HALF-WAVE POTENTIALS OF VITAMINS K, AND SOME VITAMIN B COMPLEXES

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HALF-WAVE POTENTIALS OF VITAMINS K, AND SOME VITAMIN B COMPLEXES

BY

William John Abraham

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INTRODUCTION

Polarographic methods[1] have been successfully used in the study of the kinetics of reactions; and the evaluation of the stability, and polymerization of organic substances. In numerous instances it has given definite assistance in establishing the organic structure of some complex compounds. It has been fairly well determined that there is a close relationship between the polarographic reduction potential of a compound and its chemical constitution. The polarograph is well suited for such measurements.

Much less is known of the interpretation of current-voltage curves of organic compounds than of most inorganic compounds, because, generally, reduction of organic compounds are irreversible reactions. In irreversible reductions the nature of the reduction product is often unknown, due to the exceedingly small amount formed during the electrolysis. However, much information regarding the structure of the compound can be gathered by determining the number of electrons involved in the reduction at the dropping mercury electrode.

The main difference between the oxidation or reduction of inorganic substances and organic substances is that in the reduction or oxidation of the organic substance hydrogen ions are usually involved in the electrode

reaction. The equation

$$R + nH^+ + ne \rightleftharpoons RH_n$$

represents a general case in which the oxidant and reductant are both uncharged molecules. Ordinarily n is equal to 2.

It is desirable to work with buffered solution in the studies of the reductions or oxidations of organic compounds, because electrons are involved in the electrode reactions. If buffered solutions weren't used the pH of the solution would be changing continually. Nevertheless, some compounds form better waves in unbuffered solutions and in these cases unbuffered solutions are used.

In this investigation experiments were carried out in an endeavour to determine the stability and rate of decomposition, on exposure to air, of various vitamin K solutions.

MATERIALS

The mercury which is used must be exceedingly pure, and may be used only once before being repurified. A high grade of mercury, which had been previously used in polarographic work, was used for first experiments. After being used once it was purified by first shaking vigorously with distilled water in order to remove all water soluble material. This operation is repeated four times

and then the mercury is shaken four times with 3% nitric acid. Next it is passed through a cone of filter paper with a hole in the apex and through a four foot length of glass capillary. This operation removes all solid particles not soluble in water or 3% nitric acid. The mercury is now allowed to drop in a stream of fine droplets through a five foot column of 3% nitric acid. After this the mercury is distilled in vaccuum and is then ready for use.

The nitrogen used is obtained from a commercial tank of nitrogen. Oxygen and other impurities which may be present are removed by first passing the nitrogen through a solution of pyrogallic acid, next through a solution of alkaline potassium permanganate, and finally washed by passing through water.

PROCEDURE

A Leeds and Northrup Electro-Chemograph was used in this investigation and in the following paragraph the procedure used is outlined. Mercury is poured into the dry electrolysis cell until the bottom is covered. A measured amount, usually four mililiters, of the solution to be analyzed is added. Five drops of 0.05% gelatin is added to the solution in the cell. The gelatin is added to prevent the formation of current maximas. The cell is placed in position and the electrodes inserted. Nitrogen is bubbled through the solution for three minutes, in order

to remove any dissolved oxygen. Oxygen is easily reduced at the dropping mercury electrode and if not removed it will give a reduction wave which will interfere with the wave of the substance to be analyzed. The solution is kept under an atmosphere of nitrogen throughout the electrolysis. During the time the nitrogen is bubbling through the solution the three electrical balances, described in the directions for operation of the instrument, are made. The proper shunt, depending on the concentration of the solution, is inserted. The height of the mercury column is adjusted so that the drop time is from 2 to 4 seconds. In most cases it was adjusted so that one drop of mercury fell every three seconds. The galvanometer is now released and allowed to come to rest. The polarizing unit is now started, this steadily increases the potential between the electrodes, and at the same time the chart is set in motion. The current-voltage curve is now automatically traced on the moving chart and nothing remains to be done except turn the instrument off at the conclusion of the run.

INVESTIGATION OF VITAMIN K COMPLEXES

The substances used in this investigation were: 2-methyl-1,4-naphthoquinone, 2,3-dimethyl-1,4-naphthoquinone, 1-hydroxy-2-methyl-4-naphthol amine hydrochloride, and the dimer of 2-methyl-1,4-naphthoquinone. Stock solutions of each compound were prepared by dis-

solving in either oxygen free water or oxygen free alcohol, depending on the solubility of the compound. The oxygen was removed from the water and alcohol by suction. The stock solutions were kept tightly stoppered and in the dark except when a sample was being withdrawn. When a determination was to be made a sample was withdrawn and diluted 1 to 1 with 0.1 N KCl or buffer, gelatin was added and it was then electrolyzed.

2-Methyl-1,4-naphthoquinone is practically insoluble in water, so ethyl alcohol was used as a solvent. The alcoholic solution was found to be perfectly stable. The 2-methyl-1,4-naphthoquinone gave an excellent cathodic wave in both an unbuffered KCl solution and a buffered solution of a pH of 5.0. The buffer used was composed of equal parts of 1 M $NaC_2H_3O_2$ and 1 M $HC_2H_3O_2$. The buffer alone had a pH of 4.6. When it was diluted with an equal amount of water it had a pH of 4.6. When it was diluted with an equal amount of ethyl alcohol it had a pH of 5.0. Fig. 1 shows a cathodic wave of 2-methyl-1,4-naphthoquinone, and Table 1 summarizes the results. The unbuffered solution exhibited a current maxima which could not be suppressed.

2,3-Dimethyl-1,4-naphthoquinone is quite similar to 2-methyl-1,4-naphthoquinone. It is insoluble in water but soluble in ethyl alcohol. The cathodic wave of 2,3-

dimethyl-1,4-naphthoquinone is exactly like the one obtained with 2-methyl-1,4-naphthoquinone except that it has a higher reduction potential and a higher half-wave potential as can readily be seen from Table 7. Fig. 3 shows a typical wave. Cathodic waves were formed from both buffered and unbuffered solutions. The wave from the unbuffered KCl solution was the better of the two. The wave formed from the buffered solution exhibited a current maxima which could not be suppressed by either gelatin or glue solution.

1-Hydroxy-2-methyl-4-naphthol amine hydrochloride is soluble in water thus permitting use of an aqueous solution. 1-Hydroxy-2-methyl-4-naphthol amine hydrochloride was found to be very easily oxidized upon being exposed to the air for only a very few moments. The cathodic wave formed from a buffered solution of 1-hydroxy-2-methyl-4-naphthol amine hydrochloride, pH of 5.0, changed considerably on aging. At first the reduction potential remains constant and the diffusion current increases steadily. When the solution was one hour old the diffusion current had reached a limiting value and now the reduction potential started to increase with the diffusion current remaining constant. When the solution was three hours old the reduction potential and half-wave potential had reached limiting values. After this the cathodic wave did not change as the solution continued

to age. This effect is shown in Table 4 and Fig. 5.

The results shown in Table 2 were obtained using an old solution of 1-hydroxy-2-methyl-4-naphthol amine hydrochloride in which the reduction potential and diffusion current had become constant. Good curves were formed from the buffered solution of a pH of 5.0. A slight current maxima, which could not be suppressed by either gelatin or glue, was exhibited both in the buffered and the unbuffered solution. Fig. 2 illustrates a wave from an old solution. If a sample of a fresh solution of 1-hydroxy-2-methyl-4-naphthol amine hydrochloride is taken and electrolized, then left exposed to air and electrolyzed again, etc., the same change in reduction potential and diffusion current, as described above, is noted; only this time it occurs much faster. The change depends on the time and intimacy of the contact of the solution with the air. It is probably due to oxidation of the 1-hydroxy-2-methyl-4-naphthol amine hydrochloride by the oxygen in the air.

The effect of irradiation, by ultra-violet light, on a solution of 2-methyl-1,4-naphthoquinone was studied. The solvent used was absolute ethyl alcohol. The solution was irradiated for a definite interval of time and a sample was withdrawn. The sample was diluted 1 to 1 with 0.1 N KCl and electrolyzed.

The results of this irradiation are shown in Table 8. At first the curve broke at -0.44 v. After being irrad-

iated for ten minutes there were two curves; one broke at -0.44 v. and the other at -0.74 v. The first curve disappeared gradually and the second increased as the irradiation continued. After the solution had been irradiated for a period of twenty minutes the first curve had completely disappeared and a third wave had appeared. The second wave broke at -0.70 v. and the third at -1.18 v. As irradiation continued the second curve began to lose its shape and after the solution had been irradiated for forty minutes only the third wave remained. As irradiation continued the half-wave potential of the third wave remained constant but the diffusion current increased slowly. It is readily apparent that the irradiation with the ultra-violet light changed the structure of the 2-methyl-1,4-naphthoquinone.

A solution of the dimer of 2-methyl-1,4-naphthoquinone was prepared by dissolving the dimer in absolute alcohol. Upon being reduced at the dropping mercury electrode the dimer gave a double cathodic wave. Results are given in Table 6. The dimer was diluted 1 to 1 with 0.1 N KCl before being electrolyzed.

Commercial liquid samples of 1-hydroxy-2-methyl-4-naphthol amine hydrochloride were measured. The samples were obtained in sealed ampoules; each contained 1 mg. of the compound. The ampoule was opened immediatly before it was to be electrolyzed. The contents of the ampoule was diluted 1 to 1 with acetate buffer and measured, then

left exposed to the air for a short time and remeasured.

When ampoule No. 199 (50271) was used, two cathodic waves were formed. The first increased in sized steadily until reaching a maximum value when the solution was about three hours old. The first wave had a reduction potential of -0.02 v. at first. When the solution was about two hours old the reduction potential started to increase. It reached a maximum value of -0.20 v. when the solution was six hours old. The reduction potential of the second wave was -0.70 v. at first. It increased steadily to a value of -1.0 v. by the time the solution was three hours old. The size of the second wave varied hardly at all. Table 20 summarizes the results.

When ampoule No. 199 (49771) was used, two waves were again formed, which were of about equal size at first. One broke at -0.39 v. and the other at -0.65 v. During the first one and one-half hours the first wave decreased in size while the second increased in size and the final limiting current remained approximately constant. By stating that the "wave increases in size" is meant that the diffusion current of that particular wave is increased. The reduction potential of the first wave increased to -0.45 v. whereas the reduction potential of the second did not change. At the end of four and one-half hours both full waves had increased in size. The reduction potential of the first wave fell to -0.02 v. and the sec-

ond had risen to -0.75 v. When the solution was five and one-half hours old the diffusion current of the first wave had increased slightly and the diffusion current of the second wave had remained constant. The reduction potential of the first wave had risen to -0.08 v. and the reduction potential of the second wave had risen to -0.95 v. When the solution was forty-eight hours old the values for the half-wave potential and the diffusion current of both waves were approximately the same as when the solution was only five and one-half hours old. This showed that limiting values had probably been reached. The results are tabulated in Table 21.

The solutions from the two different ampoules behaved differently on being exposed to the air but it is interesting to note that the final curves obtained from both solutions are approximately the same, as can readily be seen from a comparsion of Table 20 and Table 21. The reason for the two samples passing through a different series of steps is probably due to a difference in solvent, which would lead to a difference of pH between the two solutions. It is also to be noted that the half-wave potential of the first curve in its final form is the same as the half-wave potential of and old solution of 1-hydroxy-2-methyl-4-naphthol amine hydrochloride which was made up from the solid.

Table 1. Effect of pH on the Half-Wave Potential of 2-Methyl-1,4-Naphthoquinone

Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
100	22222	5.0	-0.34	-0.42
200	22222	5.0	-0.34	-0.43
100	50000	5.0	-0.20	-0.42
200	50000	5.0	-0.33	-0.44
100	22222	7.0	-0.44	-0.57
200	22222	7.0	-0.44	-0.58
100	50000	7.0	-0.41	-0.57
200	50000	7.0	-0.40	-0.59

Table 2. Effect of pH on the Half-Wave Potential 1-Hydroxy-2-Methyl-4-Naphthol Amine HCl

Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
100	22222	4.6	-0.24	-0.34
200	22222	4.6	-0.22	-0.33
100	50000	4.6	-0.22	-0.36
200	50000	4.6	-0.23	-0.36
100	22222	7.0	-0.37	-0.50
200	22222	7.0	-0.35	-0.52
100	50000	7.0	-0.34	-0.50
200	50000	7.0	-0.34	-0.52

Table 3. Effect of Aging on a Solution of 2-Methyl-1,4-Naphthoquinone

Conc. (mg/l)	Shunt (ohms)	Age (hrs)	Reduction Potential (volts)	Half-Wave Potential (volts)	Diffusion Current (microamp)
200	22222	0	-0.40	-0.57	3.9
200	22222	1	-0.40	-0.57	3.7
200	22222	2	-0.40	-0.57	4.0
200	22222	4	-0.40	-0.57	3.8
200	22222	8	-0.40	-0.57	3.9
200	22222	24	-0.40	-0.58	3.7

Table 4. Effect of Aging on a Solution of 1-Hydroxy-2-Methyl-4-Naphthol Amine Hydrochloride

Conc. (mg/l)	Shunt (ohms)	Age (hrs)	Reduction Potential (volts)	Half-Wave Potential (volts)	Diffusion Current (microamp)
200	50000	0	-0.02	-0.09	1.65
200	50000	1	-0.03	-0.11	2.7
200	50000	2	-0.09	-0.11	2.6
200	50000	3	-0.23	-0.33	2.6
200	50000	4	-0.24	-0.35	2.6
200	50000	6	-0.24	-0.35	2.7
200	50000	7	-0.24	-0.35	2.6
200	50000	23	-0.24	-0.36	2.75

Table 5. Effect of Aging on a Solution of 2,3-Dimethyl-1.4-Naphthoquinone

Conc. (mg/l)	Shunt (ohms)	Age (hrs)	Reduction Potential (volts)	Half-Wave Potential (volts)	Diffusion Current (microamp)
200	22222	0	-0.48	-0.65	3.3
200	22222	2	-0.48	-0.65	3.3
200	22222	6.5	-0.48	-0.65	3.5
200	22222	31.5	-0.49	-0.65	3.5
200	22222	52	-0.48	-0.65	3.7

Table 6. Measurement of the Half-Wave Potential of the Dimer of 2-Methyl-1,4-Naphthoquinone

Conc. (mg/l)	Shunt (ohms)	First Wave		Second Wave	
		Reduction Potential (volts)	Half-Wave Potential (volts)	Reduction Potential (volts	Half-Wave Potential (volts)
100	22222	-0.99	-1.06	-1.55	-1.66
100	50000	-0.98	-1.06	-1.54	-1.68
200	22222	-0.98	-1.07	-1.57	-1.69
200	50000	-0.96	-1.07	-1.57	-1.71

Table 7. Effect of pH on the Half-Wave Potential of 2,3-Dimethyl-1,4-Naphthoquinone

Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
100	22222	5.0	-0.41	-0.48
200	22222	5.0	-0.40	-0.50
100	50000	5.0	-0.40	-0.49
200	50000	5.0	-0.40	-0.52
100	22222	7.0	-0.50	-0.64
200	22222	7.0	-0.50	-0.66
100	50000	7.0	-0.51	-0.66
200	50000	7.0	-0.44	-0.67

Table 20. Effect of Aging on a Solution of 1-Hydroxy-2-Methyl-4-Naphthol Amine Hydrochloride*
Ampoule No. 50271

Shunt (ohms)	Age (hrs)	First Wave		Second Wave	
		Half-Wave Potential (volts)	Diffusion Current (microamp)	Half-Wave Potential (volts)	Diffusion Current (microamp)
22222	0	-0.05	1.1	-0.81	10.8
22222	.5	-0.06	1.9	-0.83	10.8
22222	.75	-0.07	2.6	-0.84	10.8
22222	1.5	-0.08	3.2	-0.87	10.8
22222	3.0	-0.26	3.6	-1.11	10.8
22222	6.0	-0.29	3.3	-1.12	11.0
22222	9.0	-0.31	3.8	-1.12	11.0

*Conc. is 500 mg/l.

Table 21. Effect of Aging on a Solution of 1-Hydroxy-2-Methyl-4-Naphthol Amine Hydrochloride*
Ampoule No. 49771

Shunt (ohms)	Age (hrs)	First Wave		Second Wave	
		Half-Wave Potential (volts)	Diffusion Current (microamp)	Half-Wave Potential (volts)	Diffusion Current (microamp)
22222	0	-0.48	4.2	-0.75	5.0
22222	.25	-0.48	3.2	-0.76	6.4
22222	1.5	-0.51	1.2	-0.77	8.4
22222	4.5	-0.07	2.6	-0.86	9.2
22222	5.5	-0.17	3.4	-1.08	9.2
22222	48.0	-0.32	3.7	-1.11	10.4

*Conc. is 500 mg/l.

Shunt (ohms)	Time Irradiated	First Wave		Second Wave		Third Wave	
		Half-Wave Potential (volts)	Diffusion Current (microamps.)	Half-Wave Potential (volts)	Diffusion Current (microamps)	Half-Wave Potential (volts)	Diffusion Current (microamps)
22,222	0	-0.58	3.4	-	-	-	-
"	5 min.	-0.58	2.6	-	-	-	-
"	10 "	-0.54	1.4	0.86	2.5	-	-
"	15 "	-0.49	0.8	0.87	3.4	-	-
"	20 "	-0.25	0.4	0.87	3.6	-1.28	6.2
"	30 "	-	-	0.86	3.2	-1.25	9.0
4081	40 "	-	-	-	-	-1.18	19.5
"	1 hr.	-	-	-	-	-1.19	23.5
"	1.5 "	-	-	-	-	-1.17	23.5
"	2 "	-	-	-	-	-1.20	31.0
"	3 "	-	-	-	-	-1.20	38.0
"	4 "	-	-	-	-	-1.20	47.0

Table 8

Effect of Irradiation on 2-Methyl-1,4-Naphthoquinone

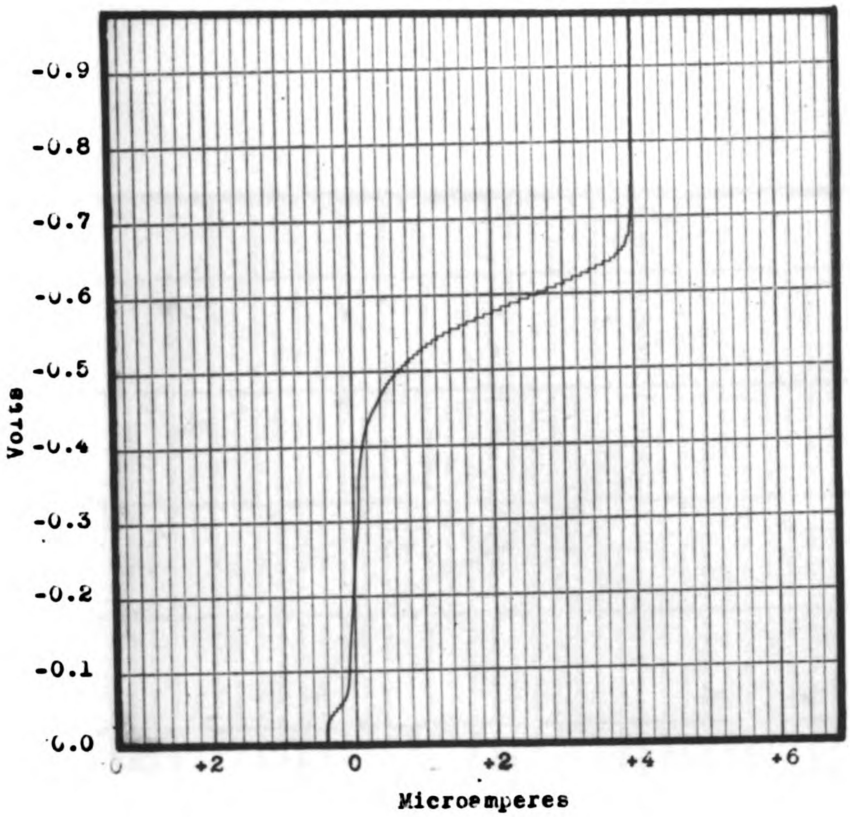


Fig. 1

2-Methyl-1,4-Naphthoquinone
200 mg. per liter
Shunt - 22,222 ohms

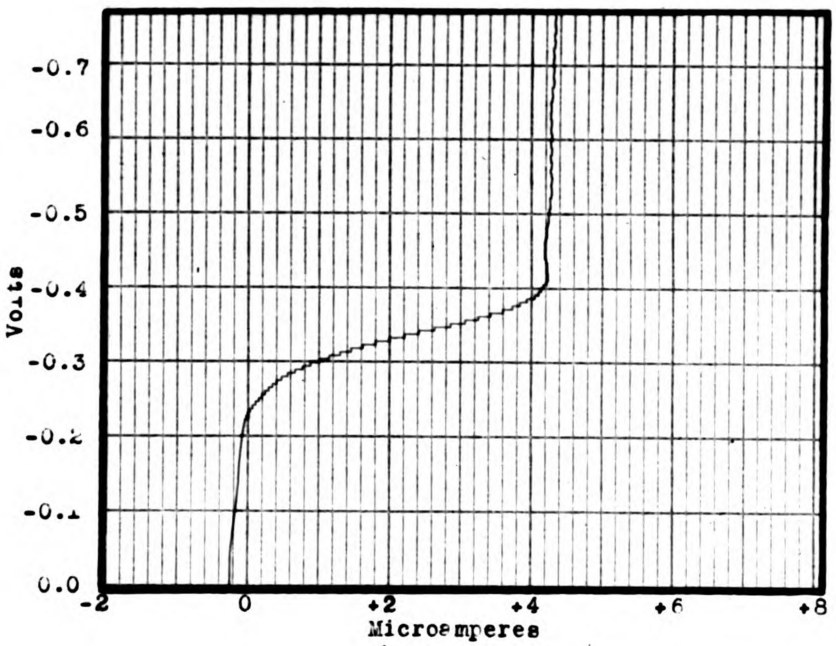


Fig. 2

1-Hydroxy-2-Methyl-4-Nephthol Amine Hydrochloride
200 mg. per liter
Shunt - 22,222 ohms

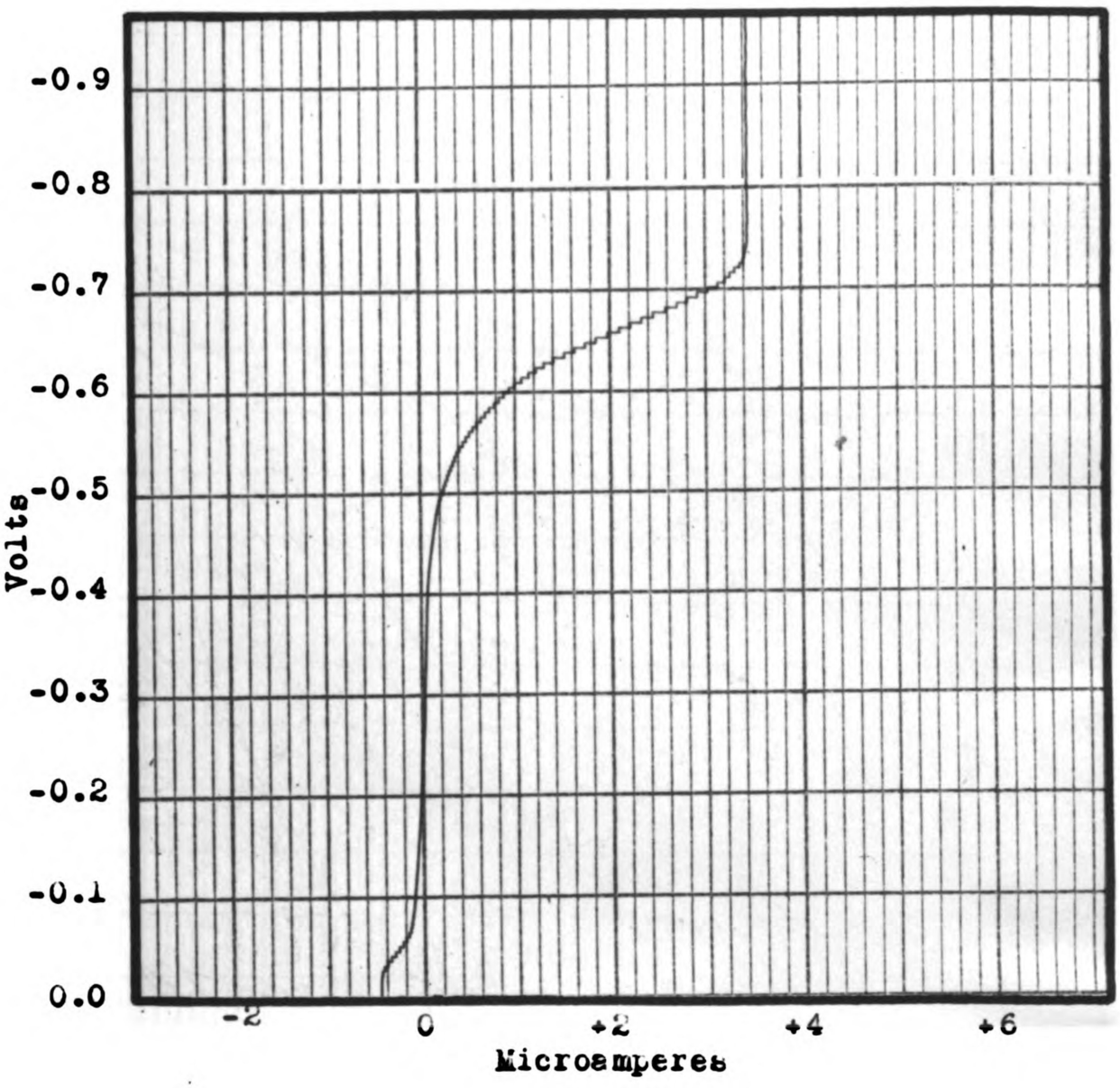


Fig. 3

2,3-Dimethyl-1,4-Naphthoquinone
200 mg. per liter
Shunt - 22,222 ohms

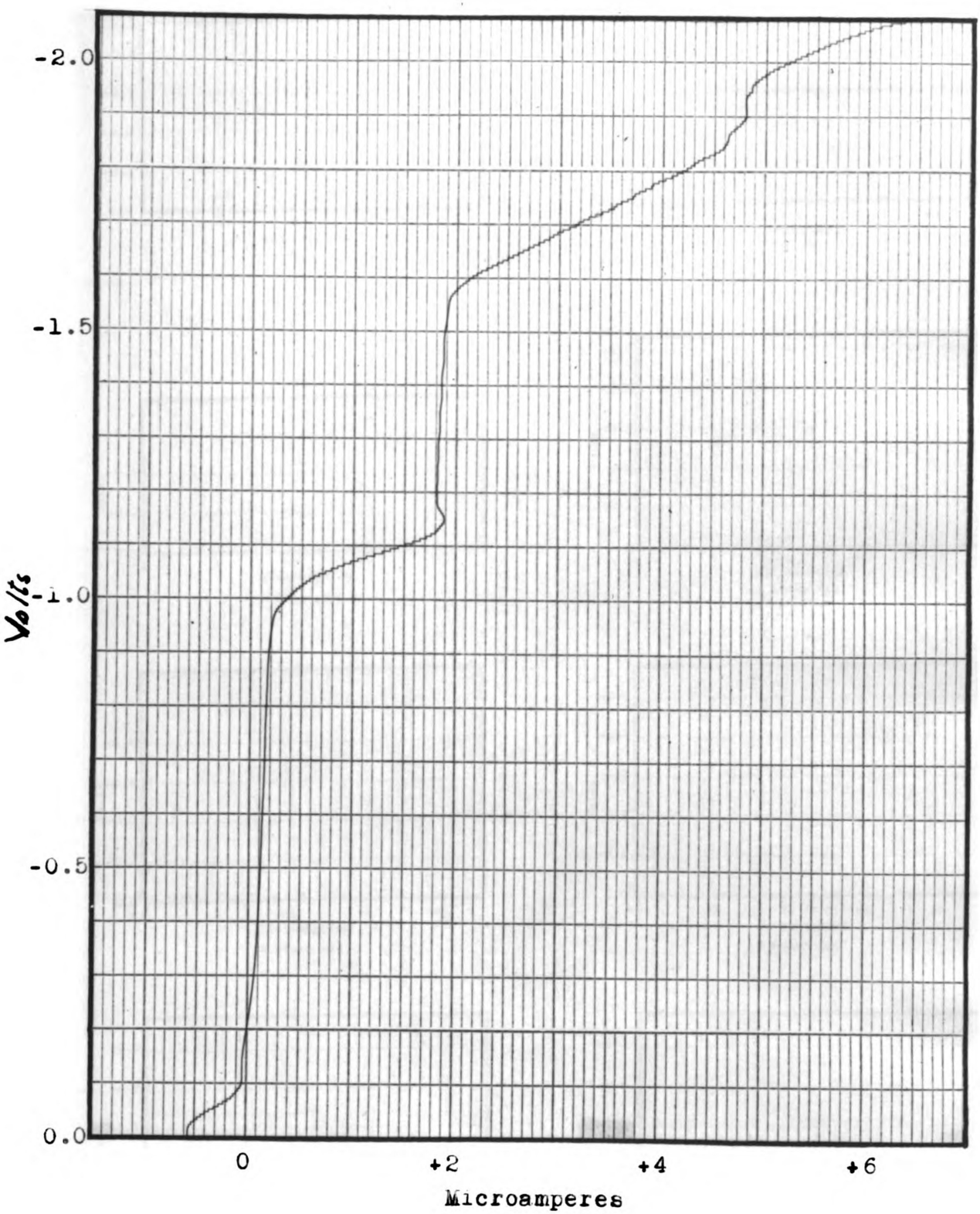
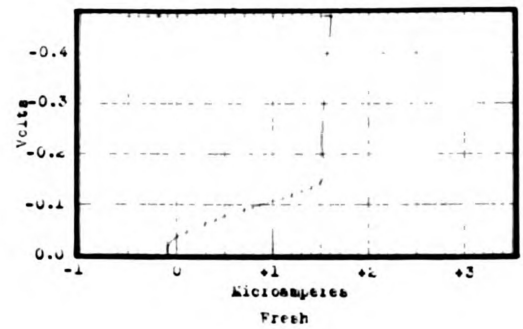
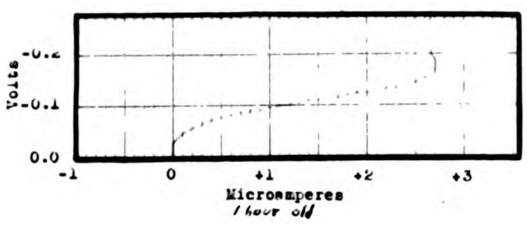


Fig. 4

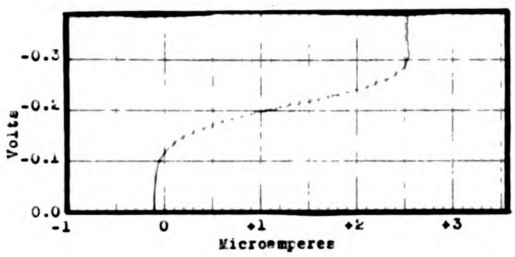
Dimer of 2-Methyl-1,4-Naphthoquinone
200 mg. per liter
Shunt - 50,000 ohms



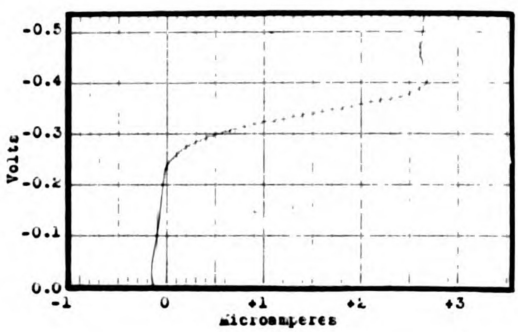
Fresh



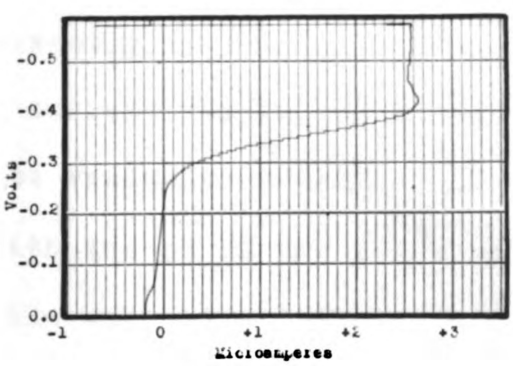
1 hour old



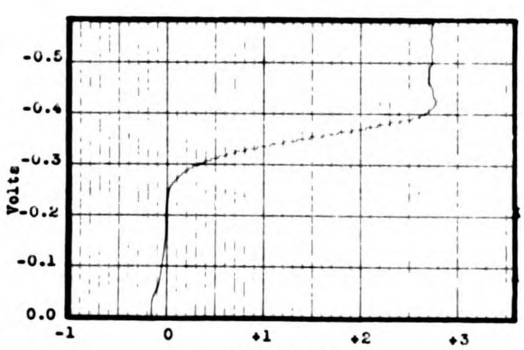
2 hours old



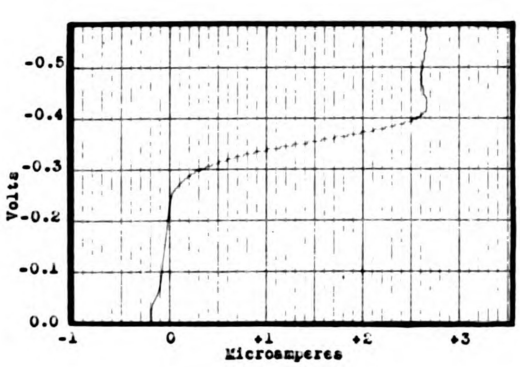
3 hours old



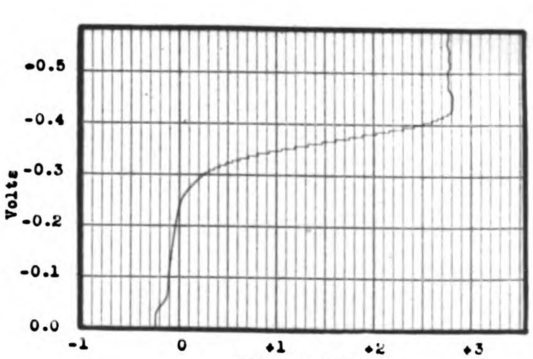
4 hours old



6 hours old



7 hours old



23 hours old

Fig. 5

1-Hydroxy-2-Methyl-4-Naphthol Amine Hydrochloride
200 mg. per liter
Shunt - 50,000 ohms

INVESTIGATION OF VITAMIN B COMPLEXES

Riboflavin, thiamin hydrochloride, nicotinic acid, and nicotinic amide were the complexes used. J.J. Lingane[2] did most of the early work in the application of the polarograph to the vitamin B complexes.

Table 9 shows some of the results obtained with riboflavin. Riboflavin was the easiest of the complexes to determine. It gave excellent cathodic waves in an unbuffered 0.1 N KCl solution. It also gave good waves in buffered solutions. The riboflavin was dissolved directly in the 0.1 N KCl. Riboflavin did not exhibit a current maxima and it was not necessary to add gelatin. The reduction potential of riboflavin was found to vary with the pH of the solution. Fig. 6 shows a good cathodic wave of riboflavin.

It was necessary to neutralize the nicotinic acid with sodium hydroxide before it would give a good cathodic wave in 0.1 N KCl. A good cathodic wave was obtained if the nicotinic acid was dissolved directly in 0.1 N $NaHCO_3$. The sodium bicarbonate neutralized the nicotinic acid and also acted as a buffer. Results are shown in Table 10. Nicotinic acid did exhibit a current maxima and so it was necessary to add gelatin as a suppressor. The curve obtained is actually the cathodic wave of sodium

nicotinate. The pH of the solution greatly affects the reduction potential of the nicotinic acid. A cathodic wave of nicotinic acid is shown in Fig. 8.

It was difficult to get a good cathodic wave of thiamin hydrochloride. Best results were obtained when the thiamin hydrochloride was dissolved directly in 0.1 N KCl. These results are given in Table 11. Fig. 7 shows a typical curve.

Nicotinic amide was easier to measure than nicotinic acid. The cathodic wave of nicotinic amide had a slightly higher half-wave potential than the wave of nicotinic acid. Fig. 9 shows a cathodic wave of nicotinic amide. This wave was obtained by dissolving the nicotinic amide directly in 0.1 N KCl. Results are given in Table 12.

Riboflavin and neutralized nicotinic acid may be simultaneously determined from the same solution. The reduction potentials of the two substances are far enough apart so that the individual waves do not interfere with each other. Only fair results, as shown by Fig. 10, were obtained. Table 13 gives the results of the determination. Table 14 summarizes results from a simultaneous determination of riboflavin and thiamin hydrochloride. Riboflavin and nicotinic amide can also be determined together. Results are given in Table 17.

A simultaneous determination of riboflavin, thiamin

hydrochloride, and nicotinic acid was made from the same solution. Fair results were obtained, as can be seen from Fig. 12 and Table 15. The results from a simultaneous determination of riboflavin, thiamin hydrochloride, and nicotinic amide were not very satisfactory; these results are given in Table 16. Great care had to be taken in the neutralization of the nicotinic acid. Either an insufficient amount or an excess of sodium hydroxide would ruin the curve. The whole determination was very sensitive to the pH of the solution. The best results were obtained at a pH of 6.8.

Table 9. Measurement of the Half-Wave Potential of Riboflavin

Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
200	50000	7.0	-0.54	-0.64
100	400000	7.0	-0.48	-0.65
87.5	200000	7.0	-0.51	-0.62
50	400000	7.0	-0.08	-0.62

Table 10. Measurement of the Half-Wave Potential of Nicotinic Acid

Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
200	200000	8.9	-1.68	-1.77
200	50000	8.9	-1.70	-1.77
100	400000	8.9	-1.66	-1.77

Table 11. Measurement of the Half-Wave Potential of Thiamin Hydrochloride

Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
200	50000	7.0	-1.20	-1.34
100	50000	7.0	-1.23	-1.35

Table 12. Measurement of the Half-Wave Potential of Nicotinic Amide

Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (Volts)
200	4081	7.0	-1.69	-1.81
50	200000	7.0	-1.67	-1.81

Table 13. Measurement of the Half-Wave Potential of a Mixture of Riboflavin and Nicotinic Acid

Substance	Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
$Riboflavin_1$	100	400000	8.9	-0.54	-0.62
$Riboflavin_2$	200	400000	8.9	-0.47	-0.59
$Nicotinic\ Acid_2$	100	400000	8.9	-1.66	-1.77
$Nicotinic_1$	200	400000	8.9	-1.64	-1.77

1 = Same Solution 2= Same Solution

Table 14. Measurement of the Half-Wave Potential of a Mixture of Riboflavin & Thiamin Hydrochloride

Substance	Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
Riboflavin	100	50000	7.0	-0.35	-0.47
Riboflavin	50	200000	7.0	-0.34	-0.45
Thiamin HCl	100	50000	7.0	-1.26	-1.34
Thiamin HCl	50	200000	7.0	-1.22	-1.36

Table 15. Measurement of the Half-Wave Potentials of a Mixture of Riboflavin and Nicotinic Acid.&Thiamin

Substance	Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (Volts)
Riboflavin	50	200000	6.8	-0.36	-0.45
Thiamin HCl	100	200000	6.8	-1.18	-1.35
Nicotinic Acid	100	200000	6.8	-1.60	-1.74

Table 16. Measurement of the Half-Wave Potentials of a Mixture of Riboflavin, Thiamin HCl & Nicotinic Amide

Substance	Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
Riboflavin	100	50000	7.0	-0.36	-0.46
Thiamin HCl	100	50000	7.0	-1.24	-1.38
Nicotinic Amide	100	50000	7.0	-1.61	-1.81

Table 17. Measurement of the Half-Wave Potentials of a Mixture of Riboflavin and Nicotinic Amide

Substance	Conc. (mg/l)	Shunt (ohms)	pH	Reduction Potential (volts)	Half-Wave Potential (volts)
Riboflavin	87.5	200000	7.0	-0.51	-0.62
Nicotinic Amide	50.	200000	7.0	-1.67	-1.81

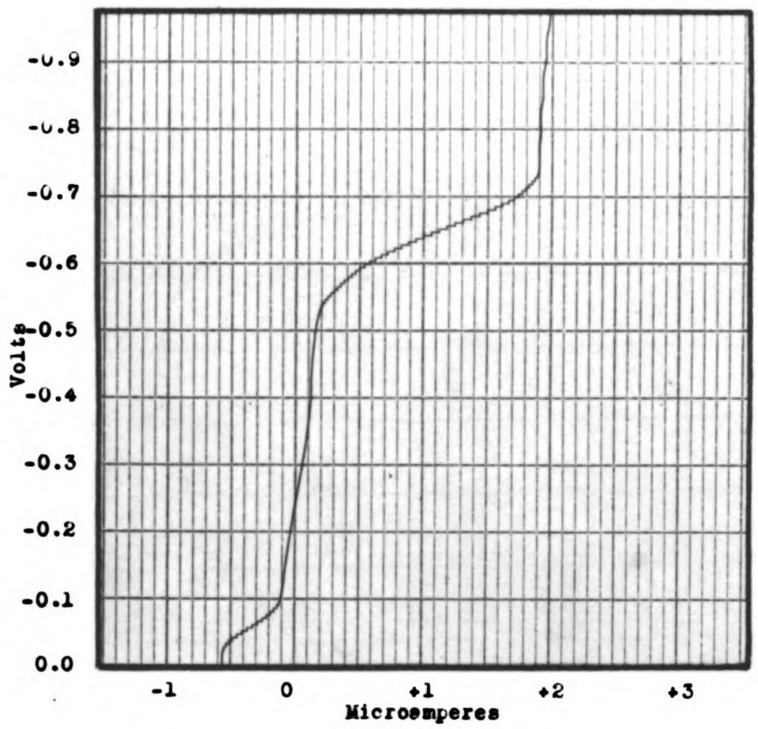


Fig. 6

Riboflavin
200 mg. per liter
Shunt - 50,000 ohms

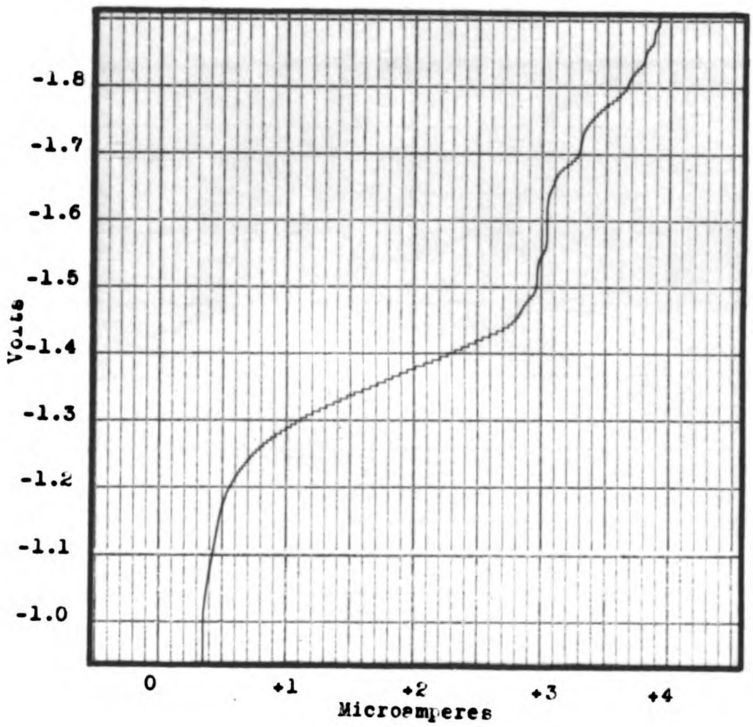


Fig. 7

Thiamin Hydrochloride
200 mg. per liter
Shunt - 50,000 ohms

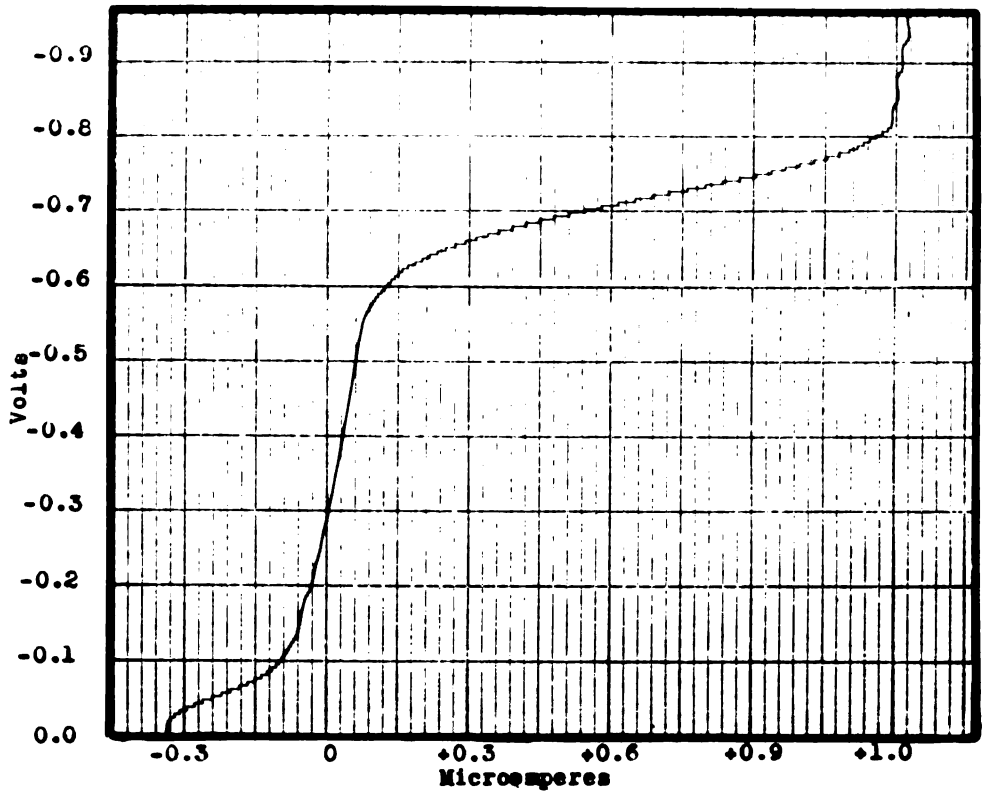


Fig. 8

Nicotinic Acid
400 mg. per liter
Shunt - 400,000 ohms

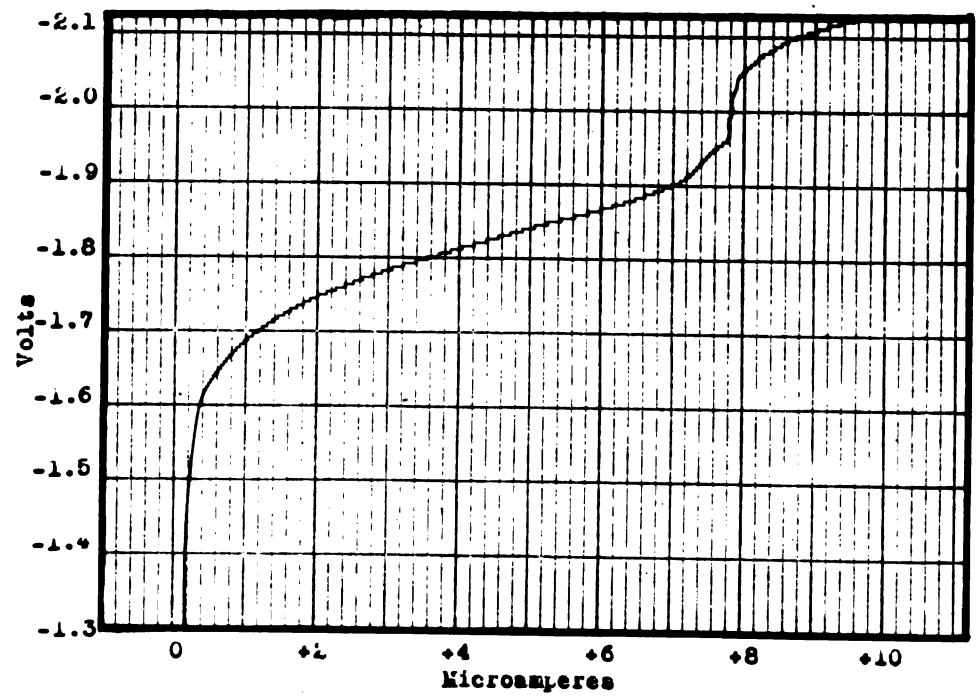


Fig. 9

Nicotinic Amide
200 mg. per liter
Shunt - 22,222 ohms

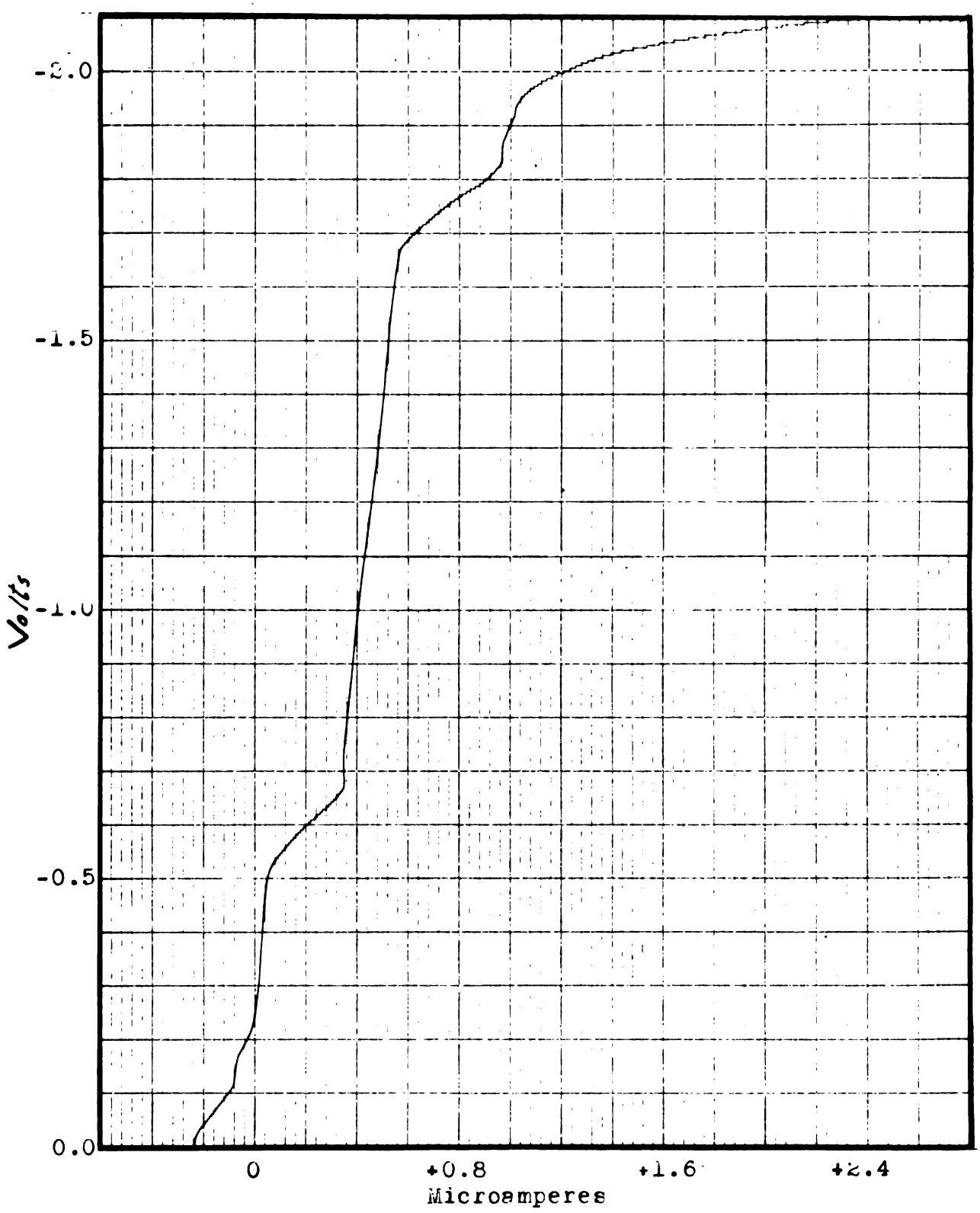


Fig. 10

Riboflavin 100 mg. per liter
Nicotinic Acid 200 mg. per liter
Shunt - 200,000 ohms

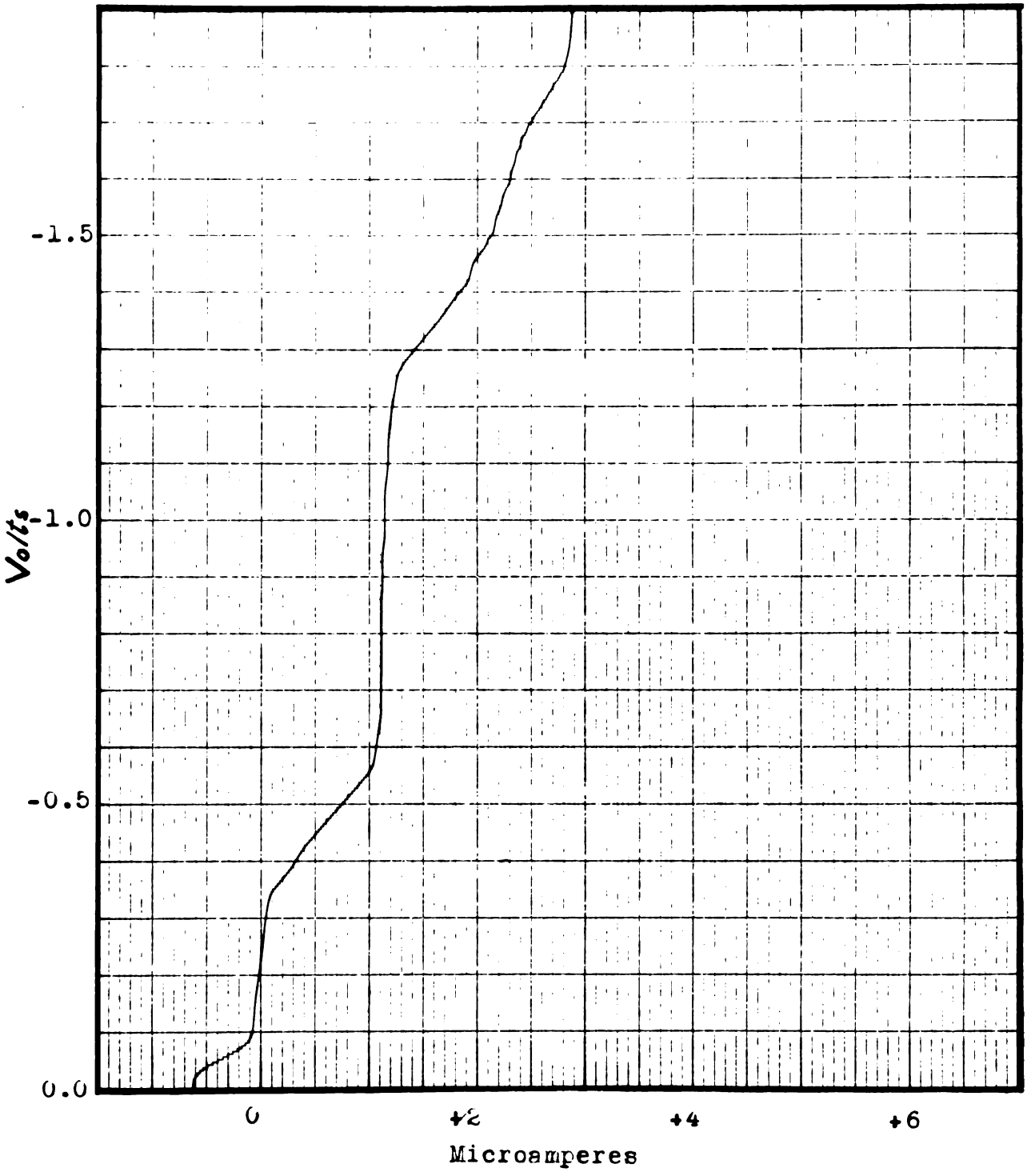


Fig. 11

Riboflavin 100 mg. per liter
Thiamin Hydrochloride 100 mg. per liter
Shunt - 50,000 ohms

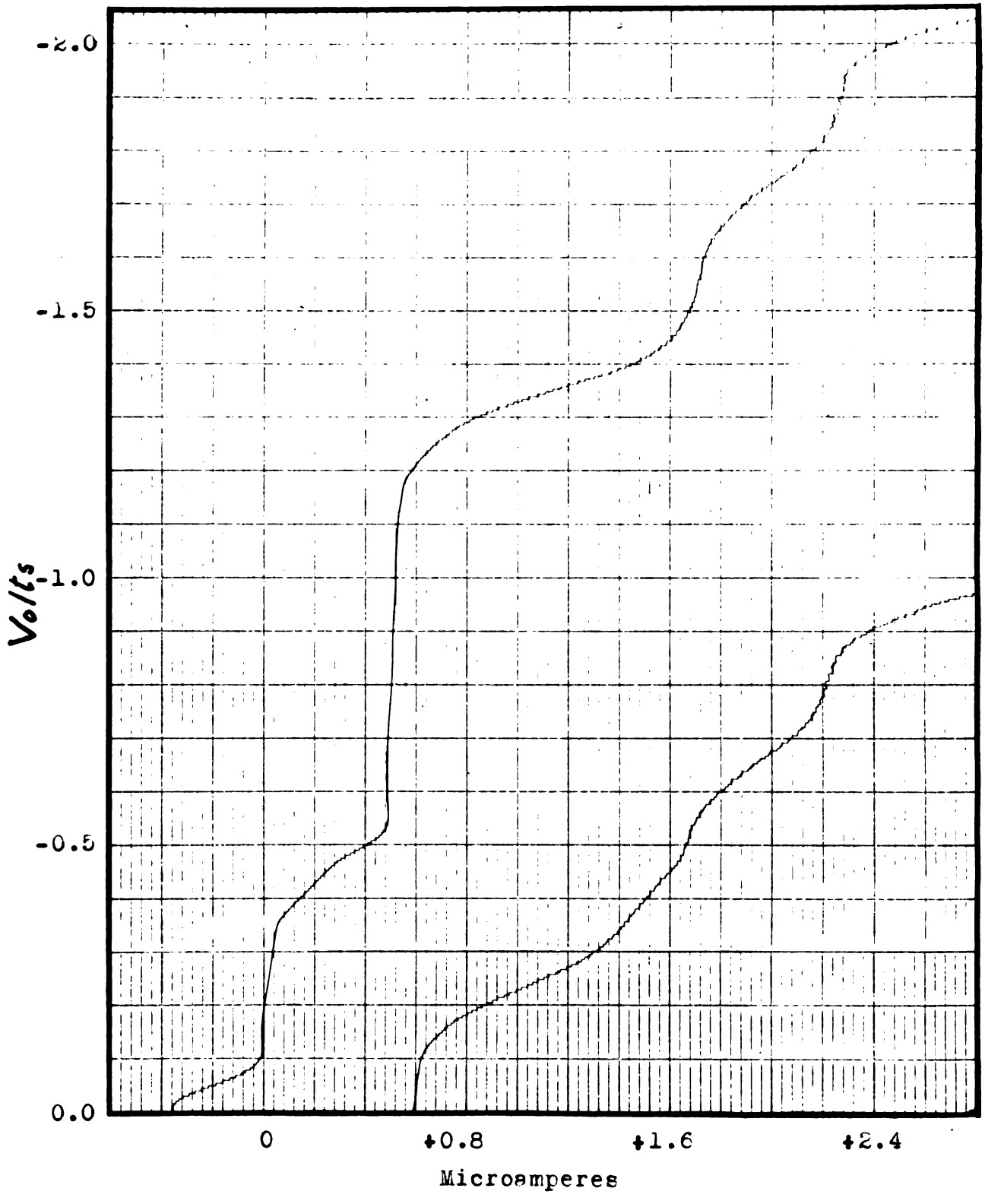


Fig. 12

Riboflavin 50 mg. per liter
Nicotinic Acid 100 mg. per liter
Thiamin Hydrochloride 100 mg. per liter
Shunt - 200,000 ohms

INVESTIGATION OF ASCORBIC ACID

Ascorbic acid did not give a cathodic wave. It is a strong reducing agent and, as a result, gives a good anodic wave. In the determination of ascorbic acid the dropping mercury electrode is the anode and the pool of mercury in the bottom of the cell is the cathode.

Ascorbic acid is soluble in water and an aqueous solution of ascorbic acid is stable. However, ascorbic acid is readily oxidized by oxygen and so oxygen must be removed from the water before the ascorbic acid is dissolved in it, and the solution must be kept in an air tight container. The oxygen was removed from the water by suction.

Sodium ascorbate is also water soluble and it gave an anodic wave almost identical with that obtained from ascorbic acid. Sodium ascorbate is, also, easily oxidized by oxygen, so the same precautions were used in the case of sodium ascorbate as were used in the preparation of a solution of ascorbic acid.

Both ascorbic acid and sodium ascorbate gave best results in a buffered solution. A buffer of pH of 7.0 and a buffer of pH 4.6 were used. The buffer of a pH of 7.0 was composed of 0.1 M NaOH and 0.1 M KH_2PO_4. The buffer of a pH of 4.6 was made up of equal parts of 1 M

$NaC_2H_3O_2$ and 1 M $HC_2H_3O_2$. The aqueous solutions of the ascorbic acid and sodium ascorbate were diluted 1 to 1 with the buffer, gelatin was added, and the solution was then electrolyzed. Excellent curves were obtained of both compounds as can be seen from Fig. 13 and Fig. 14. The buffered solution of a pH of 4.6 gave the more satisfactory results. Sodium ascorbate gave good anodic waves in both buffers. The waves of ascorbic acid in a buffer of pH of 7.0 had a poorly defined limiting current. The results obtained with these two compounds are tabulated in Table 18 and Table 19.

Table 18. Effect of pH on the Half-Wave Potential of Ascorbic Acid

Conc. (mg/l)	Shunt (ohms)	pH	Oxidation Potential (volts)	Half-Wave Potential (volts)
100	22222	4.6	0.02	0.09
100	22222	7.0	0.02	0.08
100	50000	4.6	0.02	0.09
100	50000	7.0	0.02	0.08
200	22222	4.6	0.02	0.09
200	22222	7.0	0.02	0.09

Table 19. Effect of pH on the Half-Wave Potential of Sodium Ascorbate

Conc. (mg/l)	Shunt (ohms)	pH	Oxidation Potential (volts)	Half-Wave Potential (volts)
100	22222	4.6	0.02	0.08
100	22222	7.0	0.02	0.07
100	50000	4.6	0.02	0.09
100	50000	7.0	0.02	0.08
200	22222	4.6	0.02	0.08
200	22222	7.0	0.02	0.08

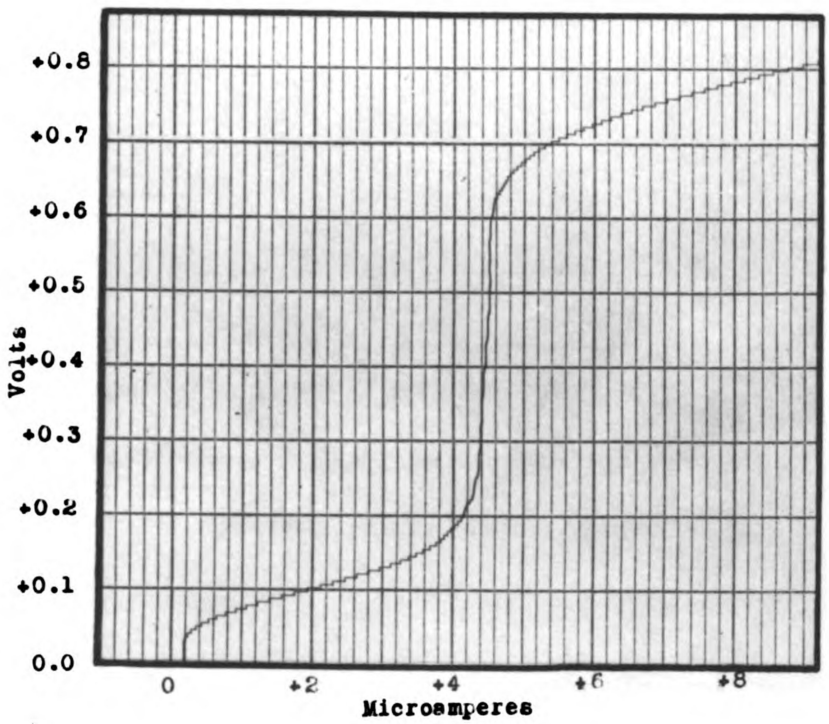


Fig. 13

Ascorbic Acid
200 mg. per liter
Shunt - 22,222 ohms

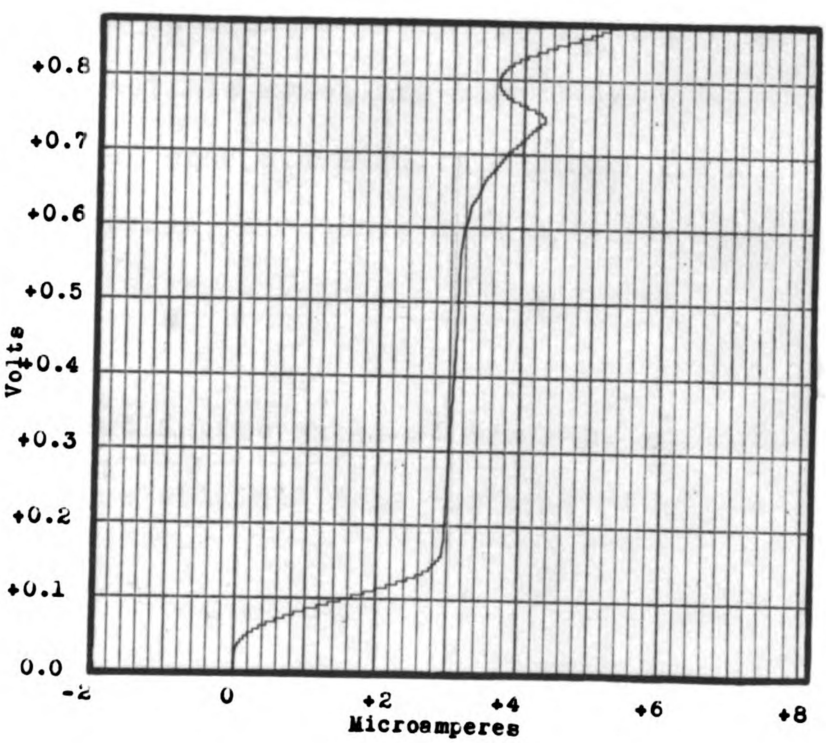


Fig. 14

Sodium Ascorbate
200 mg. per liter
Shunt - 22,222 ohms

SUMMARY

2-Methyl-1,4-naphthoquinone forms excellent cathodic waves in an unbuffered 0.1 N KCl solution. 2,3-Dimethyl-1,4-naphthoquinone, also forms excellent cathodic waves in an unbuffered 0.1 N KCl solution. 1-Hydroxy-2-methyl-4-naphthol amine hydrochloride forms good cathodic waves in a buffered solution. Solutions of 2-methyl-1,4-naphthoquinone and 2,3-dimethyl-1,4-naphthoquinone are found to be stable upon prolonged exposure to the air. A solution of 1-hydroxy-2-methyl-4-naphthol amine hydrochloride was found to be rapidly decomposed upon being exposed to the air.

The best cathodic waves of riboflavin, thiamin hydrochloride, nicotinic acid, and nicotinic amide were obtained in an unbuffered 0.1 N KCl solution. Riboflavin, thiamin hydrochloride, nicotinic acid were determined simultaneously from the same solution. Riboflavin was the easiest to determine.

Excellent anodic waves of ascorbic acid, and sodium ascorbate were obtained from buffered solutions of these compounds.

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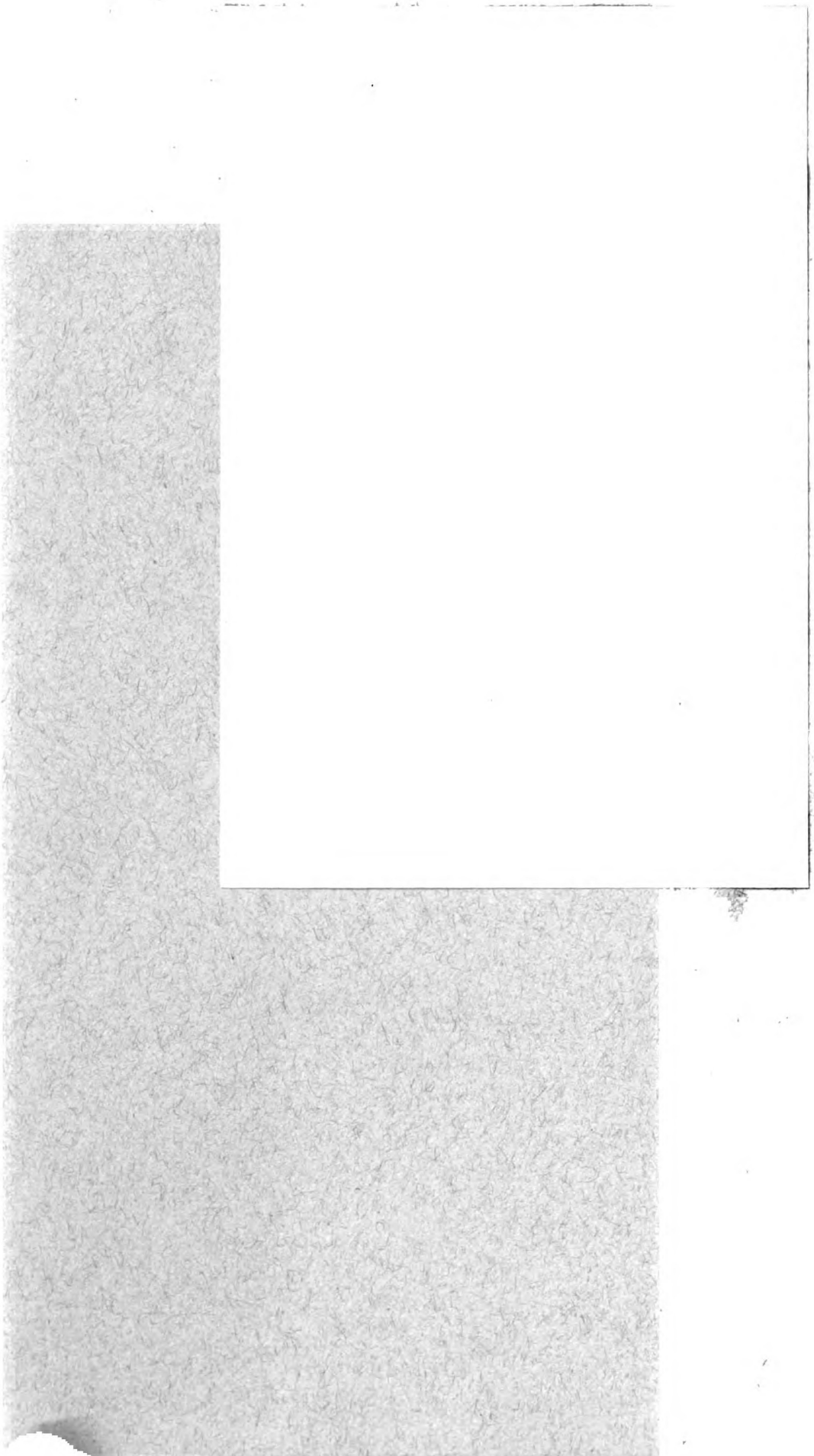

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Abraham

Half-wave potentials of vitamins K, and some vitamin B complexes.