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

Equilibrium Constants of the Reactions of Sulfite with Cystine and with Dithiodiglycolic Acid

BY W. STRICKS AND I. M. KOLTHOFF

The reaction between cystine and sulfite ($RSSR + SO_3^= \rightleftharpoons RS^- + RSSO_3^-$) is shown to be reversible in alkaline medium (pH varied between 8 and 13), the equilibrium being attained rapidly from both sides. Four equilibrium constants with reference to the various charge types of the anions of cysteine, cystine and cysteine sulfonate have been determined at 12, 25, and 37°. Neglecting the heat of dissociation of the sulfhydryl group and taking +12,000 cal. for that of the NH_3^+ group, the heat of reaction was estimated to be +7000 cal. between 12 and 37°. The reaction between dithiodiglycolate and sulfite was studied only at pH lower than 9.5 because of decomposition of dithiodiglycolate at higher pH. The equilibrium constant has been determined in the pH range between 9.4 and 6.6 at 25°. The equilibrium constant of the reaction between dithiodiglycolic acid and the anion HSO_3^- ($R_1SSR_1 + HSO_3^- \rightleftharpoons R_1SH + R_1SSO_3^-$) was also determined.

The reaction between sulfite and organic disulfides has been the subject of various investigations.

After Hefter[1] had shown that cysteine is one of the products formed in the interaction between sulfite and cystine, Clarke[2] and Lugg[3] presented conclusive evidence that the reaction between cystine and sulfite proceeds according to the equation

$$RSSR + Na_2SO_3 = RSNa + RSSO_3Na$$

where RSSR, RSNa and $RSSO_3Na$ denote cystine, cysteinate and cysteine sulfonate, respectively. Clarke[2] isolated the reaction products and described some of the properties of cysteine sulfonate. Later Schöberl[4] and Kassel, *et al.*,[5] reported on the behavior of various disulfide compounds of biological interest in the presence of sulfite.

The reaction has been made use of by Folin[6] in the colorimetric determination of cystine and cysteine and recently in the amperometric titration of traces of cysteine and cystine with silver nitrate or cupric copper as reagents.[7,8]

The reactions between sulfite and cystine, homocysteine lactone and thiazolin carboxylic acid, respectively, are also of biological interest in connection with the sulfite inactivation of the cobra venom neurotoxin.[9] A more complete understanding of the sulfite cystine system is desirable both from the theoretical and industrial (treatment of wool, hide)[10] viewpoint.

This paper deals mainly with a quantitative study of the reaction between cystine and sulfite at various pH and temperatures. It is shown that this reaction is reversible in alkaline medium. Equilibrium constants and the heat of reaction have been calculated from results of the polarographic method of analysis.

The system dithiodiglycolate–sulfite is simpler than the corresponding cystine–sulfite system since the glycolate does not contain amino groups. The equilibrium constant of the reaction between dithioglycolate and sulfite (and bisulfite) has been determined.

Materials

Cystine and sodium sulfite were C.P. reagent grade Merck products. Cysteine which was used in the form of its hydrochloride was a C.P. product from Pfanstiehl.

Thioglycolic acid was an Eimer and Amend "pure" product which was purified by two vacuum distillations. The purification of thioglycolic acid and the preparation of pure dithiodiglycolic acid were carried out by D. Leussing in this Laboratory.

All the other chemicals were C.P. reagent grade products.

Stock solutions of cystine and dithiodiglycolic acid were 10^{-2} M in RSSR and 0.1 M in hydrochloric acid. Stock solutions of sulfite were prepared with air-free water and were 0.2 to 0.5 M in sodium sulfite. Only freshly prepared sulfite solutions were used. Cysteine and cysteine sulfonate solutions were prepared in the same way as described previously.[7,11]

The stock solutions used for the preparation of the buffers were: 0.2 M disodium phosphate, 0.2 M monosodium phosphate, M sodium hydroxide, 5 M and M ammonia, respectively, 4 M and M ammonium chloride, respectively. The ionic strength was adjusted by the addition of appropriate volumes of a 3 M potassium chloride solution.

Experimental

Current-voltage curves were measured at 12, 25 and 37°, with the manual apparatus and circuit described by Lingane and Kolthoff[12] and automatically with a Heyrovsky self-recording polarograph. The diffusion current of cysteine was measured manually. All reported values of i_d were corrected for the residual current. All potentials were measured against the saturated calomel electrode (S.C.E.). Oxygen was removed from the solutions in the cell with nitrogen. During an experiment an atmosphere of nitrogen was maintained over the solution.

The characteristics of the capillary used were: $m = 1.804$ mg. sec.$^{-1}$, $m^{2/3}/t^{1/6} = 1.89$ mg.$^{2/3}$ sec.$^{-1/2}$ (open circuit); the height of the mercury column was 76 cm.

The pH was measured with a Beckman pH meter, Laboratory Model G.

Glass electrodes made of the usual 015 type electrode glasses were used for solutions with pH below 9.5 while measurements were made with the Beckman "Type E" high pH glass electrode at pH above 9.5.

Procedure.—Appropriate volumes of air-free stock solutions of cystine or dithiodiglycolic acid and sulfite (or of cysteine sulfonate, cysteine and sulfite) were added to a given volume of an air-free buffer solution which had been placed in an electrolysis cell. The total volume of the mixture was 20 ml. in each experiment. The diffusion current of cys-

(1) A. Hefter, *Chem. Zentr.*, **78**, II, 822 (1907).
(2) H. T. Clarke, *J. Biol. Chem.*, **97**, 233 (1932).
(3) J. W. H. Lugg, *Biochem. J.*, **26**, 2144 (1932).
(4) A. Schöberl and E. Ludwig, *Ber.*, **70**, 1422 (1937); A. Schöberl and F. Krumy, *ibid.*, **71B**, 2361 (1938).
(5) B. Kassel and E. Brand, *J. Biol. Chem.*, **125**, 131 (1938).
(6) O. Folin and J. M. Looney, *ibid.*, **51**, 421 (1922); O. Folin and A. D. Marenzi, *ibid.*, **83**, 103 (1929).
(7) I. M. Kolthoff and W. Stricks, THIS JOURNAL, **72**, 1952 (1950).
(8) I. M. Kolthoff and W. Stricks, *Anal. Chem.*, **23**, 763 (1951).
(9) F. Micheel and G. Bode, *Ber.*, **71B**, 2653 (1938).
(10) F. F. Elsworth and H. Phillips, *Biochem. J.*, **32**, 837 (1938); H. Phillips, *J. Soc. Dyers Colourists*, **54**, 503 (1938); F. F. Elsworth and H. Phillips, *Biochem. J.*, **35**, 135 (1941); W. R. Middlebrook and H. Phillips, *ibid.*, **36**, 294, 428 (1942); S. Blackburn, R. Consden and H. Phillips, *ibid.*, **38**, 25 (1944); H. Lindberg and H. Phillips, *ibid.*, **39**, 17 (1945); E. G. H. Carter, W. R. Middlebrook and H. Phillips, *J. Soc. Dyers Colourists*, **62**, 203 (1946); E. Elöd and H. Zahn, *Melliand Textilber.*, **27**, 68 (1946); *Chem. Zentr.*, **118**, I, 880 (1947); H. Lindley and H. Phillips, *Biochem. J.*, **41**, 34 (1947); W. Windus and H. G. Turley, *J. Am. Leather Chem. Assoc.*, **36**, 603 (1941).
(11) I. M. Kolthoff and W. Stricks, THIS JOURNAL, **73**, 1728 (1951).
(12) J. J. Lingane and I. M. Kolthoff, *ibid.*, **61**, 825 (1939).

TABLE I

ANODIC DIFFUSION CURRENTS OF 10^{-3} M CYSTEINE AND THIOGLYCOLIC ACID SOLUTIONS IN MIXTURES WITH SULFITE AT VARYING pH AND TEMPERATURES

Compound	Temperature, °C.	Na_2SO_3 M	Medium Buffer	pH	i_d at −0.4 volt (μA.)
Cysteine	12	0.1	0.1 M NaOH, 0.6 M KCl	13.26	2.36
Cysteine	25	.05	.1 M NaOH, 0.75 M KCl	12.8	2.96
Cysteine	37	.05	.1 M NaOH, 0.75 M KCl	12.41	3.49
Cysteine	25	.10	.05 M Na_2HPO_4, 0.075 M NaOH, 0.475 M KCl	12.1	2.92
Cysteine	25	.05	.075 M Na_2HPO_4, 0.05 M NaOH, 0.015 M HCl, 0.57 M KCl	11.2	3.06
Cysteine	25	.05	.075 M Na_2HPO_4, 0.025 M NaOH, 0.015 M HCl, 0.6 M KCl	10.1	3.09
Thioglycolic acid	25	.05	M NH_3, 0.1 M NH_4Cl, 0.75 M KCl	10.5	3.37
Thioglycolic acid	25	.05	M NH_3, M NH_4Cl	9.4	3.53

teine or thioglycolic acid in the mixture was measured after various periods of time until a constant value was obtained. The diffusion current of cysteine was found to be well defined and proportional to the cysteine concentration in solutions of pH 8 to 13. In agreement with the findings of Kolthoff and Barnum[13] a true diffusion current of cysteine could not be measured in solutions of pH markedly lower than 8. Thioglycolic acid gives a well defined diffusion current in the entire pH range (10.5 to 6.6) investigated.

Diffusion current constants of cysteine and thioglycolate have been determined at −0.3, −0.35 and −0.4 volt, respectively, in solutions of the same composition as the equilibrium mixtures but in the absence of the dithio- and sulfonate forms. The values obtained were used for the calculation of cysteine and thioglycolate concentrations in equilibrium mixtures. A few representative data at −0.40 v. only are given in Table I. The diffusion currents of both cysteine and thioglycolate decrease slightly with increasing pH. The temperature coefficient of the diffusion current of cysteine was found to be about 2% per degree over the temperature range from 12 to 37°. This is of the same order of magnitude as that of metal ions.[14]

The pH of each solution was determined at the temperature specified in Table I.

Results and Discussion

I. The Reaction between Cystine and Sulfite.—A complete polarogram of an equilibrium mixture obtained by the addition of 2 ml. of 0.2 M sodium sulfite solution to 18 ml. of 2.2×10^{-3} M cystine solution in an ammonia buffer of pH 10.32 is given in Fig. 1. The anodic wave is that of the cysteine formed in the reaction between cystine and sulfite. The diffusion current of this wave corresponds to a cysteine concentration of 8.3×10^{-4} M. The cathodic waves are those of the residual cystine and of the cysteine sulfonate[15]

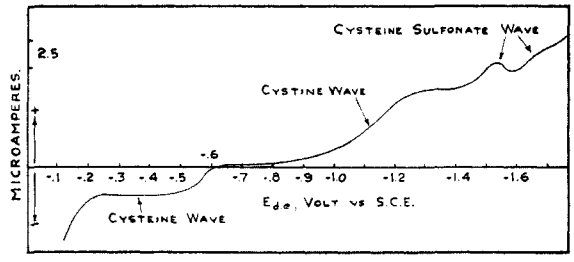


Fig. 1.—Polarograms at 25° of an equilibrium mixture (30 minutes after preparation) which was originally 2×10^{-3} M in RSSR and 0.02 M in Na_2SO_3 in an ammonia buffer (M NH_3, 0.1 M NH_4Cl, pH 10.3).

(13) I. M. Kolthoff and C. Barnum, THIS JOURNAL, **62**, 3061 (1940).

(14) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1940.

(15) Cathodic waves of cysteine sulfonate have been described in a previous paper.[11]

formed. Polarograms of the type given in Fig. 1 were also run in alkaline equilibrium mixtures of cysteine and cysteine sulfonate. Both sets of experiments indicated that reaction (1) is reversible

$$RSSR + SO_3^{=} \longrightarrow RS^- + RSSO_3^- \qquad (1)$$

and that under the experimental conditions equilibrium is attained rapidly from both sides.

Determination of the Apparent Equilibrium Constants and Heat of Reaction.—From the polarographic determination of the cysteine concentration the equilibrium constant in a given mixture can be calculated. The anodic cysteine waves in equilibrium mixtures are diffusion controlled and no indication of kinetic currents was obtained. Experimental results and calculated values of the apparent equilibrium constant K in mixtures of various temperature, pH, ionic strength and concentration of reactants are listed in Table II.

Polarographic experiments with solutions of high pH (11–13) containing only cystine revealed that this amino acid is not decomposed in strongly alkaline medium during the first hour after preparation of the solution at temperatures between 12 and 37°. This makes possible equilibrium measurements in solutions of pH as high as 13.4.

The values of the apparent equilibrium constant K in Table II have been calculated by introducing total concentrations for RSSR, RS^-, $RSSO_3^-$ and $SO_3^{=}$ in equation (2).

$$K_{app.} = \frac{[\Sigma\ RS^-][\Sigma\ RSSO_3^-]}{[\Sigma\ RSSR][\Sigma\ SO_3^{=}]} \qquad (2)$$

Actually the charge types of cysteine, cystine, cysteine sulfonate vary with the pH. At a pH greater than 9 all the sulfite may be considered present as $SO_3^{=}$, while at a pH greater than 8 the carboxyl groups of cysteine and cystine and the sulfonate group of cysteine sulfonate are present as the anions. On the other hand, the dissociation of the sulfhydryl group in cysteine and the amino groups in RS^-, RSSR and $RSSO_3^-$ varies in the pH range between 8 and 11 and this accounts for the fact that the apparent constant $K_{app.}$ varies with pH.

The acid–base constants of the various groups of cysteine and cystine are known. In the pH range listed in Table II the carboxyl group is in the ionized form and its constant need not be considered. The variation of the acid–base constants of cysteine with temperature was estimated from

TABLE II

APPARENT EQUILIBRIUM CONSTANT OF EQUATION (2) IN CYSTINE SYSTEM AT 12°, 25°, AND 37°. INITIAL CONCENTRATION OF RSSR 2.00×10^{-3} *M*

Temp. °C.	Initial concentration of sulfite (*M*)	Buffer	μ	*p*H[a]	i_d of cysteine μa.	Σ Cysteine *M*	$K_{app.} \times 10^2$	No. of expt.
12	0.15	0.2 *M* NaOH, 0.3 *M* KCl	0.95	13.40	2.17	9.24×10^{-4}	0.60	38
12	.05	0.1 *M* NaOH, 0.75 *M* KCl	1.0	13.23	1.55	6.58×10^{-4}	0.65	37
25	.05	0.1 *M* NaOH, 0.75 *M* KCl	1.0	12.78	2.34	7.91×10^{-4}	1.04	13
37	.15	0.2 *M* NaOH, 0.3 *M* KCl	0.95	12.51	4.48	1.28×10^{-3}	1.53	40
37	.05	0.1 *M* NaOH, 0.75 *M* KCl	1.0	12.41	3.25	9.30×10^{-4}	1.64	39
25	.025	0.05 *M* Na_2HPO_4 0.075 *M* NaOH, 0.7 *M* KCl	1.0	12.13	1.80	6.08×10^{-4}	1.06	10
25	.05	0.05 *M* Na_2HPO_4 0.075 *M* NaOH, 0.6 *M* KCl	1.0	12.17	2.35	7.94×10^{-4}	1.04	9
25	.10	0.05 *M* Na_2HPO_4 0.075 *M* NaOH, 0.475 *M* KCl	1.0	12.11	2.96	1.01×10^{-3}	1.03	11
12	.05	0.05 *M* Na_2HPO_4 0.075 *M* NaOH, 0.6 *M* KCl	1.0	12.39	1.55	6.44×10^{-4}	0.60	36
25	.05	0.075 *M* Na_2HPO_4 0.05 *M* NaOH, 0.75 *M* KCl	1.0	11.20	2.45	8.17×10^{-4}	0.98	15
25	.05	0.075 *M* Na_2HPO_4 0.025 *M* NaOH, 0.6 *M* KCl	1.0	10.20	3.22	1.04×10^{-3}	1.32	14
25	.05	*M* NH_3 0.1 *M* NH_4Cl, 0.75 *M* KCl	1.0	10.22	3.26	1.05×10^{-3}	1.35	18
25	.02	*M* NH_3, 0.1 *M* NH_4Cl	0.16	10.32	2.66	8.17×10^{-4}	1.76	6
25	.03	*M* NH_3, 0.1 *M* NH_4Cl	0.19	10.37	3.03	9.30×10^{-4}	1.70	7
25	.05	*M* NH_3, 0.1 *M* NH_4Cl	0.25	10.37	3.50	1.10×10^{-3}	1.68	8
25	.025	*M* NH_3, *M* NH_4Cl	1.07	9.32	3.39	1.06×10^{-3}	2.19	17
25	.05	*M* NH_3, *M* NH_4Cl	1.15	9.34	4.04	1.26×10^{-3}	1.96	16
37	.025	*M* NH_3, *M* NH_4NO_3	1.07	8.98	4.54	1.33×10^{-3}	2.94	44
37	.05	*M* NH_3, *M* NH_4Cl	1.15	9.00	5.26	1.54×10^{-3}	2.81	43
25	.025	0.1 *M* NH_3, *M* NH_4Cl	1.07	8.23	5.28	1.62×10^{-3}	6.79	20
25	.05	0.1 *M* NH_3, *M* NH_4Cl	1.15	8.27	5.83	1.79×10^{-3}	7.73	19
37	.05	0.1 *M* NH_3, *M* NH_4Cl	1.15	7.75	6.52	1.91×10^{-3}	8.9	41
25	.05[b]	0.05 *M* Na_2HPO_4 0.075 *M* NaOH, 0.6 *M* KCl	1.0	12.22	3.95	1.34×10^{-3}	(1.30)[b]	12

[a] The *p*H was measured at the temperature given in column 1. [b] Components added: 2×10^{-3}*M* $RSSO_3^-$ and 2×10^{-3}*M* RSH, value of $K_{app.}$ not reliable since concentration of $RSSO_3^-$ and Na_2SO_3 are not sufficiently accurately known.

the apparent heat of ionization $\Delta H'$ for the sulfhydryl and amino group, applying the van't Hoff equation and assuming that $\Delta H'$ is independent of temperature, over the range of measurement. $\Delta H'$ for the sulfhydryl group is assumed to be very small and thus no temperature correction was applied for the dissociation constant of this group as suggested by Cohn[16] and Borsook, *et al.*[17] $\Delta H'$ for the NH_3^+-group ranges between +10,000 and +13,300 cal. as determined for a number of amino acids and peptides.[18] Taking 12,000 cal. for $\Delta H'$ for the amino group of cysteine, approximate *pK* values of the acid–base constants at 12 and 37° are obtained from values at 25° reported by Ryklan and Schmidt.[19]

(1) Constants of cysteine at 12, 25 and 37°: The concentrations of the various species are denoted by letters above the symbols. Equation (7) was used to calculate the concentration of each of the species of cysteine at a given *p*H.

$$\overset{c}{\underset{|}{RSH}}\underset{NH_3^+}{} \rightleftharpoons \overset{d}{\underset{|}{RS^-}}\underset{NH_3^+}{} + H^+, \; pK'_A = 8.66 \text{ at } 12, 25 \text{ and } 37° \quad (3)$$

$$\overset{c}{\underset{|}{RSH}}\underset{NH_3^+}{} \rightleftharpoons \overset{b}{\underset{|}{RSH}}\underset{NH_2}{} + H^+, \; pK'_B \begin{cases} 8.99 \text{ at } 12° \\ 8.60 \text{ at } 25° \\ 8.25 \text{ at } 37° \end{cases} \quad (4)$$

$$\overset{d}{\underset{|}{RS}}\underset{NH_3^+}{} \rightleftharpoons \overset{a}{\underset{|}{RS^-}}\underset{NH_2}{} + H^+, \; pK'_C \begin{cases} 10.84 \text{ at } 12° \\ 10.45 \text{ at } 25° \\ 10.10 \text{ at } 37° \end{cases} \quad (5)$$

$$\overset{b}{\underset{|}{RSH}}\underset{NH_2}{} \rightleftharpoons \overset{a}{\underset{|}{RS^-}}\underset{NH_2}{} + H^+, \; pK'_D = 10.51 \text{ at } 12, 25 \text{ and } 37° \quad (6)$$

$$\Sigma \text{ Cysteine} = a + b + c + d = a\left[1 + \frac{[H^+]}{K'_D} + \frac{[H^+]^2}{K'_D K'_B} + \frac{[H^+]}{K'_C}\right] \quad (7)$$

(2) Constants of cystine, K'_3 and K'_4 at 12, 25 and 37° as obtained from data at 25° reported by Borsook, *et al.*[17] The constants at 12 and 37° were obtained in the same way as given for cysteine.

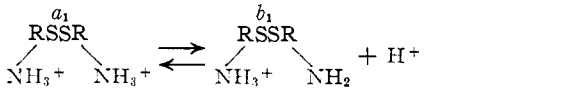
From the concentration of the total cystine in the equilibrium mixture the concentration of the different species can be calculated. The equilib-

(16) E. J. Cohn, *Ergebnisse d. Physiol.*, **33**, 781 (1931).

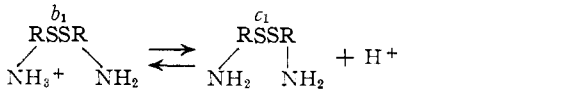
(17) H. Borsook, E. L. Ellis and H. M. Huffman, *J. Biol. Chem.*, **117**, 281 (1937).

(18) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943.

(19) L. R. Ryklan and C. L. A. Schmidt, *Arch. Biochem.*, **5**, 89 (1944).



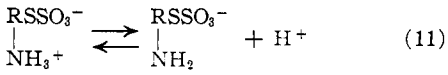
$$pK_3' \begin{cases} 8.39 \text{ at } 12° \\ 8.00 \text{ at } 25° \\ 7.65 \text{ at } 37° \end{cases} \quad (8)$$



$$pK_4' \begin{cases} 10.64 \text{ at } 12° \\ 10.25 \text{ at } 25° \\ 9.90 \text{ at } 37° \end{cases} \quad (9)$$

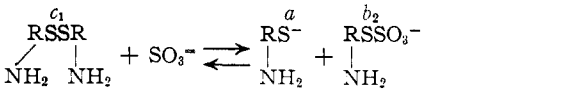
$$\Sigma \text{ Cystine} = a_1 + b_1 + c_1 = b_1\left[\frac{[H^+]}{K_3'} + 1 + \frac{K'}{[H^+]}\right] \quad (10)$$

rium concentration of the total cystine is obtained by subtracting the equilibrium concentration of the total cysteine formed from the concentration of the total cystine in the original mixture. The total concentration of cysteine sulfonate is equal to the total concentration of cysteine in equilibrium mixtures prepared from cystine and sulfite. Only the following dissociation of $RSSO_3^-$ need to be considered in alkaline medium

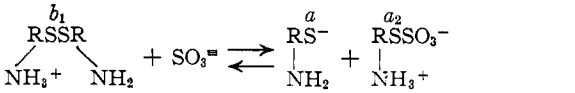


(11)

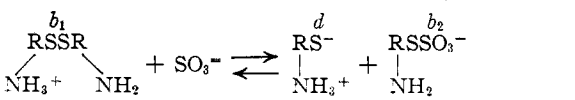
with an equilibrium constant denoted as K_S. It is now possible to calculate the equilibrium constants with reference to the various species of cystine, cysteine sulfonate and cysteine.



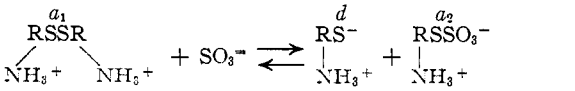
$$K_I = \frac{ab_2}{c_1[SO_3^=]} \quad (12)$$



$$K_{II} = \frac{aa_2}{b_1[SO_3^=]} \quad (13)$$



$$K_{III} = \frac{db_2}{b_1[SO_3^=]} \quad (14)$$



$$K_{IV} = \frac{da_2}{a_1[SO_3^=]} \quad (15)$$

Introducing K_S and combining equations (12) and (13) gives

$$K_{II} = \frac{K_4'}{K_S} K_I \quad (16)$$

Equations (13) and (14) combined with equations (5) and (16) give

$$K_{III} = \frac{K_4'}{K_C'} K_I \quad (17)$$

Equations (14), (15) and (17) give

$$K_{IV} = K_{III}\frac{K_3'}{K_S} = \frac{K_4'K_3'}{K_C'K_S} K_I \quad (18)$$

From equations (16), (17) and (18) it is seen that the equilibrium constants K_{II}, K_{III} and K_{IV} can be expressed in terms of K_I and K_S and of the known dissociation constants of cysteine and cystine.

The constant K_I which involves the species with uncharged amino groups is found directly from experiments with solutions of sufficiently high pH. As is to be expected, the value of the apparent constant in Table II remains practically constant in the pH range between 11.2 and 13.4. In this strongly alkaline medium $K_{app.}$ is approximately equal to K_I. Application of slight corrections by use of various equations (example *v.i.*) yields values of K_I of 0.0062, 0.0103 and 0.0159 at 12, 25 and 37°, respectively.

An example of the calculation of K_I follows

Expt. No. 13, original mixture: 2×10^{-3} *M* RSSR, 0.05 *M* Na_2SO_3; $[H^+] = 1.7 \times 10^{-13}$, $\mu = 1$

i_d of cysteine in equilibrium mixture: 2.34 μA. at − 0.4 volt

i_d of 10^{-3}*M* cysteine in the same buffer: 2.96 μA. at − 0.4 volt (see Table I)

Total cysteine at equilibrium = 7.9×10^{-4} *M*

From equation (7)

$$a = 7.83 \times 10^{-4}\ M \ldots\ldots \left[\begin{matrix}RS^- \\ | \\ NH_2\end{matrix}\right]$$

$$d = 3.65 \times 10^{-6}\ M \ldots\ldots \left[\begin{matrix}RS^- \\ NH_3^+\end{matrix}\right]$$

$\Sigma RSSO_3^-$ = total cysteine = 7.9×10^{-4} *M*

$\Sigma RSSR = 2 \times 10^{-3} - 7.9 \times 10^{-4} = 1.2 \times 10^{-3}$ *M*

$[SO_3^=] = 5 \times 10^{-2} - 7.9 \times 10^{-4} = 4.9 \times 10^{-2}$ *M*

$K_{app.} = 0.0104 = K_I$ at 25°

It is seen that d is negligibly small as compared to a, the latter being practically equal to the concentration of the total cysteine formed. Since the amino groups of cystine and cysteine sulfonate are practically all present in the uncharged form at pH 12.8 the apparent constant can be considered to be equal to K_I. The two dissociation constants of sulfurous acid are 0.017 and 6.24×10^{-8} at 25°.[20] Thus correction for the formation of bisulfite needs to be made only in solutions of pH lower than 9.

Knowing K_I the constant K_S of equation (11) can be calculated by applying equations (10) and (12) at a lower pH. An example for the calculation of K_S at 25° follows

Expt. No. 20; original mixture: 2×10^{-3} *M* RSSR, 0.025 *M* Na_2SO_3, $[H^+] = 5.9 \times 10^{-9}$

i_d of cysteine in equilibrium mixture: 5.28 μa. at −0.30 volt

i_d of 10^{-3} *M* cysteine in the same buffer: 3.25 μa. at −0.30 volt

Total cysteine: 1.62×10^{-3} *M*

$a = 2.02 \times 10^{-6}$ *M*

$d = 3.34 \times 10^{-4}$ *M*, $\Sigma RS^- = a + d = 3.36 \times 10^{-4}$ *M*

$\Sigma RSSO_3^- = 1.62 \times 10^{-3}$ *M*, $\Sigma RSSR = 3.76 \times 10^{-4}$ *M*; $[SO_3^=] = 2.14 \times 10^{-2}$ *M*

From equation (10)

$\Sigma RSSR = 3.76 \times 10^{-4}$ *M*

$b_1 = 2.35 \times 10^{-4}$ *M*, $c_1 = 2.24 = 10^{-6}$ *M*

(20) Norio Yui, *Bull. Inst. Phys. Chem. Research*, **19**, 1229 (1940); H. V. Tartar and H. H. Garretson, THIS JOURNAL, **63**, 808 (1941).

TABLE III

EQUILIBRIUM CONSTANTS, CHANGE IN FREE ENERGY ΔF AND HEAT OF REACTION ΔH OF REACTIONS I, II, III AND IV

Temp., °C.	K_I	K_{II}	K_{III}	K_{IV}	ΔF_I, cal.	ΔF_{II}, cal.	ΔF_{III}, cal.	ΔF_{IV}, cal.	ΔH, cal.
12	0.0060	0.00031	0.0095	0.086	−2900	−4600	−2600	−1400	+6900
25	.010	.00052	.016	.15	−2700	−4500	−2500	−1100	
37	.016	.00082	.025	.23	−2500	−4400	−2300	− 900	+6700

From equation (12)

$$b_2 = \frac{K_I c_1 [SO_3^=]}{a} = 2.45 \times 10^{-4}\ M$$

$$a_2 = \Sigma RSSO_3^- - b_2 = 1.38 \times 10^{-3} M$$

$$K_S = \frac{b_2[H^+]}{a_2} = 1.05 \times 10^{-9}$$

The values for K_S at 25° obtained from experiments 16, 17, 19 and 20 are 1.50, 1.06, 0.85 and 1.05×10^{-9}, the average value of K_S is 1.1×10^{-9}.

Values of K_S obtained from solutions of pH markedly greater than 10 show greater variations and are not reliable since the difference between a_2 and b_2 becomes too large as compared to the accuracy of the measurements.

Values for K_S at 37°, obtained from experiments 41, 43 and 44, are 3.3, 4.8 and 2.8×10^{-9} the average of K_S at 37° being 3.6×10^{-9}. From the K_S values at 25 and 37° a heat of dissociation $\Delta H'$ of 17,900 cal. for the NH_3^+-group of cysteine sulfonate is calculated. This value is higher but of the same order of magnitude as the corresponding values for $\Delta H'$ of other amino acids. Taking 12,000 cal. for $\Delta H'$ and 1.1×10^{-9} for K_S at 25° the constant at 37° is calculated to be 2.5×10^{-9} instead of 3.6×10^{-9}.

From equations (16), (17) and (18) the equilibrium constants K_{II}, K_{III} and K_{IV} can now be calculated. The values of these constants, the change in free energy ($\Delta F = RT \ln K$) and the heat of reaction ΔH for the reactions I, II, III and IV are given in Table III.

Assuming that ΔH for the amino groups of RSSR, RSH and $RSSO_3^-$ is the same, it is found that the heat of reaction of the four equilibrium reactions is the same ($\Delta H_I = \Delta H_{II} = \Delta H_{III} = \Delta H_{IV} = \Delta H$). ΔH was estimated from the experimental values of K_I at 12, 25 and 37° and from the apparent constants (Table II) at 25 and 37° obtained with equilibrium mixtures of pH 9.3, 8.3 and 7.75. The average values for ΔH estimated from the results of various experiments are found to be the same (about +7,000 cal.)

II. The Reaction between Dithiodiglycolic Acid and Sulfite.—Polarographic experiments with mixtures of dithiodiglycolic acid denoted as R_1SSR_1 and sulfite at pH 6 to 9 indicate that the reaction is reversible, the equilibrium being more rapidly attained in alkaline (10 to 15 minutes at pH 9.4) than in acid solutions (about 2 hours at pH 6.6). The anodic wave of thioglycolic acid and the cathodic waves of dithiodiglycolic acid and of thioglycolic acid sulfonate resemble those of cysteine, cystine and cysteine sulfonate, respectively, and polarograms obtained with equilibrium mixtures prepared from R_1SSR_1 and sulfite were similar to those given in Fig. 1.

Experiments in solutions of pH markedly higher than 9.5 cannot be carried out with dithiodiglycolic acid since this acid, in variance with cystine, decomposes rapidly in strongly alkaline medium to form thioglycolic acid. Polarograms obtained with solutions of R_1SSR_1 in buffers of pH 10.3 and 12.3 at 25°, 30 minutes after preparation are shown in Fig. 2. The higher anodic wave in the more alkaline solution indicates that the hydrolysis of R_1SSR_1 proceeds faster with increasing pH. After 30 minutes 12 and 78% of the R_1SSR_1 was decomposed in solutions of pH 10.3 and 12.3, respectively.

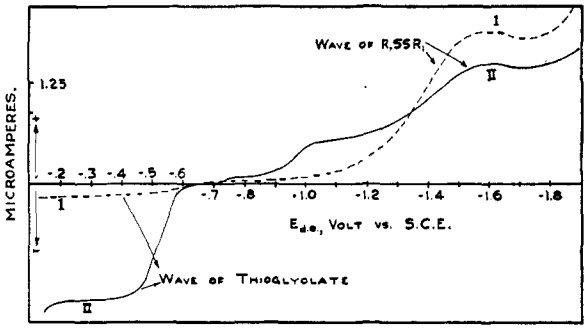


Fig. 2.—Polarograms at 25° of solutions (30 minutes after preparation) which originally had the following composition: I, 1.04×10^{-3} M R_2SSR_1 (0.1 M NH_4Cl, M NH_3, 0.9 M KCl), pH 10.3----; II, 2.3×10^{-3} M R_1SSR_1 (0.05 M Na_2HPO_4, 0.075 M NaOH, 0.6 M KCl), pH 12.3—.

Schöberl, *et al.*,[21] who carried out extensive experiments on the hydrolytic cleavage of disulfide carbonic acids isolated and identified the hydrolysis products of dithiodiglycolic acid as thioglycolic acid, hydrogen sulfide and glyoxylic acid. In agreement with our findings he also reports that cystine in alkaline solution is more stable than α-disulfide carbonic acids in the same medium. A polarographic study of the hydrolytic cleavage of various disulfide-amino acids and -peptides is planned as an aid in the understanding of the alkali denaturation of proteins.

The equilibrium constants of the reaction between dithiodiglycolic acid and sulfite can be calculated from the measured diffusion current of thioglycolic acid in an equilibrium mixture of known over-all composition and pH. The dissociation constants of thioglycolic acid have been reported by Larson[22] at 25° as $K_1 = 2.14 \times 10^{-4}$, and $K_2 = 2.1 \times 10^{-11}$. In the pH range investigated the carboxyl groups of R_1SSR_1, R_1SH (thioglycolic acid) and $R_1SSO_3^-$ (thioglycolic acid sulfonate) as well as the sulfonate group of $R_1SSO_3^-$ are present as anions. On the other hand, the dissociation of the SH group varies in the pH range studied while the sulfite is partly present as

(21) A. Schöberl, *et al.*, *Ann.*, **522**, 97 (1936); **534**, 210 (1938); **538** 84 (1934).

(22) E. Larson, *Z. anorg. allgem. Chem.*, **172**, 378 (1928).

TABLE IV

EQUILIBRIUM CONSTANT K_X OF EQUATION (19) AT 25°, IONIC STRENGTH APPROXIMATELY ONE

Components added R_1SSR_1 M	Na_2SO_3 M	Buffer	pH	Δ of thioglycolic acid μa.	Σ Thioglycolic acid M	K_X × 10⁴
2.08 × 10⁻³	0.05	M NH_3, M NH_4Cl	9.32	3.01	8.41 × 10⁻⁴	5.0
1.04 × 10⁻³	.10	0.4 M NH_3, 0.4 M NH_4Cl, 0.3 M KCl	9.37	2.30	6.43 × 10⁻⁴	5.0
1.04 × 10⁻³	.05	0.5 M NH_3, 0.5 M NH_4Cl, 0.3 M KCl	9.30	1.93	5.40 × 10⁻⁴	4.8
2.08 × 10⁻³	.05	0.1 M NH_3, M NH_4Cl	8.30	4.99	1.35 × 10⁻³	2.4
1.04 × 10⁻³	.0125	0.1 M NH_3, M NH_4Cl	8.31	1.95	5.29 × 10⁻⁴	2.2
2.08 × 10⁻³	.0125	0.1 M Na_2HPO_4, 0.01 M N. H_2PO_4, 0.65 M KCl	7.36	6.19	1.70 × 10⁻³	5.8
1.04 × 10⁻³	.1025	0.08 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.71 M KCl	7.34	3.36	9.23 × 10⁻⁴	5.0
1.04 × 10⁻³	.025	0.05 M Na_2HPO_4, 0.05 M NaH_2PO_4, 0.73 M KCl	6.69	3.72	1.01 × 10⁻³	6.9
1.04 × 10⁻³	.0063	0.05 M Na_2HPO_4, 0.05 M NaH_2PO_4, 0.78 M KCl	6.38	3.29	9.17 × 10⁻⁴	5.0
					Average	4.7

$SO_3^=$ and partly as HSO_3^-. It is possible to calculate the equilibrium constants for the R_1SSR_1–sulfite system.

$$R_1SSR_1 + SO_3^= \overset{a_1}{\underset{d}{\rightleftharpoons}} R_1S^- + R_1SSO_3^-, K_X = \frac{bb_1}{a_1d} \quad (19)$$

$$R_1SSR_1 + HSO_3^- \overset{a_1}{\underset{c}{\rightleftharpoons}} R_1SH + R_1SSO_3^-, K_Y = \frac{b_1a}{a_1c} \quad (20)$$

Denoting the dissociation constant of the sulfhydryl group of thioglycolic acid as $K_2 = b[H^+]/a$, and the second constant of sulfite as $K_4 = d[H^+]/c$ the following relation between K_X and K_Y is obtained

$$K_X = \frac{K_2}{K_4} K_Y \quad (21)$$

The experimental results and the values for the equilibrium constant K_X at 25° in mixtures of ionic strength of 1 and of various pH and concentrations of reactants are listed in Table IV.

The average value of K_X at 25° is 4.7 × 10⁻⁴ and the change in free energy ΔF_X is −4,500 cal. K_X as calculated from equation (21) is 1.40 and ΔF_Y is +200 cal. at 25°. It is of interest to note that the constant of the reaction with sulfite and cystine containing free amino groups (K_I in equation 12) is 21 times as large as that of dithiodiglycolate. The difference becomes considerably greater when the reaction between cystine, containing two NH_3^+ groups is considered (equation 15).

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[CONTRIBUTION FROM THE NAVAL RESEARCH LABORATORY]

The Size of Soap Micelles in Benzene from Osmotic Pressure and from the Depolarization of Fluorescence[1]

BY C. R. SINGLETERRY AND LORRAINE ARKIN WEINBERGER

The size of oil-soluble soap micelles in non-polar solvents may be determined from the depolarization of the fluorescence emitted from a dye adsorbed by the micelle. The depolarization results from Brownian rotation of the dye-containing micelle during the interval between light absorption and fluorescence emission, the average excited life being of the order of 5 × 10⁻⁹ second. Micelle sizes calculated from fluorescence depolarization have been compared with those obtained by osmotic pressure measurements in similar solutions and found to be slightly smaller but closely proportional to the latter. For a 0.6% solution of calcium xenylstearate in benzene, fluorescence gives a gram micellar weight of 22,500, while osmotic pressure indicates a weight of 23,700. The fluorescence technique provides a convenient and rapid tool for the investigation of very small colloidal particles or of macromolecules in any system in which a suitable fluorescent material can be quantitatively associated with the colloidal phase. It is applicable at much higher dilutions than viscometry, osmometry or light scattering. The precision of the method decreases as the product of the gram micellar volume by solvent viscosity increases. With present techniques results become mainly qualitative if ηV exceeds 1500 (poises × cm.³).

Introduction

The usefulness of fluorescent dyes as indicators of the presence of micelles of oil-soluble soaps in non-aqueous systems,[2] and the existence of a relationship between the degree of polarization of the emitted fluorescence and the volume of the micelle have been indicated in preliminary publications.[3] The phenomenon provides a convenient and powerful tool for the determination of particle size in colloidal systems. The present communication presents more detailed information concerning the spectral phenomena observed, the experimental procedures, and the factors affecting inferences of particle size from fluorescence depolarization.

When a small amount of an oil-soluble, micelle-forming soap is added to a dilute benzene solution

(1) The opinions or assertions contained in this communication are the authors' and are not to be construed as official or reflecting the views of the Navy Department. Article not copyrighted.

(2) The enhancement of the fluorescence of several dyes in aqueous systems containing soap micelles was reported by M. L. Corrin and W. D. Harkins, THIS JOURNAL, **69**, 679 (1947).

(3) Lorraine Arkin and C. R. Singleterry, *ibid.*, **70**, 3965 (1948); Lorraine Arkin and C. R. Singleterry, *J. Colloid Sci.*, **4**, 537 (1949).