in low ultraviolet to 10 millimicrons at the longest wavelength band. Berberine sulfate solutions gave the same bands as the hydrochloride.

The fact that berberine hydrochloride absorption bands were found to be identical in very acid (pH of 2) and very basic (pH of 12.5+) aqueous solutions showed that only one form of berberine existed in aqueous solutions at all pH values. If this were not the case, any change in structural form would have resulted in either a shift in wavelength or a shift in intensity of absorption bands.

On reduction two general types of changes occurred. Reduction in acid solutions yielded a colorless product whose absorption bands changed as follows: the 228-29 millimicron band stayed in the same wavelength region but its intensity was reduced to about one-third its original value; the 262-264 millimicron band disappeared and in its place a band at 285 millimicrons appeared; in one instance a new band of low intensity also appeared at 295 millimicrons; the 342-345 millimicron band stayed in the same region, but its intensity was greatly reduced; the 420-22 millimicron band

ph was similar to all other solutions measured. The solu-
tion probably caused the difficulty. An alcoholic solu-
tion of barbitone hydrochloride measured for comparison
gave absorption bands with small differences in wave-
length from 2 millimicrons in low dilution to 10 millimicrons
at the longest wavelength band. Barbitone hydrochloride
solutions gave the same bands as the hydrochloride.
The fact that barbitone hydrochloride absorption
bands were found to be identical in very acid (pH of 2)
and very basic (pH of 12.54) aqueous solutions showed that
only one form of barbitone existed in aqueous solutions at
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structural form would have resulted in either a shift in
wavelength or a shift in intensity of absorption bands.
On reduction two general types of changes occurred.
Reduction in acid solutions yielded a colorless product
whose absorption bands changed as follows: the 228-232 mil-
limicron band stayed in the same wavelength region but its
intensity was reduced to about one-third the original value;
the 262-284 millimicron band disappeared and in its place a
band at 266 millimicrons appeared; in one instance a new
band of low intensity also appeared at 288 millimicrons; the
342-348 millimicron band stayed in the same region, but its
intensity was greatly reduced; the 420-422 millimicron band

disappeared altogether.

Reduction in neutral and basic solutions yielded a partially reduced product, lighter yellow color, with changes as follows; all absorption bands remained in approximately the same regions, but the intensities were greatly reduced; the lowest wavelength band had a hypsochromic shift of about 5 millimicrons. The reduction in intensities of the absorption bands was due to both partial reduction and to partial precipitation of berberine from the solution under the conditions of the experiment. The precipitate causing cloudiness in the solutions was later found to contain occluded berberine. The current consumed during electrolysis showed that some reduction (other than catalytic waves) of berberine had taken place.

These changes are summarized in Table XXI. The table also includes the absorption bands of berberine hydrochloride heated in strong potassium hydroxide solution and a parallel solution not heated; the former should contain an equi-molar mixture of oxyberberine and dihydroberberine (compare pages 6, 28).

The absorption bands of berberine and its reduced derivatives can be correlated with the structure of berberine as represented by either the ammonium form or carbinol form.

A few factors effecting maximum absorption bands

disappeared altogether.

Reduction in neutral and basic solutions yielded a

partially reduced product, lighter yellow color, with

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reduction and to partial precipitation of barbituric from

the solution under the conditions of the experiment. The

precipitate causing cloudiness in the solution was later

found to contain occluded barbituric. The amount contained

during electrolysis showed that some reduction (other than

catalytic waves) of barbituric had taken place.

These changes are summarized in Table XII. The

table also includes the absorption bands of barbituric hy-

drochloride heated in strong potassium hydroxide solution and

a parallel solution not heated; the former should contain

an equi-molar mixture of oxybarbituric and dihydrobarbituric

(compare pages 6, 28).

The absorption bands of barbituric and its reduced de-

rivatives can be correlated with the structure of barbituric

as represented by either the ammonium form or carbonyl form.

A few factors affecting maximum absorption bands

TABLE XXI
ABSORPTION BANDS OF BERBERINE SOLUTIONS

Solution	Absorption Band millimicrons	Molecular Extinction Coefficient
Berberine hydrochloride pH, 1 to 13	228-229 262-264 342-345 420-422	27,000-30,000 27,000-30,000 25,000-27,000 5,000-6,000
Berberine hydrochloride in potassium carbonate and potassium sulfite	233*; 263; 336; 405-420* ^a .	28,000**; 20,300; 18,000; 4700.
Berberine reduced to col- orless derivative in acid solutions; tetra- hydroberberine, or ethyl- ene-dihydroberberine	227-228 285 340-345	10,500-11,500 6000 1000-1500
Berberine reduced in slightly acid solution to (a) dihydroberberine, light yellow color (b) tetrahydroberberine, colorless	228; 265; 344; 423. 227; 285; (295); 345.	18,000; 13,000; 11,000; 2600. 4700; 2500; (2000); 1200.
Berberine reduced in alkaline solutions to dihydroberberine, light yellow color (incomplete reduction)	225-229 262-265 342-344 415-417	5000-20,000 2000-20,000 3000-18,000 3000***
Berberine reacted with potassium hydroxide to mixture of dihydrober- berine & oxyberberine, equimolar, plus any unreacted berberine	229 262.5 342 415	28,100 21,350 18,700 4300

* Inflection point; **approximate; ***less than

^a Band varies with concentration.

TABLE XVI
ABSORPTION BANDS OF BERBERINE SULFATES

Wavelength mμ	Absorption Band mμ	Molecular Weight Molecular Weight
27,000-30,000 27,000-30,000 28,000-30,000 28,000-30,000	280-285 285-288 288-292 292-295	Berberine hydrochloride pH 1 to 12
28,000-30,000 28,000-30,000 28,000-30,000 28,000-30,000	280-285 285-288 288-292 292-295	Berberine hydrochloride in potassium carbonate and potassium sulfate
28,000-30,000 28,000-30,000 28,000-30,000 28,000-30,000	280-285 285-288 288-292 292-295	Berberine reduced to sol- uble derivative in acid solutions; berber- ine, or ethyl- ene-dihydroberberine
28,000-30,000 28,000-30,000 28,000-30,000 28,000-30,000	280-285 285-288 288-292 292-295	Berberine reduced in slightly acid solution to (a) dihydroberberine, light yellow color (b) tetrahydroberberine, colorless
28,000-30,000 28,000-30,000 28,000-30,000 28,000-30,000	280-285 285-288 288-292 292-295	Berberine reduced in alkaline solutions to dihydroberberine, light yellow color (incomplete reduction)
28,000-30,000 28,000-30,000 28,000-30,000 28,000-30,000	280-285 285-288 288-292 292-295	Berberine treated with potassium hydroxide to mixture of dihydrober- berine & oxyberberine, apicalar, plus any unreacted berberine

* Inflection point; ** approximate; *** free base

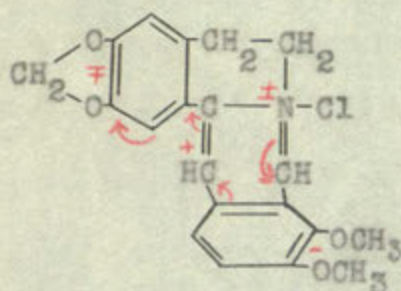
† Band varies with concentration.

might be recalled from part of the literature survey (pp. 33-34). A large electric moment connected with the electronic transition in the molecule with light absorption corresponds to strong absorption. The wavelength of maximum absorption is inversely proportional to the square root of the restoring force constant. The smaller the energy involved in producing electron displacements associated with light absorption, the longer the wavelength of maximum absorption. Resonance in the excited state lowers the potential energy of the excited state, an effect producing a bathochromic shift of the absorption band. Resonance in the ground state decreases the potential energy of the ground state, an effect producing a hypsochromic shift of the absorption band.

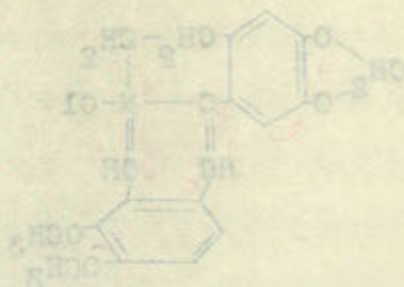
The longest wavelength absorption band of berberine, the 420-22 millimicron band, with a molecular extinction coefficient of 5000-6000, would have to be due to the structure in the excited state having the greatest resonance, and hence with the most conjugation. This could only be the two methoxy substituted benzene rings in conjugation with the ethylene connecting group, and possibly including the adjacent *C=N group, as shown in diagram.

might be recalled from part of the literature survey (25, 26-28). A large electric moment connected with the electronic transition in the molecule with light absorption corresponds to strong absorption. The wavelength of maximum absorption is inversely proportional to the square root of the restoring force constant. The smaller the energy involved in producing electron displacement associated with light absorption, the longer the wavelength of maximum absorption. Resonance in the excited state lowers the potential energy of the excited state, an effect producing a bathochromic shift of the absorption band. Resonance in the ground state decreases the potential energy of the ground state, an effect producing a hypsochromic shift of the absorption band.

The longest wavelength absorption band of porphyrins, the 420-430 millimicron band, with a molecular extinction coefficient of 5000-6000, would have to be due to the structure in the excited state having the greatest resonance, and hence with the most conjugation. This could only be the two methoxy substituted benzene rings in conjugation with the ethylene connecting group, and possibly including the adjacent $\text{C}=\text{O}$ group, as shown in diagram.



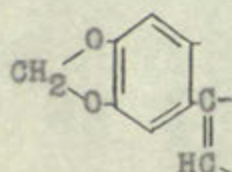
The intense yellow color of berberine is also directly connected with this conjugated chromophore, a combination of absorption and also fluorescence. As soon as the double bond in the ethylene connecting group is broken (by reduction), color, fluorescence, and this absorption band disappear, as proved by the spectral bands of the colorless reduction products obtained (Table XIX, p. 83). Tetrahydroberberine obtained from berberine by reduction with four electrons or four hydrogen-atoms per molecule, was one reduction product; in it the ethylene group has been reduced to a single bond. Another possible reduction product obtained, assuming the experimental data were not in error, is berberine reduced with two electrons or two hydrogen atoms at the ethylene double bond and not at the carbinol group, to a colorless compound, with almost the same absorption bands as the tetrahydro-derivative. The dihydro (ethylene bond) derivative has a slight bathochromic shift at the



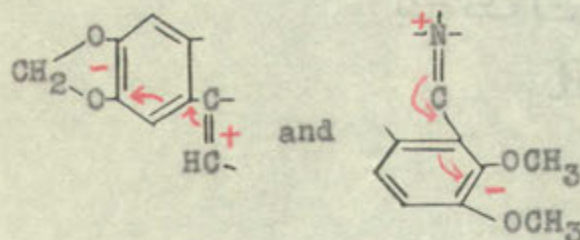
The intense yellow color of barbituric acid is also directly connected with this conjugated chromophore, a combination of absorption and also fluorescence. As soon as the double bond in the ethylene connecting group is broken (by reduction), color, fluorescence, and this absorption band disappear, as proved by the spectral bands of the colorless reduction products obtained (Table XIX, p. 85). Tetrahydrobarbituric acid obtained from barbituric acid by reduction with four electrons or four hydrogen-atoms per molecule, was one reduction product; in it the ethylene group has been reduced to a single bond. Another possible reduction product obtained, assuming the experimental data were not in error, is barbituric acid reduced with two electrons or two hydrogen atoms at the ethylene double bond and not at the carboxyl group, to a colorless compound, with almost the same absorption bands as the tetrahydro-derivative. The dihydro-derivative (bond) derivative has a slight bathochromic shift of the

shortest band. How significant these differences are is not known. They exceed experimental error shown in duplicate tests of Solution A (p. 83), and the inflection point is not present in the other reduced products.

The next longest wavelength absorption band of berberine, the 342-345 millimicron band, with a molecular extinction coefficient of 25,000-27,000, is due to the chromophoric system having the next greatest resonance in excited state. This system is the group:



or more likely a sum of the groups:



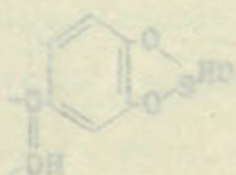
Evidence supporting this conclusion is as follows;

- (a) The longest wavelength absorption band of hydrastinine was reported in the literature as 347-392 millimicrons¹²¹

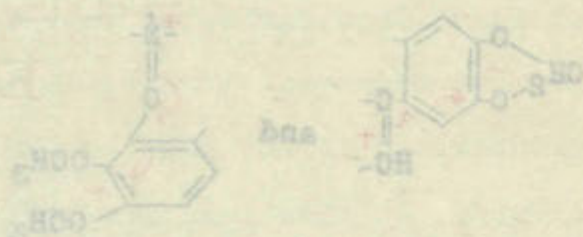
¹²¹The absorption bands of compounds measured by instruments preceding the Beckman Spectrophotometer type are much wider than measurements now possible.

shortest band. Now significant these differences are in not known. They exceed experimental error shown in duplicate tests of Solution A (p. 85), and the inflection point is not present in the other reduced products.

The next longest wavelength absorption band of pyridine, the 345-348 millimicron band, with a molar extinction coefficient of 25,000-27,000, is due to the chromophoric system having the next greatest resonance in excited state. This system is the group:



or more likely a sum of the groups:



Evidence supporting this conclusion is as follows:

(a) The longest wavelength absorption band of pyridine was reported in the literature as 345-348 millimicrons.¹²¹

¹²¹The absorption bands of compounds reported by literature preceding the present spectroscopic study are such wider than measurements now possible.

(Table VI, p. 30). A comparison of various compounds listed in Table VIII, p. 37-40, containing methylenedioxy and methoxy (or ethoxy) substituents shows that only a small difference in absorption band values exists between methylenedioxy and ortho dimethoxy compounds forming similar chromophores.

(b) The longest wavelength absorption band of 6,7-dimethoxy-2-methyl--3,4-dihydroisoquinolinium-chloride, was reported as 341-384 millimicrons¹²².

(c) The longest wavelength absorption band of cotarnine (as a salt), was reported as 328 millimicrons. Experimentally this band was found to be at 333 millimicrons with a molecular extinction coefficient of 34,100 (p. 95).

(d) The longest wavelength band reported for papaverine (in alcohol) was 304-333 millimicrons (p. 30). Experimentally two bands were found in this region; the band at 309 millimicrons, molecular extinction coefficient, 3700, and the inflection point, 325 millimicrons, molecular extinction coefficient, 3100.

(e) piperic acid (p. 38) has a broad maximum absorption band at 326 millimicrons.

Allowing for reasonable bathochromic or hypsochromic shifts (Table VII, p. 36) for the different auxochromic

¹²²See footnote 121.

(Table VI, p. 30). A comparison of various compounds listed in Table VIII, p. 37-40, containing methylenedioxy and methoxy (or ethoxy) substituents shows that only a small difference in absorption band values exists between methylenedioxy and ortho dimethoxy compounds forming similar chromophores.

(b) The longest wavelength absorption band of 6,7-dimethoxy-2-methyl-3,4-dihydroquinoline-chloride, was reported as 341-384 millimicrons¹²².

(c) The longest wavelength absorption band of cotinine (as a salt), was reported as 328 millimicrons. Experimentally this band was found to be at 333 millimicrons with a molar extinction coefficient of 34,100 (p. 35).

(d) The longest wavelength band reported for naphthalene (in alcohol) was 304-353 millimicrons (p. 30). Experimentally two bands were found in this region; the band at 309 millimicrons, molar extinction coefficient, 2700, and the inflection point, 353 millimicrons, molar extinction coefficient, 3100.

(e) Dipicric acid (p. 36) has a broad maximum absorption band at 338 millimicrons.

Allowing for reasonable bathochromic or hypsochromic shifts (Table VII, p. 35) for the different amphoteric

¹²² See footnote 121.

substituents in the different molecules, these compounds all have a band in the same wavelength region; this indicates that either or both of the two groups given as the chromophoric system are most probably the source of the 342-345 millimicron absorption band of berberine. Since the molecular extinction coefficient is large, both groups probably absorb at the same wavelength and contribute additively to the intensity of absorption. Ferguson¹²³ showed that this was a common occurrence in molecules with two insulated chromophoric systems. At longer wavelengths the groups are in conjugation but with the absorption of light of higher energy, they effectively act separately.

Further evidence supporting these groups as the source of the 342-345 millimicron band is that the partly reduced products of berberine including both oxyberberine and dihydroberberine formed by the reaction of potassium hydroxide with berberine¹²⁴ or just dihydroberberine formed by reduction in alkaline solutions involving not over two electrons per molecule, had this band with intensity reduced to one-half or less (Table XIX). The loss of the C=N bond in the second group given above, by hydrogenation,

¹²³Ferguson, Chem. Revs. 43, 398-99 (1948).

¹²⁴Tinkler, J. Chem. Soc., 99, 1346-47 (1911).

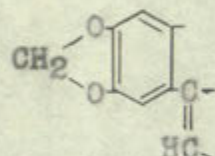
substituents in the different molecules, these compounds all have a band in the same wavelength region; this indicates that either or both of the two groups given as the chromophoric system are most probably the source of the 343-345 millimicron absorption band of berberine. Since the extinction coefficient is large, both groups probably absorb at the same wavelength and contribute additively to the intensity of absorption. Ferguson¹²³ showed that this was a common occurrence in molecules with two insolated chromophoric systems. At longer wavelengths the groups act in conjunction but with the absorption of light of higher energy, they effectively act separately.

Further evidence supporting these groups as the source of the 343-345 millimicron band is that the weakly reduced products of berberine including both oxyberberine and dihydroberberine formed by the reaction of berberine hydroxide with berberine¹²⁴ or just dihydroberberine formed by reduction in alkaline solution involving not over two electrons per molecule, had this band with intensity reduced to one-half or less (Table XIX). The loss of the 343 band in the second group given above, by hydrogenation,

¹²³Ferguson, *Chem. Rev.* 43, 355-59 (1952).

¹²⁴Tinkler, *J. Chem. Soc.* 92, 1548-49 (1911).

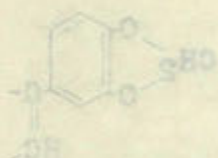
removed this group as a chromophoric system at this wavelength, leaving only the



chromophoric system to absorb the light. However, the low intensity absorption band of tetrahydroberberine (at 340-345 millimicrons, extinction coefficient, 1000 to 1500), in which there is no $-C=C-$ or $-C=N$ group adjacent to either benzene ring has not been accounted for. Piperonyl alcohol, (p. 37), has a strong band at 285.5 millimicrons, but this is out of the region of the band in question. 3-methoxy-4-hydroxy benzaldehyde has a band at 353 millimicrons, molecular extinction coefficient, 1980, and ortho-methoxy benzaldehyde has a band at 319 millimicrons, molecular extinction coefficient, 4160 (Table VIII, pp. 38-39). These are the only chromophores found in the literature search that have a band in the region of 340-345 millimicrons with methoxy substituents on the benzene ring but without a $C=C$ or $C=N$ substituent also; it is not likely that a carbonyl group is present in tetrahydroberberine, considering that as yet no evidence has been found indicating the presence of a carboxyl group in berberine (imino aldehyde form), as was pointed out in the literature survey (Chapter II). Safrole (p. 40) has a band at 278-294

removed this group as a chromophoric system at this wave-

length, leaving only the



chromophoric system to absorb the light. However, the low

intensity absorption band of tetrahydrocannabinol at 210-

245 mμ, extinction coefficient, 1000 to 1500,

in which there is no -O-C- or -C=O group adjacent to either

benzene ring has not been accounted for. Phenanthrene, alcohol,

(p. 37), has a strong band at 283.5 mμ, extinction, but this

is out of the region of the band in question. 5-methoxy-4-

hydroxy benzaldehyde has a band at 283 mμ, extinction, and

acetyl extinction coefficient, 1900, and ortho-methoxy

benzaldehyde has a band at 219 mμ, extinction, and

extinction coefficient, 4100 (Table VII, pp. 28-29).

These are the only chromophores found in the literature

which have a band in the region of 240-265 mμ.

Groups with methoxy substituents on the benzene ring but

without a C=O or C=C substituent also; it is not likely

that a carbonyl group is present in tetrahydrocannabinol,

considering that no evidence has been found indicat-

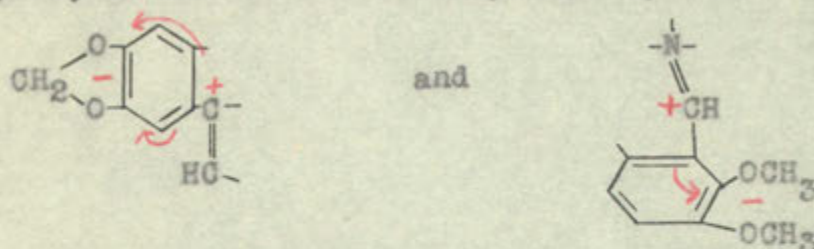
ing the presence of a carbonyl group in cannabinol (p. 37).

aldehyde form), as was pointed out in the literature survey

(Chapter II). Salvo (p. 40) has a band at 278-284

millimicrons, and the $-\text{CH}_2\text{CH}=\text{CH}_2$ group has about the same effect as a saturated alkyl substituent (p. 36). A second alkyl substituent could be expected to produce a bathochromic shift, and possibly a $-\text{CH}_2\text{-N}$ substituent, on account of the amino nitrogen, would produce a still larger bathochromic shift. Such a grouping is present in tetrahydroberberine. These shifts can be predicted on the basis of the lower ionization potentials of the di-alkyl substituted benzene compared to the benzene nucleus (p. 43). Such a chromophore might account for the 340-345 millimicron band in tetrahydroberberine if the shifts are of sufficient magnitude.

The next absorption band of berberine, the 262-264 millimicron band with a molecular extinction coefficient of 27,000-30,000, is due to the chromophoric systems



together, both absorbing at almost the same wavelength with intensities additive. Though these are the same groups that account for the 342-345 millimicron band, the $\text{C}=\text{C}$ and $\text{C}=\text{N}$ are not in full conjugation with the benzene ring but are only partially effective in influencing the

millimicrons, and the $-\text{OH}$ group has about the same effect as a saturated alkyl substituent (p. 38). A second alkyl substituent could be expected to produce a bathochromic shift, and possibly a $-\text{CH}_3$ substituent, on account of the amino nitrogen, would produce a still larger bathochromic shift. Such a grouping is present in tetrahydroberberine. These shifts can be predicted on the basis of the lower ionization potentials of the di-alkyl substituted benzene compared to the benzene nucleus (p. 43). Such a chromophore might account for the 340-350 millimicron band in tetrahydroberberine if the shifts are of sufficient magnitude.

The next absorption band of berberine, the 322-332 millimicron band with a molecular extinction coefficient of 27,000-30,000, is due to the chromophoric system



together, both absorbing at almost the same wavelength with intensive additive. Though these are the same groups that account for the 342-352 millimicron band, the $\text{O}-\text{O}$ and $\text{O}-\text{H}$ are not in full conjugation with the benzene ring but are only partially effective in influencing the

ionization state of the dimethoxy or methylenedioxy benzenoid structure. Evidence supporting this conclusion is as follows:

- (a). Iso-eugenol has been reported to have a band, at 258-259 millimicrons (p. 39); another observer (p. 39) found that it had a band at 249-275 millimicrons and an inflection point at approximately 313 millimicrons, the latter corresponding more nearly to the 342-345 band of the groups discussed above. Iso-safrole (p. 40) had an inflection point at 249-276 millimicrons.
- (b). The compound, 1-para-methoxy-phenyl-propene was reported (p. 39) to have a band at 258 millimicrons with a molecular extinction coefficient of 20,350.
- (c). The shortest wavelength absorption band of three bands reported for hydrastinine was 250-256 millimicrons (p. 30).
- (d). The next to longest wavelength absorption band of cotarnine (salts) was reported as 250 millimicrons. Experimentally this band was found to be at 252 millimicrons with a molecular extinction coefficient of 27,500. (p. 95).
- (e). The compound phenyl-propene has been found to have three absorption bands, the shortest and strongest being at 249 millimicrons with a molecular extinction coefficient of 14,700. Other substituents could easily shift this into the 262-264 millimicron region.
- (f). The compound, benzal methylamine was reported to have

location state of the diastereomer or asymmetrically ben-
zenoid structures. Evidence supporting this conclusion is

as follows:

(a). 1,2-dibromobenzene has been reported to have a band at 835-

839 millimicrons (p. 38); another observer (p. 38) found

that it had a band at 835-839 millimicrons and an inflec-

tion point at approximately 835 millimicrons. The latter

corresponding more nearly to the 845-845 band of the groups

discussed above. 1,2-dibromobenzene (p. 40) had an inflection

point at 845-849 millimicrons.

(b). The compound, 1,2-dibromo-3-phenylpropane was re-

ported (p. 38) to have a band at 858 millimicrons with a

molecular extinction coefficient of 20,300.

(c). The shortest wavelength absorption band of three bands

reported for hydrazine was 850-858 millimicrons (p. 38).

(d). The next to longest wavelength absorption band of cop-

erine (salts) was reported as 850 millimicrons. Exper-

imentally this band was found to be at 853 millimicrons with

a molecular extinction coefficient of 27,500. (p. 38).

(e). The compound phenylpropane has been found to have

three absorption bands, the shortest and strongest being at

849 millimicrons with a molecular extinction coefficient of

14,700. Other substituents could easily shift this band

the 850-854 millimicron region.

(f). The compound, benzal hydrazine was reported to have

the band located at 247 millimicrons with a molecular extinction coefficient of 17,200. Further substitution on the nitrogen-atom, by inference from ionization potential changes with increased substitution (pp. 43,44), could bring this band to coincide with the above compound (e) and other substituents on the benzene ring could shift the band to the 262-264 millimicron region.

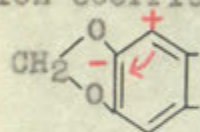
Small bathochromic shifts due to the differences of substituents in the above compounds and berberine could place them all in the 262-264 millimicron region, thus giving good evidence of the existence of the chromophoric groups in berberine as described for this band. In addition, the partially reduced derivatives of berberine (involving not over two electrons or two H-atoms per molecule) were found to have an absorption band at 262-265 millimicrons, molecular extinction coefficient, 2000 to 20,000, whose intensity was generally less than half of the original intensity, indicating that one of the two groups contributing equally to the band was gone; this was to be expected with the $-C=N$ group changed to $-CH_2-N$, the dihydro compound. The fully reduced compound (four hydrogen atoms per molecule) did not have this band at all, which was to be expected, since the auxochromic groups $-C=C$ and $-C=N$ were reduced and thus changed to different auxochromic groups.

The shortest wavelength absorption band of berberine,

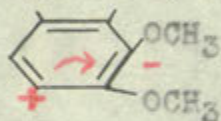
the band located at 647 millimicrons with a molar extinction coefficient of 17,200. Further examination on the nitrogen atom, by interference from ionization potential changes with increased substitution (7.45, 6.45), could bring this band to coincide with the above compound (c) and other substituents on the benzene ring could shift the band to the 662-684 millimicron region.

Small bathochromic shifts due to the difference of substituents in the above compounds and derivatives could place them all in the 662-684 millimicron region, thus giving good evidence of the existence of the chromophoric group in derivative as described for this band. In addition, the partially reduced derivatives of peroxide (11-12), the fully reduced compound (13) hydrogen atom involving not over two electrons or two H-atoms per molecule) were found to have an absorption band at 662-684 millimicrons, molar extinction coefficient, 2000 to 30,000, whose intensity was generally less than half of the original intensity, indicating that one of the two groups contributing equally to the band was gone; this was to be expected with the -O-O- group changed to -O-H, the diphenyl compound. The fully reduced compound (14) hydrogen atom per molecule) did not have this band at all, which was to be expected, since the azo group -O-O- and -O-H were reduced and thus changed to different chromophoric groups. The shortest wavelength absorption band of peroxide,

located at 228-229 millimicrons with a molecular extinction coefficient of 27,000-30,000, was due to the groups



and



. Evidence sup-

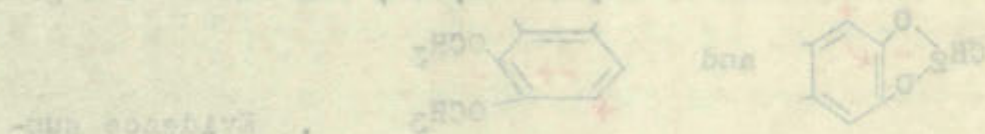
porting this follows:

(a) Absorption bands reported on the compounds (pp. 37-38); benzene, band at 203.5 millimicrons, extinction coefficient 7400; anisole, band at 217 millimicrons, extinction coefficient, 6400; ortho-dimethoxy benzene, or ortho-veratrole, band at 227 millimicrons, extinction coefficient, 5000; and piperonyl alcohol with an inflection point at 220-238 millimicrons, indicate the simple dimethoxy and methylenedioxy benzene nuclei to be the responsible chromophoric groups for the band in question with intensities additive.

(b) The auxochromic group $-C=C-CH_3$ on benzene nucleus changed to the group $-CH_2-CH=CH_2$ or $-R$ (saturated alkyl), would result in a hypsochromic shift of 20-30 millimicron, thus a 262 millimicron band could go to a 232 to 242 millimicron band (p. 36).

(c) The chromophore group, $C=N$, has an absorption band at 230 millimicrons (p. 36). It is probable that this group, present in the ammonium form of berberine, contributes also to the intensity of the 228-229 millimicron band of

located at 298-329 millimicrons with a molar extinction coefficient of 27,000-30,000, was due to the groups



porting this follows:

- (a) Absorption bands reported on the compounds (pp. 27-28): benzene, band at 298.5 millimicrons, extinction coefficient 27,000; anisole, band at 315 millimicrons, extinction coefficient 6400; ortho-dimethoxy benzene, or ortho-veratrole, band at 327 millimicrons, extinction coefficient 5000; and piperonyl alcohol with an extinction coefficient of 290-328 millimicrons, indicate the single dimethoxy and methylenedioxy benzene nuclei to be the responsible chromophoric groups for the band in question with intensities additive.

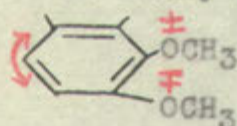
- (b) The chromophoric group -O-CH₂-CH₂-O- on benzene nucleus changed to the group -CH₂-CH₂-CH₂-O- or -O- (saturated alkyl), would result in a hypsochromic shift of 20-30 millimicrons, thus a 298 millimicron band could go to a 268 to 268 millimicron band (p. 28).

- (c) The chromophoric group, O-CH₂, has an absorption band at 330 millimicrons (p. 28). It is probable that this group, present in the ammonium form of piperonyl, contributes also to the intensity of the 298-329 millimicron band of

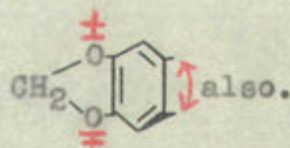
berberine, since the intensity of the band is fairly high.

The reduced derivatives have the same band but with reduced intensity. However, this band is the strongest of all the absorption bands in either the partially reduced (two hydrogen atoms per molecule) or the fully reduced (four hydrogen atoms per molecule) derivatives. The methylenedioxy and dimethoxy benzene nuclei are still present in reduced compounds, but the C=N group is no longer present.

The band at 285 millimicrons, molecular extinction coefficient, 6000, formed in place of the 262-264 millimicrons band in the colorless reduced berberine derivative is due to the fully conjugated group



and possibly



Evidence of this conclusion follows, according to results obtained from literature reports;

- (a) Ortho-dimethoxy benzene or ortho-veratrole (p. 38) has an absorption band at 278 millimicrons, extinction coefficient 2000.
- (b) Safrole, (p. 40) has an absorption band at 278-294 millimicrons.
- (c) Eugenol, (p. 40) has an absorption band at 278-286 millimicrons.
- (d) 6,7-Dimethoxy-2-methyl-tetrahydro-isoquinoline, has an absorption band at 286-294 millimicrons.

barbitone, since the intensity of the band is fairly high.

The reduced derivatives have the same band but with

reduced intensity. However, this band is the strongest of

all the absorption bands in either the partially reduced

(two hydrogen atoms per molecule) or the fully reduced (four

hydrogen atoms per molecule) derivatives. The asymmetrical

oxy and dimethoxy benzene nuclei are still present in re-

duced compounds, but the $\text{C}=\text{C}$ group is no longer present.

The band at $288 \text{ m}\mu$ is characteristic of molecular extinction

coefficient, 6000 , formed in place of the $282\text{--}284 \text{ m}\mu$

absorption band in the colorless reduced barbitone derivative

is due to the fully conjugated form



and possibly



Evidence of this conclusion follows, according to results

obtained from literature reports:

(a) Ortho-dimethoxy benzene or ortho-veratrole (p. 28) has

an absorption band at $278 \text{ m}\mu$ characteristic, extinction coeffi-

cient 2000 .

(b) Veratrole (p. 40) has an absorption band at $278\text{--}284$

$\text{m}\mu$.

(c) Eugenol (p. 40) has an absorption band at $278\text{--}284$

$\text{m}\mu$.

(d) 3,5-Dimethoxy-2-methyl-4-oxo-1,2,3,4-tetrahydro-2H-pyridine, has

an absorption band at $286\text{--}284 \text{ m}\mu$.

(e) Hydrocotarnine, (p. 30) has an absorption band at 285.5 millimicrons.

(f) Tetrahydroberberine, tetrahydropapaverine, corydaline, laudanosine (p. 30), all have an absorption band at 282 millimicrons.

(g) Hydrohydrastinine (p. 54) has an absorption band at 285-297 millimicrons. These compounds all have the groups considered to be the chromophoric origin of the 285 millimicron band in the fully reduced derivative of berberine, and allowing for small differences due to the different auxochromic groups in the different compounds, all fall within the same range.

This band only occurs with the colorless reduced derivative of berberine, and so must be due to the methylenedioxybenzene nucleus in the absence of an attached ethylene or unsaturated group.

The contribution of the C=N group to some of the absorption bands of aqueous (or alcoholic) berberine solutions shows that the ammonium form structure of berberine exists in these solutions. Reduced derivatives, without the C=N group must have the carbinol form structure or the other alternative, an imine-primary alcohol form structure.

The major absorption bands of berberine and its reduced derivatives have thus been correlated to accepted structures of berberine.

CHAPTER X

SUMMARY AND CONCLUSION

A. SUMMARY

An analytical study of the alkaloid, berberine, has been made. A quantitative analysis of the particular lot used for the study was first performed and some special glass apparatus required for the study was constructed. Following preliminary work to establish optimum polarographic conditions, berberine solutions were analyzed polarographically to determine factors effecting the reduction of the alkaloid. Two series of experiments were then carried out to reduce berberine under controlled conditions. Both the original solutions and the various solutions containing the reduced products connected with these experiments were measured spectrophotometrically. An analysis of data obtained was made to correlate the properties of berberine observed with its known structural features.

Spectral bands and corresponding molecular extinction coefficients observed for berberine in aqueous solutions at all pH values were: (1) 228-229 millimicron, 27,000-30,000; (2) 262-264 millimicron, 27,000-30,000; (3) 342-345 millimicron, 25,000-27,000; and (4) 420-422

CHAPTER V

SUMMARY AND CONCLUSION

A. SUMMARY

An analytical study of the alkaloid, barbitone, has been made. A quantitative analysis of the alkaloid for use in the study was first carried out and some special glass apparatus required for the study was constructed. Following preliminary work to establish optimum photographic conditions, barbitone solutions were analyzed colorimetrically to determine factors affecting the reduction of the alkaloid. Two series of experiments were then carried out to reduce barbitone under various conditions. Both the original solutions and the various reduction products were analyzed colorimetrically. An analysis of the reduced products connected with these experiments was made to correlate the properties of barbitone observed with its known structural features.

Spectral bands and corresponding molar extinction coefficients observed for barbitone in aqueous solution at all pH values were: (1) 225-235 millimicrons, 27,000-30,000; (2) 235-245 millimicrons, 27,000-30,000; (3) 245-255 millimicrons, 25,000-27,000; and (4) 255-265 millimicrons, 25,000-27,000.

millimicron, 5,000-6,000. Bands for the partially reduced, light yellow colored compounds and involving two electrons or hydrogen atoms per molecule, were found to be in the same wavelength regions but of considerably reduced intensities. Bands for the reduced, colorless compounds, involving four electrons per molecule, or two under certain conditions, differed from the original bands as follows: the 228-229 millimicron band was the same with reduced intensity; the 262-264 millimicron band was replaced by a band at 285 millimicrons, molecular extinction coefficient, 6,000; the 342-345 millimicron band was the same with greatly reduced intensity; and the 420-422 millimicron band disappeared entirely. In addition, the colorless reduced products lost all fluorescence.

The spectral bands of cotarnine chloride, papaverine hydrochloride, and hydrastine were measured for the purpose of comparison with berberine.

B. CONCLUSION

It is known that berberine salts have a deep yellow color; that they fluoresce under some conditions; that berberine has some properties in common with other alkaloids of the isoquinoline group; that some changes occur in the spectral absorption characteristics of berberine upon reduction; and that the reactions of this compound indicate

millimicrons, 5,000-8,000. Bands for the partially reduced, light yellow colored compounds and involving two electrons or hydrogen atoms per molecule, were found to be in the same wavelength regions but of considerably reduced intensity. Bands for the reduced, colorless compounds, involving four electrons per molecule, or two under certain conditions, differed from the original bands as follows: the 328-329 millimicron band was the same with reduced intensity; the 382-384 millimicron band was replaced by a band at 385 millimicrons, molecular extinction coefficient, 5,000; the 342-345 millimicron band was the same with greatly reduced intensity; and the 420-422 millimicron band disappeared entirely. In addition, the colorless reduced products lost all fluorescence.

The spectral bands of cotinine chloride, papaverine hydrochloride, and pyrazine were measured for the purpose of comparison with berberine.

B. CONCLUSION

It is known that berberine salts have a deep yellow color; that they fluoresce under some conditions; that berberine has some properties in common with other alkaloids of the isoquinoline group; that some changes occur in the spectral absorption characteristics of berberine upon reduction; and that the reactions of this compound indicate

more than one structure.

It has been shown that berberine has four absorption bands in the visible and ultraviolet regions of the spectrum, three being in the ultraviolet range and of considerable intensity. Partially reduced products have the same bands but with much smaller intensity. Completely reduced berberine has three bands in the ultraviolet region, two the same as the original and a new band in place of one of the original bands; all have smaller intensities than the original bands of berberine, and there is no band in the visible range.

Reduction occurs in strongly acid solutions to a colorless product, either ethylenedihydroberberine or tetrahydroberberine; the tetrahydroberberine is more likely to be formed. In both compounds it seems logical to conclude that the ethylene group between the two benzene rings has been reduced.

In slightly acid solutions reduction takes place in two stages, dihydroberberine being formed at the first stage, and tetrahydroberberine at the second.

In alkaline solutions only partial reduction involving two electrons per molecule occurs; the product formed apparently is dihydroberberine, with the ethylene group

more than one specimen.

It has been shown that the specimens are not identical.

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unreduced. Oxyberberine and dihydroberberine, obtained by the reaction of potassium hydroxide with berberine upon heating, have the same absorption bands as dihydroberberine but with greater intensity. This indicates that oxyberberine also has the same absorption bands.

The contribution of the C=N group to some of the absorption bands of berberine in aqueous solutions indicates that in such solutions berberine exists as the ammonium form. Only reduced derivatives would have the carbinol form.

The correlation of each absorption band observed for berberine and its reduction products with the structure as represented by the ammonium or carbinol form has been made. Each band is caused by a definite chromophoric system in the molecule responding to light absorption; the various chromophoric systems involved have been described.

unreduced. Oxypiperine and dihydropiperine, obtained by the reaction of potassium hydroxide with piperine upon heating, have the same absorption bands as dihydropiperine but with greater intensity. This indicates that oxypiperine also has the same absorption bands.

The contribution of the C-H group to some of the absorption bands of piperine in aqueous solution indicates that in such solutions piperine exists as the monomer form. Only reduced derivatives would have the carbonyl form.

The correlation of each absorption band observed for piperine and its reduction products with the structure as represented by the number of carbonyl form has been made. Each band is caused by a definite electronic system in the molecule responding to light absorption; the various chromophoric systems involved have been described.

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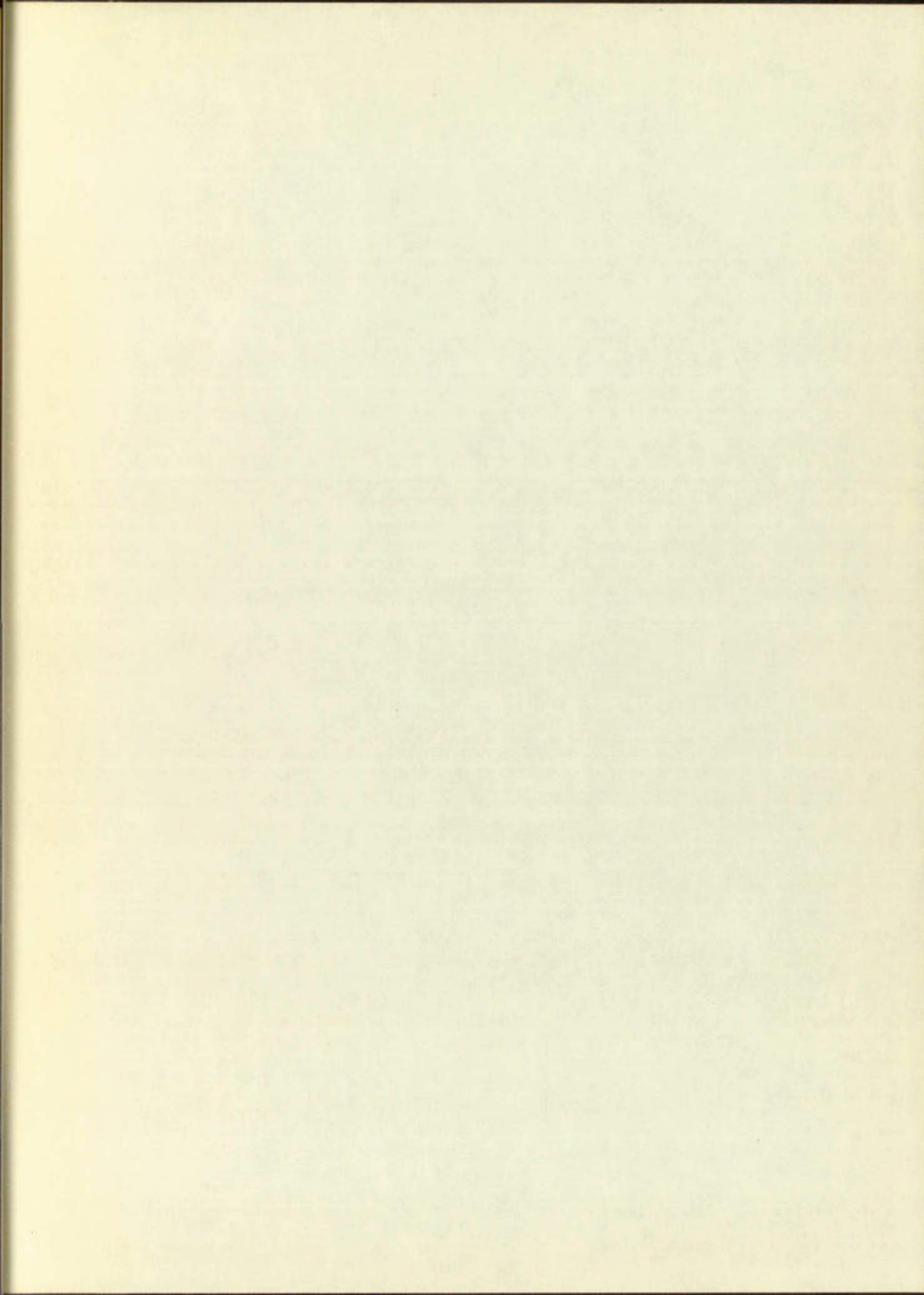
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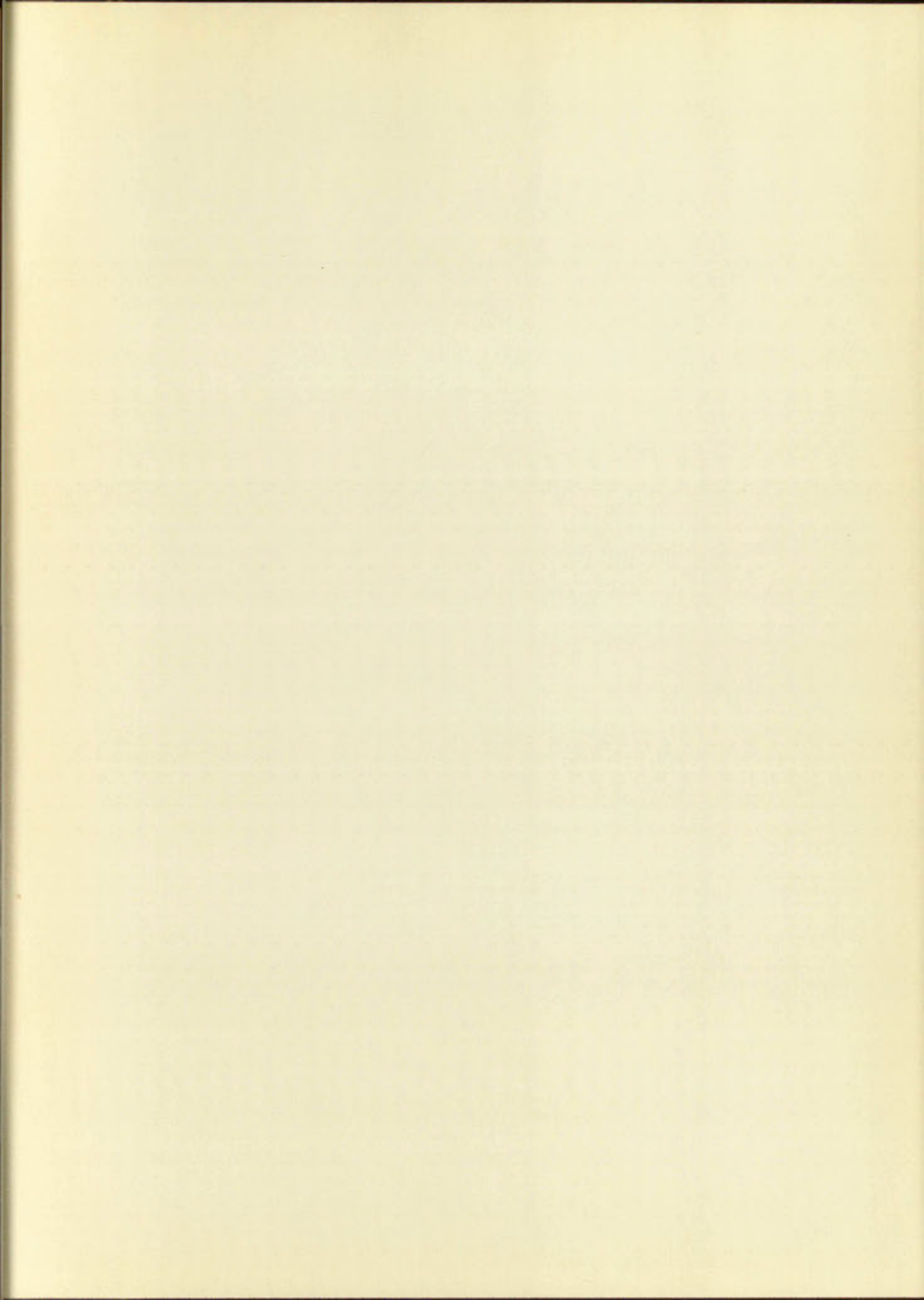
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