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63-2-4

Annual Progress Report for the Period February 1962 to January 1963

Principal Investigator: Walter Stricks

Institution: Department of Chemistry
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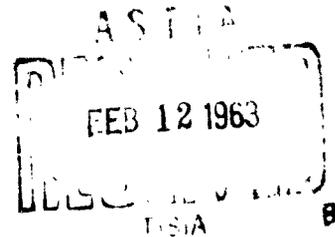
Subject: Amperometric Titrations and Polarographic Studies of
Antiradiation Drugs

Contract Number: DA-49-193-MD-2146 P.R. No. 241

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Abstract

1. Preparing Institution: Department of Chemistry, Marquette University
2. Title of Report: Amperometric Titrations and Polarographic Studies of Antiradiation Drugs
3. Principal Investigator: Walter Stricks
4. Number of pages, illustrations and date:
74 Pages
20 Illustrations
5 February, 1963
5. Contract Number: DA-49-193- MD-2146 P.R. No. 241
6. Supported by: U.S. Army Medical Research and Development Command,
Department of the Army, Washington 25, D.C.

The work deals with amperometric and polarographic studies of antiradiation drugs and consists of three parts. Part I deals with amperometric mercurimetric and argentimetric titrations of mercaptoethylguanidine, mercaptopropylguanidine and mercaptoethylamine at the rotated platinum electrode as indicator electrode. Procedures are given which allow the accurate titration of these three compounds with mercuric chloride as titrating agent. Argentimetric titrations are considerably more complex with respect to the reactions involved and to the surface conditions of the electrode. Silver can form various complexes with mercaptans and the endpoint depends largely on the experimental conditions. From an analytical point of view argentimetric titrations of mercaptans are therefore less satisfactory than mercurimetric determinations.

Part II deals with the polarography of 2-aminoethane thiosulfuric acid, RSSO_3H and 2-aminoethaneselenosulfuric acid, RSeSO_3H . RSSO_3H , which is reduced at a considerably more negative potential than RSeSO_3H , gives a single wave while RSeSO_3H is reduced in two steps, all waves being irreversible at the dropping mercury electrode. At alkaline and neutral pH the heights of the waves of RSSO_3H and RSeSO_3H are independent of pH. In acid solution the height of the RSSO_3H wave and the second step of RSeSO_3H increase with decreasing pH. This is explained by the reduction in acid medium of sulfite which is one of the products of the electrochemical reduction of RSSO_3H and RSeSO_3H at the dropping mercury electrode. The behavior of RSeSO_3H in the presence of surface active agents at the rotated dropping mercury electrode is different from that at the conventional electrode. An explanation for this behavior is given.

Part III deals with catalytic effects which are observed in ammoniacal cobalt solutions in the presence of minute quantities of sulfhydryl or disulfide containing compounds. When electrolyzed at the dropping mercury electrode, many of these compounds give, under proper experimental conditions, high current peaks at potentials of -1.4 to -1.9 volts vs. the saturated calomel

electrode. Because of their abnormal height, these peaks are attributed to catalytic phenomena. Some 18 sulfhydryl and disulfide compounds were investigated in order to find which sulfur compounds gave a catalytic effect, and at which concentrations, since not all mercaptans investigated gave this effect. The ability of these compounds to give the effect was found to be greatly affected by the kind and number of functional groups in the compounds. Also, the effect of the buffer composition was studied. These catalytic effects can be helpful for a study of the interaction of sulfhydryl compounds with serum proteins before and after irradiation. It was with this aim in mind that these studies were initiated.

Part I

Mercurimetric and Argentimetric Titrations of Disulfides
and Sulfhydryl Compounds

Mercurimetric and Argentimetric Titrations of Disulfides
and Sulfhydryl Compounds

In previous work¹ it was shown that the difference in rate of the hydrolytic fission of oxidized glutathione and cystine can be made the basis of an amperometric titration of oxidized glutathione in presence of cystine. Conditions have been established under which the hydrolytic fission of oxidized glutathione according to the over-all equation,



can be made to run to completion by removing the GS^- with mercury which reacts fast and stoichiometrically with GS^- .

In the present report the alkaline fission of diguanidinodipropyl-disulfide ($\text{R}_{\text{II}}\text{SSR}_{\text{II}}$) and dihydroxydiethyl-disulfide ($\text{R}_{\text{IV}}\text{SSR}_{\text{IV}}$) in the presence of mercury has been investigated. In connection with these investigations it was necessary to explore the feasibility of mercurimetric amperometric titrations of guanidinopropyl mercaptan ($\text{R}_{\text{II}}\text{SH}$), guanidino ethyl mercaptan ($\text{R}_{\text{I}}\text{SH}$) and aminoethyl mercaptan ($\text{R}_{\text{III}}\text{SH}$). The amperometric mercurimetric titration of mercaptoethanol ($\text{R}_{\text{IV}}\text{SH}$) was studied by Kolthoff and al.² Since we also intended to study the hydrolytic fission of disulfides in the presence of other metals such as silver we extended our experiments also to amperometric titrations with silver nitrate in various media.

Materials. Aminopropylisothiuronium bromide hydrobromide was obtained from the Oak Ridge National laboratories. Aminoethylisothiuronium bromide hydrobromide and mercaptoethylamine hydrochloride were obtained from the Walter Reed Army Research Center. 2-mercaptoethanol was an Eastman White Label product. The solutions of dipropylguanidino disulfide ($\text{R}_{\text{II}}\text{SSR}_{\text{II}}$),

(1) W. Stricks and I. M. Kolthoff; Anal. Chem. 25, 1050 (1953).

(2) I. M. Kolthoff and J. Eisenstädter; Anal. Chim. Acta, 24, 280 (1961).

diethylaminodisulfide ($R_{III}SSR_{III}$) and diethylhydroxydisulfide ($R_{IV}SSR_{IV}$) were $10^{-2}M$. They were prepared by air oxidation of the corresponding isothiuronium salts or sulfhydryl compounds as described in previous reports. All other reagents were commercial C. P. products.

Methods. Current voltage curves were obtained automatically with a Sargent model XXI polarograph and manually with the apparatus and circuit described by Lingane and Kolthoff³. The pH of all solutions was measured with a Beckman Zeromatic pH meter with a type 41260 glass electrode which can be used for the entire pH range. Ten ml. and 0.5 ml. semimicroburets with 0.01-ml. divisions were used for the titrations.

The platinum wire electrode was rotated at a speed of 600 r.p.m. A silver coated platinum electrode was used for most of the experiments. A new platinum electrode was cleaned with warm concentrated nitric acid, rinsed with distilled water and electroplated in a $10^{-3}M$ silver nitrate solution which was 0.1M in ammonia and 0.1M in ammonium chloride at -0.3 volt vs. a saturated calomel electrode (S.C.E.) for 1 hour. When not in use the platinum electrode was kept in distilled water.

The titration vessel was a 100 ml. beaker into which was placed an appropriate volume of buffer solution. The beaker was fitted with a rubber stopper with holes for the nitrogen inlet tube, electrode, buret, and salt bridge. The solution was made air free by passing 99.996% pure nitrogen (Linde) through whereupon the residual current was measured at the proper potential. A given volume of mercaptan or disulfide solution was now added and the solution titrated with mercuric chloride or silver nitrate. The initial volume of the titration mixture was 40 ml. in most experiments. All current values were corrected for this volume. The end point of a titration was determined in the usual way by the interaction of excess reagent line with the line representing the residual current.

(3) J. J. Lingane and I. M. Kolthoff, J. Am. Chem. Soc. 61, 825 (1939).

Results and Discussion

(1) Mercurimetric Titrations of R_{II}SSR_{II} and R_{IV}SSR_{IV}.

Titration of R_{II}SSR_{II} were performed with mixtures which were 0.2M in sodium hydroxide and 0.5M in potassium chloride at -0.3 volt vs. S.C.E. R_{IV}SSR_{IV} was titrated at two different sodium hydroxide concentrations (0.02M and 0.2M NaOH) all solutions being 0.05M in KCl. With both R_{II}SSR_{II} and R_{IV}SSR_{IV} a current of about 3 μ A was measured after the addition of the first increment of mercuric chloride (10^{-3} M) indicating that mercury did not react with the titration mixture. Since from previous experiments it is known that the reaction between mercaptan and mercury,



is instantaneous the hydrolysis of the disulfides (equation 1) must be slow or does not take place at all. From the data of Table I it is seen that the current corresponding to the reduction of mercuric chloride decreases when the solution is allowed to stand. Apparently mercury is used up slowly, indicating that the hydrolysis of the two disulfides investigated is slow and, in contrast with oxidized glutathione, can not be made the basis for a titration of these disulfides.

(2) Mercurimetric Titrations of Mercaptans.

In mercurimetric titrations the electrode becomes virtually an amalgam electrode in the course of the titration, since mercury is deposited at the electrode. This is evidenced by the appearance of an anodic current before the end point, the mercaptan reacting anodically with the mercury. The anodic current does not decrease linearly with the volume of mercuric chloride added, and therefore the reaction line is curved. Similar observations have been made in titrations of mercaptans at the dropping mercury electrode and a clear cut explanation for this peculiar behavior cannot be given at the present time. In all mercurimetric titrations the excess reagent line was

Table I

Amperometric mercurimetric titration at -0.3 volt vs. S.C.E. of 4 ml. of 10^{-3} M $R_{IV}SSR_{IV}$ in 40 ml. solution of various NaOH concentrations. KCl concentration was 0.05M in each case.

| ml 10^{-3} M $HgCl_2$ added | <u>0.02M NaOH</u> | |
|-------------------------------|-------------------|--|
| | current, μa | Time (minutes) elapsed between addition of $HgCl_2$ and measuring of current |
| 0.00 | 0.05 | 10 |
| | 0.05 | 15 |
| 0.50 | 3.22 | 2 |
| | 2.65 | 7 |
| 3.00 | 16.70 | 3 |
| | 15.67 | 10 |
| | <u>0.2M NaOH</u> | |
| 0.00 | 0.05 | 0 |
| 1.00 | 5.25 | 1 |
| | 4.99 | 5 |
| 3.00 | 15.88 | 2 |
| | 14.32 | 8 |

straight and well defined.

A few mercaptans were also titrated with ethylmercury chloride at the rotated dropping mercury electrode (R.D.M.E.) as indicator electrode.⁴ In all instances the agreement between the results obtained at the R.D.M.E. and those at the platinum electrode was within 1%. From this we conclude that the results obtained with the platinum electrode are reliable.

Figure 1 gives titration curves for mercaptoethyl guanidine ($R_{I}SH$), mercaptopropylguanidine ($R_{II}SH$) and mercaptoethylamine ($R_{III}SH$). It is seen that the curves are similar in shape although the slope of the excess reagent line varies to some extent in spite of the fact that a synchronous motor was used for the rotation of the platinum electrode.

Tables II and III give the results obtained in mercurimetric titrations of $R_{I}SH$ and $R_{II}SH$. It is seen that $R_{I}SH$ and $R_{II}SH$ can be titrated with an average error of 4.8 and 0.3% respectively. Since the results agree well with those obtained at the R.D.M.E. it is justified to assume that the products obtained are not 100% pure and that the "errors" listed in Table II and III really give the impurities of the products analyzed.

3. Argentimetric Titrations of Mercaptans.

Argentimetric titrations of mercaptans were the subjects of many investigations. Cysteine in ammonia and tris- (trihydroxyethylaminomethane) buffers give results which are 30 to 50% high.⁵ With thioglycolic acid the positive error is about one half of that found with cysteine.⁶ Sluyterman⁵ suggested that both the carboxylate and aminogroup in cysteine are responsible for the binding of silver by $RSAg$, while the smaller error in thioglycolic acid is due to the carboxylate group. In the absence of silver binding

(4) W. Stricks, S. K. Chakravarti, *Anal. Chem.* 33, 194 (1961)

(5) L. A. Ac. Sluyterman, *Biochem. et Biophys. Acta*, 25, 402 (1957).

(6) I. M. Kolthoff and J. Eisenstädter, *Anal. Chim. Acta*, 24, 83 (1961).

Figure 1. Amperometric titration of three mercaptans in 0.02M NaOH, 0.05M KCl with $10^{-3}M$ mercuric chloride at the rotated platinum wire electrode at -0.3 volt vs. S.C.E. (A) 1 ml. of $2 \times 10^{-3}M$ R_ISH, (B) 2 ml. of $2 \times 10^{-3}M$ R_{II}SH (C) 4 ml. of $2 \times 10^{-3}M$ R_{III}SH.

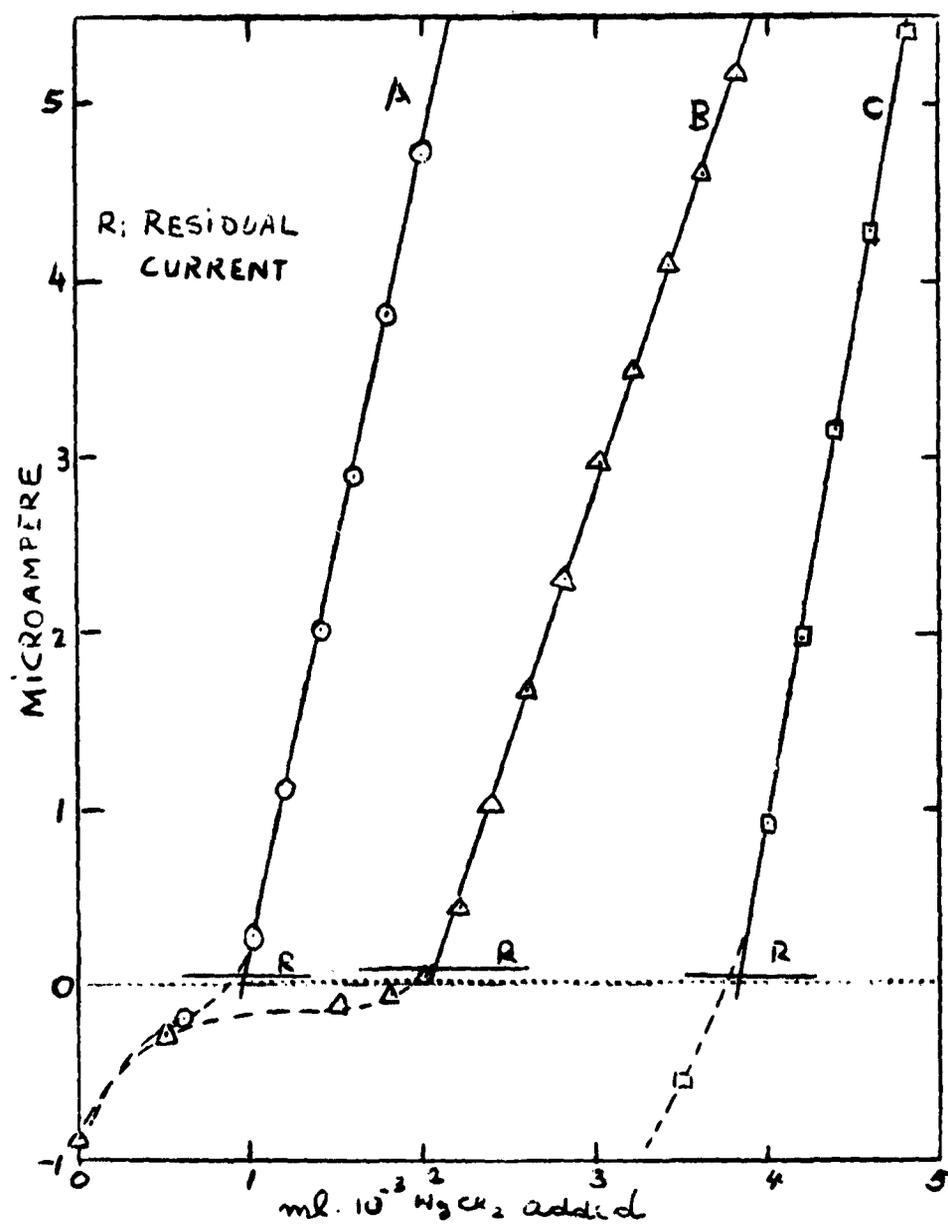


Table II

Amperometric mercurimetric titrations of R_1SH at the rotated platinum electrode in 0.02M NaOH, 0.05M KCl (pH 12.1) at -0.3 volt vs. S.C.E. if not stated otherwise. The titrant was $10^{-3}M$ $HgCl_2$.

| R_1SH , Mg | | Error % |
|--------------|-------|----------|
| Taken | Found | |
| 0.476 | 0.471 | - 1.0(a) |
| 0.476 | 0.451 | - 5.0(a) |
| 0.476 | 0.467 | - 2.0(a) |
| 0.714 | 0.690 | - 4.0 |
| 0.952 | 0.932 | - 3.8 |
| 0.714 | 0.690 | - 4.0 |
| 1.160 | 1.110 | - 5.0 |
| 1.160 | 1.102 | - 5.8 |
| 1.328 | 1.261 | - 5.6 |
| 0.476 | 0.452 | - 6.0 |
| 0.952 | 0.940 | - 3.0(b) |

(a) at -0.2 volt vs. S.C.E.

(b) buffer: 0.1M NH_3 , 0.1M NH_4NO_3

Table III

Amperometric Titration of $R_{II}SH$ and $R_{III}SH$ in 0.02M NaOH, 0.05M KCl
(pH 12.0) at -0.3 volt vs. S.C.E. with $10^{-3}M$ $HgCl_2$.

| $R_{II}SH, Mg$ | | Error % |
|----------------|-------|---------|
| Taken | Found | |
| 1.068 | 1.134 | + 6.0 |
| 1.068 | 1.068 | 0.0 |
| 1.068 | 1.020 | - 4.4 |
| 1.068 | 1.057 | - 1.0 |
| 1.068 | 1.048 | - 1.8 |
| 1.068 | 1.063 | - 0.5 |
| 0.534 | 0.529 | + 3.0 |

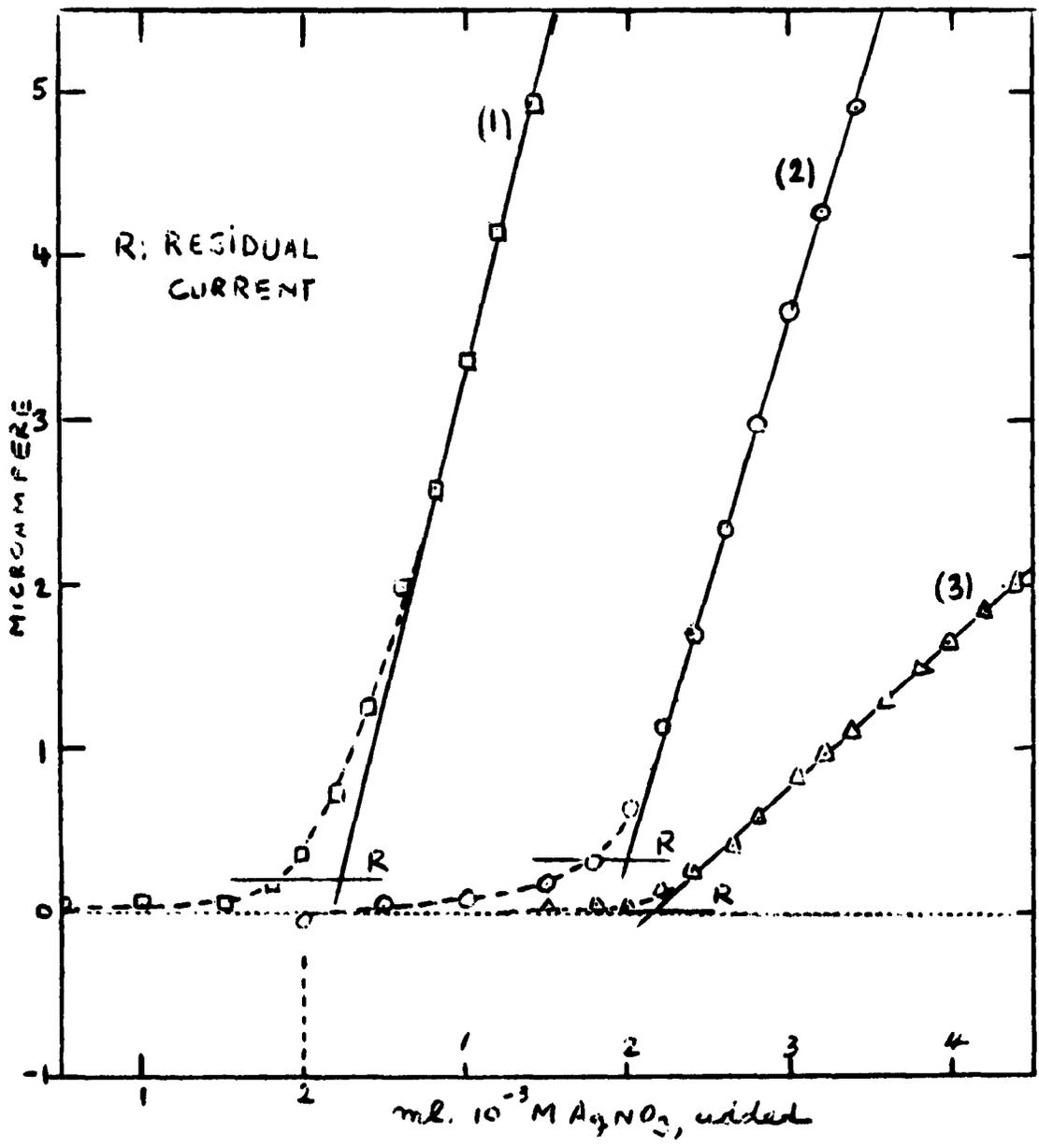
| $R_{III}SH, Mg$ | | Error % |
|-----------------|-------|---------|
| Taken | Found | |
| 0.624 | 0.601 | - 3.8 |
| 0.624 | 0.601 | - 3.8 |

groups (unsubstituted mercaptans, mercaptoethanol) the end point is found at a molar ratio of sulfhydryl to silver 1:1.⁶

The mercaptans studied so far contained amino and carboxyl groups. It appeared of interest to study mercaptans with an amino group only such as mercaptoethylamine and mercaptans with a group more basic than the amino groups, such as the guanidine group. Because of its larger pK value the guanidine group may be charged at a pH at which the amino group is present in the uncharged state. In solutions of such a pH (for instance, pH 11) the amino group can combine with silver while the guanidine group would be less likely to react with silver. It was of interest to study these two compounds and to show whether Sluyterman's postulate could be verified in these cases. We titrated mercaptopropylguanidine and mercaptoethylamine in various buffers in the presence of various complexing agents for silver in the assumption that such agents, if properly chosen, may preclude or diminish the complex formation of silver with the amino or guanidine group. Also the pH was varied from 12 to 7 in order to study the effect of the charge on the basic group.

Titration lines of titrations of R_2IISHS in ammoniacal medium are illustrated in Figure 2. A comparison of line 1 (clear platinum wire electrode) with line 2 (silver coated platinum wire) indicates that the result as well as the shape of the titration curve depend on the surface properties of the electrode. At the region around the end point the line obtained with the blank platinum wire is considerably more curved than the line obtained with the silver coated wire. The end point obtained with the coated electrode is therefore better defined than that given by the blank wire. It is concluded that silver coated wire is preferable to a blank wire. Ammoniacal media gave straight titration lines and no precipitate was observed in the course of the titration. Tris buffers gave well defined titration lines but the current after the end point was considerably smaller than that measured in

Figure 2. Amperometric argentimetric titration of 1 ml. of $2 \times 10^{-3}M$ RIIISH in (1) 0.1M NH_3 , 0.1M NH_4NO_3 at the clean platinum electrode, (2) 0.1M NH_3 , 0.1M NH_4NO_3 at the silver coated platinum electrode, (3) 0.1M Tris, 0.1M CH_3COONa at the silver coated platinum electrode. All titrations were performed at -0.3 volt vs. S.C.E.



the presence of ammonia buffers. This is seen from a comparison of line 2 with line 3 of Figure 2. Apparently the diffusion coefficient of the silver-Tris - complex is much smaller than that of the silver amino complex. Experiments were also performed with titration mixtures containing large amounts of chloride which, at relatively large concentrations, can also act as a complexing agent for silver. From Figure 3 it is seen that the titration lines are curved in the proximity of the end point, the extent of curvature increasing with increasing chloride concentration.

In the presence of surface active agents such as 0.005% gelatin or 0.5% Triton X-100 two excess reagent lines were generally obtained and a well defined end point was therefore not obtainable. An example of a titration line obtained in the presence of a surface active agent is given in Figure 4, which represents the titration of 1 ml. of $2 \times 10^{-3}M$ RIISH in 40 ml. $1M$ KCl, $0.1M$ CH_3COONa , 0.5% Triton X-100 with $10^{-2}M$ $AgNO_3$ as titrant. The end points obtained with the first straight line correspond to errors of +2 to -2% while the second straight line gave end points which were 25 to 16% in error. A blank titration with a $4M$ KCl solution ($0.1M$ CH_3COONa) gave a perfectly straight line and no precipitation upon addition of silver nitrate to the concentrated chloride solution was observed.

When RIISH was titrated in the presence of chloride the formation of a precipitate was observed before or after the end point, depending on the experimental conditions. In $4M$ KCl ($0.1M$ CH_3COONa) a precipitate was sometimes observed when the amount of silver added was 10 to 20% less or more than the stoichiometric amount. Apparently precipitate formation is dependent on the rate of addition of titrant. In the presence of ammonia ($4M$ KCl, $0.1M$ NH_3) a precipitate was not observed until 50% of excess silver was added. At smaller chloride concentrations (1 to $2M$) a precipitate was formed only after addition of a 100 to 140% excess of silver. The peculiar fact that no

Figure 3. Amperometric titrations of 1 ml. of $2 \times 10^{-3}M$ $R_{II}SH$ in (1) 1M KCl, 0.1M CH_3COONa , (2) 2M KCl, 0.1M CH_3COONa , (3) 4M KCl, 0.1M CH_3COONa with $10^{-2}M$ silver nitrate at the rotated silver coated platinum electrode at -0.2 volt vs. S.C.E.

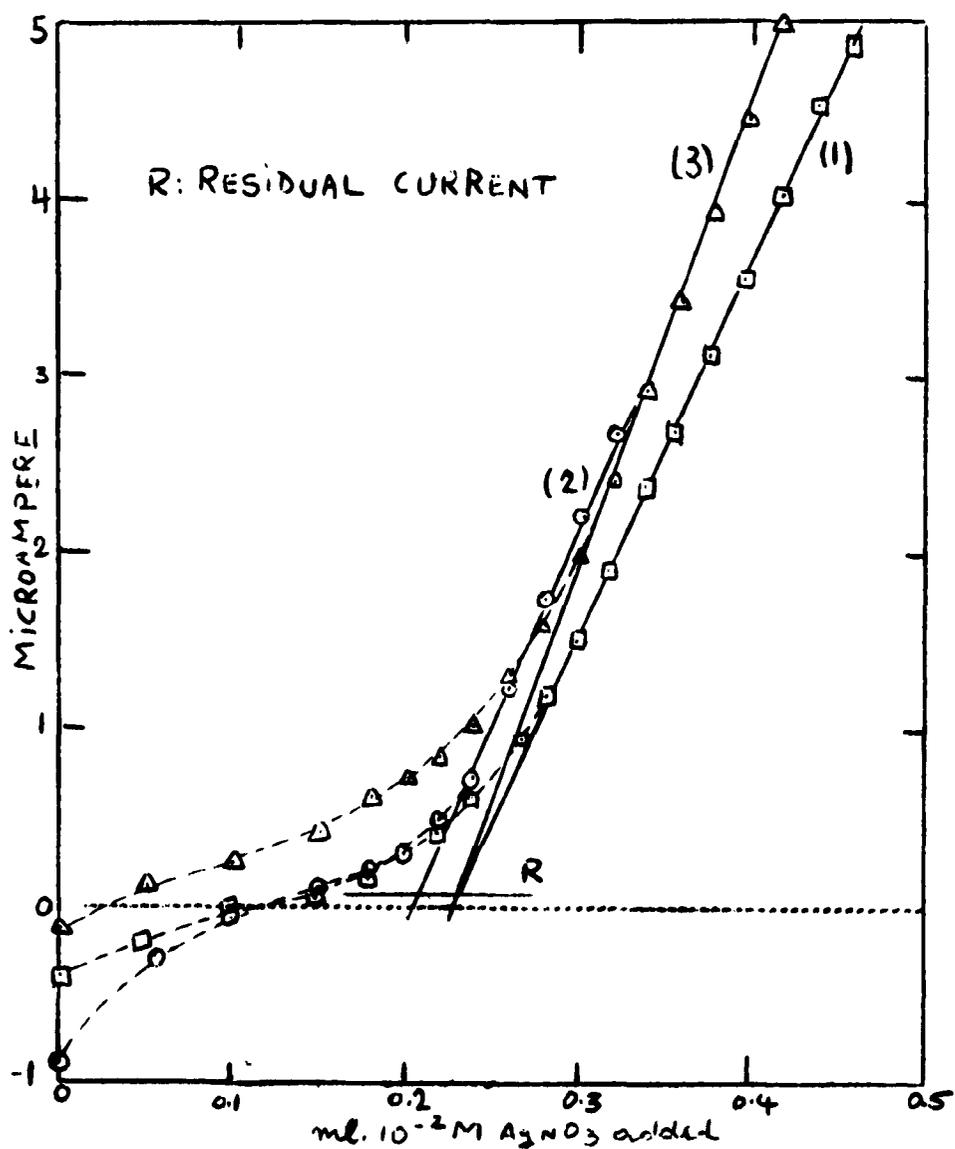
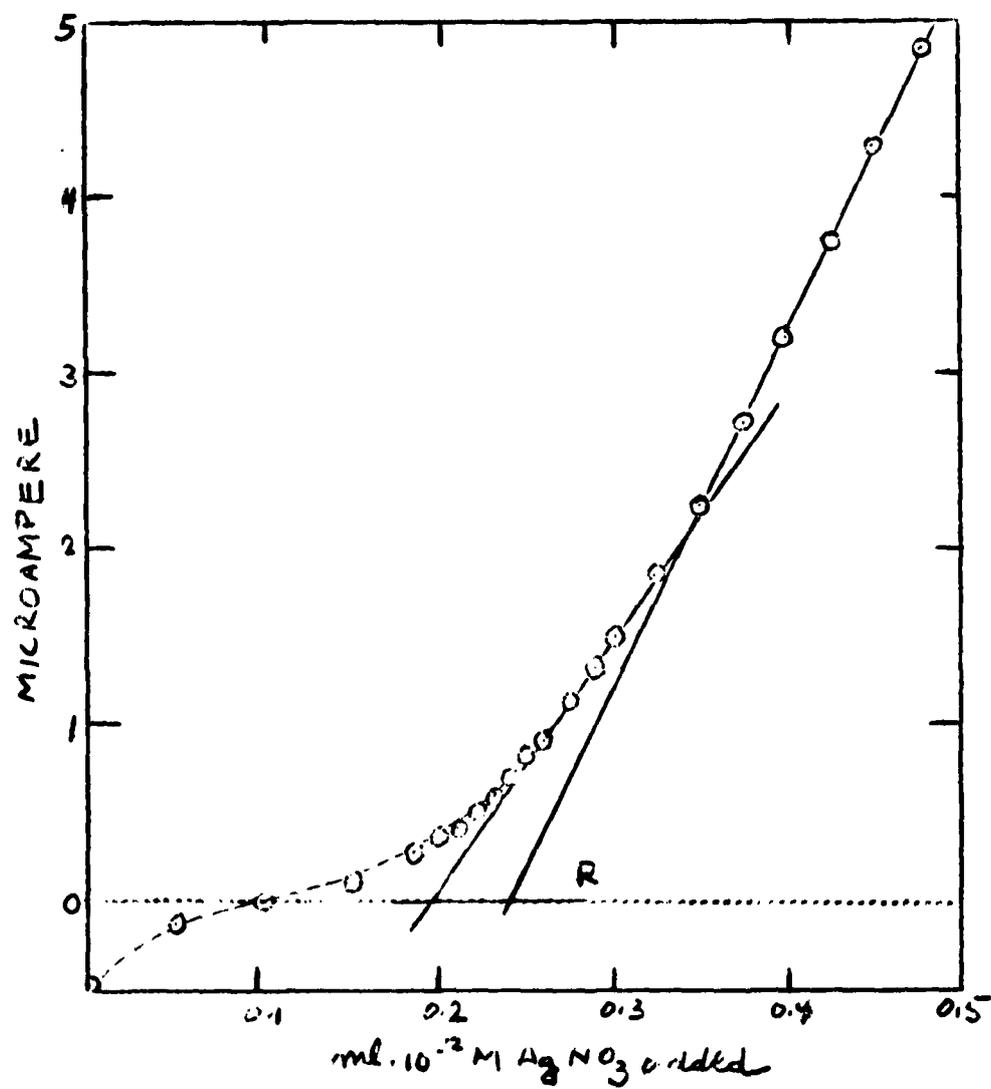


Figure 4. Amperometric titration of 1 ml. of $2 \times 10^{-3}M$ RIISH in 1M KCl, 0.1M CH_3COONa , 0.5% triton X-100, 2 drops of M-octyl alcohol at the rotated silver coated platinum electrode at -0.2 volt vs. S.C.E.



precipitate is observed in ammoniacal or Tris-solutions while precipitates are observed in the presence of chloride can tentatively be explained by assuming that the silver thiolcomplex has the ability to form an amine complex, $RSAg(NH_3)_2$ or a Tris complex which is soluble in water. In the presence of chloride no such complex can be formed, but the complex can be hydrated and soluble in this form. At a large concentration of potassium chloride ($4M$) the complex becomes dehydrated, the water being taken up by the chloride. This explains why a precipitate is formed in the vicinity of the end point at large chloride concentrations while such precipitation ensues much slower at smaller chloride concentrations. An inspection of the results obtained in these titrations will show that the reactions involved in these titrations are rather complicated and it must be assumed that more than one complex is formed in the titration mixture. Tables IV and V give the results of titrations of $R_{11}SH$ in various buffers. It is seen that the results obtained in ammonia buffers with $10^{-3}M$ $AgNO_3$ as titrant exhibit errors of 2 to -9% while the errors in titrations with $10^{-2}M$ $AgNO_3$ vary from 8 to 28%, the results in these titrations being always high. The reason for this peculiar difference between the titrants of different concentrations seems to be the different rate of addition of reagent. When the concentration of the titrant is larger the rate of addition is faster and local high concentrations of silver may be built up. This may give rise to complex compounds of the formula $(RSAg)Ag_x$ and therefore to higher end points. Whether or not this explanation is correct could be tested by more extensive experiments in which titrants of the same concentration are added at different rates. If higher results are obtained at faster rates our explanation would be confirmed. Experiments along this line are planned. All experiments with tris buffer gave high results with errors varying from 8 to 29%. Apparently formation of compounds $(RSAg)Ag_x$ occurs, in which the silver is likely to be bound by the guanidine group. Ammonia buffers can, under proper experimental conditions,

Table IV.

Amperometric titrations of RIISH in ammonia and Tris- buffers with $10^{-2}M$ silver nitrate at -0.2 volt vs. S.C.E.

| Buffer | pH | RIISH, Mg. | | Error, % |
|--|--------------------|------------|-------|----------|
| | | Taken | Found | |
| 0.1M NH ₃ , 1.0M NH ₄ NO ₃ | 8.6 | 0.267 | 0.282 | + 5.0(a) |
| 0.1M NH ₃ , 0.1M NH ₄ NO ₃ | 9.3 | 0.267 | 0.342 | +28.0 |
| " " | 9.3 | 0.267 | 0.288 | + 8.0 |
| " " | 9.3 | 0.267 | 0.325 | +23.0 |
| " " | 9.3 ^(b) | 0.267 | 0.273 | + 2.0(a) |
| " " | 9.3 ^(b) | 0.267 | 0.252 | - 5.0(a) |
| " " | 9.3 | 0.267 | 0.258 | - 3.0(a) |
| 1.0M NH ₃ , 0.1M NH ₄ NO ₃ | 10.3 | 0.267 | 0.240 | - 9.0(a) |
| 0.1M NH ₃ , 0.1M KNO ₃ | 11.0 | 0.267 | 0.273 | + 2.0(a) |
| 0.1M Tris, 0.1M CH ₃ COONa, 0.092M CH ₃ COOH | 7.5 | 0.267 | 0.291 | + 8.0 |
| 0.1M Tris, 0.1M CH ₃ COONa, 0.092M CH ₃ COOH | 7.5 | 0.267 | 0.312 | +15.0 |
| 0.1M Tris, 0.1M CH ₃ COONa | 10.2 | 0.267 | 0.291 | + 8.0 |
| 0.1M Tris, 0.0018M NaOH | 11.3 | 0.267 | 0.354 | +29.0 |

(a) Titrant, $10^{-3}M$ AgNO₃

(b) Titration performed at -0.3 volt

Table V.

Amperometric titrations of $R_{11}SH$ in chloride solutions with $10^{-2}M$ silver nitrate at -0.2 volt vs. S.C.E.

| Buffer | pH | $R_{11}SH$, Mg. | | Error, % |
|--------------------------|---------------------|------------------|-------|-----------------------|
| | | Taken | Found | |
| 1M KCl, 0.1M CH_3COONa | 7.4 | 0.267 | 0.273 | + 2.0 |
| " " | 7.4 | 0.267 | 0.276 | + 3.0 |
| " " | 7.4 | 0.134 | 0.141 | + 5.0 |
| 2M KCl, 0.1M CH_3COONa | 7.4 | 0.267 | 0.282 | + 5.0 |
| 4M KCl, 0.1M CH_3COONa | 7.4 | 0.267 | 0.273 | + 2.0 |
| " " | 7.4 | 0.267 | 0.297 | + 10.0 |
| " " | 7.4 | 0.267 | 0.312 | + 15.0 |
| " " | 7.4 | 0.267 | 0.306 | + 13.0 |
| " " | 7.4 | 0.267 | 0.321 | + 18.0 |
| 4M KCl, 0.1M NH_3 | 11.1 ^(a) | 0.267 | 0.297 | + 10.0 ^(b) |
| 4M KCl, 0.02M NaOH | 12.2 ^(a) | 0.267 | 0.306 | + 13.0 ^(b) |

(a) Titration performed at -0.3 volt vs. S.C.E.

(b) Titrant, $10^{-3}M$ $AgNO_3$

give better results. The reason seems to be that ammonia forms a complex with silver which is strong enough, so that it can compete with the guanidine for the binding of silver. This is not the case in Tris solutions which cannot bind silver strongly enough so as to prevent it from combining with the guanidine group to form $(RSAg)Ag_x$. From the data in Table IV it is seen that a variation of pH from 9.3 to 11.0 in an ammonia buffer and from 7.5 to 11.3 in a Tris buffer has little effect on the results. In these buffers the guanidine group is mostly present in the charged form. As indicated by our results the charged guanidine group appears to have the ability to react with silver by replacement of a proton by the metal. From Table V it is seen that also chloride ion can be used to react with silver to form complexes $AgCl_x^{1-x}$ which can compete with the guanidine group. However, the chloride concentration should not be markedly larger than 2M. At larger chloride concentrations precipitation is observed near the end point and adsorption of silver ion on the precipitate, which may be colloidal in the beginning, is possible. This gives rise to high results.

Table VI gives results of titrations of $R_{III}SH$ (mercaptoethylamine) in various buffers with $10^{-3}M$ silver nitrate as titrant. With the exception of one result, all results are 13 to 38% high. It is of interest to note that while mercaptopropylguanidine can give good results in ammonia or chloride buffers no correct results can be obtained with mercaptoethylamine in these buffers. It appears that the amino group has a stronger complexing power for silver than the guanidine group. We also have to consider that at the pH of our solutions the amino group is uncharged while the guanidino group is charged. Experiments with $R_{III}SH$ at pH 7.7 (0.1M $NaNO_3$, 0.1M CH_3COONa) gave a curved titration line and no end point could be determined. In a $4M$ KCl -0.1M CH_3COONa -solution (1 ml. of $10^{-3}M$ $R_{III}SH$) no silver nitrate was used up by the solution and after addition of the first increment of $AgNO_3$ a current was measured which increased upon further addition of $AgNO_3$.

Table VI.

Amperometric Titrations of $R_{III}SH$ in various buffers with $10^{-3}M$ silver nitrate
at -0.3 volt.

| Buffer | pH | $R_{III}SH$, Mg. | | Error, % |
|--|------|-------------------|-------|----------|
| | | Taken | Found | |
| 0.1M NH_3 , 0.1M NH_4NO_3 | 9.4 | 0.156 | 0.232 | + 38.0 |
| 0.1M NH_3 , 0.05M $NaNO_3$ | 10.0 | 0.312 | 0.390 | + 26.0 |
| " " " " | 10.0 | 0.312 | 0.384 | + 24.0 |
| 0.1M Tris, 0.1M $NaNO_3$ | 10.2 | 0.156 | 0.222 | + 33.0 |
| 2M KCl, 0.02M NaOH | 12.0 | 0.156 | 0.182 | + 13.0 |
| 4M KCl, 0.02M NaOH | 12.1 | 0.156 | 0.160 | + 2.0 |
| " " " " | 12.1 | 0.156 | 0.212 | + 28.0 |
| " " " " | 12.1 | 0.156 | 0.214 | + 29.0 |
| " " " " | 12.1 | 0.156 | 0.214 | + 29.0 |
| 0.01M EDTA, 0.03M NaOH 0.05M $NaNO_3$ | 12.0 | 0.156 | 0.224 | + 34.0 |

An explanation for this behavior cannot be given at the present time. In a few instances (electrolyte: 2M KCl, 0.05M Borax) we found that after the end point the current was not constant, but decreased continuously. Generally, precipitate formation after or at the end point was followed by a marked decrease in current due to the excess reagent. This was also observed in titrations of $R_{II}SH$ and can probably be explained by adsorption of excess silver on the precipitate or by a reaction of excess silver with silver mercaptide to form an insoluble poly-silver mercaptide $(RSAg)_x$. The formation of a deposit at the electrode and a decrease of its sensitivity and following decrease in current cannot be excluded as a possibility in this case. Our present experiments show that an argentimetric titration of $R_{II}SH$ in the buffers investigated is not feasible.

Summarizing our results, it can be stated that amperometric mercurimetric titrations of mercaptans at the platinum electrode can be successfully performed under proper conditions of pH and buffer. The reactions between mercaptans and mercuric chloride are stoichiometrically simple and the results are reproducible. The platinum electrode which in the course of a mercurimetric titration becomes virtually a mercury electrode can be used repeatedly without treatment for many titrations and its sensitivity does not change upon use. As mentioned in the experimental part, the platinum electrode was usually silverplated and amalgamated before use in order to have a uniform surface. Argentimetric titrations are considerably more complex with respect to the reactions involved and to the surface conditions of the electrode. It has been shown by our experiments and by other investigators, that silver can form various complex compounds with mercaptans, the kind of compound formed being dependent on the experimental conditions such as pH, kind of buffer, concentration of reagents and rate of addition of silver to the mercaptan. The stoichiometric end point therefore depends upon these factors. Silver is deposited at the electrode in the form of

crystals, the size and shape of which can be affected by the conditions of the electrolysis and of the electrolyte. The properties of the deposit determine the sensitivity of the electrode, its response to small currents, and therefore indirectly the sharpness of the end point. These considerations make it clear why argentimetric titrations of mercaptans are generally less satisfactory than mercurimetric determinations.

In spite of these difficulties we found it possible that, to a limited extent, certain mercaptan titrations with silver are possible. Further experiments are planned with the intention to obtain more insight into the reactions between silver and mercaptans and the electrolysis processes involved. The effect of the rate of addition of silver nitrate to the mercaptan solution should be studied. Also, several complexing agents for silver, such as sulfite, which can compete with the binding to the basic group, should be explored. Since the surface conditions of the electrode are important various treatments of the electrode will be studied. In the present studies the variation of concentrations of mercaptan was rather limited and an extension of this line of experiments is therefore planned.

Part II

This part is identical with a manuscript entitled "Polarography of 2-Aminoethanethiosulfuric Acid and 2-Aminoethaneselenosulfuric Acid" by Walter Stricks and R. G. Mueller, submitted to the Analytical Chemistry section of the American Chemical Society for presentation at the 144th National Meeting, Los Angeles, California, March 31-April 5, 1963.

Polarography of 2-Aminoethanethiosulfuric Acid
and
2-Aminoethaneselenosulfuric Acid

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A polarographic study of the two antiradiation drugs 2-aminoethanethiosulfuric acid ($\text{H}_2\text{N}-\text{CH}_2-\text{CH}_2-\text{S}-\text{SO}_3\text{H}$) denoted as RSSO_3H and 2-aminoethaneselenosulfuric acid, ($\text{H}_2\text{N}-\text{CH}_2-\text{CH}_2-\text{Se}-\text{SO}_3\text{H}$) denoted as RSeSO_3H is reported in this paper. The results obtained with the two compounds are compared.

Experimental

Materials. RSSO_3H and RSeSO_3H were obtained from the Walter Reed Army Institute of Research, Department of Radiology. All other chemicals were commercial analytical grade products. All solutions were prepared with double distilled water.

The stock solutions used for the preparation of the mixtures to be electrolyzed were; 5M ammonia, 2M ammonium chloride, 2M potassium chloride, 1M hydrochloric acid, 1M acetic acid, 0.2M disodium phosphate, 0.2M monosodium phosphate, 0.5% gelatin, 0.5% polyacrylamide, 10^{-2}M RSSO_3H and 10^{-2}M RSeSO_3H . Stock solutions of RSSO_3H , RSeSO_3H and of gelatin were kept refrigerated if not in use. The gelatin solution contained one drop of toluene in 100 ml. solution to protect it from bacterial attack.

Methods. Current voltage curves were measured at $25^{\circ} \pm 0.1^{\circ}\text{C}$ with the manual apparatus and circuit described by Lingane and Kolthoff(5) and automatically with a Sargent Model XXI automatic recording polarograph. All potentials were measured against a saturated calomel electrode (S.C.E.). Oxygen was removed from the solution in the cell with a stream of Linde Nitrogen (99.996%) pure. The nitrogen was passed through three bottles containing solutions of the same composition as that of the buffers used for the electrolysis mixture. During an experiment an atmosphere of nitrogen was maintained over the solution. Corrections were made for the residual current.

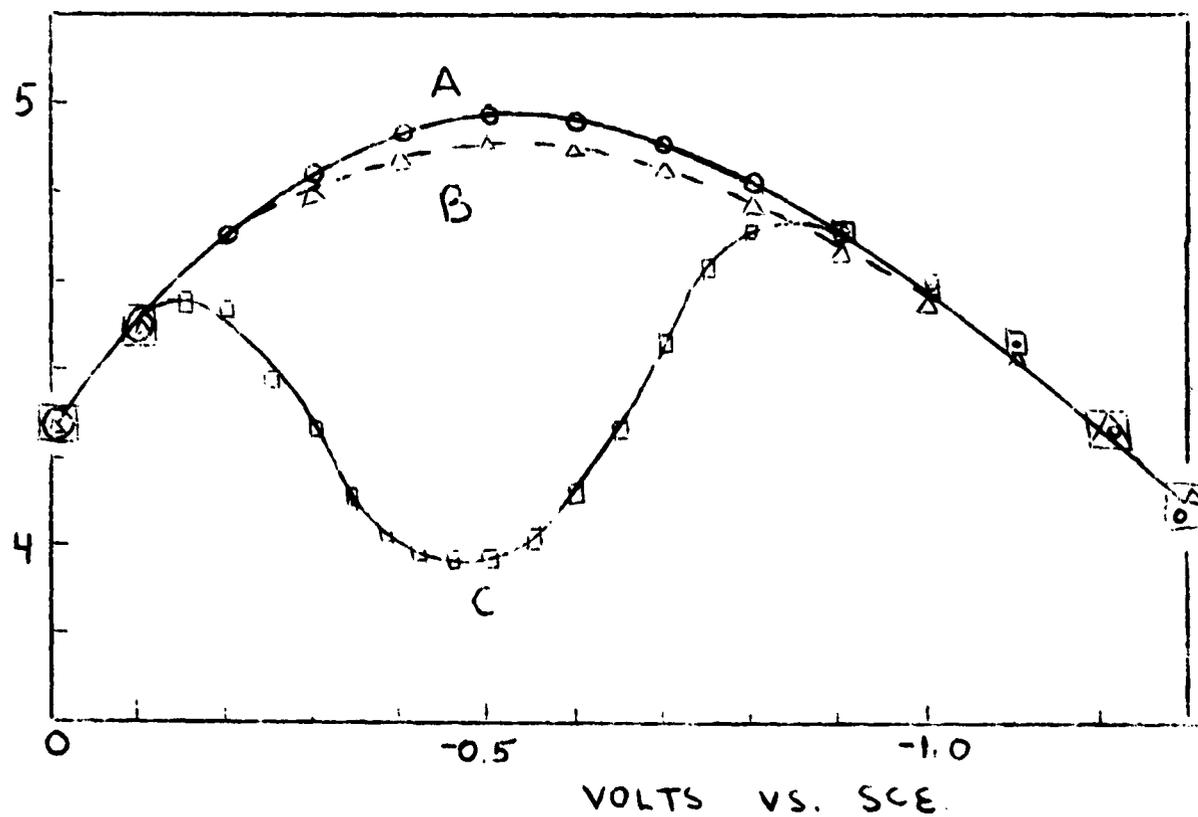
The characteristics of the capillary of the dropping mercury electrode, (D.M.E.) were: $m=1.98 \text{ mg sec}^{-1}$, $t=4.07 \text{ sec}$. (open circuit, 0.1M KCl, $h=67.8 \text{ cm}$.)

The rotated dropping mercury electrode (R.D.M.E.) had the following characteristics: speed of rotation 225 r.p.m., $m=15.28 \text{ mg sec}^{-1}$, $t=3.9 \text{ sec}$. (open circuit 0.1MKCl, $h=52 \text{ cm}$.)

The pH was measured with a Beckman Zeromatic pH meter with a glass electrode of the 41260 type, usable for the entire pH range.

Results.

RSSO₃H. Polarograms were taken with solutions of various concentrations in RSSO₃H in buffers from pH 1 to 11. All RSSO₃H solutions gave a well defined cathodic wave. In strongly alkaline medium (0.05M NaOH, 0.95M KCl) RSSO₃H decomposes slowly. After the first hour of standing of 10^{-3}M RSSO₃H in this solution the polarogram was found to be unchanged. However, after 11 hours the height of the wave was reduced from 7.2 to 4.9 μa and the main wave ($E_{\frac{1}{2}} = -1.16 \text{ volt}$) was preceded by a small prewave ($E_{\frac{1}{2}} = 0.72 \text{ volt}$) of a height of 0.86 μa . An anodic wave was not detected in the partially decomposed solution, indicating that mercaptoethylamine (RSH) is not formed in the course of the decomposition. RSSO₃H has hardly any effect on the surface tension of mercury as indicated by the electrocapillary curve (drop time against potential) obtained in a phosphate buffer at pH 7, (See Figure 1).

Electrocapillary Curves of RSSO_3^- and RSeSO_3^- A) Supporting Electrolyte: 0.05 M Na_2HPO_4 , 0.005 M NaH_2PO_4 ,0.85 M KCl , pH 7.4B) with 10^{-3} M RSSO_3H C) with 10^{-3} M RSeSO_3H 

The diffusion current and half wave potential of a $10^{-3}M$ $RSSO_3H$ solution at various pH are listed in Table I. It is seen that two pH regions can be distinguished. Between pH 11 and 7 the diffusion current is practically constant at various pH and the half wave potential becomes more negative with increasing pH.

In the acid pH region the diffusion current increases markedly with decreasing pH and the half wave potential is unaffected by a change of pH in this region. The presence of sulfite (0.2 to 0.02M) in alkaline medium has a slight suppressive effect on the diffusion current of $RSSO_3H$ but no effect on the half wave potential. Experiments with a $10^{-3}M$ $RSSO_3H$ solution in a phosphate solution in a phosphate buffer at pH 5.4 were performed with varying heights of the mercury column (48 to 101 cm) and the ratio i/\sqrt{h} obtained after correction for back pressure was found to be constant. The wave can thus be considered to be diffusion controlled.

$RSeSO_3H$. From curve C of fig. 1 it is seen that $RSeSO_3H$, in contrast with $RSSO_3H$ is capillary active at the mercury solution interface. Current voltage curves of $RSeSO_3H$ were taken with solutions at pH ranging from 1.0 to 12.4 and ionic strengths from 0.1 to 1.0. Examples of current voltage curves of $RSeSO_3H$ are illustrated in Fig. 2 which for comparison also gives a C-V-curve of $RSSO_3H$. In contrast with the single wave of $RSSO_3H$, $RSeSO_3H$ gives polarograms which essentially consist of two waves. In the pH range between 5.4 and 9.3 the C-V curve exhibits a high peak at a potential of about -1.6 volt. In the presence of 0.00125% gelatin the peak is suppressed and a well defined wave is observed at this potential. It is interesting to note (Fig. 2) that $RSeSO_3H$ is reduced at a considerably more positive potential than $RSSO_3H$. Thus in a phosphate buffer at pH 7.4 the zero current potential of $RSeSO_3H$ is about -0.2 volt while that of $RSSO_3H$ is -0.8 volt. Halfwave potentials and wave heights of $RSeSO_3H$ are listed in Table II. At pH lower than 3 the plateau

Polarograms of (A) 10^{-3} M RSSO_3H ; (B), (C), (D) 10^{-3} M RSeSO_3H
Supporting Electrolyte

(A), (B) 0.05 M Na_2HPO_4 , 0.005 M NaH_2PO_4 , 0.85 M KCl , pH 7.4

(C) 0.005 M Na_2HPO_4 , 0.05 M NaH_2PO_4 , 0.85 M KCl , pH 5.4

(D) 0.1 M HCl , 0.9 M KCl , pH 1.1

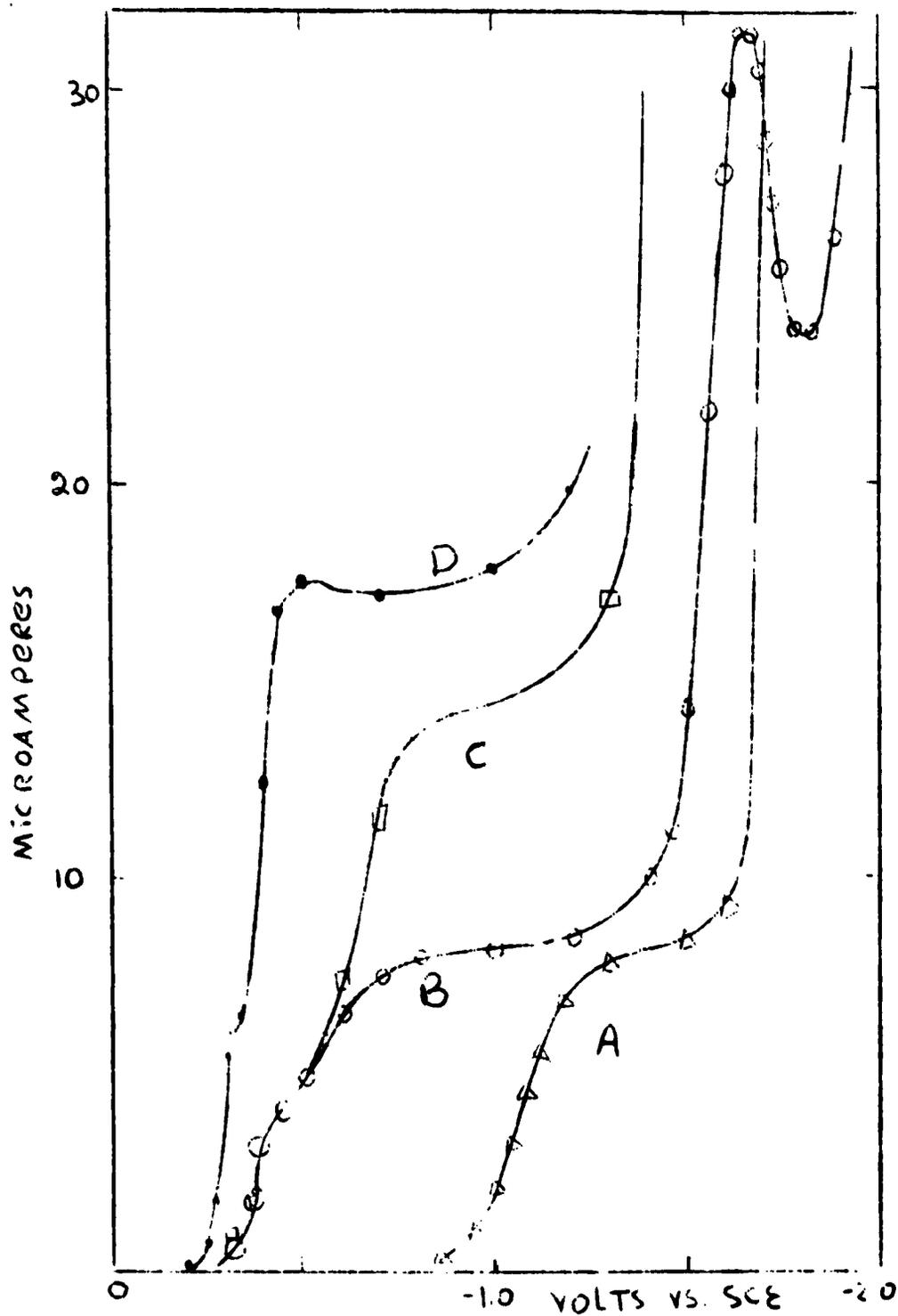


Table I. Diffusion current and halfwave potential of $10^{-3}M$ $RSSO_3H$ in various buffers.

| Buffer | pH | i_d , ua | $E_{1/2}$ |
|---|-------|------------|-----------|
| 0.1M NH_3 , 0.4M KCl, 0.2M Na_2SO_3 | 10.94 | 6.80 | -1.198 |
| 0.1M NH_3 , 0.7M KCl, 0.1M Na_2SO_3 | 10.94 | 6.85 | -1.197 |
| 0.1M NH_3 , 0.94M KCl, 0.02M Na_2SO_3 | 10.84 | 7.08 | -1.176 |
| 0.1M NH_3 , 1.0M KCl | 10.86 | 7.72 | -1.176 |
| 0.1M NH_3 , 0.1M NH_4Cl | 9.20 | 7.18 | -1.139 |
| 0.05M Na_2HPO_4 , 0.005M NaH_2PO_4 0.85M KCl | 7.32 | 7.92 | -1.065 |
| 0.005M Na_2HPO_4 , 0.05M NaH_2PO_4 0.85M KCl | 5.38 | 10.02 | -1.070 |
| 0.1M CH_3COOH , 1.0M KCl | 2.90 | 14.60 | -1.065 |
| 0.1M HCl, 0.9M KCl | 1.08 | 17.00 | -1.06 |

Table II. Wave heights i_1 , i_2 , and i_3 and halfwave potentials, $E_{\frac{1}{2}}$, $2E_{\frac{1}{2}}$, $3E_{\frac{1}{2}}$ of $10^{-3}M$ $RSeSO_3H$ in various buffers. All solutions contain 0.00125% gelatin and have ionic strength one if not indicated otherwise.

Current in μa , halfwave potentials in volt vs. S.C.E.

| Buffer | pH | First wave | | Second wave | | Third wave | |
|---|-------|------------|--------------------|-------------|--------------------|------------|--------------------|
| | | i_1 | $1E_{\frac{1}{2}}$ | i_2 | $2E_{\frac{1}{2}}$ | i_3 | $3E_{\frac{1}{2}}$ |
| 0.1M HCl, 0.9M KCl | 1.04 | 5.91 | -0.29 | 11.03 | -0.38 | - | - |
| 0.1M CH_3COOH , 1M KCl | 2.86 | 5.05 | -0.33 | 10.50 | -0.51 | - | - |
| 0.1M CH_3COOH , 0.01M CH_3COONa 1.0M KCl | 3.60 | 4.00 | -0.36 | 9.67 | -0.61 | - | - |
| 0.1M CH_3COOH , 0.1M CH_3COONa 0.9M KCl | 4.56 | 3.70 | -0.35 | 9.50 | -0.56 | - | - |
| 0.005M Na_2HPO_4 , 0.05M Na H_2PO_4 0.85M KCl | 5.36 | 3.80 | -0.36 | 9.40 | -0.56 | 155.0 | -(1) |
| 0.01M CH_3COOH , 0.1M CH_3COONa 0.9M KCl | 5.54 | 3.65 | -0.36 | 9.95 | -0.66 | 35.0 | -1.45 |
| 0.1M CH_3COONH_4 , 0.9M KCl | 7.12 | 4.00 | -0.35 | 4.04 | -0.58 | 22.2 | -1.63 |
| 0.05M Na_2HPO_4 , 0.005M Na H_2PO_4 0.85M KCl | 7.40 | 3.65 | -0.39 | 3.36 | -0.57 | 18.0 | -1.59 |
| 0.05M Na_2HPO_4 , 0.005M Na H_2PO_4 0.05M KCl (2) | 7.76 | 4.39 | -0.36 | 2.85 | -0.57 | 18.9 | -1.56 |
| 0.05M $Na_2B_4O_7$, 0.85M KCl | 9.00 | 3.36 | -0.41 | 3.23 | -0.62 | 4.3 | -1.74 |
| 0.1M NH_3 , 0.1M NH_4Cl (3) | 9.28 | 3.90 | -0.41 | 3.39 | -0.58 | 7.35 | -1.69 |
| 0.1M NH_3 , 0.1M NH_4Cl 0.9M KCl | 9.32 | 3.75 | -0.42 | 3.38 | -0.59 | 2.45 | -1.69 |
| (4) 0.1M NH_3 , 0.1M KCl | 10.80 | 4.15 | -0.48 | 3.15 | -0.65 | - | - |
| 0.1M NH_3 , 1M KCl | 10.88 | 3.80 | -0.48 | 3.34 | -0.65 | - | - |
| 0.05M NaOH, 0.95M KCl (5) | 12.40 | 2.28 | -0.47 | 2.66 | -0.64 | - | - |

(1) i_3 is the height of a peak at -1.9 volt, hence no halfwave potential is given

(2) ionic strength is 0.35

(3)(4), ionic strength is 0.10

(5) polarogram taken 5 minutes after addition of $RSeSO_3H$ to the buffer.

of the first wave is poorly defined and its height is larger than that observed at less acid pH. Over the pH range 10.9 to 3.6 the limiting current of the first wave is practically constant at the same ionic strength and independent of the composition of the buffer (compare the data with phosphate and acetate buffer at pH 5.36 and 5.54 respectively). The ionic strength has little effect on the height i_1 , of the first wave as indicated by experiments with ammonia buffers at ionic strengths 0.1 and 1.0. The low currents obtained in solutions at pH 12.4 can be explained by the alkaline decomposition of $RSeSO_3H$ which is much less stable than $RSSO_3H$ in this medium. When a solution of $10^{-3}M$ $RSeSO_3H$ in $0.05M$ $NaOH$, $0.95M$ KCl was allowed to stand, the height of the first wave decreased from 2.32 to 0.38 μa after 4 and 690 minutes respectively, while the height of the second wave decreased only from 2.85 to 2.46 μa in the same time. Apparently the second wave corresponds to the reduction of a new substance formed during alkaline decomposition of $RSeSO_3H$.

The variation of the height of the second wave with pH is similar to that of the $RSSO_3H$ wave. In the alkaline pH region (7.1 to 10.9) the height of the second wave is independent of pH and practically equal to the height of the first wave. In the acid pH range the second wave (12) becomes markedly larger than i_1 .

The height of the third wave decreases markedly with increasing pH. Its variation with the ionic strength appears to be affected by the composition of the buffer. Thus in phosphate buffers (pH 7.40 and 7.76) of ionic strengths 1 and 0.35, i_3 is almost the same (18.0 and 18.9 μa respectively) while in ammonia-ammonium chloride buffers (pH 9.32 and 9.28) of ionic strengths 1 and 0.1 the values of i_3 were 2.45 and 7.35 μa respectively. A comparison of the height of this wave in a borate buffer (pH 9.0) and ammonia-ammonium chloride buffer (pH 9.32) both of ionic strength 1 ($i_3=4.3$ μa and 2.4 μa respectively) shows that it can be affected by the composition of

the buffer. The halfwave potentials of the three waves are little affected by the pH of the solutions, indicating that these waves are of an irreversible nature.

Polarograms taken with solutions of various RSeSO_3H concentrations (5×10^{-4} to $1.4 \times 10^{-3}\text{M}$) at 5.4, 7.4 and 9.2 revealed that the height of the first and second wave are proportional to the concentration while the i_3/C value of the third wave decreases markedly with increasing concentration. Thus at RSeSO_3 concentrations of 2.7×10^{-4} and 10^{-3}M (phosphate buffer, pH 7.4) the values of i_3/C (ua liters mmoles^{-1}) were 58.2 and 19.2 respectively, indicating that the third wave is not diffusion controlled.

In order to obtain more insight into the nature of the waves, polarograms were also taken at various heights of the mercury column and at various temperatures. The ratios i_1/\sqrt{h} and i_2/\sqrt{h} were constant at various h (32.5 to 101.6 cm) in alkaline, neutral and acid media. The temperature effect on the heights of the waves was studied at 10, 25, and 50°C with a 10^{-3}M RSeSO_3H solution at pH 7.4 (phosphate buffer). From Table III it is seen that the temperature coefficients of the wave heights are within the order of magnitude of those ordinarily observed with diffusion controlled currents. It is of interest to note that the temperature coefficient of the first wave is lower than that of the second and third wave in the lower and higher temperature region. All temperature coefficients in the lower temperature range are greater than those observed in the higher temperature range. This is in contrast with findings for metal ions whose temperature coefficients increase with increasing temperature, (3). Table III also shows that the halfwave potentials of the first and second step are shifted to more positive values with increasing temperature. In the temperature region between 10 and 25°C the shift for the first and second wave is 1.33 millivolts per degree and in the range between 25 and 50°C the shifts for the two waves are 1.33 and 1.6 millivolts per degree. These values agree favorably with results reported by Stricks and Kolthoff(8) for

Table III. Effect of temperature on limiting current (i_1, i_2, i_3) and half wave potential ($1E_{\frac{1}{2}}, 2E_{\frac{1}{2}}$, volt vs. S.C.E.) of $10^{-3}M RSeSO_3H$ in $0.05M Na_2HPO_4$, $0.005M Na H_2PO_4$, $0.85M KCl$, 0.00125% gelatin, pH 7.42. Temperature coefficient α (per cent/ $^{\circ}C$) = $\Delta i \times 100/i_{25} \times \Delta t$.

| Temperature t, $^{\circ}C$ | 10 | 25 | 50 |
|-----------------------------|-------|-------|-------|
| i_1 | 3.32 | 4.04 | 4.60 |
| i_2 | 2.72 | 3.92 | 5.68 |
| i_3 | 12.4 | 19.2 | 24.3 |
| $i_1 + i_2$ | 6.04 | 7.96 | 10.28 |
| α of i_1 | | 1.19 | 0.56 |
| α of i_2 | | 2.04 | 1.24 |
| α of i_3 | | 2.36 | 1.06 |
| α of ($i_1 + i_2$) | | 1.61 | 1.17 |
| $1E_{\frac{1}{2}}$ | -0.39 | -0.37 | -0.35 |
| $2E_{\frac{1}{2}}$ | -0.59 | -0.57 | -0.53 |

oxidized glutathione. They found that the halfwave potential of oxidized glutathione was shifted to more positive values as the temperature was increased from 25 to 50°C, the shift being 1.24 millivolt per degree. Apparently the ease of breaking the bond between selenium and sulfur in $RSeSO_3H$ and the disulfide bond in oxidized glutathione increases with increasing temperature. In this connection it should be mentioned that the temperature coefficient of halfwave potentials of metal ions is negative(3), a behavior which is in contrast with that of the compounds considered in this paper.

Polarograms taken with $RSeSO_3H$ solutions in an ammonia buffer (pH 11) with varying sulfite concentrations (0.02 to 0.1M) showed that neither the halfwave potentials nor the limiting currents are affected by the presence of sulfite.

Surface active compounds such as gelatin at concentrations of 0.00125 to 0.01% have no effect on the polarogram of $RSeSO_3H$. This was found for neutral as well as for acid solutions.

At the R.D.M.E. the shape of the polarograms of $RSeSO_3H$ is the same as that observed at the D.M.E. in acid, neutral and alkaline solutions in the absence of surface active agents. The two steps at the R.D.M.E. occur at more negative potentials than at the D.M.E., an observation which is usually made with irreversible waves. Thus in a phosphate buffer at pH 7.4 the halfwave potentials of the two steps at the R.D.M.E. are -0.46 and -0.82 volts respectively as compared to the corresponding values of -0.39 and -0.57 volt at the D.M.E. These differences are less pronounced at lower pH. In neutral or alkaline medium the second step is higher than the first step. Thus at pH 7.4 the first and second step of a $10^{-4}M$ $RSeSO_3H$ solution were 4.7 and 5.8 μA respectively. This is at variance with the behavior at the D.M.E. at which the two steps are of equal height in these media. Also, at acid pH the ratio i_1/i_2 is larger at the R.D.M.E. than at the D.M.E. The explanation can be

obtained from an inspection of the electrocapillary curve (Fig. 1) from which it is seen that $RSeSO_3H$ is adsorbed at the electrode over a potential range of -0.12 to -0.85 volt. The first reduction step of $RSeSO_3H$ which occurs within this potential range is, as a maximum of the second kind(9), completely suppressed while the second step which occurs at or beyond the desorption potential of $RSeSO_3H$ is not or only partially suppressed by $RSeSO_3H$. When gelatin or polyacrylamide is added to $RSeSO_3H$ solutions the first wave remains unchanged while the second wave is suppressed. However, the suppression of the second wave is much more extensive than that observed previously with other ions(9). Thus in a $10^{-4}M$ $RSeSO_3H$ solution (phosphate buffer, pH 7.4) a gelatin concentration of 0.0025% is sufficient to reduce the height of the second wave to zero and at pH 5.4 0.0125% gelatin reduced the second step from 9.59 ua (no gelatin) to 0.60 ua. At pH 2.88 the suppressive effect of gelatin is smaller and the second step of $10^{-4}M$ $RSeSO_3H$ is reduced from 9.5 (without gelatin) to 4.1 ua. An explanation for this peculiar behavior will be given shortly.

Discussion.

In neutral and alkaline media the diffusion current of $RSSO_3H$ is practically unaffected by pH. Assuming a two electron reduction in this pH region, a diffusion coefficient of $9.9 \times 10^{-6} \text{cm}^2 \text{sec}^{-1}$ is calculated which compares favorably with that of 2-mercaptoethylamine ($8.9 \times 10^{-6} \text{cm}^2 \text{sec}^{-1}$)(7). Thus it may be safely concluded that the cathodic wave of $RSSO_3H$ in neutral and alkaline solutions involves 2 electrons. The electroreduction obviously takes place at the sulfur-sulfur bond and the overall reaction in alkaline or neutral medium can be expressed by

$$RSSO_3^- + 2e = RS^- + SO_3^- \quad (1)$$

The products of reaction (1) are mercaptoethylamine and sulfite which is not reduced at the mercury electrode in alkaline or neutral medium(4).

In the acid pH region the diffusion current of $RSSO_3H$ increases with

decreasing pH. This can be explained by the fact that sulfite is reduced in acid media and therefore contributes to the increased limiting current of RSSO_3H in these solutions. Moreover, it is seen from Fig. 3 that the sulfite wave in acid solution behaves similar to the RSSO_3H wave in that its limiting current also increases with decreasing pH. The mechanism of the electro reduction of sulfite has been discussed by Kolthoff and Miller(4) and more recently by Cermak(2). Assuming that the two electron reduction of RSSO_3H according to equation (1) gives approximately the same wave height in alkaline and acid medium the contribution of the reduction of sulfite to the overall height of the RSSO_3H wave can be calculated at various acid pH by deducting the height of the RSSO_3H wave at pH 7.4 (no sulfite reduction) from the overall heights of the RSSO_3H waves at acid pH. For 10^{-3}M RSSO_3H solutions one obtains 2.1, 6.7 and 9.1 μa at pH 5.4, 2.9 and 1.1 respectively. These values are of the same order of magnitude as the heights of the sulfite waves (3.7, 6.6 and 8.8 μa) obtained with 10^{-3}M sulfite solutions at the same pH (Fig. 3). This agreement supports the assumption that sulfite is one of the products of the electroreduction of RSSO_3H according to equation (1).

If the electrode reaction according to equation (1) were reversible the plot $\log (i_d - i)/i^2$ versus the potential should yield a straight line with slope of 0.0295. An example of such a plot obtained with a 10^{-3}M RSSO_3H solution (phosphate buffer, pH 7.4) is given in Fig. 4. The plot is a straight line with slope 0.089. Similar results were found in solutions at pH 10.9 and 2.9. The plot $\log (i_d - i)/i$ versus E which is also given in Fig. 4 is a straight line of slope 0.137 which has no theoretical meaning. Since sulfite is one of the products of reaction (1) it was thought that the presence of an excess of sulfite may change the characteristics of the wave. Polarograms were therefore analyzed of ammoniacal solutions which were 0.2M, 0.1M and 0.02M in sulfite. For a reversible wave the slope $\log (i_d - i)/i$ vs. E would be 0.0295. However, straight lines were obtained which had slopes of 0.16. All these

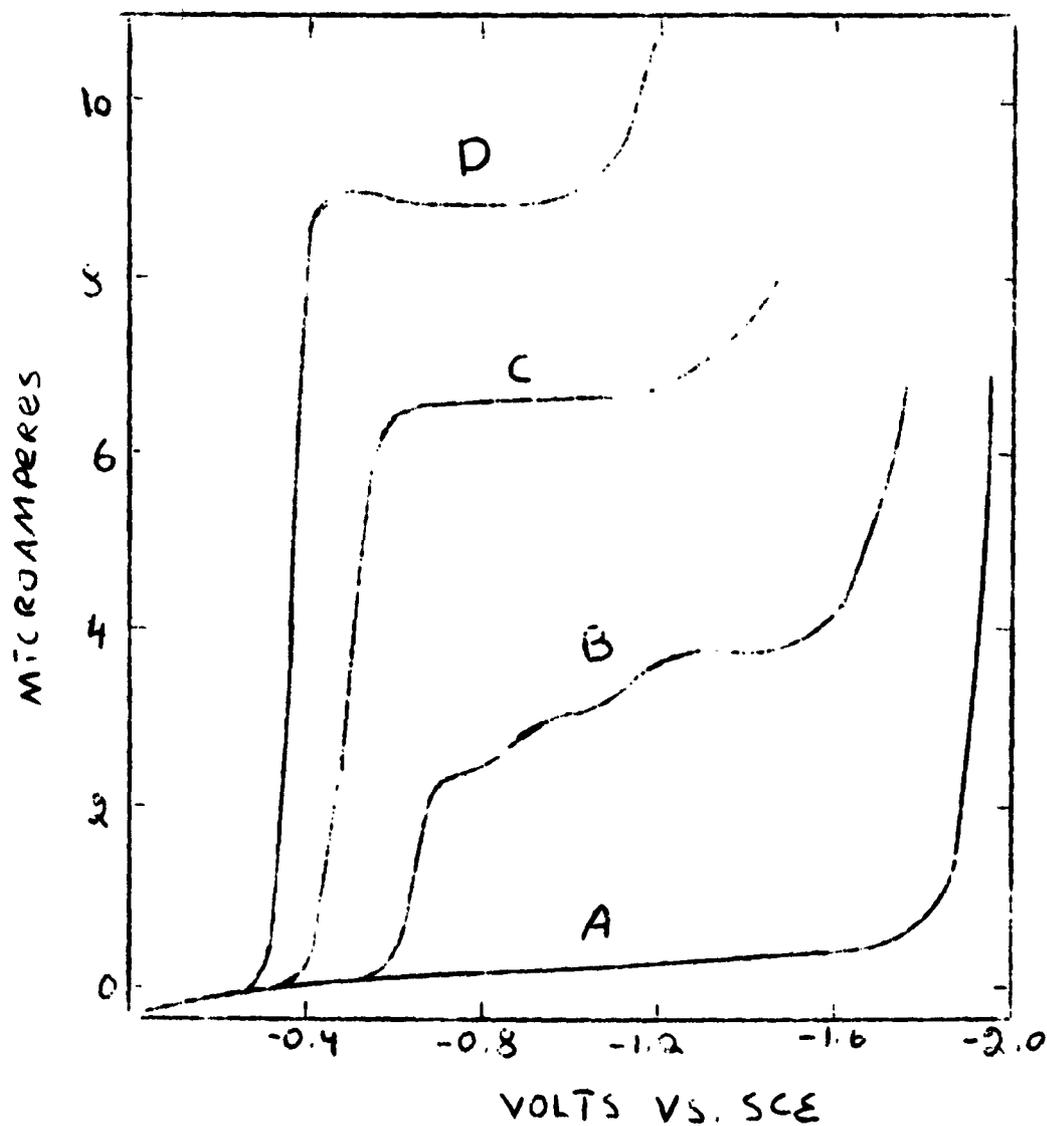
Polarograms of 10^{-3} M Na_2SO_3 in

A) 0.05 M Na_2HPO_4 , 0.005 M NaH_2PO_4 , 0.85 M KCl , pH 7.4

B) 0.005 M Na_2HPO_4 , 0.05 M NaH_2PO_4 , 0.85 M KCl , pH 5.4

C) 0.1 M CH_3COOH , 1 M KCl , pH 2.9

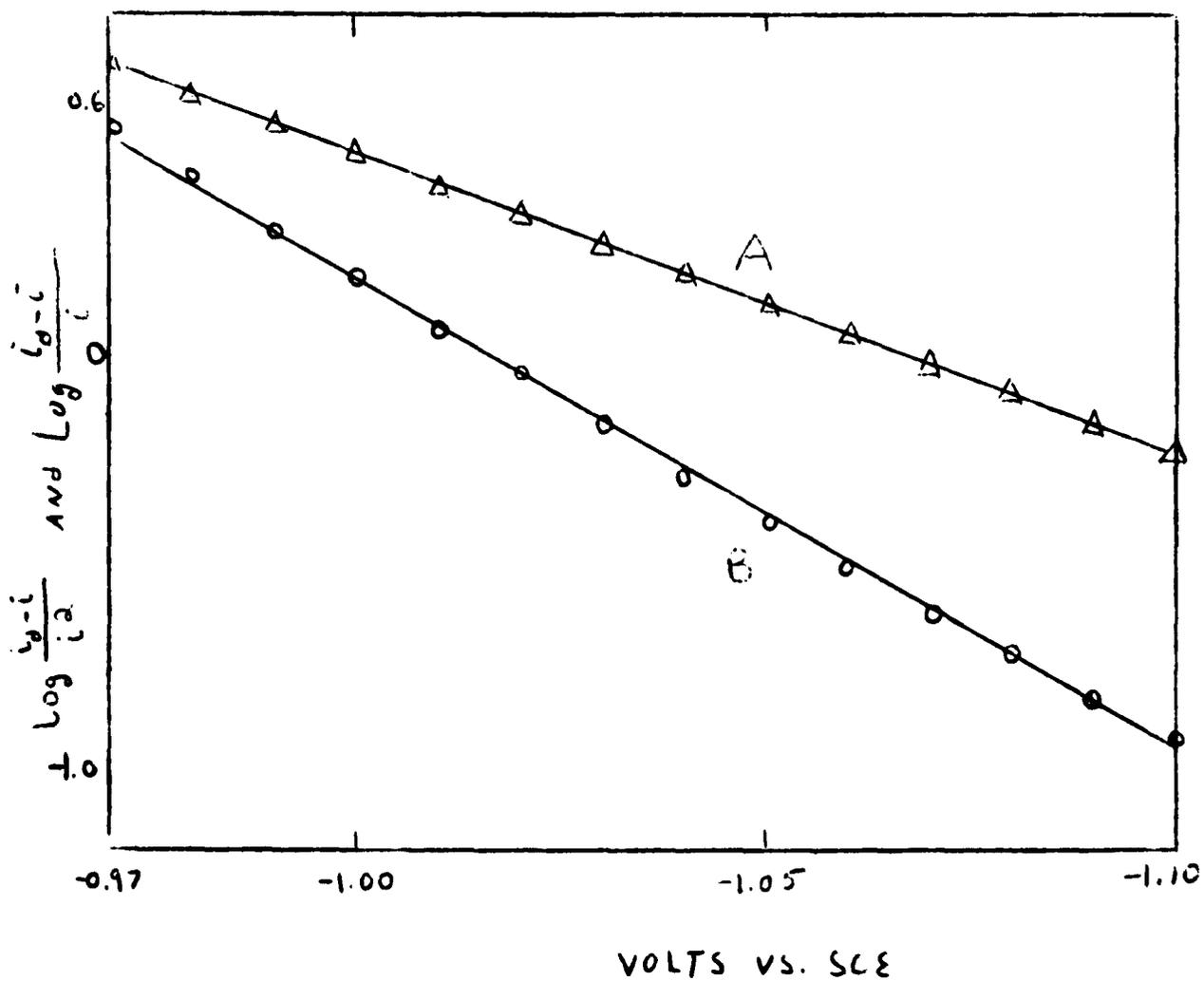
D) 0.1 M HCl , 0.9 M KCl , pH 1.1



(Analysis of the Wave of 10^{-3} M RSSO_3H in Phosphate Buffer, pH 7.4

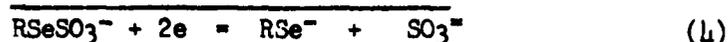
A) plot $\log \frac{i_d - i}{i}$ versus E

B) plot $\log \frac{i_d - i}{(i)^2}$ versus E



observations as well as the behavior of the wave at various pH point to the fact that RSSO_3H is reduced irreversibly at the D.M.E.

The sum of the height of the two steps of RSeSO_3H is about equal to the height of the RSSO_3H wave in the same pH region, indicating that the two steps of RSeSO_3H correspond each to a one electron reduction. Apparently a free radical is involved and the two reduction steps in neutral or alkaline medium can be represented by the following equations:



the overall reaction being analogous to that of equation (1). The increase of the height of the second wave with decreasing pH can, of course, be explained by the reduction of sulfite in acid medium. A quantitative evaluation of the contribution of the sulfite reduction to the height of the second wave can hardly be made with reasonable accuracy since the plateau of the first wave becomes ill defined as the acidity is increased. The halfwave potentials of the second wave of RSeSO_3H in acid solutions (-0.38, -0.51 and -0.56 volt at pH 1.1, 2.9 and 5.4 respectively) are approximately equal to those of the sulfite waves (-0.36, -0.51 and -0.64 volt) in solutions at the same pH. This is the reason why a separate sulfite wave cannot be detected in polarograms of RSeSO_3H in acid solutions. The double wave obtained with a 10^{-3}M RSeSO_3H solution in an ammonia-ammonium chloride buffer (pH 9.2) was analyzed. The first wave gave a plot $\log (i_d - 1)/i$ vs. E which consisted of two parallel straight lines of slope 0.057 while the plot $\log (i_d - 1)/i^2$ vs. E was an S-shaped curve. The plot $\log (i_d - 1)/i$ vs. E of the second wave was a straight line of slope 0.079 and the plot $\log (i_d - 1)/i^2$ vs. E for this wave was a curved line. This indicates that the waves of RSeSO_3H are irreversible.

The reactions involved in the third wave which occurs in the pH region of

5 to 9 is not known. Apparently the wave which is extremely high at low pH and low at high pH is of a catalytic kinetic nature.

The disappearance or partial elimination of the second wave of $RSeSO_3H$ in the presence of gelatin at the R.D.M.E. can be explained by the slow rate of the electrode process at the second step at this electrode. Apparently in the presence of gelatin the reduction is still more sluggish. It is thus reasonable to assume that the free radical $SO_3^{\cdot-}$, instead of being reduced, polymerizes according to

$$2SO_3^{\cdot-} = S_2O_6^{\cdot-} \quad (5)$$

to form dithionate. Dithionate is inactive at the mercury electrode(10), (6) and the second wave is therefore eliminated. As the acidity of the solution is increased the suppressive effect of gelatin becomes less pronounced and the second wave is only partially suppressed at pH 2.88. A tentative explanation would be the assumption that the polymerization of the free radical SO_3H^{\cdot} to form H_2SO_6 is slower than the electrode process. One also has to consider that $S_2O_6^{\cdot-}$ can yield sulfite(1) according to: $S_2O_6^{\cdot-} + H_2O = H_2SO_3 + SO_4^{\cdot-}$ (6) The reduction of chemically formed sulfite being responsible for the second step of the polarogram. Separate experiments with sulfite at pH 2.88 at the R.D.M.E. showed that the sulfite wave is suppressed by gelatin in a normal fashion.

Acknowledgement. This investigation was supported by the U.S. Army Medical Research and Development Command, Department of the Army, under Research Contract No. DA-49-193-MD-2146.

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Part III

Catalytic Polarographic Phenomena in Solutions of Organic Sulfur
Compounds

Introduction

This part deals with investigations of a number of peculiar phenomena observed at the dropping mercury electrode in ammoniacal solutions containing cobalt and sulfhydryl or disulfide or thiosulfate compounds. When electrolyzed at the dropping mercury electrode, many of these compounds give high current peaks at potentials of -1.4 to -1.9 volts versus the saturated calomel cell. Because of their abnormal height, these peaks were attributed to catalytic phenomena. Since its discovery some 30 years ago, a tremendous amount of work in this field has been published in the literature all over the world. However, the mechanism of the electrode reactions involved in these phenomena is still unexplained. In spite of this fact, the catalytic current produced in the presence of sulfhydryl and disulfide compounds of biological interest such as proteins and amino acids, were applied widely as a test in the diagnosis of cancer, and aroused wide interest in the biological and biochemical fields.

In recent years, sulfhydryl and other sulfur compounds were found to have radiation protective properties, and many investigations of these compounds are now under way. In this connection the catalytic effects produced by these compounds at the dropping mercury electrode became again of great interest. The polarographic effects can be helpful for a study of the interaction of these compounds with serum proteins before and after irradiation, a study which may throw some light into the mechanism involved in the radiation protective activity of these compounds. It was with this aim in mind that the present studies were initiated, this work consisting of three series of inter-related studies.

The first section deals with the study of just which sulfur compounds give a "catalytic wave", and at which concentrations, since not all the mercaptans investigated give waves.

The second part consists of the variation of experimental conditions.

Variables such as concentration of buffer (ammonia, ammonium chloride), the presence of the metal ions of mercury(II), copper(II) and silver(I) were found to effect the "catalytic wave".

The third section of this work is the description of a few preliminary experiments which were performed at the rotated dropping mercury electrode. This electrode proved to be as versatile as the dropping mercury electrode, and enabled the investigation to be extended to concentrations which were lower than those performed at the conventional dropping mercury electrode by a factor of ten.

CATALYTIC WAVES

It has been known for some time that the presence of small amounts of certain mercaptans will markedly change the shape of the current-voltage curves of the reduction of cobalt and nickel ions at the DME.

The normal current-voltage curve for the electrolysis of cobalt(II) ammine complex (see Fig. 1A) is characterized by a decomposition potential of about -1.1 volts versus SCE, and by a large maximum at about -1.25 volts vs. SCE. The current at potentials more negative than -1.4 volts corresponds to the normal diffusion current. This flat diffusion region continues to about -1.9 volts vs. SCE, where the reduction of hydrogen ions begins to take place, causing a sharp increase in current.

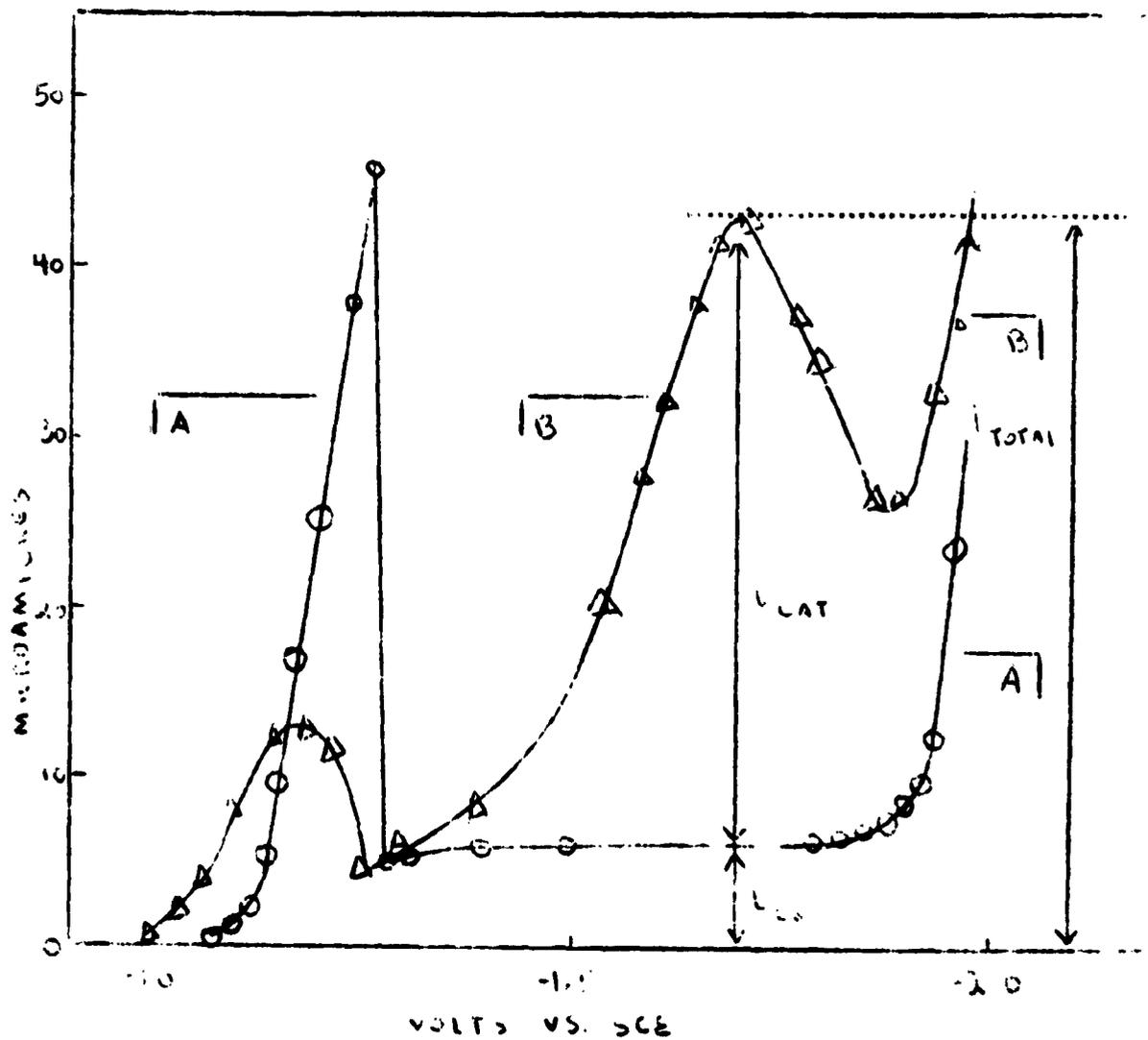
Now if the cobalt(II) solution mentioned above is made 10^{-6} to 10^{-5} M in mercaptan, for example cysteine (CSH), an entirely different current-voltage curve will be obtained. (See Fig. 1B). The decomposition potential of the cobalt(II) ammine complex is shifted to a more positive potential, indicating that cysteine catalyzes the decomposition of cobalt (8). Most notable, however, is the change brought about in the diffusion current region of the cobalt(II) ammine wave.

The relatively constant diffusion current, which was one of the characteristics of the cobalt(II) wave, is replaced by a "peak" or large increase, in

Effects of Cysteine on the Polarogram of Cobalt (II) Sulfate
Polarogram of:

A) 10^{-3} M CoSO_4 , 0.1 M NH_3 , 0.1 M NH_4Cl ○

B) 10^{-3} M CoSO_4 , 0.1 M NH_3 , 0.1 M NH_4Cl , and 10^{-5} M CSH Δ



current, which is many times higher than the diffusion current of cobalt(II).

This peak, or wave begins at about -1.3 volts vs. SCE, passes through a maximum in the range -1.7 to -1.9 volts, then falls off, until hydrogen is evolved. The relative height of the peak or wave (the names will be used interchangeably in this report) is indicated by i_{cat} in Figure 1B, and is determined by subtracting the current due to the reduction of the cobalt(II) ion, i_{Co} from the total current at the peak, i_{total} , or,

$$i_{cat} = i_{total} - i_{Co} \quad (1)$$

The final increase in current at -1.9 volts versus SCE is again due to the evolution of hydrogen.

The results of the first comprehensive study of this peak on the cobalt wave⁽³⁾ indicated that the height of this wave depends on the concentration of the mercaptan and cobalt ion, and is affected by the concentrations of the buffer and any other electrolytes present in the solution. The reaction involved, according to this investigation (3) is the catalytic evolution of hydrogen. Brdicka (3) therefore called these waves "catalytic hydrogen waves".

Brdicka considered two possible mechanisms for hydrogen evolution, involving two different "catalysts". First, the production of a cobalt amalgam, which acts as a catalyst, or, second, the production of an activated form of the mercaptan, which acts as a catalyst.

Ilkovic (7) found that the polarographic wave of cobalt(II) chloride, in the absence of any trace of electrolytes, does not show the normal diffusion current of cobalt, but rather, that the current increases almost linearly with increasing applied potential. Ilkovic explained this effect⁸ due to the evolution of hydrogen, catalyzed by the cobalt amalgam formed. According to Brdicka, this effect (catalytic evolution of hydrogen by means of cobalt amalgam) cannot occur in the case of the catalytic hydrogen waves caused by mercaptans because of the presence of large amounts of electrolytes; in fact, these catalytic waves will not occur in the absence of electrolytes. Ilkovic's

mechanism must also be discarded, according to Brdicka, because cobalt(III) ions do not give the catalytic waves with cysteine, which they should if the formation of an amalgam is necessary.

Later work has cast some doubt on the validity of the cobalt amalgam catalyst theory. It has been found (8) that cobalt(III) ion does indeed give a catalytic wave with cystine.

The mechanism considered by Brdicka (3) is that of the reduction of the mercaptan, or formation of an activated form of mercaptan. According to this theory, the sulfhydryl group (RS^-) is supplied to the electrode-solution interface by some cobalt(II) complex. Once at the interface, the sulfhydryl group is attracted to, and is activated by, the potential gradient near the electrode, the activated sulfhydryl group (RS^{*-}) then acquires a proton from a well buffered solution; it is this proton, attached to the activated sulfhydryl group, which is reduced.



where RSH refers to the general formula of mercaptans. The RS^- formed in reaction (4) rapidly re-cycles through the first step of the mechanism, and the concentration of hydrogen ions near the electrode is maintained by the buffer. The overall reaction is then the decomposition of water.

The main trouble with the mechanism of Brdicka is that it does not explain the role of the cobalt ion; once at the electrode surface, the RS^- is re-cycled through the process, and cobalt ion does not enter into the picture anymore.

In a more recent study, Brezina (1), using the hanging drop mercury electrode, indicates that the formation of a cobalt amalgam is not involved. In his series of experiments, Brezina allowed cobalt amalgam to form on the

mercury drop by electrolyzing for several minutes at -1.1 volts versus SCE before taking the polarogram of the catalytic maximum. It was found that a decrease, rather than an increase, in height of the catalytic wave occurred. Brezina also found that ethylenediaminetetracetic acid could remove the catalytic wave; this is probably due to the effective removal of cobalt from the reaction series by the EDTA.

Brezina reports (1) that certain amino acids, namely histamine, histidine, and uric acid, give catalytic waves, and states that "almost all sulfhydryl compounds give the catalytic effect in cobaltous solution, whereas only a few nitrogen compounds without sulfhydryl groups are catalytically active". Brezina also states that the cobalt(II) and nickel(II) ions form chelates with proteins and amino acids, and in doing so, facilitate the electrode reduction of hydrogen. Since Brezina's theory requires a chelate at the potential at which the maxima occurs, he has to assume that the metal "remains in the catalytically active complex for some time after its reduction". Thus, cobalt in oxidation state zero would have to form a complex which is stable enough to act as a catalyst.

Kalous (8) also studied the catalytic deposition of hydrogen at the hanging mercury electrode, and came to the conclusion that it cannot be excluded that cobalt, reduced from some complex other than the hexamine, takes part in the catalytic reaction.

Fraser, Owen, and Shaw (4), in a study of a series of mercapto-acids, found that only the alpha-mercapto acids gave catalytic waves in the usual sense (a recognizable peak at $10^{-5}M$ concentration), the beta-mercapto acids giving only a poorly defined inflection point under the same conditions. Also, it was reported by Shaw et al. that the presence of an amino group greatly increases the height of the maxima which a compound will produce. For example, these authors found that 3-mercaptopropionic acid does not give a peak, while homocysteine (gamma-mercapto-alpha-aminobutyric acid) gave a wave. These

authors also concluded that a compound would not give a catalytic peak unless it contained a carboxyl group.

Up until this point, all the theories discussed considered the catalytic maxima to be due to the evolution of hydrogen. Weronki (13), however, proved that the catalytic peak was due to an increase in the rate of the reduction of cobalt, not the evolution of hydrogen. Weronki passed equal currents through identical solutions ($10^{-3}M$ cobalt(II) chloride, $10^{-5}M$ cystine, $0.1M$ ammonia, and $0.1M$ ammonium chloride), at two different potentials, -1.35 volts, and -1.65 volts. The only reaction occurring at -1.35 volts is the reduction of cobalt(II) ions while at -1.65 volts, the potential of the catalytic peak, there are two possible reactions. First, the deposition of cobalt alone, or, second, the combined deposition of cobalt and hydrogen. After the passage of equal amounts of electricity through the solutions at the two different potentials, it was found that the cobalt waves were reduced by the same amount in both cases. Thus, the same reaction must have taken place at both potentials i.e., deposition of cobalt. Weronki goes on to suggest that the cobalt ions are probably transported to the electrode surface in a manner similar to that observed in a polarographic maxima, that is, by movement of the solution at the solution-electrode interface.

Stricks and Kolthoff (1) have devised a rotated dropping mercury electrode (RDME), the sensitivity of which is about ten or more times as great as the conventional DME.

This electrode consists, essentially, of a rotated U tube from which mercury droplets are dislodged upwards into the solution. The speed of rotation is between 100 and 300 revolutions per minute.

Currents at this electrode are actually maxima of the second kind, that is, currents caused by motion of the mercury in the drop. As currents at the RDME are usually controlled by stirring, it will be of interest to see whether catalytic waves also occur at this electrode.

MATERIALS AND EQUIPMENT

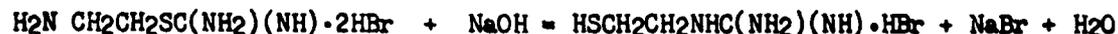
Sargent Model XXI Automatic Recording Polarograph, Constant Temperature Water Bath, Beakers and Pipets of various sizes, Volumetric Flasks, 1000, 500, 100, and 50 ml.

| Reagents | Sources | Molarity of Stock Solution |
|--|------------------------------|----------------------------|
| $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ | Mallinckrodt | 10^{-2} , 10^{-3} |
| Ag_2SO_4 | Mallinckrodt | 10^{-3} |
| HgCl_2 | Merck | 10^{-3} |
| $\text{CoSO}_4 \cdot 7 \text{H}_2\text{O}$ | Mallinckrodt | 10^{-2} |
| $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$ | " | 10^{-2} |
| $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ | G. Frederick Smith Chem. Co. | 10^{-2} |
| $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$ | Mallinckrodt | 10^{-2} |
| NH_4Cl | Baker | 2 |
| KCl | Mallinckrodt | 2 |
| KI | " | 2 |
| KNO_3 | " | 2 |
| NH_3 | du Pont | 5 |
| Gelatin | Atlantic Gelatin Co. | 0.5% |

Mercaptans Used: (all stock solutions of mercaptans being 10^{-3}M).

Aminoethylisothiuronium bromide hydrobromide, $\text{H}_2\text{NCH}_2\text{CH}_2\text{SC}(\text{NH}_2)(\text{NH}) \cdot 2\text{HBr}$
Walter Reed Institute of Research, representation: AET

Note: the compounds aminoethylisothiuronium bromide hydrobromide and aminopropylisothiuronium bromide hydrobromide undergo rearrangement in alkaline medium to form the respective mercaptoalkyl guanidines, mercaptoethyl guanidine and mercaptopropyl guanidine:



Aminopropylisothiuronium bromide hydrobromide - $\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{SC}(\text{NH}_2)(\text{NH}) \cdot 2\text{HBr}$
Walter Reed Institute of Research, representation: APT

Mercaptoethylamine hydrochloride - $\text{HCl} \cdot \text{H}_2\text{NCH}_2\text{CH}_2\text{SH}$
Walter Reed Institute of Research - representation: MEA

2-aminoethanethiosulfuric acid - $\text{H}_2\text{NCH}_2\text{CH}_2\text{SSO}_3\text{H}$
Walter Reed Institute of Research, representation: RSSO₃H

2-aminoethaneselenosulfuric acid - $\text{H}_2\text{NCH}_2\text{CH}_2\text{SeSO}_3\text{H}$

Walter Reed Institute of Research, representation: RSeSO_3H

2-mercaptoethanol - $\text{HOCH}_2\text{CH}_2\text{SH}$

Eastman Organic Chemicals

3-mercaptopropylamine hydrochloride - $\text{HCl}\cdot\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{SH}$

Walter Reed Institute of Research

N-decylaminoethane thiol - $(\text{C}_{10}\text{H}_{21})\text{HNCH}_2\text{CH}_2\text{SH}$

Walter Reed Institute of Research - Note: the sample was not soluble, but gave a colloidal suspension after treatment of 0.1087 g. of the sample with 10 ml. of 1M sodium hydroxide and dilution to 500 ml.

N,N'-bis(mercaptoacetyl) hydrazine - $\text{HSCH}_2\text{CONHNHCOCH}_2\text{SH}$

Walter Reed Institute of Research

Diethyldithiocarbamic acid (sodium salt) - $(\text{C}_2\text{H}_5)_2\text{NCSSNa}\cdot 3\text{H}_2\text{O}$

Eastman Organic Chemicals

L(+) cysteine monohydrochloride monohydrate - $\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOH}\cdot\text{HCl}\cdot\text{H}_2\text{O}$

Pfanstiehl, representation: CSH

3-mercaptopropionic acid - $\text{HSCH}_2\text{CH}_2\text{COOH}$

Eastman Organic Chemicals, representation: PSH

Mercaptoacetic acid, or, thioglycolic acid - HSCH_2COOH

Eastman Organic Chemicals, representation: TSH

Thiomalic acid - $\text{HOOCCHSHCH}_2\text{COOH}$

Evans Chemetics

Thiolactic acid - $\text{CH}_3\text{CHSHCOOH}$ -- Evans Chemetics

Thiosalicylic acid, or, O-hydroxybenzoic acid - $\text{HO C}_6\text{H}_4\text{COOH}$, Evans Chemetics

N,N-dimethylaminoethanethiol hydrochloride - $\text{HCl}\cdot(\text{OH}_3)_2\text{NCH}_2\text{CH}_2\text{SH}$

Evans Chemetics

2-amino-1-pentanethiol - $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CH}_2\text{SH}$

Walter Reed Institute of Research

The characteristics of the DME used are: $m = 1.985$ mg/sec

$t = 4.07$ sec.

$h = 67.8$ cm.

These characteristics were measured in 0.1M potassium chloride, at 25°C , with no potential impressed on the DME.

The characteristics of the RDME used are: $m = 15.28$ mg/sec

$t = 3.9$ sec.

$h = 52.0$ cm.

These characteristics were measured in 0.1M potassium chloride at 25°C, with no potential impressed on the RDME.

PROCEDURES

Preparation of Solutions:

All solutions were prepared with double distilled water. Mercaptan solutions were prepared in water which had been made oxygen free by passing a stream of nitrogen (Linde, 99.996% pure) through for 15 minutes; these solutions were preserved by continuous passage of nitrogen through them. Fresh solutions were prepared daily. These precautions were not taken with the disulfides and the Bunte salts, RSSO_3H and RSeSO_3H , as these are not oxidized by oxygen.

Sample Experiment: Into a beaker were added enough ammonia, ammonium chloride, inert electrolyte (if any was added) gelatin, and water to make the final volume of solution, including cobalt(II) or any other metal ion and mercaptan, exactly 40.00 ml.

The background electrolyte (without metal ion or mercaptan) was deaerated with nitrogen which had been passed through three bottles of ammonia-ammonium chloride solution of the same concentration as that of the solution to be electrolyzed. To the deaerated electrolyte was then added, by means of a pipet, the proper amount of cobalt(II), or any other metal ion solution. The electrolyte-metal ion solution was then deaerated, and the proper amount of oxygen free mercaptan solution was added.

The potential of the DME was varied continuously from 0 to -2.0 volts vs. SCE, and the current was recorded automatically with a Sargent model XXI Polarograph. The current was then measured at the appropriate potentials. The drop time of the electrode and the pH of the solution were measured after each experiment.

RESULTS AND DISCUSSION

The effect on the cobalt(II) ammine wave of the mercaptans and Bunte salts, mercaptoethyl guanidine, mercaptoethyl guanidine, 2-mercaptoethylamine, 2-aminoethanethiosulfuric acid, 2-aminoethaneselenosulfuric acid, 2-mercaptoethanol, 3-mercaptoethylamine, N-decylaminoethane thiol, N,N'-bis(mercaptoacetyl) hydrazine, diethyldithiocarbamic acid, cysteine, 3-mercaptopropionic acid, mercaptoacetic acid, thiomalic acid, thiolactic acid, thiosalicylic acid, N,N-dimethylaminoethane thiol, and 2-amino-1-pentane thiol were investigated.

The compounds listed in Table I gave catalytic maxima at concentrations of $4 \times 10^{-6}M$ or greater, while the other compounds investigated gave catalytic maxima only at concentrations of the order of $10^{-4}M$ or greater. (See Table IV)

The particular effects noted for the eight compounds found to give catalytic maxima will be discussed in the order of conditions varied.

Variation of Concentration of Mercaptan: The polarograms obtained with the various mercaptans listed in Table I are all similar in appearance, and resemble that given in Fig. 1, as well as those determined by Brdicka (3) for cysteine.

The concentration range over which these mercaptans were able to induce the catalytic effect was found to begin at about $10^{-6}M$ and to continue to about $10^{-4}M$.

In general, it was found that peak height varied linearly with concentration of mercaptan over the range $2 \times 10^{-6}M$ to $2 \times 10^{-5}M$, although there is some variation among the compounds themselves. For example, the peak height due to thiomalic acid varies linearly with concentration up to a concentration of $4 \times 10^{-5}M$, while that of CSH was linear only up to $10^{-5}M$. (See Fig. 2).

Concentrations higher than $7 \times 10^{-5}M$ were not investigated in the cases of those compounds which gave a peak at low concentrations because the height of the catalytic peak becomes independent of the mercaptan concentration at these high concentrations; this is illustrated by the levelling off of the concentration versus peak height curve. (See Fig. 2).

Figure 2, Part III

35 A.

Effect of Concentration of Mercaptan on Peak Height

All Solutions: 0.1 M Ammonia, 0.1 M Ammonium Chloride, and Variable

A) N,N-dimethylaminoethane thiol, 10^{-3} M CoCl_2 , and 0.00125% Gelatin

B) 2-amino-1-pentane thiol, 10^{-3} M CoCl_2 , and 0.00125% Gelatin

C) MEA, 10^{-3} M CoSO_4 , and no Gelatin

D) MEA, 10^{-3} M CoSO_4 , and 0.00375% Gelatin

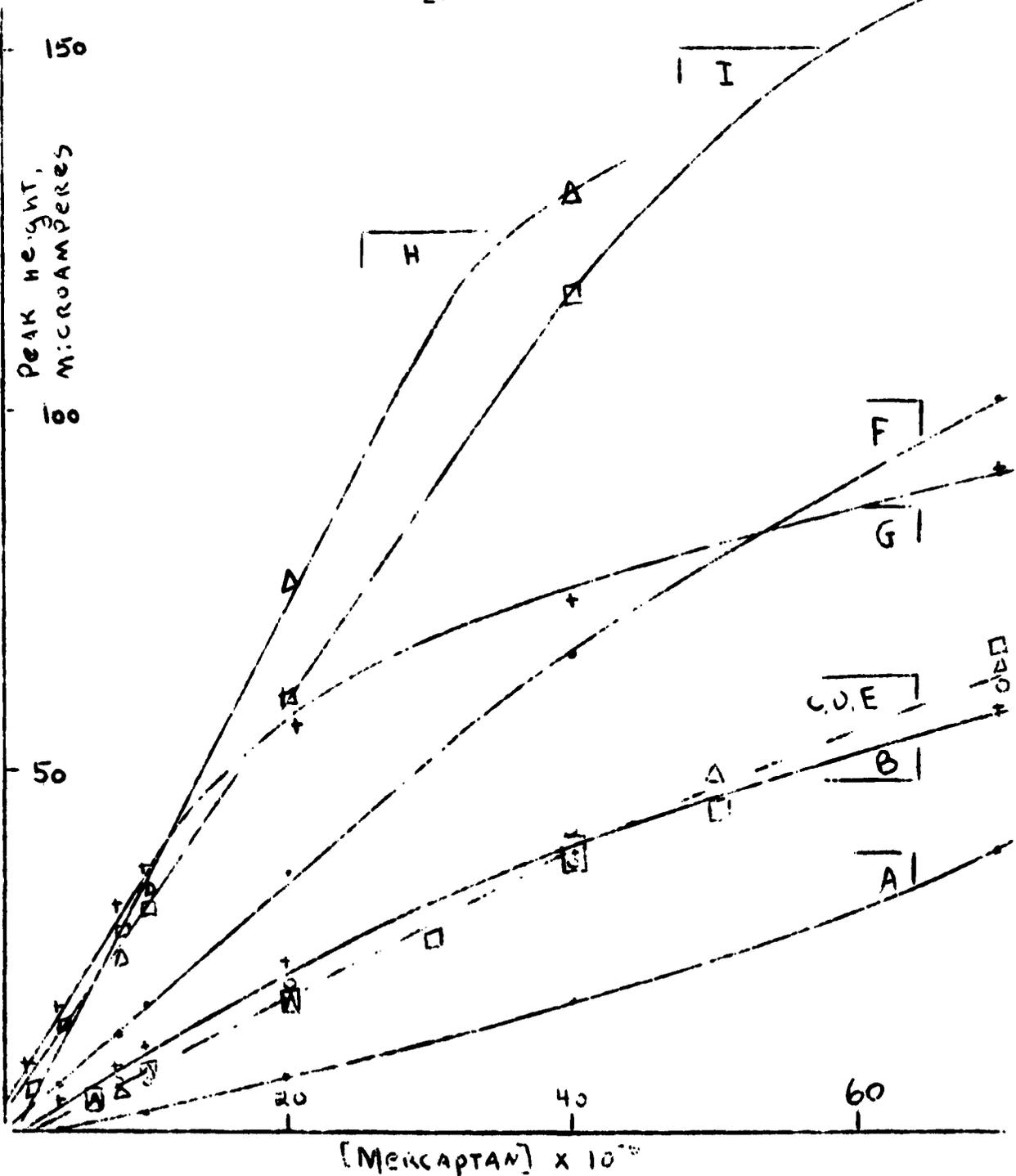
E) RSSO_3H , 10^{-3} M CoSO_4 , and no Gelatin

F) Thiolactic Acid, 10^{-3} M CoCl_2 , and 0.00125% Gelatin

G) CSH, 10^{-3} M CoCl_2 , and 0.00125% Gelatin

H) TSH, 10^{-3} M CoCl_2 , and 0.00125% Gelatin

I) Thiomalic Acid, 10^{-3} M CoCl_2 , and 0.00125% Gelatin



It will be noticed that the graphs of Figure 2 do not pass through the origin as would be expected. This effect was also noted by Brdicka (3) who attributed it to the absorption of mercaptan on the walls of the apparatus.

For a given mercaptan, the potential at which the peak occurs, denoted here as "peak potential", shifts to more negative values as the concentrations of mercaptan increases. (See Table I).

For different mercaptans at the same concentration, the peak potential shifts to more negative values as the height of the wave increases. Thus, at a given mercaptan concentration, the mercaptan which gives the highest peak probably will have the most negative peak potential.

If one combines the trend in the peak potential with variation of mercaptan concentration, and with that of variation of the specific mercaptans, the peak potential becomes more negative as one goes from left to right, or, from top to bottom in Table I. In general, the trends indicate that the higher the peak, the more negative the peak potential.

It happens quite often, because of the large currents involved in catalytic maxima, that the current-voltage curve, as recorded by the automatic instrument is quite irregular. This situation was usually remedied by the addition of a trace (0.00125%) of gelatin. It was found that gelatin, in concentrations of up to 0.00375%, has very little or no effect on the height or peak potential of MEA. (See Table II).

The relatively small effect of gelatin indicates that the process controlling the catalytic maxima is not the same as that in other polarographic maxima, since polarographic maxima are usually reduced in height by the presence of trace amounts of gelatin.

It should also be mentioned that the anion of the cobalt salt (either sulfate or chloride) has no observable effect on the height or peak potential of the peak of MEA. (See Fig. 2, curves C and D).

It was found that the height of the peaks produced by RSSO_3H and MEA are

Table II.

Effect of Gelatin on the Height of the Catalytic Maximum of MEA

All Solutions: $10^{-3}M$ $CoSO_4$, $0.1M$ NH_3 , $0.1M$ NH_4Cl , and Various MEAConcentrations in the Absence of Gelatin and in the
Presence of 0.00375% Gelatin.

| MEA X 10^{-6} | 4 | 6 | 10 | 20 | 30 | 40 | 50 | 70 |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| ua, no Gelatin | 2.4 | 4.6 | 8.2 | 18.8 | 29.4 | 38.0 | 49.6 | 63.7 |
| st... volts | -1.60 | -1.64 | -1.64 | -1.69 | -1.72 | -1.74 | -1.76 | -1.77 |
| ua, 0.00375% Gelatin | 3.0 | 4.4 | 8.2 | 18.0 | 27.4 | 39.4 | 45.8 | 65.6 |
| st... volts | -1.64 | -1.64 | -1.66 | -1.70 | -1.70 | -1.74 | -1.75 | -1.78 |

quite similar. The Bunte salt can be reduced to MEA, the proposed reaction being (12)



the halfwave potential of this reaction being about -1.1 volts versus SCE. Thus, RSSO_3H is converted to MEA at the electrode surface, and the MEA is the actual species involved in the catalysis. The amount of MEA present at the electrode surface will be controlled by the differences in the rate of diffusion of RSSO_3H to the electrode surface and the rate of diffusion of MEA away from the surface. Because of the similarity of MEA and RSSO_3H , and probable small difference in diffusion coefficients, the rate of diffusion to and from the electrode will be about equal. It follows then that the system with RSSO_3H near the electrode surface can be treated the same as if the bulk of the solution were a solution of MEA of the same concentration as the actual concentration of RSSO_3H . Thus, provided that the diffusion coefficients are nearly equal, MEA and RSSO_3H should give catalytic waves of the same height.

Concluding, it can be seen that the catalytic maxima depends on the species of mercaptan at the electrode surface, and not on the species in the bulk of the solution.

It is of interest to note that experiments with 2-aminoethaneselenosulfuric acid (RSeSO_3H) revealed that this compound does not give a catalytic effect. The effect thus seems to be specific for sulfur compounds.

The formulas of the compounds found to give catalytic waves are given in Table III.

The most interesting and obvious difference in behavior of the compounds investigated can be seen by comparing the data for 2-mercaptoethylamine and thioglycolic acid with that of the next highest homologs, 3-mercaptoethylamine and 3-mercaptoacetic acid. At a concentration of $2 \times 10^{-5}\text{M}$, the first two compounds give peaks of 18.8 and 76.8 μs , respectively, while at the same

Table III.

Formulas of the Mercaptans Found to Give Catalytic Peaks:

| | |
|---|--------------------------------|
| $\text{H}_2\text{NCH}_2\text{CH}_2\text{SH}$ | 2-mercaptoethylamine |
| $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CH}_2\text{SH}$ | 2-amino-1-pentane thiol |
| $\text{H}_2\text{NCH}_2\text{CH}_2\text{SSO}_3\text{H}$ | 2-aminoethanethiosulfuric acid |
| $(\text{CH}_3)_2\text{NCH}_2\text{CH}_2\text{SH}$ | N,N-dimethylaminoethane thiol |
| $\text{HOOCCH}(\text{NH}_2)\text{CH}_2\text{SH}$ | cysteine |
| HOOCCH_2SH | thioglycolic acid |
| $\text{CH}_3\text{CHSHCOOH}$ | thiolactic acid |
| $\text{HOOCCHSHCH}_2\text{COOH}$ | thiomalic acid |

concentration, the second two do not even cause any irregularities in the cobalt diffusion wave.

The compounds which give a catalytic wave all have one thing in common with each other, and one thing different from the compounds which do not give a catalytic wave, and that is an ethyl "stem" with a mercaptan group on the number one carbon atom, and a carboxyl or an amino group on the number two carbon atom, respectively. The compounds 2-mercaptoethylamine and thioglycolic acids are of this form. On the other hand, the compounds 3-mercaptopropylamine and 3-mercaptopropionic acid do not give a catalytic peak, and they do not have the ethyl stem with sulfhydryl and amino or carboxyl groups on adjacent carbon atoms.

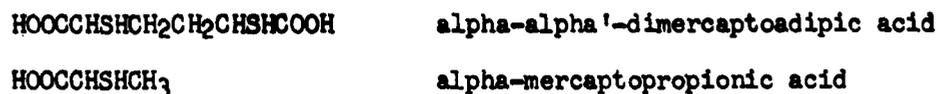
The addition of a -COOH group increases the height of the catalytic peak. For example, the catalytic peak of cysteine (stem plus -COOH) is three times as high as that of 2-mercaptoethylamine (plain stem), the heights being 57.2 and 18.8 μ a respectively, for solutions which were $2 \times 10^{-5}M$ in mercaptan, $10^{-3}M$ in cobalt(II), 0.1M in ammonia and ammonium chloride, and 0.00125% in gelatin.

This increase in current caused by the addition of a carboxyl group or an amino group is in agreement with the results published by Frazer, Owen, and Shaw (4), and Brezina (1).

Frazer, Owen, and Shaw found that all the alpha-mercapto acids investigated, namely alpha-mercaptopropionic acid, mercaptosuccinic acid, alpha-mercapto-glutaric acid, alpha-mercaptoadipic acid, and alpha-alpha'-dimercaptoadipic acid all gave catalytic peaks in the range -1.8 to -1.9 volts vs. SCE. This study (4) also showed that the beta-mercapto acids gave only a slight inflection point at -1.8 volts vs. SCE.

It is also of interest that the alpha-alpha'-dimercaptoadipic acid (4) which has two clusters of functional groups, each having one sulfhydryl group and one carboxyl group in close proximity, gave a wave which was about twice

as high as that of alpha-mercaptopropionic acid, which has just one such cluster. The structural formulas of these compounds are:



These authors (4) state that homocysteine (gamma-mercapto-alpha-aminobutyric acid) gives curves almost identical to those of cysteine. From these data, the authors (4) concluded that the presence of the amino group greatly increases the sensitivity of the catalyzed reaction of the mercapto acids.

In the second study (1) it was found that the compounds histamine, histidine, and uric acid (none of which contain an -SH group) gave catalytic maxima, although these maxima were considerably lower than those compounds containing an -SH group.

Our results, and those of Frazer, Owen, and Shaw, and Brezina are in agreement. The data of all three studies indicate that the amino group not only increases the sensitivity of the catalysts, as in (4) but can actually bring about the reaction. For example, 3-mercaptopropionic acid does not give a wave, whereas cysteine (2-amino-3-mercaptopropionic acid) does. All three of these studies indicate a general rule, and that is; the species causing the catalytic peak contains at least two or three different types of functional groups, the possible groups being the sulfhydryl, amino, and carboxyl groups. If the compound contains only two functional groups, they must be in the alpha position to each other, as in the case of 2-mercaptoethylamine. If the species contains more than two of the three possible functional groups, these groups need not be in the alpha position, but can be further apart from each other, especially apart from the mercapto group, as in homocysteine (alpha-amino-gamma-mercaptobutyric acid).

As a qualitative rule governing the relation between the height of the catalytic wave and the structure of the compounds, it can be stated that the more carboxyl, amino, and sulfhydryl groups the compound contains, and the

closer these groups are together in the molecule, the higher will be the catalytic peak.

Apparently, the stability and kind of complex formed between cobalt and the sulfhydryl compound greatly affects the height of the catalytic peaks.

The behavior of four mercaptans which do not give waves at low concentrations (10^{-6} to $10^{-5}M$), namely mercaptoethyl guanidine, 3-mercaptopropionic acid, 3-mercaptopropylamine, and thiosalicylic acid were studied at higher (up to $10^{-3}M$) concentrations. It was found that all four compounds gave rise to a catalytic maxima if the concentrations were high enough. (See Table IV). The study of the compounds which give waves at high concentration only was limited to the investigation of the effect of mercaptan concentration on peak height. The effects noted are described in the following paragraphs.

The effect of concentration of AET on the height of the catalytic maxima cannot be determined because the wave is small, and merges with the normal hydrogen wave at an AET concentration of $7.5 \times 10^{-4}M$.

3-mercaptopropionic acid gives a catalytic wave whose height varies linearly with concentration over the range 7×10^{-5} to $6 \times 10^{-4}M$; at higher concentrations, the height of the peak becomes independent of concentration of thiosalicylic acid.

The peak potentials of the catalytic waves of this second group of substances was found to shift to more negative values as the concentration increased. This phenomena is similar to that observed for substances which give catalytic waves at low ($10^{-5}M$) concentration.

It is interesting to note that 3-mercaptopropylamine gives rise to two catalytic waves. This behavior is similar to that of AET in the presence of hexaminocobaltichloride, to be discussed later. (See Figures 9, 10 for a comparison).

Table IV.

Mercaptans Which give Catalytic waves at High Concentration Only.

Heights of the Catalytic Waves and "Peak Potentials" at Various Mercaptan Concentrations.

All Solutions: $10^{-3}M$ $CoCl_2$, $0.1M$ NH_3 , $0.1M$ NH_4Cl , and 0.00125% Gelatin.

1. Mercaptoethyl guanidine

| | | | | |
|------------------------|-------|-------|------|--|
| $[RSH] \times 10^{-5}$ | 2.50 | 50.0 | 75.0 | |
| ua | 3.0 | 5.6 | | |
| a at... volts | -1.44 | -1.42 | | |

2. 3-mercaptopropionic acid

| | | | | | |
|------------------------|-------|-------|-------|-------|-------|
| $[RSH] \times 10^{-5}$ | 6.855 | 9.753 | 19.59 | 39.17 | 58.76 |
| ua | 2.8 | 4.4 | 8.8 | 18.2 | 28.8 |
| at... volts | -1.82 | -1.82 | -1.80 | -1.88 | -1.90 |

3. Thiosalicylic acid

| | | | | | | | |
|------------------------|-------|-------|-------|-------|-------|-------|-------|
| $[RSH] \times 10^{-5}$ | 6.95 | 13.9 | 33.5 | 66.9 | 99.3 | 198.6 | 297.9 |
| ua | 1.6 | 3.4 | 6.2 | 8.6 | 8.0 | 8.2 | 9.0 |
| at... volts | -1.54 | -1.56 | -1.54 | -1.56 | -1.54 | -1.56 | -1.58 |

4. 3-Mercaptopropylamine

| | | | | | |
|--------------------------|---|-------|-------|-------|-------|
| $[RSH] \times 10^{-5}$ | 2 | 3.986 | 10 | 19.48 | 39.86 |
| ua, first Step .. | 0 | 0 | 1.8 | 2.0 | 6.6 |
| at... volts, first wave | - | - | -1.60 | -1.40 | -1.38 |
| ua, Second Step | 0 | 0 | 0 | 5.8 | 22.6 |
| at... volts, second wave | - | - | - | -1.64 | -1.68 |

Variation of Ammonia and Ammonium Chloride Concentration:

The effect of the concentration and composition of the buffer on the height of the catalytic peaks of RSSO_3H , CSH , and TSH were noted. The results are given in Figures 3 and 4.

Examination of Figure 3 shows that although there is some vertical separation of the graphs of the compounds because of their "inherent" ability to cause waves of different heights, the similarities of the trends of the three compounds is unmistakable.

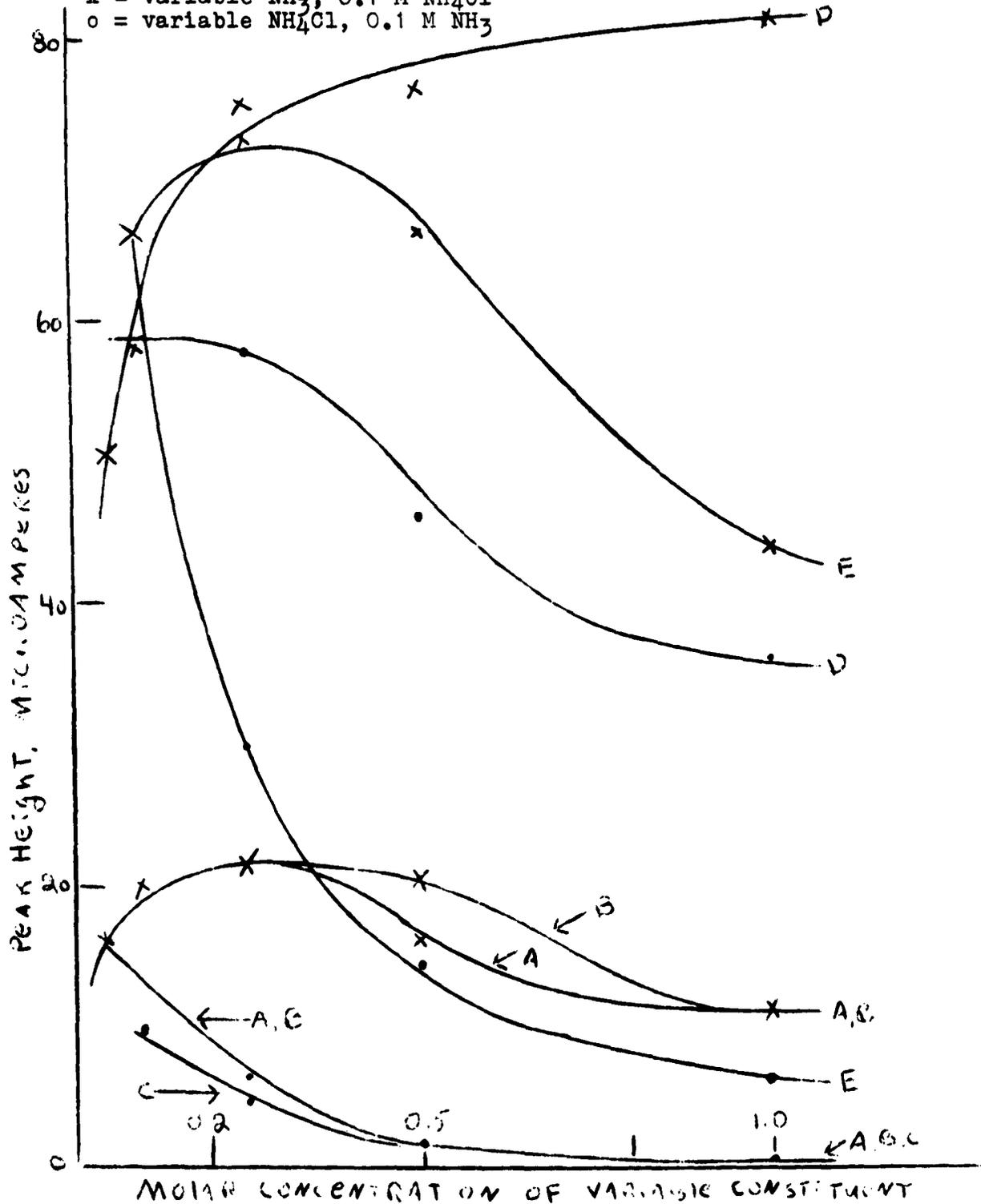
Increases in ammonium chloride concentration, at constant ammonia, cobalt (II) chloride, and mercaptan concentrations, causes a decrease in the height of the catalytic peaks of CSH , TSH , and RSSO_3H , at ammonium chloride concentrations greater than 0.1M. Ammonium chloride concentrations lower than 0.1M were not investigated because of precipitate formation, probably $\text{Co}(\text{OH})_2$, which occurred in 0.05M ammonium chloride, 0.1M ammonia. The decrease of the peak height due to ammonium chloride concentration is most noticeable for TSH , (see Fig. 3, curve E), the peak height being reduced from 65 to 30 μa by a change of 0.1 to 0.25M ammonium chloride concentration. The peak of RSSO_3H is also severely reduced by increasing ammonium chloride concentration (see Fig. 3, curves A, B, C), the peak being effectively removed by 1.0M ammonium chloride.

The peak due to CSH is not as severely reduced as those of TSH and RSSO_3H , especially at low concentrations. It was seen that the peak height of TSH was more than halved by a change from 0.1 to 0.25M ammonium chloride; that of CSH is reduced from 58.4 to 57.6 μa for the same concentration change.

It can be seen from Figure 3 that there is some buffer concentration which will give maximum height to the catalytic waves. Comparing and compromising the data for the three compounds investigated, this optimum buffer concentration was found to be near 0.1M ammonia and 0.1M ammonium chloride.

Effect of Variation of Ammonia and Ammonium Chloride Concentration on Peak Height.

- A) 2×10^{-5} M RSSO_3H , 10^{-3} M CoSO_4 , and no Gelatin
 - B) 2×10^{-5} M RSSO_3H , 10^{-3} M CoSO_4 , and 0.00125% Gelatin
 - C) 2×10^{-5} M RSSO_3H , 10^{-3} M CoCl_2 , and 0.00125% Gelatin, Ionic Strength 1 (constant)
 - D) 2×10^{-5} M GSH , 10^{-3} M CoSO_4 , and 0.00125% Gelatin
 - E) 2.044×10^{-5} M TSH , 10^{-3} M CoCl_2 , and 0.00125% Gelatin
- x = variable NH_3 , 0.1 M NH_4Cl
 o = variable NH_4Cl , 0.1 M NH_3



It was suspected by Brdicka that the effect of buffer concentration was not strictly a concentration effect, but also, a p^H effect. The effect of p^H on peak height can best be seen by re-plotting the data used to construct Figure 3 on axes of p^H versus peak height, as was done in Figure 4. However, only in one case (Figure 4, curve B) was the ionic strength kept constant, and the other curves, therefore, represent data at varying p^H and varying ionic strength. Inspection of Figure 4, curve C, shows that there is a definite trend in peak height with change in p^H in the case of CSH, at variable ionic strength, and $RSSO_3H$ at constant ionic strength (the ionic strength being adjusted to unity by addition of the proper amount of potassium chloride), the height of the wave increasing with increase in p^H .

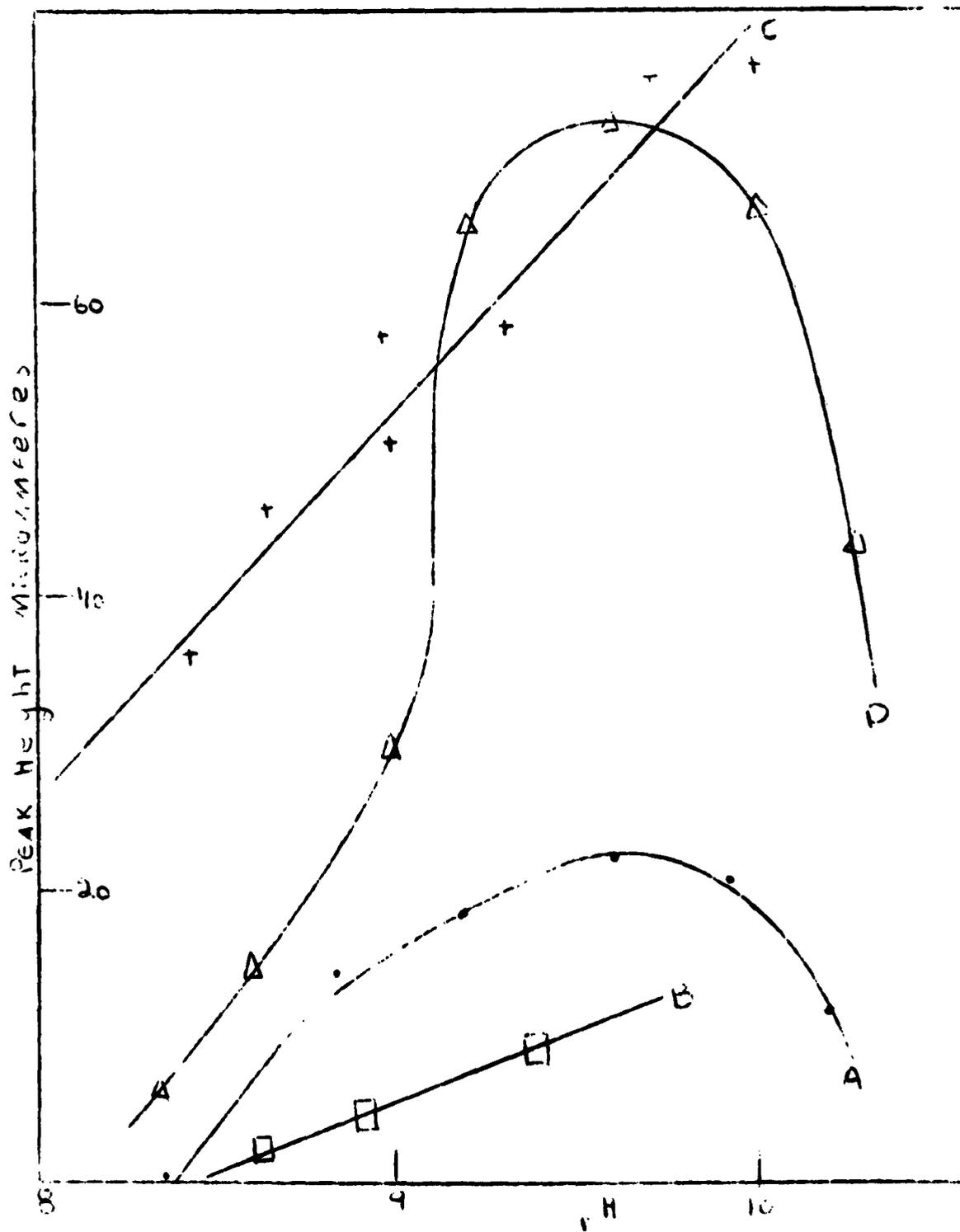
The results for $RSSO_3H$ and TSH, both at variable ionic strength, are less conclusive. The heights of the waves of these two compounds increase with increasing p^H up to p^H 9.6, and then decrease with further increase in p^H .

Effect of Other Electrolytes: Potassium Chloride, Potassium Iodide, and Potassium Nitrate on the Peak of $RSSO_3H$.

It was found that the height of the peak of $RSSO_3H$ was reduced by about 50% by the addition of potassium chloride or iodide at a concentration of 0.9 M. The height of the peak of $RSSO_3H$ was also reduced to about 3% of the "optimum" value (in 0.1 M ammonia and 0.1 M ammonium chloride) when the ammonium chloride concentration was increased from 0.1 to 1.0. It can be seen from this that at constant

Effect of pH on Peak Height

- A) 2×10^{-5} M RSSO_3H , 10^{-3} M CoCl_2 , and 0.00125% Gelatin
 B) 2×10^{-5} M RSSO_3H , 10^{-3} M CoCl_2 , and 0.00125% Gelatin, $\mu = 1$
 C) 2×10^{-5} M CSH , 10^{-3} M CoSO_4 , and 0.00125% Gelatin
 D) 2.044×10^{-5} M TSH , 10^{-3} M CoCl_2 , and 0.00125% Gelatin



ionic strength, the height of the catalytic wave is affected not only by the electrolyte, but also by the pH.

The catalytic peak of RSSO_3H was hardly affected by low (10^{-5} , $2 \times 10^{-5}\text{M}$) concentration of potassium nitrate. The addition of an amount of nitrate ion equimolar to the RSSO_3H present merely reduced the peak height by about 2% of the optimum value in the absence of nitrate ion. The chief interference due to nitrate is caused by the reduction of nitrate ion (8) which occurs at a potential near the peak potential of the catalytic wave. (See Figure 5). If the concentration of nitrate is high enough (0.1 M), its cathodic current will completely mask any other waves which occur at a similar potential, that is, nitrate ion present in this concentration will entirely eliminate the catalytic peak.

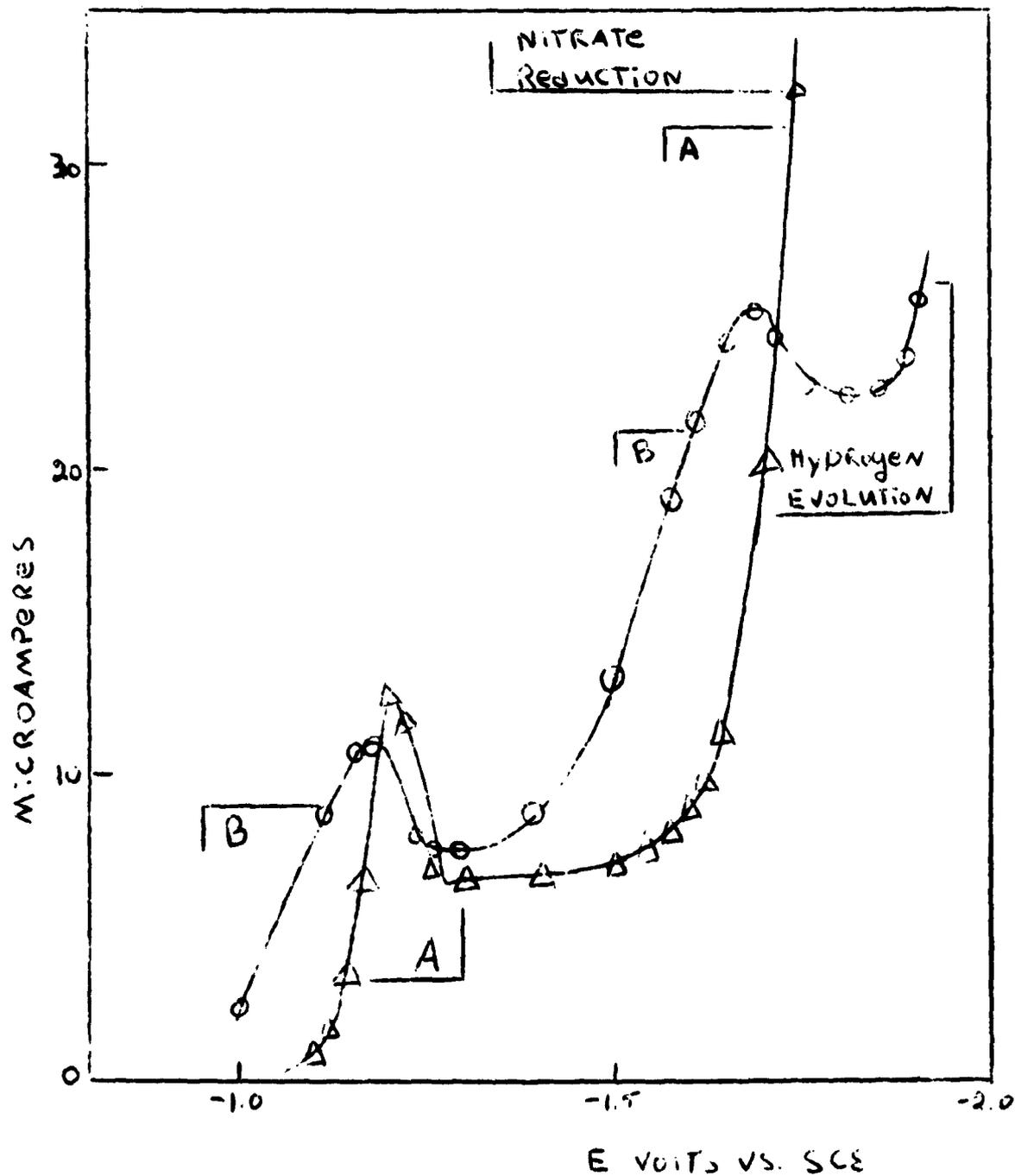
Effect of Metals on the Height of the Catalytic Maxima; Effect of Cobalt and Nickel.

The effect of concentration of cobalt (II) sulfate on the height of the MEA peak, and the effect of concentration of cobalt (II) chloride and nickel (II) chloride on the height of the RSSO_3H peak were investigated, and graphs of peak height versus concentration of metal were prepared. (See Figure 6).

Upon examination of Figure 6, it can be seen that the effect of cobalt (II) sulfate on the MEA wave, and the effect of cobalt (II) chloride on the RSSO_3H wave are quite similar; this is not surprising, as the species (probably) involved in the reaction, namely Co^{++} and MEA are the same in both cases.

Effect of Nitrate Ion on the Shape of Polarograms

- A) Polarogram of 10^{-3} M CoSO_4 , 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, and 0.1 M KNO_3
- B) Polarogram of 10^{-3} M CoSO_4 , 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, 2×10^{-5} M RSSO_3H , and 0.1 M KCl



The curve indicating concentration of cobalt (II) versus peak height is a smooth, almost linear, curve over the concentration range 10^{-4} to 10^{-3} M. A well defined peak could not be obtained at concentrations lower than 10^{-4} M cobalt (II), while the height of the peak seems to become independent of concentration at concentrations greater than 10^{-3} M.

The variation of peak height with concentration of nickel (II) chloride shows the same trend as the variation of peak height with the concentration of cobalt (II) chloride, an increase with increasing concentration in the range 10^{-4} to 10^{-3} M, and independence of peak height from concentration of nickel (II) at concentrations greater than 10^{-3} M, as reflected by the levelling off of the peak height versus concentration curve. (See Figure 6).

The greatest difference noted in the behavior of these two metals is in their "catalyzing ability". A given concentration of cobalt (II) will cause a peak which will be two to two and one half times as high as the peak caused by the same concentration of nickel (II).

Also of interest is the less than additive nature of the "catalyzing ability" of the metal ions, cobalt (II) and nickel (II). For example, it can be seen in Figure 6, that a solution 10^{-3} M in cobalt (II) chloride, 4×10^{-5} M in RSSO_3H , 0.1 M in ammonia, 0.1 M ammonium chloride, and 0.00125% in gelatine will give a peak height of 42 ua, and the same solution, and 10^{-3} M in nickel (II) chloride will give a peak of 17 ua. From this, it could be expected that the same solution, 10^{-3} M in both cobalt (II) chloride and nickel

Effect of Concentration of Cobalt and Nickel on Peak Height

- A) All Solutions: 4×10^{-5} M MEA, 0.1 M NH_3 , 0.1 M NH_4Cl , and various CoSO_4 Concentrations
 B) All Solutions: 4×10^{-5} M RSSO_3H , 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, and various CoCl_2 Concentrations
 C) All Solutions: 4×10^{-5} M RSSO_3H , 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, and various NiCl_2 Concentrations

Points: All Solutions: 4×10^{-5} M RSSO_3H , 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, and
 $X_1 \cdot 10^{-3}$ M CoCl_2 , 10^{-3} M NiCl_2 , $\text{Co}^{+2} + \text{Ni}^{+2} = 2 \times 10^{-3}$ M
 $X_2 \cdot 10^{-3}$ M CoCl_2 , 5×10^{-4} M NiCl_2 , $\text{Co}^{+2} + \text{Ni}^{+2} = 1.5 \times 10^{-3}$ M
 $X_3 \cdot 5 \times 10^{-4}$ M CoCl_2 , 5×10^{-4} M NiCl_2 , $\text{Co}^{+2} + \text{Ni}^{+2} = 10^{-3}$ M

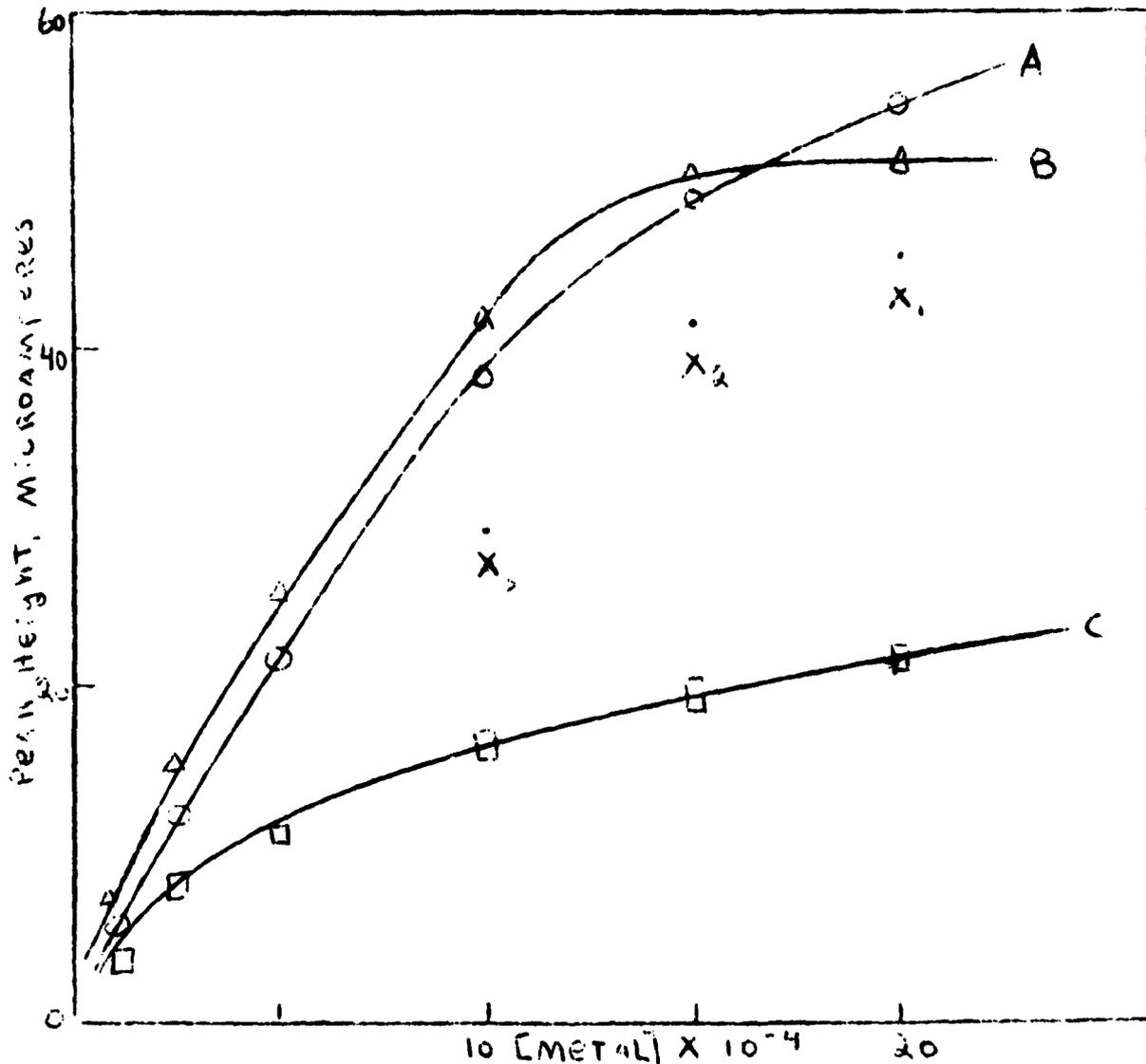


Table V.

Effect of Other Electrolytes on the Height of the Catalytic Maxima of RSSO_3H .

All Solutions: 2×10^{-5} M RSSO_3H , 10^{-3} M CoSO_4 , 0.1 M NH_3 , 0.1 M NH_4Cl and Various Amounts of Other Electrolytes

| | | | | | | |
|---------------------------------|-------|-------|-------|------------|-------|-------|
| $[\text{KCl}]$ | 0 | 0 | 0 | 0 | 0 | 0.90 |
| $[\text{KI}]$ | 0 | 0 | 0 | 0 | 0.90 | 0 |
| $[\text{KNO}_3] \times 10^{-5}$ | 0 | 1 | 2 | 10^4 | 0 | 0 |
| ua | 20.0 | 19.8 | 19.6 | No wave | 10.8 | 4.4 |
| At...volts | -1.70 | -1.70 | -1.72 | - | -1.70 | -1.70 |

(II) chloride would give a peak of $42 + 17$, or, 69 ua. The observed peak height of a solution of this composition is 46 ua. Actually, the height of the wave produced by this solution (10^{-3} M in both cobalt (II) and nickel (II)) is closer to the height of the peak produced by a solution which is 2×10^{-3} M in cobalt (II) chloride (51 ua). This observation, along with the previous one that peak height becomes independent of cobalt or nickel concentration at concentrations greater than 10^{-3} M, seems to indicate that the total metal ion concentration has an optimum value at around 10^{-3} M.

Effect of Metals on the Height of the Catalytic Maxima: Effect of Metals Other than Cobalt and Nickel.

The effect of copper (II) sulfate, mercury (II) chloride, and silver (I) sulfate on the catalytic peaks were determined. It was found necessary to plot the data on a logarithmic scale because of the large concentration ranges investigated (2×10^{-6} to 8×10^{-4} M). The data are given in Figures 7 and 8.

Examination of the data (Figures 7 and 8) indicates that the effect of a given metal is almost the same on the two mercaptans investigated. This can be seen from the very similar shapes of the curves of Figures 7 and 8.

Although all three of the metal ions under consideration form complexes with mercaptans, it was not expected that these metals would decrease the height of the catalytic peak. The reason for this is that the reaction



Figure 7, Part III
Effect of Metals on Peak Height of the Catalytic CoCl_2 -CSH Peak

Polarograms of 10^{-3} M CoCl_2 , 0.1 M NH_3 , 0.1 M NH_4Cl , and (A) 0.00125% Gelatin, (B) 0.00375% Gelatin

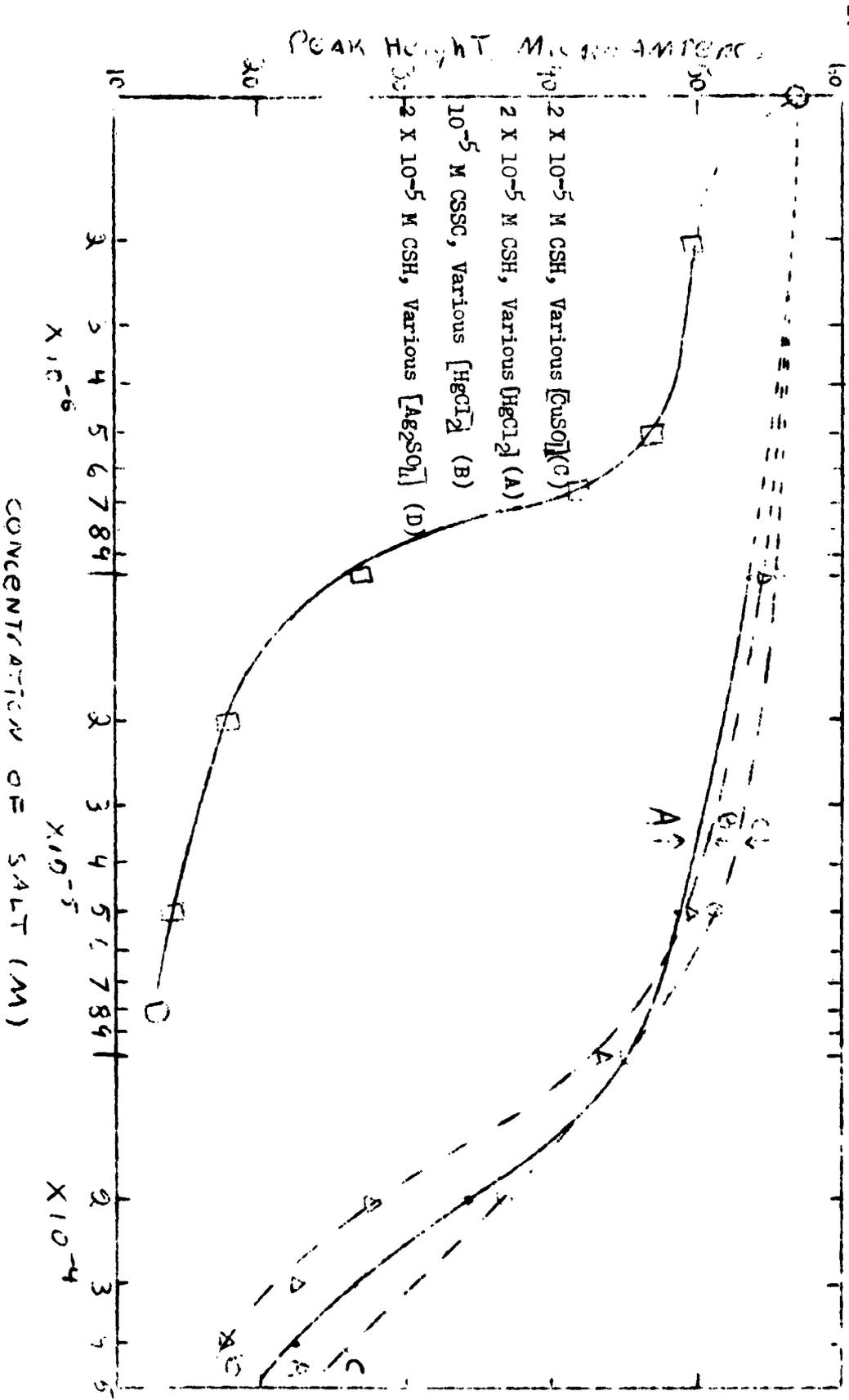
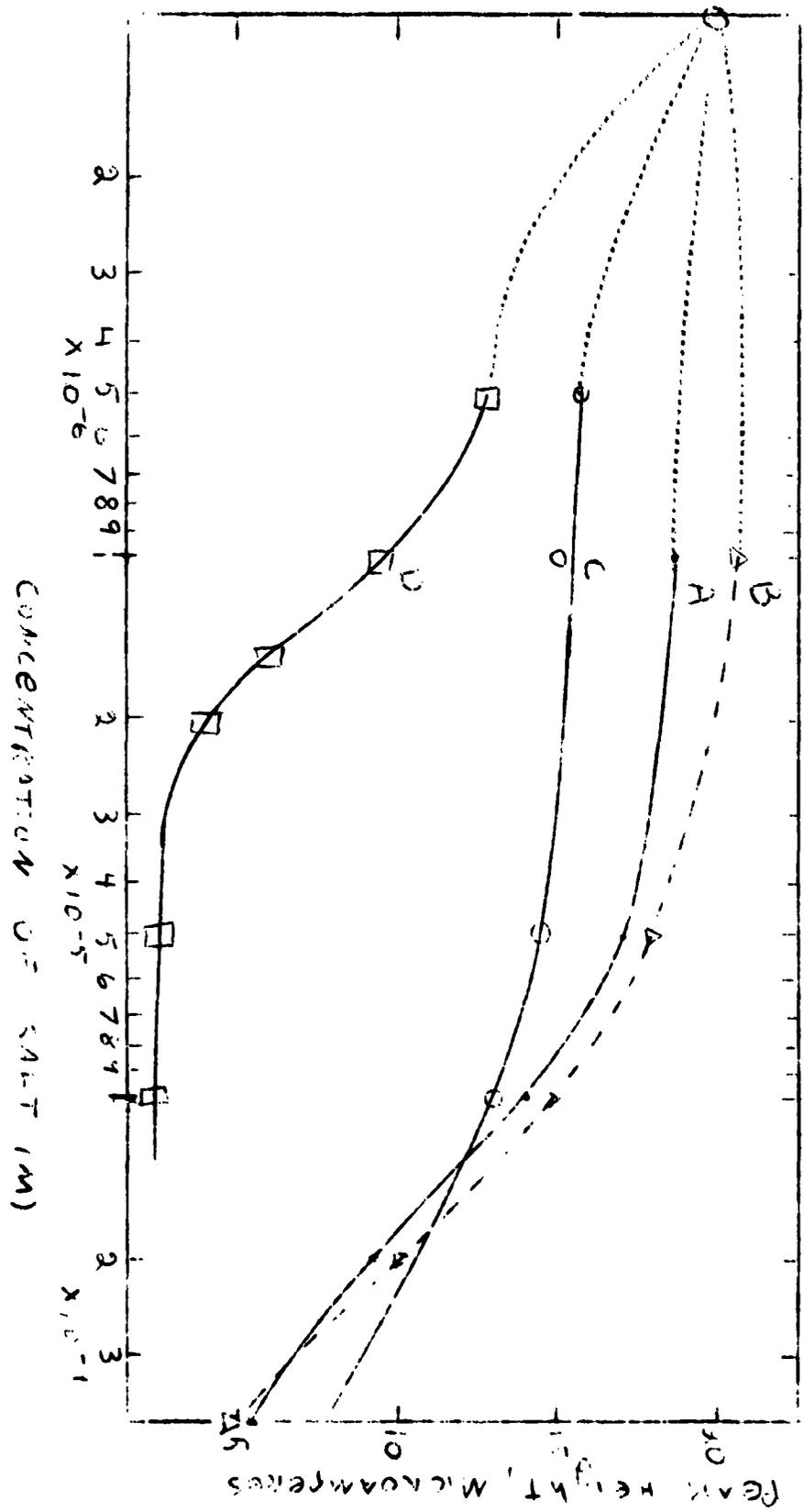


Figure 8, Part III

B. Effect of Metals on Peak Height of the Catalytic CoCl_2 -MEA and RSSO_3H Peaks
 50 Polarograms of 10^{-3} M CoCl_2 , 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, and

- (A) 2×10^{-5} M MEA, and various $[\text{HgCl}_2]$
- (B) 2×10^{-5} M RSSO_3H , and various $[\text{HgCl}_2]$
- (C) 2×10^{-5} M MEA, and various $[\text{CuSO}_4]$
- (D) 2×10^{-5} M MEA, and various $[\text{Ag}_2\text{SO}_4]$



where M-S-R represents the metal complex, M the free metal, and RS⁻ the mercaptan, occurs at a potential more positive than that necessary to cause the catalytic maxima. In this case, the uncomplexed mercaptan would be present at the electrode surface, and should still be able to cause the catalytic peak. This expectation, however, was not realized.

The suppressing effect of the metal ions investigated on the catalytic peaks does not appear to depend on complex formation in the bulk of the solution. Thus, the lowering in peak height for the Bunte acid, RSSO₃H, and for the disulfide, CSSC, which do not form complexes with the metals, is the same as the lowering of the peak height for the mercaptans MEA and CSH, which do form complexes.

As already mentioned, complex formation at the electrode surface cannot be involved because the complexes are reduced at the negative potential at which the catalytic maxima occur, and hence, the complexes could not exist at the electrode surface. In general, it may be said that the metal ions copper (II), mercury (II), and silver (I) reduce the height of the catalytic waves, the effect of silver (I) being greater than that of copper (II) and mercury (II), which are about equal in suppressive effect.

The reason for the decrease of the catalytic peaks in the presence of these metals appears to be the displacement of cobalt by the metals at the surface of the electrode, cobalt being the only metal which is responsible for the catalytic effect.

Catalytic Peaks observed in Alcoholic Medium:

Polarograms were taken of solutions which were 10^{-3} M in cobalt (II) chloride, 0.1 M in ammonia, 0.1 M in ammonium chloride, 0.00125% in gelatin, and of various mercaptain concentration. (See Table VI).

The heights and the peak potentials of the waves were of the same order of magnitude in water and in 75% ethyl alcohol.

This observation casts some doubt on Brdick's theory according to which only catalytic discharge of hydrogen ions is responsible for the appearance of the waves. If the catalytic discharge of hydrogen ions were responsible, such a drastic change in solvent would be expected to produce a change in peak height and potential.

Catalytic Maxima in the Presence of Cobalt (III) Chloride:

It had been previously noted (Table IV) that both mercaptoethyl guanidine and 3-mercaptopyrrolamine give poorly defined waves in the presence of cobalt (II) chloride, even at high (2.5×10^{-4} M) concentration. (See Figures 9 and 10 for a comparison).

In contrast to this, it has been found that both of these substances give well defined, two step catalytic maxima in the presence of 10^{-3} M hexaminocobalt (III) chloride, in the concentration range 5×10^{-5} to 3×10^{-3} M. See Tables VII and VIII).

The height of the catalytic peaks of LET in the presence of cobalt (III) ion were found to increase almost linearly with concentration at concentrations up to 1.2×10^{-3} , and then become independent

Catalytic Maxima of AET

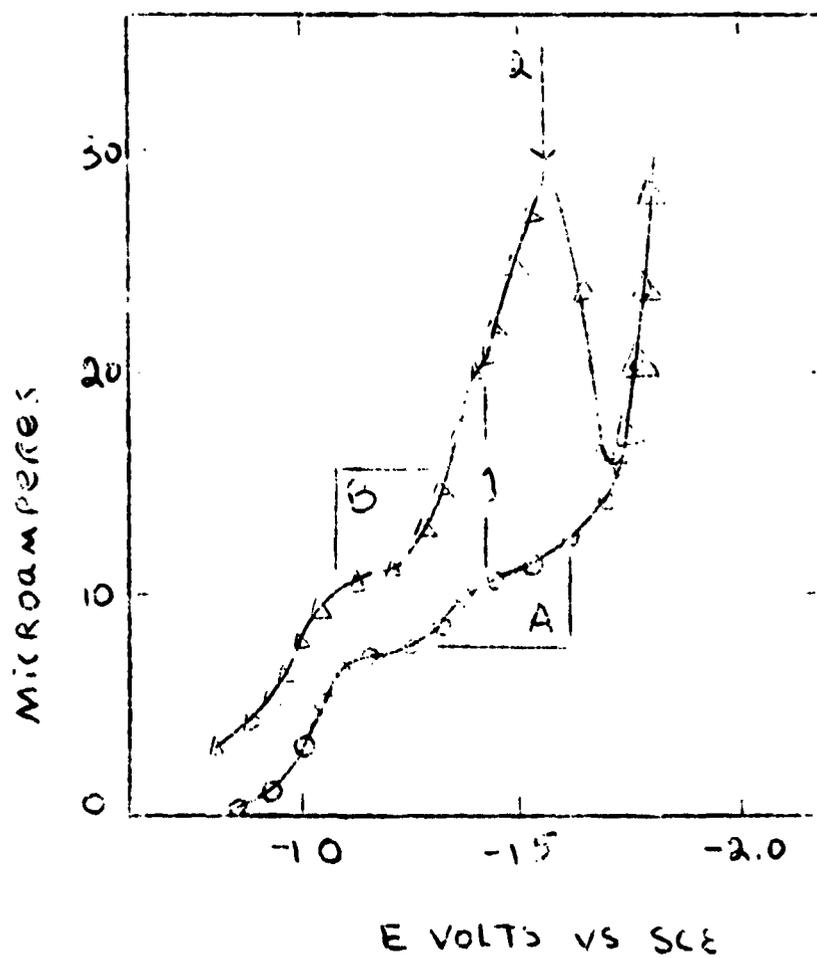
All Solutions 2.5×10^{-4} M AET, 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, and

A) 10^{-3} M CoCl_2

B) 10^{-3} M $\text{Co}(\text{NH}_3)_6\text{Cl}_3$

(1) First Peak

(2) Second Peak



Catalytic Maxima of 3-mercaptopropylamine

Both Solutions: 1.948×10^{-4} M 3-mercaptopropylamine, 0.1 M NH_3 ,0.1 M NH_4Cl , 0.00125% Gelatin, andA) 10^{-3} M CoCl_2 B) 10^{-3} M $\text{Co}(\text{NH}_3)_6\text{Cl}_3$

(1) First Peak

(2) Second Peak

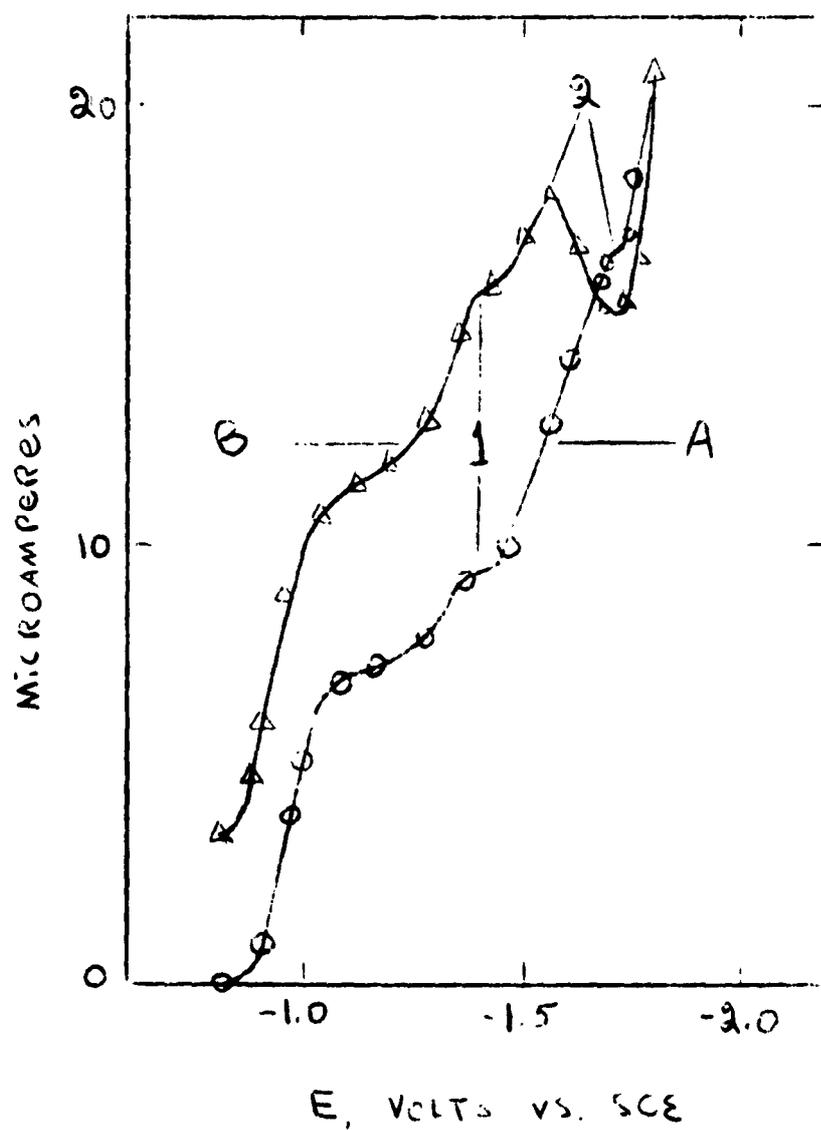


Table VI.

Catalytic Waves Observed in Alcoholic Medium.

All Solutions: 75% ethanol, 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125%
Gelatin 10^{-3} M CoCl_2 , and Various Mercaptan
Concentrations.

| Mercaptan | RSSO ₃ H | CSH | TSH | AET | R _{IV} SH | DDTC |
|----------------------------|---------------------|-------|-------|-------|--------------------|---------|
| [RSH] X 10 ⁻⁵ | 2 2 ⁽²⁾ | 2 | 4 | 2 7 | 2 7 | 2 7 |
| ua, 75% ethanol | 19.8 11.4 | 61.6 | 61.2 | - 2.0 | - - | - - |
| at...volts, 75% ethanol | -1.62 -1.56 | -1.81 | -1.87 | - - | - - | (1) (1) |
| ua, in 100% water | 21.4 - | 57.2 | 76.8 | - - | - - | - - |
| at...volts, 100% water | -1.68 - | -1.77 | -1.89 | - - | - - | - - |

Hyphen "-" indicates no wave

(1) Cobalt wave very irregular in the presence of DDTC

(2) Cobalt concentration = 5×10^{-4} M, not 10^{-3} M.

of AET concentration at higher concentration, a behavior similar to that of the mercaptans which give catalytic waves with cobalt (II) chloride at low (10^{-5} M) concentration. (See Table VII).

The first catalytic wave becomes indistinguishable from the second wave at AET concentrations equal to or higher than 5×10^{-4} M.

It is seen in Table IV that the catalytic wave of the AET-cobalt (II) system merges with the normal hydrogen wave at a concentration of 7.5×10^{-4} M, and that the peak potentials and the height of the first wave in the AET-cobalt (III) systems and those of the only wave in the AET-cobalt (II) systems both at 2.5×10^{-4} M AET, 0.1 M AET, 0.1 M ammonia, 0.1 M ammonium chloride, and 0.00125% gelatin are -1.41 and -1.44 volts, respectively, while the heights are 3.0 and 0.4 μ a, respectively. Thus, although the peaks are of considerably different height, the similarity of the peak potentials is striking.

3-mercaptopyrrolamine was found to give rise to two catalytic maxima in the presence of both cobalt (III), (one at -1.40 volts, the other at -1.58 volts) and cobalt (II), (one at -1.40 volts, the other at -1.70 volts. (See Table VII and Figure 9).

The height of both waves, and the sum of the two waves, of 3-mercaptopyrrolamine varies linearly with concentration of mercaptan at low (10^{-3} M or lower) concentration, but becomes independent of concentration at higher concentration.

The effect of variation of the height of the mercury column on

Table VII.

Effect of Variation of Concentration of .ET on the Catalytic Peak.

All Solutions: 10^{-3} M $\text{Co}(\text{NH}_3)_6$, 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125%

Gelatin, and the .ET concentrations Given Below.

| $\frac{[\text{RSH}]}{x 10^{-5}}$ | First Wave | | Second Wave | | Total Wave |
|----------------------------------|------------|-----------|-------------|-----------|------------|
| | Ua | at..Volts | ua | at..Volts | ua |
| 5.0 | 2.6 | -1.40 | 1.6 | -1.56 | 4.2 |
| 10.0 | 4.66 | -1.41 | 3.4 | -1.57 | 8.0 |
| 25.0 | 9.4 | -1.41 | 8.6 | -1.57 | 18.0 |
| 50.0 | (1) | | 34.4 | -1.61 | 34.4 |
| 80.0 | | | 48.4 | -1.64 | 48.4 |
| 120.0 | | | 64.0 | -1.64 | 64.0 |
| 300 | | | 104 | -1.74 | 104 |

(1) The first wave merged with the second wave at concentration equal to or greater than 5×10^{-4} M .ET.

Table VIII.

Effect of Variation of Concentration of 3-Mercaptopropylamine on the Catalytic Peak.

All Solutions: 10^{-3} M $\text{Co}(\text{NH}_3)_6\text{Cl}_3$, 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin, and the 3-Mercaptopropylamine Concentrations Given Below.

| $[\text{RSH}]$ $\times 10^{-5}$ | First Wave | | Second Wave | | Total Wave |
|------------------------------------|------------------------|----------|-------------|----------|------------|
| | ua | at-volts | ua | at-volts | ua |
| 5.0 | 1.0 | -1.38 | 0.5 | -1.56 | 1.5 |
| 10.0 | 1.7 | -1.36 | 1.5 | -1.56 | 3.2 |
| 19.5 | 3.8 | -1.37 | 2.8 | -1.56 | 6.6 |
| 39.0 | 7.1 | -1.40 | 5.9 | -1.59 | 13.0 |
| 60.0 | 10.6 | -1.42 | 9.1 | -1.60 | 19.7 |
| 80.0 | 13.1 | -1.41 | 11.3 | -1.60 | 24.4 |
| 120.0 | 17.9 | -1.41 | 16.1 | -1.60 | 34.1 |
| 300.0 | only one wave observed | | 50.0 | -1.62 | 50.0 |

the heights of the catalytic peaks of the cobalt (III)-AET and cobalt (III)-3-mercaptopropylamine systems was studied.

It was found that the value of $i/h^{1/2}$ for the first peak of both the mercaptoethyl guanidine and 3-mercaptopropylamine systems was a constant. (See Tables IX and X). This indicates that the electrode reaction producing the first catalytic peak is not kinetically controlled. In view of the high mercaptan concentrations, it seems possible that this first "catalytic peak" is in reality the polarographic wave of a cobalt-mercaptan complex.

The values of $i/h^{1/2}$ for the second peaks of both the cobalt (III)-mercaptoethyl guanidine and 3-mercaptopropylamine systems are not constant, increasing as concentration increases. This variable $i/h^{1/2}$ indicates a kinetically controlled current.

The value of $i/h^{1/2}$ for the combined waves of both systems are also included in Tables IX and X. These values merely indicate the sum of the constant value for the $i/h^{1/2}$ of the first peak, and the variable value for the second peak.

Catalytic Waves at the Rotated Dropping Mercury Electrode (RDME)

The chief concern of this series of experiments was to see whether or not catalytic waves similar to those obtained at the DME could be obtained at the RDME.

Table IX.

Effect of Variation of the Height of the Mercury Column on the Height of the Catalytic Peak of Mercaptoethyl Guanidine.

All Solutions: 10^{-3} M $\text{Co}(\text{NH}_3)_6 \text{Cl}_3$, 10^{-4} M AET, 0.1 M NH_3 , 0.1 M NH_4Cl , and 0.00125% Gelatin.

| H cm | $H^{\frac{1}{2}}$ $\text{cm}^{\frac{1}{2}}$ | First Wave | | | Second Wave | | | Total Wave | |
|---------|--|------------|-------|----------------------|-------------|-------|----------------------|------------|----------------------|
| | | ua | at..V | $ua/H^{\frac{1}{2}}$ | ua | at..V | $ua/H^{\frac{1}{2}}$ | ua | $ua/H^{\frac{1}{2}}$ |
| 48.1 | 6.95 | 3.8 | -1.42 | 0.547 | 2.2 | -1.58 | 0.317 | 6.0 | 0.864 |
| 62.3 | 7.90 | 4.5 | -1.41 | 0.570 | 3.3 | -1.57 | 0.418 | 7.8 | 0.988 |
| 67.8 | 8.23 | 4.6 | -1.40 | 0.559 | 3.4 | -1.57 | 0.413 | 8.0 | 0.972 |
| 76.0 | 8.72 | 4.8 | -1.41 | 0.551 | 4.1 | -1.57 | 0.470 | 8.9 | 1.021 |
| 89.5 | 9.46 | 5.8 | -1.41 | 0.550 | 4.6 | -1.58 | 0.486 | 9.8 | 1.036 |
| 101.6 | 10.14 | 5.4 | -1.40 | 0.532 | 5.3 | -1.58 | 0.522 | 10.7 | 1.054 |

Table X.

Effect of Variation of the Height of the Mercury Column on the Height of the Catalytic Peak of 3-Mercaptopropylamine.

All Solutions: 10^{-3} M $\text{Co}(\text{NH}_3)_6 \text{Cl}_3$, 8×10^{-4} M 3-Mercaptopropylamine
 0.1 M NH_3 , 0.1 M NH_4Cl , 0.00125% Gelatin.

| H cm | $H^{\frac{1}{2}}$ $\text{cm}^{\frac{1}{2}}$ | First Wave | | | Second Wave | | | Total Wave | |
|---------|--|------------|-------|----------------------|-------------|-------|----------------------|------------|----------------------|
| | | ua | at..V | $ua/H^{\frac{1}{2}}$ | ua | at..V | $ua/H^{\frac{1}{2}}$ | ua | $ua/H^{\frac{1}{2}}$ |
| 48.1 | 6.95 | 12.0 | -1.42 | 1.72 | 7.9 | -1.60 | 1.14 | 19.9 | 2.86 |
| 62.3 | 7.90 | 13.2 | -1.43 | 1.67 | 9.3 | -1.60 | 1.18 | 22.5 | 2.85 |
| 67.8 | 8.23 | 13.1 | -1.41 | 1.59 | 11.3 | -1.60 | 1.37 | 24.4 | 2.97 |
| 76.0 | 8.22 | 14.2 | -1.42 | 1.63 | 13.4 | -1.60 | 1.56 | 27.6 | 3.19 |
| 89.5 | 9.46 | 15.1 | -1.42 | 1.60 | 14.6 | -1.61 | 1.54 | 29.7 | 3.24 |
| 101.6 | 10.14 | 15.9 | -1.43 | 1.57 | 15.3 | -1.61 | 1.51 | 31.2 | 3.08 |

Two experiments were carried out at the RDME, using CSSC and cobalt (II) sulfate. The polarograms obtained are given in Figures 11 and 12 below, along with similar curves obtained from solutions of different concentrations at the DME, for comparison.

Figure 11 clearly indicates the greater sensitivity of the RDME. The two graphs of Figure 11 were obtained from two solutions whose cobalt (II) concentrations were in a ratio of ten to one, and whose CSSC concentrations were in a ratio of 2.5 to one, yet, the peaks are of comparable height.

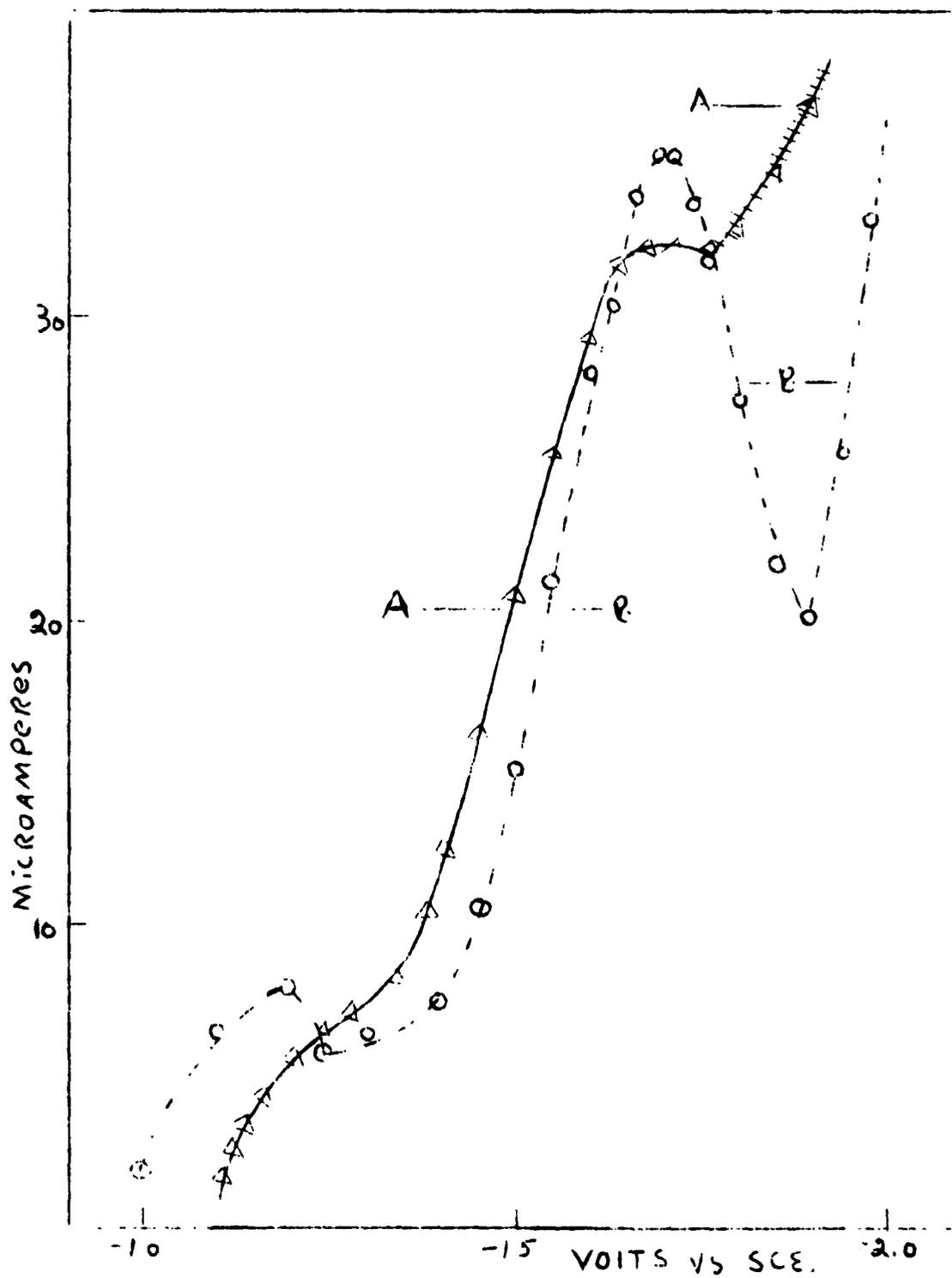
It should be noted (See Figure 11) that the current-voltage curves measured at the RDME becomes irregular in the potential range -1.8 to -2.0 volts versus SCE, as indicated by the crossmarked line. This irregularity is due to changes of the electrode surface caused by hydrogen evolution.

Figure 12 shows the shape of the catalytic peak of MEA in the presence of 0.010% gelatin, the wave being quite regular at this gelatin concentration. A polarogram of a cobalt (II) - MEA system is included for comparison.

Preliminary investigations thus show that catalytic maxima occur at the RDME as well as at the DME, the RDME being about ten times as sensitive as the DME.

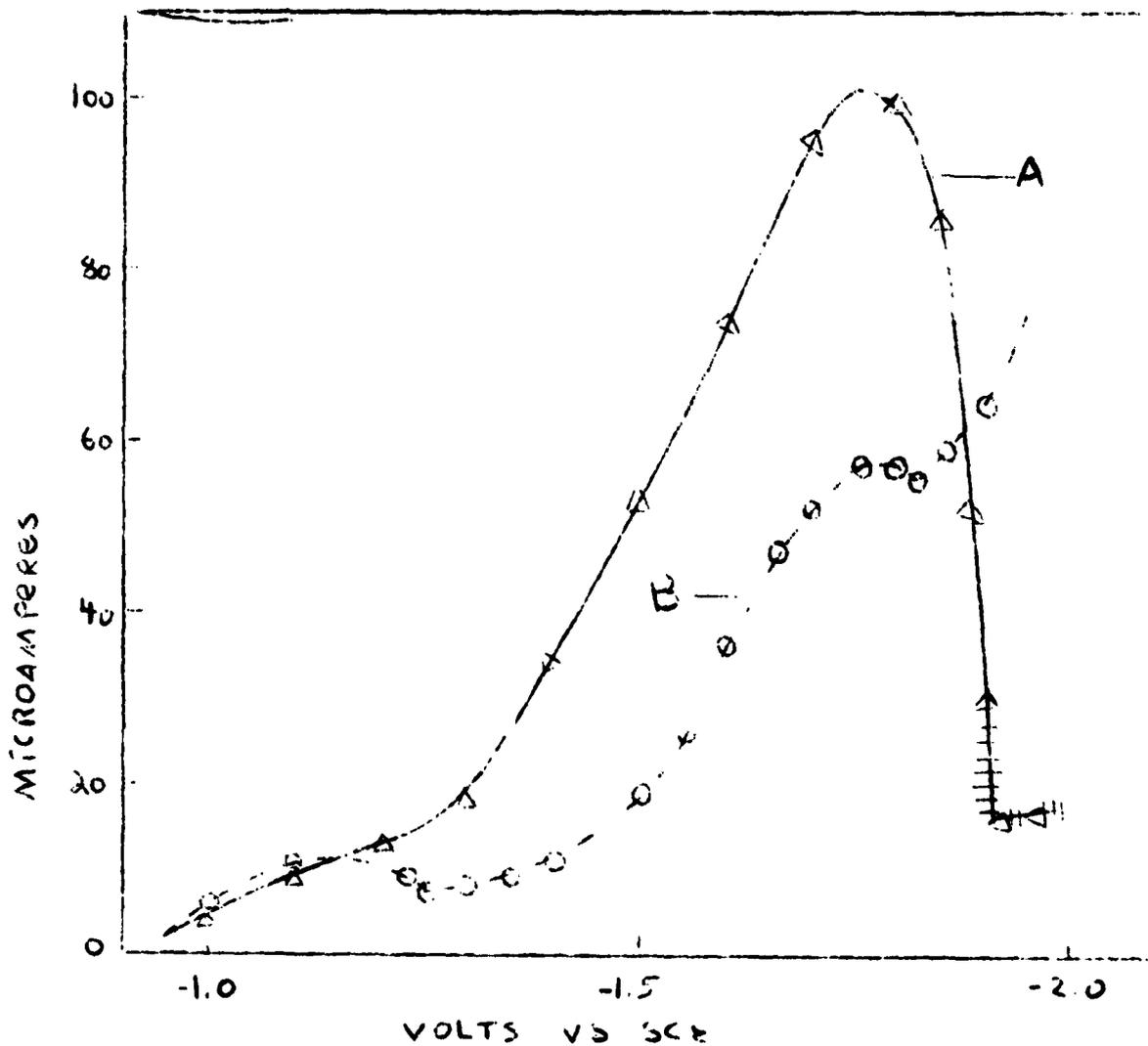
Comparison of the Height of the Catalytic Peak of CSSC at the R.D.M.E. and at the D.M.E.

- A) 10^{-4} M CoSO_4 , 2×10^{-6} M CSSC, 0.1 M NH_3 , 0.1 M NH_4Cl , and no Gelatin, at the R.D.M.E.
- B) 10^{-3} M CoSO_4 , 2×10^{-6} M CSSC, 0.1 M NH_3 , 0.1 M NH_4Cl , and 0.00375% Gelatin at the D.M.E.



Comparison of the Height of the Catalytic Peak of MEA at the D.M.E.
and at the R.D.M.E.

- A) 2.5×10^{-4} M CoSO_4 , 5×10^{-5} M MEA, 0.1 M NH_3 , 0.1 M NH_4Cl , and 0.01000% Gelatin at the R.D.M.E.
- B) 10^{-3} M CoSO_4 , 5×10^{-5} M MEA, 0.1 M NH_3 , 0.1 M NH_4Cl , and no Gelatin, at the D.M.E.



basic stem seems to increase the catalyzing ability of the mercaptan. For example, cysteine gives a higher wave than 2-mercaptoethylamine, and thiomalic acid gives a higher wave than mercaptoacetic acid.

The two carbon atoms seem to be very specific. For example, 2-mercaptoethylamine and mercaptoacetic acid give waves at low (10^{-5} M) concentrations, while 3-mercaptopropylamine and 3-mercaptopropionic acid do not.

The relation between concentration and peak height for a given mercaptan was found to be almost linear in the range 2.5×10^{-5} M down to zero; this system then seems to offer some possibilities for quantitative analysis, once the proper calibration curves have been determined.

Disulfides and thiosulfates are indirectly able to produce a catalytic peak if they can react at the electrode to form a mercaptan which gives a catalytic peak. The active species in this case is the mercaptan formed at the electrode.

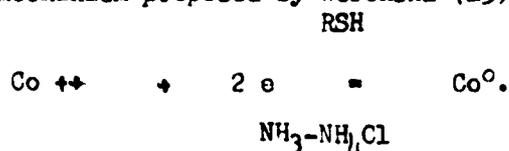
The height of the catalytic peak is apparently dependent on both p^H and ionic strength, the highest peaks being obtained with solutions 0.1 M in both ammonia and ammonium chloride for most mercaptans, with a trace of gelatin present to minimize current irregularities. The presence of substances whose reduction waves may mask the catalytic peak, such as nitrate, should be avoided.

The height of the catalytic peak increases almost linearly with concentration of cobalt (II) and nickel (II) at concentrations less

than 10^{-3} M. The "catalyzing ability" of cobalt (II) and nickel (II), in solutions containing both, is not strictly additive.

The compounds mercury (II) chloride, copper (II) sulfate, and silver (I) sulfate all have the ability to lower catalytic peaks, the effect being most marked for silver (I) sulfate.

In view of the fact that the metal-mercaptan complexes formed would be unstable at the electrode surface at potentials where the catalytic peaks occur, it may be said that the lowering of the catalytic peaks by these metal ions is not caused by complex formation, either in the bulk of the solution, or, at the electrode surface. Catalytic peaks were observed in 75% alcoholic medium. The height of the wave was suppressed not more than 10%, and the peak potential was virtually the same, as in 100% aqueous medium. This data supports the mechanism proposed by Weronki (13), i.e.,



The substances mercaptoethyl guanidine and 3-mercaptoethylamine, which do not give catalytic peaks with cobalt (II) chloride, give well defined, two step, catalytic peaks at concentrations as low as 5×10^{-5} M in the presence of hexamino-cobalt (III) chloride. Preliminary investigations indicate that the first of these two waves, that is, the one which occurs at the more positive potential, is not a kinetic wave, but rather, is diffusion controlled.

Catalytic waves were also observed at the rotated dropping mercury electrode.

The two proposed mechanisms for the reaction which produces the catalytic peaks of the cobalt-sulfhydryl system, namely those of Brdicka and Weronski, do not fully explain the data which have been collected. The Brdicka theory, involving the catalyzed evolution of hydrogen, neglects to explain the role played by the cobalt or nickel ion, while the Weronski theory neglects the role played by the mercaptan.

It is reasonable to assume that a cobalt maximum can occur only if the cobalt occurs at the electrode in the form of a mercaptide of the proper structure and composition.

The reduction process of the cobalt or nickel at the electrode, and therefore the peak, seems to be highly affected by the nature of this complex. This explains our observations that only certain mercaptans which fulfill the conditions for the formation of the "proper" complex, and at only the proper ammonia and ammonium chloride concentrations, allow the full development of the peak.

Although the phenomena involved cannot be explained yet in a quantitative way, experiments of the kind reported in this report are helpful in the qualitative deduction of the mechanisms involved in the catalytic peaks.

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First Year

Polarographic studies of several mercaptoalkyl compounds show that these compounds behave in a similar way as mercaptans studied previously, such as cysteine or reduced glutathione. The polarographic behavior of the disulfides differs to a greater extent. Guanidinoethyldisulfide behaves in the same way as oxidized glutathione, while the other disulfides studied are reduced irreversibly at the dropping mercury electrode. The dissociation constants of the silver mercaptides of the three mercaptans in question are of the same order of magnitude as that of silver cysteinolate. The same was found for the dissociation constants of the cuprous mercaptides. The studies of the equilibrium reactions between disulfides and sulfite reveal that the equilibrium constants of the reactions of the three disulfides compare favorably with the corresponding constant for the reaction between cystine and sulfite.

Second Year

Amperometric mercurimetric titrations of mercaptans such as mercaptoethyl guanidine, mercaptopropyl guanidine and mercaptoethyl sine indicate that these compounds form well defined complexes with mercury in the stoichiometric ratio: two mercaptans to one mercury. The reactions between mercaptans and silver are much more complicated and the kind and stability of the silver complex formed depends on the functional groups in the mercaptan and on the experimental conditions.

2-aminoethanesulfonic acid (RSSO_3H) and 2-aminoethaneselenosulfonic acid (RSsSO_3H) are both reduced irreversibly at the dropping mercury electrode, RSSO_3H giving one wave while

RSeSO_3H exhibits a two wave polarogram. The products of the electrochemical reduction of the two compounds are sulfite and mercaptan (RSH) and sulfite and hydrogen selenide (RSeH) respectively. In acid medium sulfite is reduced at the dropping mercury electrode, thus giving rise to an increase in the height of the thio- and selenoacid waves at acid pH.

The catalytic effects of various mercaptans, disulfides, and thiosulfates in ammoniacal cobalt solutions have been studied at the dropping mercury electrode. The ability of a mercaptan to give a catalytic wave depends largely on the structure of the mercaptan and on the kind and number of functional groups, such as amino or carboxyl groups. Disulfides are reduced at the electrode and also give catalytic waves, the height of which depends on the nature of the mercaptan produced at the electrode. 2-aminoethanethiosulfuric acid is also reduced at the dropping electrode and the height of its catalytic wave is the same as that of mercaptoethylamine, which is one of the reduction products of the 2-aminoethanethiosulfuric acid. At a cobalt concentration less than 10^{-3} M the height of the catalytic peak is almost proportional to the cobalt concentration. Variation of the ammonia and ammonium chloride concentrations indicate that the highest catalytic peaks can be obtained with a buffer which is 0.1 M in both ammonia and ammonium chloride. The presence of metal ions such as mercury, copper and silver in the solution decreases the height of the peaks, the effect being most pronounced for silver. Catalytic waves are also observed in a medium containing 75% ethyl alcohol. These observations support the assumption that the catalytic peaks are rather the result of an excessive cobalt deposition than that of hydrogen evolution at the electrode.

B) Significance of Results

In order to understand the mechanism of action of anti-radiation drugs it is necessary to study first some of the physical chemical properties of these compounds. These properties can then be compared with the prophylactic activities of these drugs.

With this aim in mind the polarographic studies of several antiradiation drugs have been performed and it was found that although the mercaptans behave alike a remarkable difference exists in the polarography of their disulfides.

Since the action of these drugs may be affected by their reaction with biologically important trace metals, a study of the interaction of such metals with mercaptan was initiated with the copper mercaptan system. Studies of mercaptan-mercury systems resulted in the development of a rapid and accurate method for the quantitative determination of mercaptans and disulfides.

The catalytic mercaptan waves were studied for two reasons. Under favorable experimental conditions these waves can be applied to the quantitative determination of traces of mercaptans, disulfides, and thiosulfates. Still more important is the application of the catalytic waves to studies on the interaction between radiation protective drugs and proteins which also give catalytic waves. This may be best illustrated by an example. Preliminary experiments on the ultraviolet irradiation of crystallized horse serum albumin indicated that the catalytic waves of this protein markedly decrease and even disappear upon prolonged ultraviolet irradiation. When albumin is irradiated in the presence of mercaptoethylguanidine, which does not give catalytic waves at the concentrations used, the height of the catalytic albumin wave remains unchanged upon irradiation. This

seems to indicate that mercaptoethylguanidine precludes denaturation of albumin through ultraviolet irradiation. Catalytic waves thus represent a new approach to the study of denaturation problems.

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5, 1963

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