average of the total number of oxygen molecules absorbed until time t and an average of the total number of cuts that have occurred until time t. A scission ratio would be more exactly defined by the ratio of the *rate* of oxygen absorption at a given time to the *rate* of scission at the same time. The present calculations, which are sufficiently accurate for the data at hand, represent an average value of this scission ratio.

In other studies on pure unvulcanized natural rubber, Farmer and Sundralingham[29] have observed scission ratios as high as 75 during photo-autoxidation at 35°. The number of cuts in this case was measured by the decrease in molecular weight of the polymer during oxidation in benzene solution.

It would be of great importance to ascertain whether the scission reaction can be directly associated with any of the steps in the autoxidation chain reaction, *e. g.*, initiation, propagation, or termination, or whether it is to be regarded as a side reaction. Our results are not sufficient to completely elucidate the nature of the scission reaction, which will be achieved only if more kinetic studies and more chemical evidence as regards the nature of the oxidation products become available. On the other hand, the observed fact that the scission ratio is close to unity under conditions of maximum rate, but larger than unity in the thermal reaction at relatively low temperatures should be of aid in delimiting the possible scission reactions.

For example, if chain scission were to occur in every initiation step (*i. e.*, as a result of hydroperoxide decomposition) then the scission ratio would be an exact measure of the oxidation chain length. If this were true, then during a typical autocatalytic oxidation the scission ratio should be high at first and approach unity as the maximum rate is approached. We have not observed this to occur under the specified conditions of the present studies.

(29) E. H. Farmer and A. Sundralingham, *J. Chem. Soc.*, 125 (1943).

Summary

Experimental data on the relationship between moles of oxygen absorbed and moles of chain cleavage during oxidation of three dimensional macromolecular hydrocarbons in the range 50–130° are presented. For the six different natural and synthetic rubbers studied, it is found that the ratio of oxygen absorbed to chain cleavages is close to unity when a steady rate of oxidation is observed. Under conditions of autoxidation, however, up to 30 moles of oxygen per mole of chain cleavage are found. An expression is derived to obtain the total number of chain cleavages from stress relaxation measurements.

BROOKLYN 2, N. Y. RECEIVED JUNE 28, 1949

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA]

Argentometric Amperometric Titration of Cysteine and Cystine

BY I. M. KOLTHOFF AND W. STRICKS

Introduction

In the present studies it has been shown potentiometrically, polarographically and by solubility experiments that cysteine forms one or more compounds with silver ions in which the silver is bound very strongly. The chemistry of these compounds is unknown. From preliminary work which will be presented in a subsequent paper it appears to be very complex.

In this paper it is shown polarographically that at a *p*H in the neighborhood of 9, a slightly soluble silver cysteine compound is formed upon addition of silver nitrate to a buffered cysteine solution. This compound which is designated in this paper as RSAg gives a reduction wave at the dropping mercury electrode at a very negative potential indicating that the silver ion activity of the solution of the complex is extremely small. This behavior can be made the basis of an amperometric titration using the dropping mercury electrode as indicator electrode. Titrations with the dropping electrode do not give accurate and precise results at concentrations of 10^{-4} M or greater. Especially for the analysis of cysteine in biological materials it is desirable to have a rapid method for the determination of this substance. From the polarographic results it was concluded that under suitable conditions it should be possible to titrate traces of cysteine (further designated as RSH) with silver nitrate using the rotating platinum wire electrode in a way similar to that given by Kolthoff and Harris[1] for mercaptans.

When the present work was finished a paper appeared by Benesch and Benesch[2] who applied without essential change the procedure given by Kolthoff and Harris to the determination of sulfhydryl groups in amino acids and proteins. They mostly used solutions of high alcohol content as is required in the mercaptan determination.

In the present paper it is shown that accurate results can be obtained in aqueous medium if the titration is carried out under proper conditions.

The proposed method can also be applied for

(1) I. M. Kolthoff and W. E. Harris, *Ind. Eng. Chem., Anal. Ed.*, **18**, 161 (1946).

(2) R. Benesch and R. E. Benesch, *Arch. Biochem.*, **19**, 35-45 (1948).

the determination of cystine (designated as RSSR) after quantitative reduction to RSH. For the present purpose this reduction can be carried out conveniently with sodium amalgam. Moreover, it has been found very expedient to make use of the reaction between sodium sulfite and RSSR. The sodium sulfite not only converts RSSR into RSH but also inhibits air oxidation of the cysteine in alkaline medium.

Materials.—Cysteine which was used in the form of its hydrochloride was a Paragon reagent grade product. Cystine and silver nitrate were Merck reagent grade products. The silver nitrate was dried at 110° before use. Shinohara[3] and Sullivan, *et al.*,[4] pointed out that cysteine hydrochloride is hygroscopic. It is necessary to keep the compound in a desiccator over phosphorus pentoxide or magnesium perchlorate. The weighings must be done as rapidly as possible. With these precautions it was possible to obtain a good agreement between the weighed amount of cysteine hydrochloride and the amounts found by potentiometric and amperometric titrations.

Stock solutions of cysteine hydrochloride of different concentrations (from 10^{-2} M to 5×10^{-4} M) were prepared in air-free conductivity water. The stock solutions were kept under nitrogen. It was found that the cysteine content of these solutions diminishes considerably on standing. A 2×10^{-3} M cysteine hydrochloride solution which was 2×10^{-3} M in hydrochloric acid (all cysteine stock solutions were made to be 10^{-2} to 10^{-3} M in hydrochloric acid) was found to have its cysteine content reduced by 2.4% after twenty-four hours standing at room temperature. The relative losses in cysteine are still greater in more dilute solutions. For reliable work it is necessary to make up fresh stock solutions every day.

Stock solutions of 5×10^{-4} to 10^{-3} M cystine in 0.01 to 0.025 M sulfuric acid were stable for several months.

Experimental Methods

Polarographic current–voltage (C–V) curves were obtained manually with equipment similar to that suggested by Lingane and Kolthoff[5] and automatically with a Heyrovsky self recording polarograph. All potentials were measured against the saturated calomel electrode (S.C.E.).

Most of the experiments with the rotating platinum electrode were carried out manually. The apparatus used for the amperometric titrations was similar to that described by Kolthoff and Harris.[1] A 5-ml. microburet divided into hundredths of a ml. was used in all titrations.

Titrations with the Dropping Mercury Electrode as Indicator Electrode.—The polarography of cysteine and cystine in different media has been studied by Kolthoff and Barnum.[6,7] A well-defined anodic diffusion current of cysteine is obtained in ammonia buffers. In a buffer which is 0.1 M in ammonium nitrate, 0.1 M in ammonia and 10^{-3} M in cysteine, the half-wave potential is −0.52 v.

The anodic diffusion current of cysteine in this ammonia buffer decreases on the addition of silver ions while a new cathodic wave appears at about −0.52 v. The diffusion current of cysteine disappears if silver and cysteine are present in a mole ratio of approximately 1:1; at this point only a cathodic wave is observed. The height of this wave is much smaller than that corresponding to the silver ammine complex at the same molar concentration of silver. No doubt, this cathodic wave is due to the reduction of silver in a practically undissociated silver cysteine compound. Plots of $\log i/(i_d - i)$, as well as $\log i/(i_d - i)^2$ *versus* applied e. m. f. do not give straight lines, indicating that the RSAg wave is irreversible. Assuming a one-electron reduction, the diffusion coefficient as calculated from the Ilkovic equation was found to be $D = 3.8 \times 10^{-6}$ cm.^{2} sec.$^{-1}$. The diffusion coefficient for cysteine in the same ammonia buffer was found to be 7.43×10^{-6} cm.^{2} sec.$^{-1}$, about twice that of RSAg. This difference, combined with other observations not reported here, indicates that dissolved RSAg is associated.

In connection with the interpretation of the various results obtained from amperometric titrations it is of interest to mention that polarographic, potentiometric and solubility measurements indicate that the RSAg can combine with silver ions to form at least two complex ions $[Ag(RSAg)_2]^+$ and $[Ag(RSAg)_3]^+$. More details about the behavior of RSAg will be given in a subsequent paper.

The reaction between cysteine, dissolved in an ammonia buffer, and silver ion was used as the basis of an amperometric titration of cysteine with silver nitrate. A few titrations of this kind were done with the dropping mercury electrode at applied potentials of −0.3 and −0.4 v., respectively. At both potentials the anodic diffusion current of cysteine is measured while in the presence of an excess of silver the diffusion current of the silver ammine ion is found. The RSAg is not reduced at −0.3 to −0.4 v. In one instance 5 ml. of a 0.01 M RSH solution was added to 14 ml. of an ammonia buffer (0.1 M in ammonia and 0.1 M in ammonium nitrate) and titrated with a 0.02 M silver nitrate solution. The end-point was taken as the amount of silver added when the current was zero. The mercury was collected under chloroform in order to prevent it from reacting with the silver in the solution. The titration line of this experiment is given in Fig. 1. The lines are corrected for a dilution effect. The dotted line in Fig. 1 gives the ideal titration line as calculated from the known diffusion constants of cysteine and silver ammine ion. This line would be observed in titrations of cysteine with silver nitrate if RSAg would

(3) K. Shinohara, *J. Biol. Chem.*, **112**, 671 (1935–1936).

(4) M. X. Sullivan, W. C. Hess and H. W. Howard, *ibid.*, **145**, 621 (1942).

(5) J. J. Lingane and I. M. Kolthoff, THIS JOURNAL, **61**, 825 (1939).

(6) I. M. Kolthoff and C. Barnum, *ibid.*, **62**, 3061 (1940).

(7) I. M. Kolthoff and C. Barnum, *ibid.*, **63**, 520 (1941).

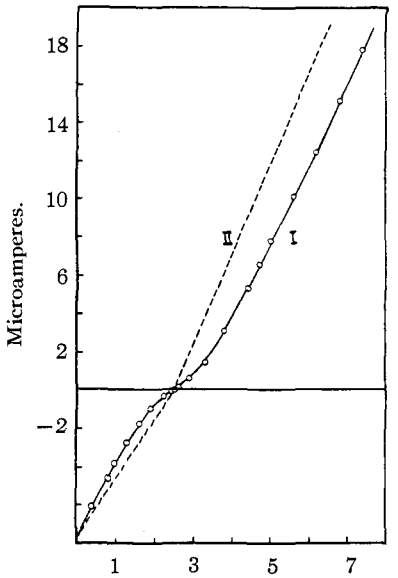

Fig. 1.—Amperometric titration of cysteine hydrochloride with the dropping mercury electrode as indicator electrode: 5 ml. 10^{-2} *M* RSH·HCl in 19 ml. ammonia buffer (0.1 *M* NH_3, 0.1 *M* NH_4NO_3) with 2 × 10^{-2} *M* silver nitrate at −0.4 v. *vs.* S. C. E.; I, real titration line; II, ideal titration line.

not form complex compounds with cysteine or silver ion. It is seen that the experimental titration lines deviate most from the ideal lines close to the end-point. The reason seems to be that RSAg can form complex compounds with cysteine, as a result of which the diffusion current of the residual cysteine becomes less. Similarly the silver ammine ion can react with RSAg to form various cationic complex compounds.

The composition of these compounds may tentatively be represented by the formulas $RSAg(RSH)_x$ and $[Ag(RSAg)_x]^+$. There is evidence that the extent to which these compounds are formed depends on the experimental conditions, like *p*H, cysteine concentration and rate of addition of the silver ion to the cysteine solution. Thus the rapid addition of silver nitrate suppresses the formation of $RSAg(RSH)_x$ and favors the formation of $[Ag(RSAg)_x]^+$. The results of titrations are high if carried out very rapidly. If the silver nitrate is added slowly and in very small increments the RSAg has an opportunity to react with the excess of cysteine which is present before the end-point and compounds of the type $RSAg(RSH)_x$ are formed which seem to react slowly with silver ammine. Consequently, low results are found upon slow titrations. The experiment given in Fig. 1 was carried out with 2 × 10^{-3} *M* RSH solution. Better results are obtained with more dilute cysteine solutions (= $<10^{-3}$ *M*), but even in these solutions the accuracy and precision obtained are not better than about 1%.

Accurate results can be obtained with the rotating platinum wire electrode as indicator electrode. Especially in the particular instance where we are dealing with complicated reactions between *X* moles of RSAg (or $[RSAg]_x$) and silver ammine the rotated platinum wire electrode has great advantages over the dropping electrode. The titrations can and should be carried out at great dilutions. The extent of complex formation $X\ RSAg + Ag^+ \rightleftarrows [Ag(RSAg)_x]^+$ decreases exponentially (exponent *X*) with increasing dilution. Thus we have a unique case here in which the accuracy of a titration increases with increasing dilution. At the proper applied potential no current flows through the titration system before the end-point. Under ideal conditions the diffusion current of the silver ammine ion is measured after the end-point. The end-point is found in the usual way as the point of intersection between two straight lines.

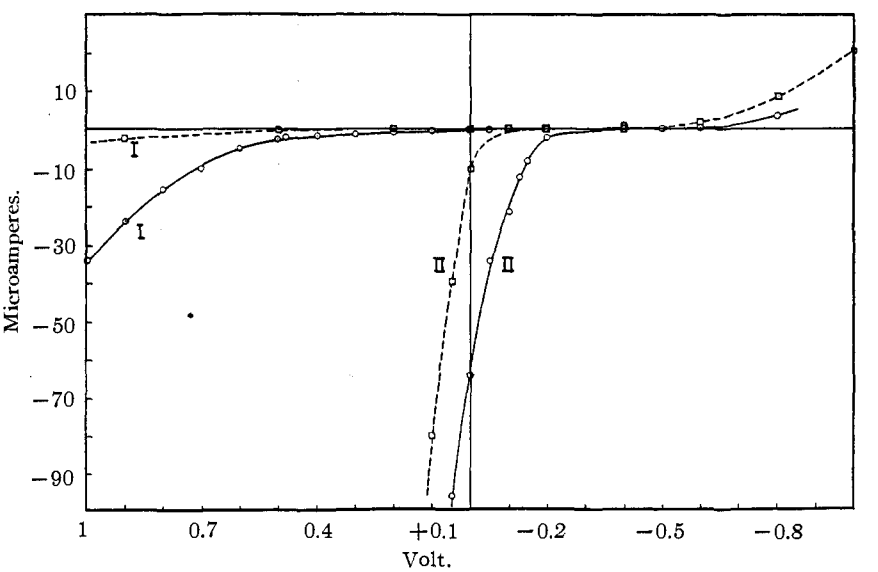

Fig. 2.—Current-voltage curves of 5 × 10^{-4} *M* cysteine in an ammonia buffer (0.1 *M* NH_3, 0.1 *M* NH_4NO_3) at the rotating platinum electrode: ⊡, blank; ⊙, 5 × 10^{-4} *M* RSH; I, at rotating platinum electrode; II, at rotating silver coated platinum electrode.

Titration with the Rotating Platinum Electrode.—It is seen from the C–V curves in Fig. 2 that cysteine, dissolved in an ammonia buffer,

does not give an anodic diffusion current up to +1 v. if electrolyzed at the rotating platinum wire electrode. Comparing the C–V curve of the blank (ammonia buffer without cysteine) with that of the same buffer, containing 5×10^{-4} *M* RSH, it is seen that the cysteine solutions give greater anodic currents than the blank at the same potentials, both at the rotating platinum and at the rotating silver coated platinum electrodes. No diffusion current of cysteine is observed at these electrodes. An ammoniacal silver solution gives a well defined cathodic wave with a half-wave potential of about −0.07 v. at the rotating platinum or silver electrode. An ammonia buffer containing equimolar amounts of cysteine and silver does not give an appreciable current at potentials between −0.2 and −0.4 v. where the diffusion current of silver ammine ion is measured. The diffusion current of RSAg appears at about −0.7 v. and is much smaller than that of the silver ammine ion at the same molar concentration of silver. The addition of an excess of silver ion to the ammoniacal solution of RSAg results in the appearance of the diffusion current of the silver ammine complex. Under proper conditions and within a limited range of excess of silver this current is proportional to the concentration of the silver ammine ion. Use is made of these observations in the amperometric titration of cysteine. The best results were obtained at an applied potential of −0.3 v. (*vs.* S.C.E.) at which potential most of the titrations were carried out. A great number of titrations was carried out under varying conditions. The effects of the concentration of ammonia and ammonium nitrate and of the cysteine concentration were studied. Cysteine is oxidized relatively rapidly in alkaline solutions. At cysteine concentrations in the range between 8×10^{-4} and 10^{-4} *M* it is not necessary to work in air-free solutions. At lower concentrations of cysteine the oxygen must be removed either by bubbling nitrogen through the solution or by the addition of sodium sulfite. At concentrations of 0.025 to 0.05 *M* the sulfite not only removes oxygen but also improves greatly the sharpness of the end-point. The addition of alcohol which was used by Kolthoff and Harris as a solvent for mercaptans is not required in the analysis of cysteine.

Cystine does not react with silver under the conditions of the titrations and it does not interfere. In order to determine cystine by amperometric titration it must be reduced to cysteine. This is best done with either sodium sulfite in ammoniacal medium or with sodium amalgam in acid solution.

The action of sulfite on cystine was studied by Clarke[8] who found that the reaction proceeds according to the equation $R\text{–}SS\text{–}R + Na_2SO_3 = R\text{–}SNa + R\text{–}SSO_3Na$. Thus, one mole of cystine yields one mole of cysteine. This we also found by amperometric titration.

(8) H. T. Clarke, *J. Biol. Chem.*, **97**, 233 (1932).

With sodium amalgam as reducing agent one mole of cystine yields two moles of cysteine.

The reduction of cystine to cysteine should not be carried out with zinc or cadmium or their amalgams as reducing agents since these metals interfere with the titration of cysteine.

Procedures

Determination of Cysteine.—In a 150-ml. beaker put 25 to 100 ml. of a solution which is 0.1 *M* in ammonia and 0.2 to 0.3 *M* in ammonium nitrate. Add so much of the cysteine solution that its concentration in the mixture is between 1 and 5×10^{-4} *M*. If the concentration of the solution to be titrated is less than 10^{-4} *M* in cysteine the electrolyte solution should be made air-free before adding the sample of cysteine. This is done either by bubbling nitrogen through the solution or by making the solution 0.025 to 0.05 *M* in sodium sulfite. Sodium sulfite may be used only if the sample does not contain cystine which reacts with sodium sulfite (see procedure below). Immerse the salt bridge and the rotating platinum electrode in the solution and titrate with silver nitrate of suitable concentration (0.005 to 0.001 *M*) at an applied potential of −0.3 v. *vs.* S.C.E.

Determination of Cystine. 1. Reduction with Sodium Sulfite.—In a 150-ml. beaker place 25 to 100 ml. of a solution which is 0.2 to 0.3 *M* in ammonium nitrate, add the cystine sample so that its concentration in the mixture is between 5×10^{-4} and 10^{-4} *M*. Pass nitrogen through the solution, make the solution 0.1 *M* in ammonia and make 0.1 to 0.025 *M* in sodium sulfite and titrate with 10^{-3} *M* silver nitrate at an applied potential of −0.3 v. *vs.* S.C.E.[9]

2. Reduction with Sodium Amalgam.—Place the sample, containing 1 to 0.3 mg. of cystine (corresponding to 0.27 to 0.08 mg. of titratable sulfur) into a small test-tube. Make it 0.05 *M* in sulfuric acid and add about a drop of 1% sodium amalgam. Let react for about half an hour while shaking frequently. Transfer the solution quantitatively into a 150-ml. beaker containing about 25 ml. of air-free ammonia buffer (0.1 *M* in NH_3, 0.2 *M* in NH_4NO_3) and proceed as in the analysis of cysteine.

Results

Tables I and II give the results of the amperometric titrations of cysteine and cystine, respectively.

If the cysteine concentration of the solution is markedly greater than 5×10^{-4} *M* the end-point is not sharp and the results are not accurate. Thus from Table I it is seen that the titration of 3.1 mg. RSH·HCl in 25 ml. solution (8×10^{-4} *M* in RSH) gives a result which is 1.7% high while the same quantity of cysteine dissolved in 100 ml. of the buffer (solution is only 2×10^{-4} *M* in RSH) gives an average error of +0.4% only. As stated for the experiments with the dropping mercury electrode it seems that the formation of vari-

(9) Care must be taken to have ample excess of sodium sulfite in the solution (at least 0.025 *M*). The reaction between cystine and sulfite goes to an equilibrium state and does not seem to become complete without silver ion present. Upon the addition of silver ion the equilibrium is shifted by the formation of the practically undissociated silver-cysteine compound. If too little sodium sulfite has been added it is found that the current increases abruptly upon each addition of silver nitrate and then decreases until a constant value is attained. The explanation is that the initial excess of silver nitrate is being used up by the cysteine which is formed continuously by the reaction $RSSR + Na_2SO_3 \rightleftarrows RSNa + RSSO_3Na$. When too little sulfite is added the end-point is not sharp. It is of interest to mention that the rate of the above reaction from left to right could be determined by measuring amperometrically the rate with which the silver disappears.

TABLE I

AMPEROMETRIC TITRATION OF CYSTEINE

Detns.	Init. cysteine concn. of soln. titrated M (approx.)	Cysteine hydrochloride taken, mg.	Cysteine hydrochloride found, mg.	Average error, %	Volume of soln., ml.	Remarks
1	8×10^{-4}	3.16	3.22	+ 1.7	25	Presence of air
3	2×10^{-4}	3.14	3.15	+ 0.4	100	Presence of air
7	2×10^{-4}	1.59	1.59 to 1.57	± 0.4	50	Presence of air
4	2×10^{-4}	1.66	1.68 to 1.65	+ 0.6 − 0.4	50	Air-free solution
7	2×10^{-4}	1.66	1.66	0.0	50	0.05 M Na_2SO_3
1	2.8×10^{-4}	2.21	2.22	+ 0.4	50	Air-free solution (0.5 N NH_4NO_3, 0.1 N NH_3)
1	4.2×10^{-4}	1.66	1.66	0.0	25	0.05 M $NaSO_3$
3	10^{-4}	1.64	1.63	− 0.3	100	Air-free solution
3	1.4×10^{-4}	1.10	1.10	0.0	50	0.025 M Na_2SO_3
1	7×10^{-5}	1.10	1.10	0.0	100	Air-free solution
2	1.2×10^{-4}	0.936	0.937	+ 0.1	50	0.025 M Na_2SO_3
2	1.6×10^{-4}	.630	.628	− 0.3	25	.025 M Na_2SO_3
1	1.2×10^{-4}	.473	.473	0.0	25	.025 M Na_2SO_3
2	10^{-4}	.394	.390	− 1.0	25	.025 M Na_2SO_3
1	8×10^{-5}	.316	.308	− 2.5	25	.025 M Na_2SO_3
1	2×10^{-4}	1.581	1.764	+11.6	50	No NH_4NO_3 0.1 N KNO_3, 0.1 N NH_3
1	2×10^{-4}	1.581	1.576	− 0.3	50	0.1 N NH_4NO_3, 0.5 N NH_3 Large anodic current

TABLE II

DETERMINATION OF CYSTINE

Number of detns.	Init. cystine concn. of soln., M	Cystine taken, mg.	Cystine found, mg.	Average error, %	Treatment	Vol. of soln., ml.
1	10^{-4}	1.201	1.198	−0.3	Sodium sulfite	50
6	8×10^{-5}	0.962	0.964–0.958	−0.3 +0.2	Sodium sulfite	50
1	4×10^{-5}	.962	.945	−1.8	Sodium sulfite	100
6	1.2×10^{-4}	.722	.728–0.718	+0.3	Sodium sulfite	25
2	6×10^{-5}	.722	.728	+0.8	Sodium sulfite	50
2	1.6×10^{-4}	.962	.957	−0.5	Sodium amalgam	25
2	6.4×10^{-5}	.384	.384	0.0	Sodium amalgam	25

ous silver cysteine complexes which is favored in more concentrated solutions may also be the cause of erroneous titration results with the rotating platinum electrode when the concentration of cysteine is relatively large. Figures 3 and 4 give the titration lines obtained with solutions which were 8×10^{-4} M and 10^{-4} M in RSH, respectively. It is seen that the end-point in the more dilute solution is much sharper. The slope of the excess reagent line (corresponding to the diffusion current of the silver ammine ion) is 4.9 for the concentrated and 10.7 for the dilute solution. The results indicate that a considerable fraction of the excess of silver ammine is tied up into a complex silver–silver cysteinate in the titration of the more concentrated solution (Fig. 3). All the experimental points in Figs. 3 and 4 have been corrected for a dilution effect.

A quantitative interpretation of the excess of silver lines, especially in the more concentrated solutions cannot be given yet. From a practical point of view it is important to note that the end-point can be found graphically without difficulty when the initial concentration of cysteine is less than 5×10^{-4} M, the end-point becoming sharper with increasing dilution. In a concentration range between 5×10^{-4} and 1×10^{-4} M the accuracy and precision are of the order of 0.3%. When the concentration becomes less than 10^{-4} M the accuracy and precision decrease as a result of air oxidation of the cysteine. A concentration of 10^{-4} M cysteine in a titration volume of 25 ml. corresponds to 0.4 mg. of cysteine hydrochloride. By using smaller titration vessels and smaller volumes much smaller quantities can be determined.

The titration is affected by the ammonia and ammonium nitrate concentration of the solution. A greater ammonium nitrate concentration (*e. g.*, 0.5 M NH_4NO_3, 0.1 M NH_3, $2.8.10^{-4}$ M RSH) usually results in a steeper excess reagent line, while a greater ammonia concentration (*e. g.*, 0.1 M NH_4NO_3, 0.5 M NH_3, 2.10^{-4} M RSH) causes a considerable anodic current before the end-point, and thus decreases the precision of the result. It is seen from Table I that also with this ammonia concentration (0.5 M) a relatively good result can

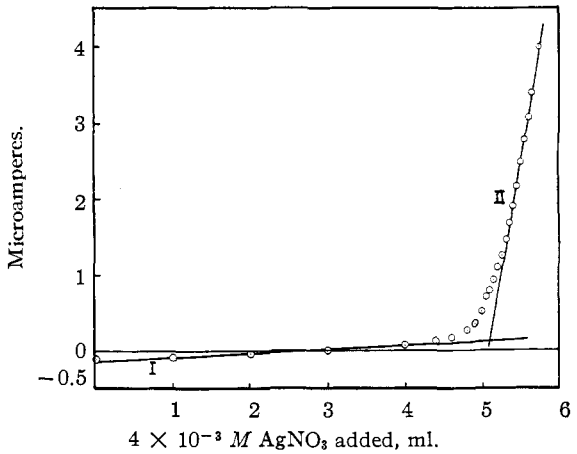

Fig. 3.—Amperometric titration of 3.16 mg. RSH·HCl at the rotating platinum electrode: solution is 8×10^{-4} M in RSH; I, reaction line; II, excess reagent line.

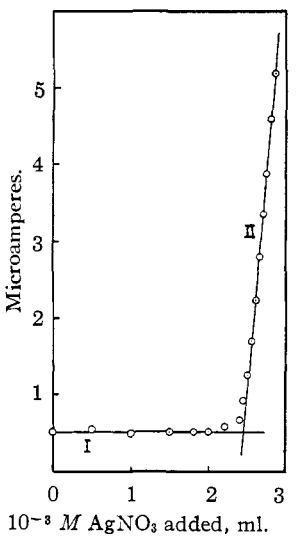

Fig. 4.—Amperometric titration of 0.39 mg. RSH·HCl: solution is 10^{-4} M in RSH; I, reaction line; II, excess reagent line.

be obtained in the presence of 0.1 M NH_4NO_3, if the cysteine concentration is low enough (2×10^{-4} M). If ammonium nitrate was replaced with potassium nitrate erroneous results were found. From Table I it is seen that the titration of 1.58 mg. of cysteine hydrochloride in 50 ml. of a 2×10^{-4} M solution which was 0.1 M in potassium nitrate and 0.1 M in ammonia gave a result which was 12% high. Potentiometric investigations which will be reported in a subsequent paper show that the pAg of the silver cysteine compounds formed during the titration is greatly affected by the pH of the solution. Amperometric titrations gave best results with solutions of an ammonium nitrate normality twice or three times that of the ammonia. The presence of sodium sulfite improves the sharpness of the end-point. In the presence of sulfite the current at the end-point is usually reduced while the diffusion current of the excess of silver is increased. This remarkable effect of sodium sulfite needs further investigation.

The rate of addition of silver nitrate affects the appearance of the titration line. This was also found in titrations with the dropping mercury electrode, as stated above. Upon very slow addition of silver nitrate (time of titration two to four hours) low results are obtained and it becomes difficult to find the end-point unambiguously. An example of a slow titration of 8×10^{-4} M cysteine is given in Fig. 5. After the end-point two straight

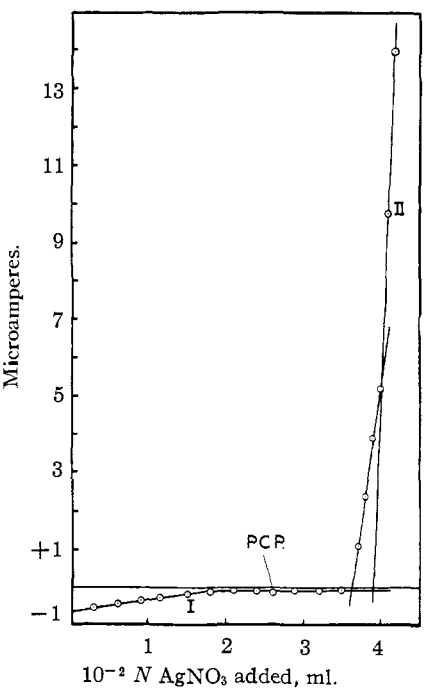

Fig. 5.—Slow amperometric titration of 6.31 mg. RSH·HCl: time of titration four hours; solution is 8×10^{-4} M in RSH; I, reaction line; II, excess reagent line.

lines can be constructed. Part of the silver amino ion added immediately after the end-point is bound in the form of a silver–silver cysteinate complex. For this reason the point of intersection of the first excess of silver line with the reaction line gives an end-point which is 9% low. Using the point of the intersection of the second excess of silver line gives an end-point which is 2% low. After the addition of 65% of the equivalent amount of silver a white precipitate was formed while no precipitate was formed in the rapid titration. The precipitate dissolved gradually on the addition of an excess of silver. The solution at the end of the titration, containing an excess of silver, did not contain cystine as shown polarographically. Hence the low results cannot be ascribed to oxidation of cysteine. A slow titration of 4×10^{-4} M cysteine also gave two end-points with errors of −5.5% and −0.6%, respectively. These errors are considerably less than those found in

the slow titration of 8×10^{-4} M RSH. This shows that the effect of the rate of addition of silver nitrate is the greater the greater the cysteine concentration. No precipitate was formed during the slow titration of 4×10^{-4} M cysteine.

Separate experiments have indicated that the reactions between RSAg and cysteine and excess of silver, respectively, are slow. Fortunately, an amperometric titration with the rotated electrode is always carried out rapidly. Thus under normal practical conditions the above peculiarities observed in slow titrations are not found.

Generally it was found by Kolthoff and Harris that the precision of the mercaptan titration can be influenced by the surface conditions of the electrodes. Electrodes which have been used for a long time often give an appreciable anodic current at the beginning of the titration and sometimes excessively large currents after the end-point. These electrodes can be used again after cleaning with nitric acid. Freshly cleaned electrodes when they have been allowed to stand in dilute ammonia for a few hours often proved to be insensitive in the first one or two titrations but responded normally thereafter. These peculiarities were found to be more pronounced with thick platinum electrodes than with electrodes of a small diameter.

Interferences.—Substances which react with silver in ammoniacal medium affect the titration. Iodide reacts with silver in ammoniacal medium and interferes. For example, a solution which was 5×10^{-3} M in potassium iodide, 2×10^{-4} M in cysteine, 0.1 M in NH_3 and 0.1 M in NH_4NO_3, did not show an end-point until more than 100% excess of silver nitrate had been added. At this point the excess of silver line appeared. The solution remained perfectly clear after the addition of a 300% excess of silver nitrate. At greater iodide concentrations (5×10^{-2} M) a precipitate was formed. Experiments carried out under various conditions showed that iodide does not interfere if its concentration does not exceed one-fifth of the molar cysteine concentration and if the latter is not greater than 10^{-4} M. In all other cases the results were found to be high.

Metal ions like zinc, cobalt, cadmium and copper interfere and cause low results in the analysis as they form slightly dissociated compounds with cysteine, like silver. Generally, the interference is reduced but not eliminated if sodium sulfite (0.05 M) is present in the solution. Under these conditions zinc and cadmium do not interfere if their molarities are not greater than one-tenth of the molarity of the cysteine. The concentration of copper and cobalt should be less than 1% of the cysteine molarity which should not exceed 2×10^{-4}. Sodium sulfite has no effect on the interference by cobalt; but it must be added if a trace of copper is present. The concentration of metals and iodide in biological materials in which cysteine is to be determined is usually so small that no interference of iodide and the above metals is to be expected. If separations must be made it will be profitable to first oxidize the cysteine to cystine and reduce the latter again after the separation.

It was found polarographically that copper forms a strong complex with cysteine in ammoniacal medium. It will be the subject of further work to make use of this complex for another method of amperometric determination of cysteine. The application of the amperometric titration of cysteine and cystine in the analysis of native and denatured proteins, and to protein hydrolysates is planned.

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Summary

The reaction between silver and cysteine in a suitable ammonia buffer can be used for a simple and accurate amperometric titration of cysteine using the rotated platinum wire electrode as indicator electrode. Cystine is converted into cysteine by means of sodium sulfite or sodium amalgam.

The accuracy and precision are 0.3% with cysteine concentrations between 5×10^{-4} and 10^{-4} M.

Iodide ion interferes if its molarity is greater than one-fifth of that of the cysteine. Metals like zinc, cadmium, cobalt and copper interfere.

Upon rapid titration of dilute solutions the endpoint corresponds to the formation of silver cysteinate (RSAg). This compound reacts with ammino silver and apparently also with cysteinate with the formation of complexes of a very complicated nature. Equilibrium is established slowly in these reactions.

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