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Reactions between Mercuric Mercury and Cysteine and Glutathione. Apparent Dissociation Constants, Heats and Entropies of Formation of Various Forms of Mercuric Mercapto-Cysteine and -Glutathione

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Reactions of cysteine and of reduced glutathione with mercuric mercury in media of various *p*H have been studied polarographically. In the absence of chloride, and in the *p*H range between 3 and 9, cysteine and glutathione can form at least three compounds with mercury: $Hg(RS)_2$, $Hg_2(RS)_2$ and $Hg_3(RS)_2$, in which mercury is bound firmly as mercaptide. In the presence of much chloride the formation of the compounds $Hg_2(RS)_2$ and $Hg_3(RS)_2$ is suppressed by the complex $HgCl_4^{--}$. The formation of compounds of the type RSHgCl could not be demonstrated polarographically. The apparent dissociation constants of three charge types of the mercaptides of cysteine and glutathione were calculated from e.m.f. measurements of mercury in equilibrium with solutions of the mercaptides in the entire *p*H range in the presence of a large excess of either cysteine or glutathione. The constants of the mercaptide species with two charged amino groups are found to be of the same order of magnitude for both cysteine and glutathione, while the constants of other species differ to a greater extent. From the values of the dissociation constants at 12 and 25° the heats and entropies of formation were calculated.

The stable bond between mercury and sulfur of sulfhydryl groups has been made wide use of in biology. Thus mercury compounds have long been used as specific reagents for sulfhydryl groups in biological materials.[1-5] Mercury has also found application in the isolation of proteins like mercaptoalbumin.[6] More recently analytical use of mercury compounds was made in the quantitative mercurimetric determination of sulfhydryl and disulfide groups in proteins, peptides and amino acids.[7,8]

Considering the wide biochemical applicability of mercury as a sulfhydryl reagent, a more complete knowledge of the reactions between mercury and sulfhydryl containing amino acids, peptides and proteins is desirable.

This paper deals with a quantitative study of the mercuric–cysteine (RSH) and the mercuric–glutathione (GSH) systems at various *p*H and temperatures. It is shown polarographically that mercury can form at least three compounds with RSH and GSH, respectively. The mercury bound to the sulfur is very slightly dissociated. Apparent dissociation constants, heat and entropy of formation of the mercaptides of cysteine and glutathione have been calculated from e.m.f. measurements. The results obtained with the amino acid (RSH) are compared with the data of the peptide (GSH).

Materials.—Cysteine used as free base was a product from Mann, glutathione was a Pfanstiehl product. The purity of these products was 99% as determined by titration with cupric copper.[9] Stock solutions of these compounds (0.01 and 0.2 *M*) were prepared in air-free water. Only freshly prepared stock solutions of the amino acid and peptide were used. The stock solution of mercuric acetate (a C.P. product from Powers–Weightman–Rosengarten Co.) was 0.05 *M* in mercury and 0.08 *M* in acetic acid. The concentration of the stock solution of mercuric chloride (a Mallinckrodt product, better than 99.5% pure) was 0.05 *M* in Hg(II). All the other chemicals used were C.P. reagent grade products.

The stock solutions for the preparation of the buffers were: *M* hydrochloric acid, *M* acetic acid, *M* sodium acetate, 0.2 *M* disodium phosphate, 0.2 *M* monosodium phosphate, 0.1 *M* borax, 0.4 *M* boric acid, 2.6 *M* sodium hydroxide, *M* ammonia, *M* ammonium nitrate. The ionic strength was adjusted by addition of appropriate volumes of 2 *M* potassium nitrate or 4 *M* potassium chloride solutions.

Experimental Methods.—Current–voltage curves were determined at 25.0 ± 0.1° with the manual apparatus and circuit described by Lingane and Kolthoff[10] and automatically with a Heyrovsky self-recording polarograph. The characteristics of the capillary were: $m = 1.56$ mg. sec.$^{-1}$, $t = 4.82$ sec. (open circuit); $m^{2/3}t^{1/6} = 1.748$ mg.$^{2/3}$ sec.$^{-1/2}$; $h = 80$ cm. A layer of chloroform at the bottom of the cell was used to prevent the mercury metal from reacting with free mercury in solution. As maximum suppressor 10^{-3} to 2×10^{-3}% gelatin was used. Dissociation constants of the mercuric mercaptides of cysteine and glutathione were calculated from e.m.f. measurements of mercury metal in equilibrium with mercuric mercaptides in the presence of a large excess of uncomplexed thiol. Under these conditions all the mercury in the solution is present in the mercuric form. Mercurous cysteinate and glutathionate are unstable and readily decompose to mercury metal and the mercuric compounds.[11] The mercuric ion concentration was calculated from the measured potential. All potentials were measured with a Leeds and Northrup student potentiometer against the saturated calomel electrode (S.C.E.) which was kept at the same temperature as the test solution (25.0 ± 0.1° and 12 ± 0.1°) using a salt bridge with saturated potassium chloride solution. The potential of the S.C.E. against the hydrogen electrode was calculated by the expression[12]

$$E_{S.C.E.} = -0.2420 + 0.00076\,(t - 25) \qquad (1)$$

where t is the temperature in °C. Oxygen was removed from the solution in the cell with a stream of oxygen-free nitrogen which was purified by bubbling through vanadous sulfates.[13] An atmosphere of nitrogen was maintained over the solution during the measurements. The *p*H was measured with a Beckman *p*H meter, Laboratory Model G, using the Beckman "General Purpose" glass electrode for the entire *p*H range. *p*H measurements at above 10 were corrected for the sodium ion concentration.

The cell for the e.m.f. measurements was provided with a rubber stopper with holes for a nitrogen inlet tube, for a tube with sintered glass bottom which contained the salt bridge, and with a hole which served for introducing solutions into the cell by means of a pipet. Ten to 20 ml. of pure mercury was placed into the cell and a given volume of buffer solution

(1) L. Hellerman, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 165 (1939).

(2) H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Revs.*, **34**, 157 (1944).

(3) M. L. Anson, *Advances Protein Chem.*, **2**, 361 (1945).

(4) H. S. Olcott and H. Fraenkel-Conrat, *Chem. Revs.*, **41**, 151 (1947).

(5) G. E. S. Barron, *Advances Enzymol.*, **11**, 201 (1951).

(6) W. L. Hughes, Jr., THIS JOURNAL, **69**, 1836 (1947); *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 79 (1949).

(7) W. L. Hughes, Jr., "Proceedings, First Conference on Cancer Diagnostic Tests," p. 26, 1950.

(8) W. Stricks and I. M. Kolthoff, *Anal. Chem.*, **25**, 1050 (1953).

(9) I. M. Kolthoff and W. Stricks, *ibid.*, **23**, 763 (1951).

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

(11) W. Stricks and I. M. Kolthoff, *ibid.*, **74**, 4646 (1952).

(12) S. Glasstone, "Textbook of Physical Chemistry," D. Van Nostrand and Co., Inc., New York, N. Y., 1940, p. 922.

(13) L. Meites and T. Meites, *Anal. Chem.*, **20**, 984 (1948).

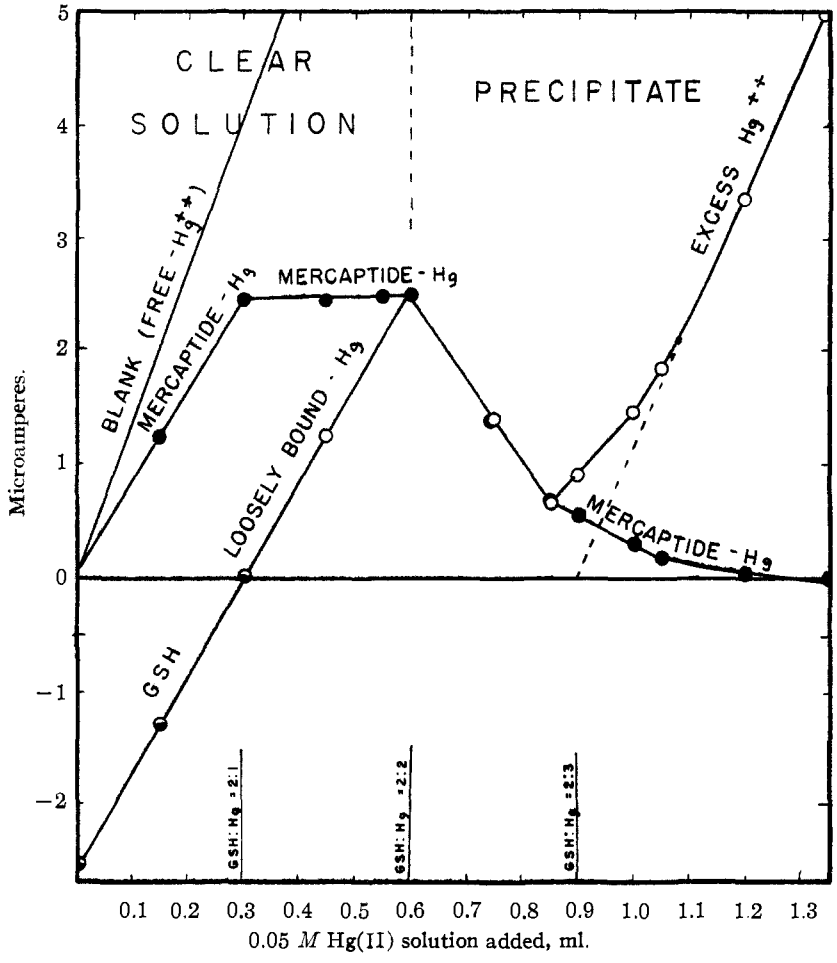


Fig. 1.—Titration of 30 ml. of 10^{-3} *M* GSH (acetate buffer, *p*H 5.6, 1.7×10^{-3}% gelatin) with 0.05 *M* mercuric acetate solution. Plot of diffusion currents *versus* ml. of Hg(II) solution added: ○,●; i_d measured at −0.10 v., ●, i_d measured at −0.65 volt.

added. Nitrogen was bubbled through and appropriate volumes of air-free stock solutions of cysteine or glutathione and of mercuric acetate were added to the air-free buffer, the total volume being either 10 or 20 ml. The sintered glass tube with the salt bridge was lowered into the solution and the e.m.f. measured. The *p*H of each solution was determined at the temperature specified in Tables I to IV.

I. Polarographic Measurements

To air-free 10^{-3} *M* RSH or GSH solutions in the absence and presence of chloride ion and at various *p*H were added known volumes of 0.05 or 0.025 *M* mercuric solutions and complete polarograms were taken after each addition of mercury. From the known polarographic characteristics of cysteine, glutathione and their mercuric mercaptides[11,13a] the diffusion currents (corrected for residual current and change in volume) of the various compounds present in the course of a titration could be plotted. As an example such a plot is presented in Fig. 1, which gives the various diffusion currents in a titration of a 10^{-3} *M* GSH solution in an acetate buffer at *p*H 5.6 in the absence of chloride ion. From Fig. 1 it is seen that the anodic diffusion current of GSH decreases on the addition of mercuric ions and disappears when GSH and mercury are present in a mole ratio 2:1 (first end-point). At this point only one cathodic wave is observed, starting at a potential of −0.3 v., corresponding to the reduction of mercury in the slightly dissociated mercaptide $Hg(GS)_2$. On further addition of mercury the height of the mercaptide wave remains constant and another cathodic wave starts at a more positive potential of about +0.2 v. This wave increases in height along a straight line which is parallel to the line of the mercaptide wave. The slope of these lines is considerably smaller than that of the blank line, obtained by adding mercuric ion to an acetate buffer in the absence of GSH. The location of the various waves is made clear in Fig. 2 which illustrates polarograms obtained during a titration of GSH at *p*H 5.6 with mercury. All observations indicate that most of the mercury added after the first end-point is not present as free mercuric ion but is bound to the mercaptide. When mercury has been added in the mole ratio GSH:Hg = 1:1 the two cathodic waves are of the same height. Apparently a compound $Hg_3(GS)_2$ is formed in which one mercury is bound firmly as mercaptide and one much more loosely, probably by way of carboxyl groups.

The addition of more mercury after the second end-point gives rise to the formation of a white precipitate which results in a reduction in height of the two cathodic waves to the same extent. Thus the change in height of the two waves on the addition of increasing amounts of mercury can now be represented by the same line as is illustrated in Fig. 1. Since no new wave appears in the polarogram it is concluded that the mercury added after the second end-point, combines with $Hg_3(GS)_3$ to form a slightly soluble compound $Hg_3(GS)_2$. When the amount of mercury added is in the vicinity of the mole ratio GSH:Hg = 2:3 (third end-point) the cathodic wave at the more positive potential increases in height again but starts now at +0.28 v. instead of +0.20 v. It is seen from Fig. 1 that this "excess reagent" line is curved at first, becomes steeper after the third end-point and finally attains a slope which is practically equal to that of the blank line. The mercaptide wave, however, decreases continuously along a curved line and finally disappears in the presence of an excess of mercury. Apparently precipitation of the compound $Hg_3(GS)_2$ is not complete at the third end-point and therefore a mercaptide wave is still observed at this point. Addition of more mercury decreases the solubility of $Hg_3(GS)_2$ and gives rise to a further decrease and finally disappearance of the mercaptide wave. The slow precipitation of $Hg_3(GS)_2$ also explains why the excess of mercury line is not parallel to the free mercury line until a considerable excess of reagent is present.

The white precipitate formed during the titration was centrifuged, washed with air-free acetate buffer (*p*H 5.6) and suspended in an air-free acetate buffer of the same *p*H. Part of the precipitate dissolved and the resulting solution was polarographed. The polarograms showed two cathodic waves starting at +0.22 and −0.3 v. with diffusion currents of 0.613 μamp. (measured at −0.1 v.) and of 0.232 μamp. (measured at −0.65 v.) corresponding to the reduction of loosely bound and mercaptide mercury, respectively. The ratio of the heights of the first and the second waves was found to be 2.6 indicating an appreciable dissociation of $Hg_3(GS)_2$ into Hg^{++} and $Hg_2(GS)_2$, the wave of free mercuric ions overlapping with the first wave of $Hg_2(GS)_2$.

Figure 3 presents the results of a titration of a 10^{-3} *M* GSH solution in an acetate buffer (*p*H 5.6) which was 2 *M* in potassium chloride. In the presence of chloride ion no precipitate is formed and only one end-point corresponding to the formation of the mercaptide can be detected. After this end-point the excess mercury line is slightly less steep than the blank line but on further addition of mercury becomes parallel to the blank. Evidently the complex $HgCl_4^{--}$ in the presence of 2 *M* chloride is stable enough to suppress the reaction of excess mercury with the mercaptide.

(13a) I. M. Kolthoff and C. Barnum, THIS JOURNAL, **62**, 306 (1940).

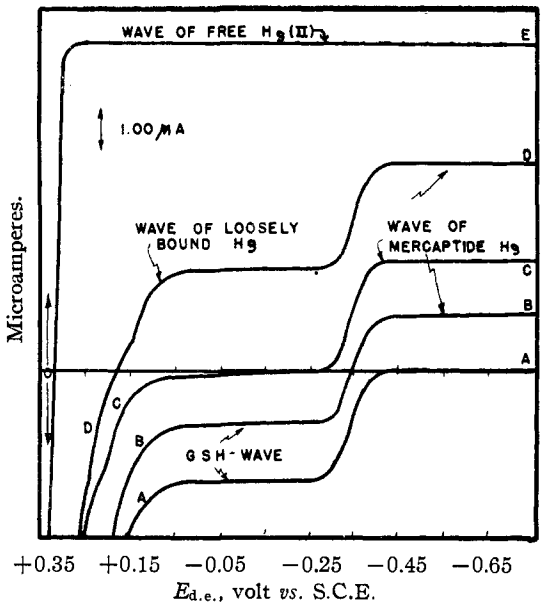


Fig. 2.—Polarograms taken during a titration of 30 ml. of 10^{-3} *M* glutathione (acetate buffer, *p*H 5.6, 1.7 × 10^{-3}% gelatin) with a 0.05 *M* mercuric acetate solution. A, before addition of mercury. After addition of (B) 0.15 ml., (C) 0.3 ml., (D) 0.6 ml. 0.05 *M* Hg(II) solution. (E) Blank: 10^{-3} *M* Hg(II) in acetate buffer in the absence of GSH.

Since the dissociation constant of $HgCl_4^{--}$ is 8.3 × 10^{-16},[14] the constants for mercury bound by carboxyl groups of glutathione must be considerably larger.

Titrations of glutathione carried out at *p*H 9 and *p*H 3 in the absence and presence of chloride ion gave results which were similar to those presented in Figs. 1 and 3. At *p*H markedly lower than 3 no precipitate is formed during the titration in a chloride-free solution and chloride ion has no effect on the titration lines. Thus a titration of 10^{-3} *M* GSH in 0.1 *M* perchloric acid, 0.1 *M* sodium perchlorate gives only the mercaptide end-point and the diagram of the titration is practically identical with that in Fig. 3.

Mercurimetric titrations of cysteine indicate that the amino acid qualitatively behaves in the same way as the peptide in its reactions with mercury. A titration of 10^{-3} *M* RSH in a borax buffer (*p*H 9.06) in the absence of chloride is presented in Fig. 4. The results indicate that cysteine can also form three compounds with mercury: $Hg(RS)_2$, $Hg_2(RS)_2$ and $Hg_3(RS)_2$ in which one mercury atom is bound firmly to sulfur. In the presence of chloride a diagram is obtained which is practically identical with that in Fig. 3.

Polarographic experiments thus indicate that cysteine as well as glutathione can form at least three compounds with mercury in which all (in $Hg(RS)_2$) or a part (in $Hg_2(RS)_2$ and $Hg_3(RS)_2$) of the mercury is bound firmly as a mercaptide. No polarographic evidence can be obtained for the formation of compounds of the type RSHgCl in which one mercury is bound by sulfur as well as by chloride or any other anion.

The polarographic findings on the system cysteine–mercury substantiate and extend Barnum's[15] results on the reactions between cysteine and mercuric chloride. Barnum mixed equimolar solutions of cysteine and mercuric chloride and observed the precipitation of a white amorphous compound which was analyzed and

(14) A. Johnson, I. Quartfort and L. Sillén, *Acta Chem. Scand.*, I, 46, 473 (1947).

(15) C. P. Barnum, Jr., Ph.D. Thesis, University of Minnesota (1940).

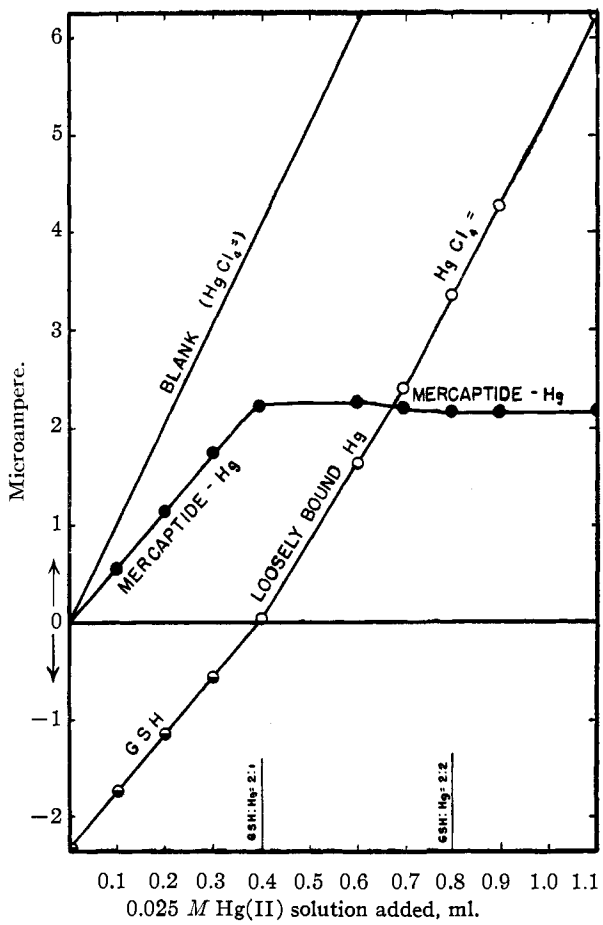


Fig. 3.—Titration of 20 ml. of 10^{-3} *M* GSH (acetate buffer, *p*H 5.57, 2 *M* KCl, 2.5 × 10^{-3}% gelatin) with 0.025 *M* mercuric chloride solution. Plot of diffusion currents *versus* ml. of Hg(II) solution added. ○,◒; i_d measured at −0.15 v.; ●, i_d measured at −0.70 volt.

found to contain one cysteine residue and one mercury, thus corresponding to the formula $Hg_2(RS)_2$. When he mixed cysteine and mercuric chloride in the mole ratio 2:1 the amorphous white precipitate first formed disappeared on stirring, but on standing of the clear solution a white precipitate separated which microscopically was found to consist of transparent needle shaped crystals. Analysis showed this compound to be $Hg(RS)_2$. When an excess of mercuric chloride was added to cysteine a white amorphous precipitate was formed the composition of which corresponded to the formula $Hg(RS)_2 \cdot 2HgCl_2$. The formation of the compounds $Hg(RS)_2$ and $Hg_2(RS)_2$ was also demonstrated by potentiometric titrations with the dropping mercury electrode as indicator electrode. In a potentiometric titration of 20 ml. of 6 × 10^{-3} *M* RSH in a buffer of *p*H 4.48 with 2 × 10^{-2} *M* mercuric chloride Barnum obtained two endpoints corresponding to the compounds $Hg(RS)_2$ and $Hg_2(RS)_2$.

II. Determination of the Apparent Dissociation Constants. Heats and Entropies of Formation of the Mercuric Mercaptides of RSH and GSH

From the mercuric ion concentration determined in a given mixture of mercury(II) and an excess of

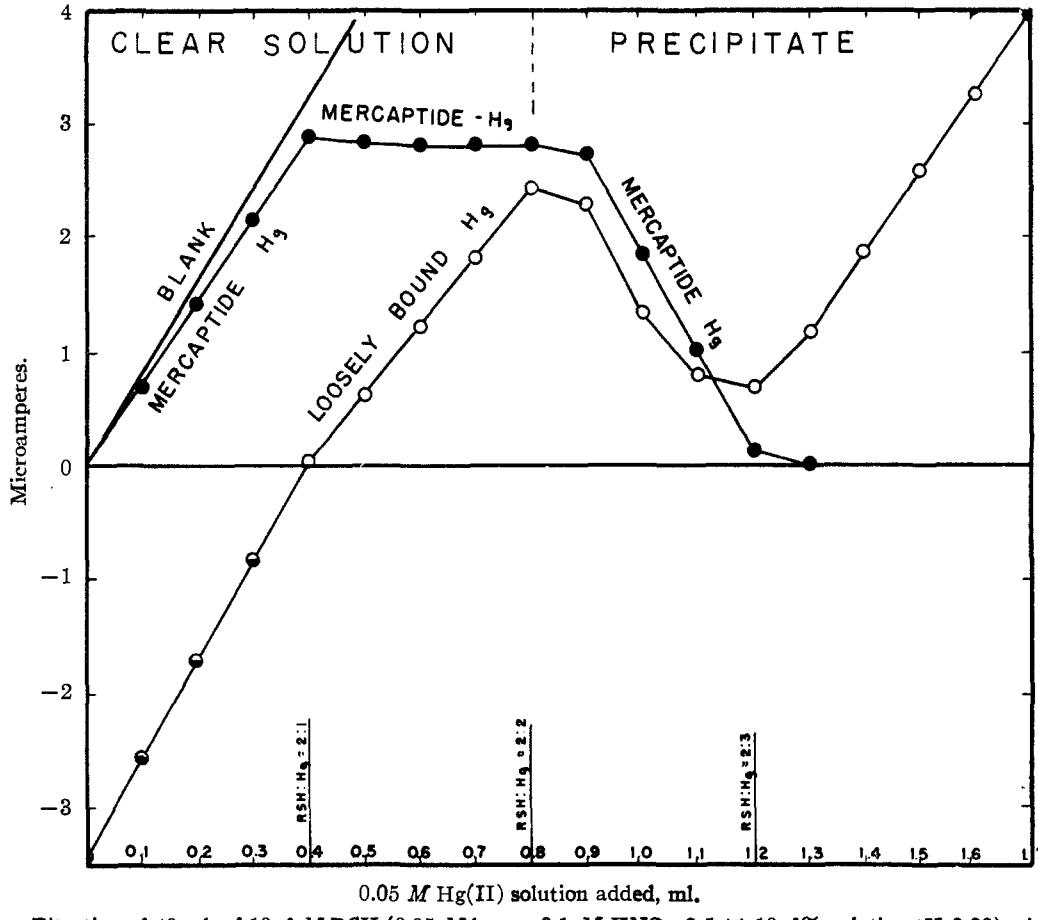


Fig 4.—Titration of 40 ml. of 10^{-3} *M* RSH (0.05 *M* borax, 0.1 *M* KNO_3, 2.5 × 10^{-3}% gelatin, *p*H 9.06) with 0.05 *M* mercuric acetate solution. Plot of diffusion currents *versus* ml. of Hg(II) solution added: ○,●, i_d measured at −0.3 v.; ●, i_d measured at −0.80 volt.

RSH or GSH the apparent dissociation constant of the mercaptide can be calculated. All measurements were carried out at an ionic strength of 1 ± 0.1 and concentrations instead of activities of the various ionized forms of RSH and GSH were used in the calculations. The values of the apparent dissociation constants *K* have been calculated by introducing the proper concentration values in equation 2

$$K_{app.} = [a_{Hg^{++}}][RS^-]^2/[Hg(RS)_2] \quad (2)$$

The mercuric ion activity was calculated from e.m.f. measurements *vs.* S.C.E. using the equation

$$\text{e.m.f.} = E_0 + \frac{RT}{2F} \ln [a_{Hg^{++}}] - 0.2420 + 0.00076\,(t - 25) \quad (3)$$

where E_0 = 0.854 volt,[16] the standard potential (*vs.* N.H.E.) of the mercury–mercuric system.

The charge forms of cysteine and glutathione as well as of the mercaptides vary with the *p*H. The acid–base constants of the various groups of cysteine are known and their variation with temperature was estimated previously.[17] The experimental data in this paper indicate that the charge of the carboxyl groups in RSH and GSH has no effect on the dissociation constants of the mercaptides and therefore the constants of the carboxyl groups need not be considered.

Constants of cysteine at 12 and 25° are given in equations 4, 5, 6 and 7. The concentrations of the various species are denoted by letters above the symbols.

$$\overset{c}{\text{RSH}}\,|\,\text{NH}_3^+ \rightleftharpoons \overset{d}{\text{RS}^-}\,|\,\text{NH}_3^+ + \text{H}^+,\ pK'_A = 8.66 \text{ at } 12 \text{ and } 25° \quad (4)$$

$$\overset{c}{\text{RSH}}\,|\,\text{NH}_3^+ \rightleftharpoons \overset{b}{\text{RSH}}\,|\,\text{NH}_2 + \text{H}^+,\ pK'_B \begin{cases} 8.99 \text{ at } 12° \\ 8.60 \text{ at } 25° \end{cases} \quad (5)$$

$$\overset{d}{\text{RS}^-}\,|\,\text{NH}_3^+ \rightleftharpoons \overset{a}{\text{RS}^-}\,|\,\text{NH}_2 + \text{H}^+,\ pK'_C \begin{cases} 10.84 \text{ at } 12° \\ 10.45 \text{ at } 25° \end{cases} \quad (6)$$

$$\overset{b}{\text{RSH}}\,|\,\text{NH}_2 \rightleftharpoons \overset{a}{\text{RS}^-}\,|\,\text{NH}_2 + \text{H}^+,\ pK'_D = 10.51 \text{ at } 12 \text{ and } 25° \quad (7)$$

$$\Sigma \text{ Cysteine} = a + b + c + d = a\left[1 + \frac{(H^+)}{K'_D} + \frac{(H^+)^2}{K'_D K'_B} + \frac{(H^+)}{K'_C}\right] \quad (8)$$

Equation 8 was used to calculate the concentration of each of the species of cysteine at a given *p*H.

The individual constants of glutathione are not known. However, approximate values of the con-

(16) W. M. Latimer, "The Oxidation States of the Elements and their Potentials in Aqueous Solutions," Prentice-Hall, Inc., New York, N. Y., 1952, p. 179.

(17) W. Stricks and I. M. Kolthoff, THIS JOURNAL, 73, 4569 (1951).

centrations of the various charge species can be calculated, assuming that in analogy to cysteine and homocysteine,[18] the values for glutathione of pK'_A and pK'_B are practically identical at 25°. This assumption is reasonable considering the fact that the measured dissociation constants of the peptide $K'_3 = 2.2 \times 10^{-9}$ (NH_3^+) and $K'_4 = 7.6 \times 10^{-10}$ (SH)[19] differ to a much smaller extent than the corresponding constants of cysteine ($K'_2 = 4.7 \times 10^{-9}$, $K'_3 = 1.7 \times 10^{-11}$).[19] The constants K'_3 and K'_4 as determined directly by pH titration are related as follows to the individual constants[18,20]

$$K'_3 = \frac{[H^+](d+b)}{c} = K'_A + K'_B \qquad (9)$$

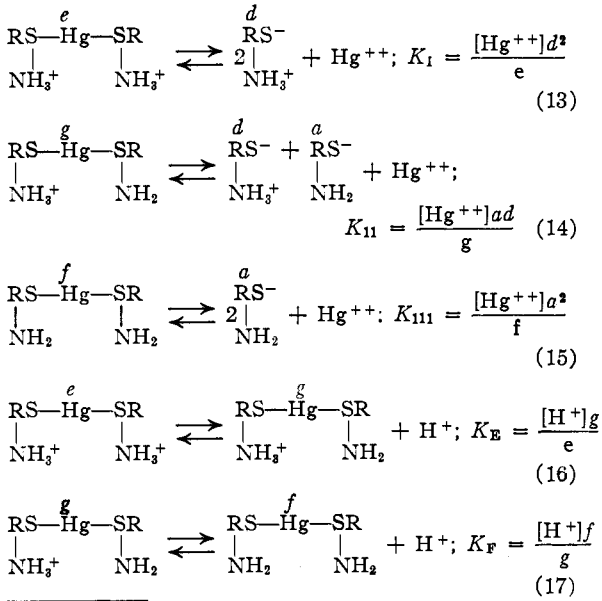
$$K'_4 = \frac{[H^+]a}{(d+b)};\ \frac{1}{K'_4} = \frac{1}{K'_C} + \frac{1}{K'_D} \qquad (10)$$

$$K'_3K'_4 + K'_AK'_C = K'_BK'_D \qquad (11)$$

The concentration of the total glutathione is given by

$$\Sigma\text{glutathione} = a + b + c + d = a\left\{1 + \frac{[H^+]}{K'_4} + \frac{[H^+]^2}{K'_3K'_4}\right\} \qquad (12)$$

where $a[H^+]/K'_4 = b + d$. The small letters refer to the same charge species as indicated for cysteine. Equality of K'_A and K'_B means that b and d are also equal. The values of b and d can be calculated from equation 12. Using equations 9, 10 and 11 and the known values of K'_3 and K'_4, the approximate pK values of the individual constants of glutathione are found to be $pK'_A = pK'_B = 8.96$ and $pK'_C = pK'_D = 8.82$ at 25°. At 12° $pK'_A = 8.96$, $pK'_B = 9.35$, $pK'_C = 9.21$ and $pK'_D = 8.82$. It is now possible to calculate the dissociation constants of the mercaptides with reference to the various species of cysteine and glutathione, respectively.



$$K_I = \frac{[Hg^{++}]d^2}{e} \qquad (13)$$

$$K_{II} = \frac{[Hg^{++}]ad}{g} \qquad (14)$$

$$K_{III} = \frac{[Hg^{++}]a^2}{f} \qquad (15)$$

$$K_E = \frac{[H^+]g}{e} \qquad (16)$$

$$K_F = \frac{[H^+]f}{g} \qquad (17)$$

Combining equations 13 with 14 and 14 with 15 and substituting from equation 6 gives

$$K_E = \frac{K_I}{K_{II}}K'_C \qquad (18)$$

and

$$K_F = \frac{K_{II}}{K_{III}}K'_C \qquad (19)$$

From equations 18 and 19 it is seen that the dissociation constants of the amino groups in the mercaptide can be expressed in terms of the constants K_I, K_{II}, K_{III} and of the individual constant K'_C of the amino acid or peptide.

The constant K_{III} which involves the species with uncharged amino groups is found directly from experiments with solutions of sufficiently high pH. An example of the calculation of K_{III} for cysteine follows: original mixture: 4×10^{-2} M RSH, 5×10^{-4} M Hg(II), pH 13.3, $\mu = 1$, $t = 12°$, e.m.f. = −0.6968 v. *vs.* S.C.E.

From equation 3: $[Hg^{++}] = 1.2 \times 10^{-46}$. Σ uncombined cysteine = total cysteine added $-2 \times$ total mercury added $= 4 \times 10^{-2} - 10^{-3} = 3.9 \times 10^{-2}$.

From equation 8: $a = 3.88 \times 10^{-2}$, $d = 1.28 \times 10^{-4}$. It is seen that d is negligibly small as compared to a, while a is practically equal to the concentration of total uncombined cysteine. Therefore e and g can be neglected as compared to f, while f is equal to the concentration of the total mercury added (5×10^{-4} M), the mercuric ion concentration being negligibly small.

From equation 15: $K_{III} = 3.6 \times 10^{-46}$. This simplified calculation of K_{III} can be applied for cysteine at pH higher than 11.5 at 12 and 25° and for glutathione at pH higher than 10.9 and 9.5 at temperatures of 12 and 25°, respectively. It is seen from Tables I to IV that the values of K_{III} as calculated in the above way are practically constant within these pH ranges. Below is given an example of the calculation of K_I: original mixture: 4×10^{-2} M RSH, 5×10^{-4} M Hg(II), pH 2.97, $\mu = 1$, $t = 12°$, e.m.f. = −0.2737 volt *vs.* S.C.E., $[Hg^{++}] = 1.1 \times 10^{-31}$. Σ Uncombined cysteine: $4 \times 10^{-2} - 2 \times 5 \times 10^{-4} = 3.9 \times 10^{-2}$. $a = 1.073 \times 10^{-15}$, $d = 7.95 \times 10^{-8}$, $e = \left[Hg\left(\begin{matrix}RS\\|\\NH_3^+\end{matrix}\right)_2\right] = 5 \times 10^{-4}$ M.

From equation 13: $K_I = 1.4 \times 10^{-42}$. The concentration of the species with uncharged amino groups can be neglected over a pH range which extends for RSH from pH 0 to pH 7 at 12° and from pH 0 to pH 5.5 at 25°, and for GSH from pH 0 to pH 7.5 both at 12 and 25°. Beyond these pH ranges the species with uncharged amino groups must be considered. The constant K_{II} can now be calculated from measurements in solutions in the higher pH range and from K_I and K_{III}. An example for the calculation of K_{II} follows: original mixture: 5×10^{-2} M RSH, 7.5×10^{-4} M Hg(II), pH 9.10, $\mu = 1$, $t = 25°$, e.m.f. = −0.6338 v. *vs.* S.C.E., $[Hg^{++}] = 7.4 \times 10^{-43}$. Σ Uncombined cysteine $= 5 \times 10^{-2} - 1.5 \times 10^{-3} = 4.85 \times 10^{-2}$ M. $a = 8.48 \times 10^{-4}$, $d = 1.90 \times 10^{-2}$.

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

(19) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 85.

(20) J. T. Edsall and M. H. Blanchard, THIS JOURNAL, **55**, 2337 (1933).

TABLE I

APPARENT DISSOCIATION CONSTANTS OF THE THREE CHARGE TYPES OF MERCURIC MERCAPTO CYSTEINATE ($Hg(RS)_2$) AT 25°

RSH added, M	Hg(II) added, M	Electrolyte	pH	$p[Hg^{++}]$	$K_I \times 10^{41}$	$K_{II} \times 10^{44}$	$K_{III} \times 10^{44}$
2×10^{-2}	5×10^{-4}	0.2 M NaOH, 0.8 M KNO_3	>13	43.36	...	...	3.1
3×10^{-2}	5×10^{-4}	.2 M NaOH, .8 M KNO_3	>13	43.81	...	...	2.6
4×10^{-2}	5×10^{-4}	.5 M NaOH, .5 M KNO_3	>13	44.04	...	...	2.8
10^{-2}	10^{-3}	.2 M NaOH, .8 M KNO_3	>13	42.25	...	...	3.6
2×10^{-2}	10^{-3}	.2 M NaOH, .8 M KNO_3	>13	43.03	...	...	3.0
2×10^{-2}	10^{-3}	.3 M NaOH, .8 M KNO_3	>13	43.02	...	...	3.1
2×10^{-2}	10^{-3}	.3 M NaOH, .8 M KCl	>13	43.03	...	...	3.0
3×10^{-2}	10^{-3}	.3 M NaOH, .8 M KCl	>13	43.47	...	...	2.7
4×10^{-2}	10^{-3}	.3 M NaOH, .8 M KNO_3	>13	43.81	...	...	2.2
6×10^{-2}	10^{-3}	.3 M NaOH, .7 M KNO_3	>13	44.27	...	...	1.8
6×10^{-2}	2×10^{-3}	.3 M NaOH, .7 M KNO_3	>13	43.91	...	...	2.0
4.44×10^{-2}	2.2×10^{-3}	.3 M NaOH, .7 M KNO_3	>13	43.41	...	...	2.8
2×10^{-2}	10^{-3}	.05 M Na_2HPO_4, .078 M NaOH, .8 M KNO_3	11.61	43.02	...	1.2	...
2×10^{-2}	10^{-3}	.08 M Na_2HPO_4, .078 M NaOH, .7 M KNO_3	11.24	42.92	...	1.6	...
2×10^{-2}	10^{-3}	.08 M Na_2HPO_4, .039 M NaOH, .7 M KNO_3	10.23	42.31	...	1.7	...
2×10^{-2}	10^{-3}	.1 M NH_4NO_3, .2 M NH_3, .9 M KNO_3	9.40	41.28	...	2.8	...
2×10^{-2}	10^{-3}	.05 M Borax, .026 M NaOH, .85 M KCl	9.15	40.81	...	4.3	...
5×10^{-2}	7.5×10^{-4}	.05 M Borax, .052 M NaOH, .8 M KCl	9.10	42.13	...	1.6	...
2×10^{-2}	10^{-3}	.0175 M Borax, .13 M boric acid, .026 M NaOH, .9 M KCl	8.22	39.45	...	3.1	...
3×10^{-2}	10^{-3}	.015 M Borax, .14 M boric acid, M KCl	7.59	38.57	...	1.9	...
2×10^{-2}	5×10^{-4}	.08 M Na_2HPO_4, .75 M KNO_3, .01 M NaH_2PO_4	7.27	37.51	...	4.9	...
2×10^{-2}	10^{-3}	.08 M Na_2HPO_4, .75 M KNO_3, .01 M NaH_2PO_4	7.16	36.99	...	2.1	...
2×10^{-2}	10^{-3}	.05 M Na_2HPO_4, .05 M NaH_2PO_4, .76 M KNO_3	6.40	35.31	...	2.8	...
2×10^{-2}	10^{-3}	.18 M CH_3COONa, .02 M CH_3COOH, .8 M KNO_3	5.52	33.50	5.4	...	...
3×10^{-2}	10^{-3}	.1 M CH_3COONa, .1 M CH_3COOH, .9 M KNO_3	4.62	32.07	5.6	...	.
4×10^{-2}	10^{-3}	.2 M CH_3COOH, M KNO_3	3.07	29.21	5.9	...	...
3×10^{-2}	5×10^{-4}	.2 M CH_3COOH, M KNO_3	3.00	29.17	5.5	...	...
2×10^{-2}	10^{-3}	.2 M CH_3COOH, M KNO_3	2.90	28.35	4.4	...	...
6×10^{-2}	2×10^{-3}	.2 M HCl, .8 M KCl	1.00	25.11	5.8	...	...
4×10^{-2}	10^{-3}	.2 M HCl, .8 M KNO_3	0.88	24.80	6.3	...	...
			Av. K:		5.6	2.5	2.7

From equations 13 and 15 and the known values of K_I and K_{III}, given in Table I

$$e = \frac{[Hg^{++}]d^2}{K_I} = 4.79 \times 10^{-6}, f = \frac{[Hg^{++}]a^2}{K_{III}} = 1.95 \times 10^{-5}$$

$\Sigma Hg(II) = e + f + g$; hence $g = \Sigma Hg(II) - e - f = 7.5 \times 10^{-4} - 4.79 \times 10^{-6} - 1.95 \times 10^{-5} = 7.26 \times 10^{-4}$

From equation 14: $K_{II} = 1.6 \times 10^{-44}$. Experiments were carried out over the entire pH range with mixtures of varying mercury and RSH(GSH) concentrations, respectively. The experimental data and the constants K_I, K_{II} and K_{III} are listed in Tables I, II, III and IV. The variation of the mercuric ion concentration with pH for a mixture of the same original concentration in mercury (10^{-3} M) and RSH(GSH) (2×10^{-2} M) is presented graphically in Fig. 5. It is seen that the $p[Hg^{++}]$ values of the particular mixtures are identical for RSH and GSH in the lower pH range but differ by about two units for mixtures in the higher pH range at which the $p[Hg^{++}]$ becomes independent of the hydrogen ion concentration. The relation between hydrogen ion and mercuric ion concentration can be given by the equation

$$\left(1 + \frac{[H^+]}{K'_3} + \frac{[H^+]^2}{K'_2K'_3}\right)^2 = [Hg^{++}]\frac{(A - 2B)^2}{B}\left\{\frac{[H^+]^2}{(K'_C)^2K_I} + \frac{1}{K_{III}} + \frac{[H^+]}{K'_CK_{II}}\right\} \quad (20)$$

where A is the original concentration of cysteine (glutathione) and B the concentration of mercury added. K'_2 and K'_3 are the titration dissociation constants of RSH (the corresponding constants of

TABLE II

APPARENT DISSOCIATION CONSTANTS OF THE THREE CHARGE TYPES OF MERCURIC MERCAPTO CYSTEINATE AT 12°

RSH added, M	Hg(II) added, M	pH	p-[Hg^{++}]	$K_I \times 10^{42}$	$K_{II} \times 10^{40}$	$K_{III} \times 10^{40}$
2×10^{-2}	5×10^{-4}	13.20	45.18	...	..	4.7
4×10^{-2}	5×10^{-4}	13.32	45.93	...	..	3.6
2×10^{-2}	10^{-3}	13.20	44.85	...	..	4.6
5×10^{-2}	10^{-3}	13.20	45.86	...	..	3.2
6×10^{-2}	2×10^{-3}	13.26	45.63	...	..	3.7
2×10^{-2}	10^{-3}	12.64	44.79	...	..	5.0
2×10^{-2}	10^{-3}	11.37	44.67	...	..	3.4
2×10^{-2}	10^{-3}	10.42	44.10	...	3.8	...
2×10^{-2}	10^{-3}	9.52	42.90	...	7.8	...
4×10^{-2}	10^{-3}	9.32	43.58	...	4.04	...
2×10^{-2}	10^{-3}	8.32	41.12	...	5.7	...
2×10^{-2}	10^{-3}	7.19	38.47	1.1	..	...
2×10^{-2}	10^{-3}	6.40	36.77	1.6	..	...
2×10^{-2}	10^{-3}	5.49	34.90	1.9	..	...
2×10^{-2}	10^{-3}	4.57	33.09	1.7	..	...
2×10^{-2}	10^{-3}	3.60	31.25	1.4	..	...
5×10^{-2}	10^{-3}	3.00	30.94	1.3	..	...
4×10^{-2}	5×10^{-4}	2.97	30.97	1.4	..	...
2×10^{-2}	10^{-3}	2.86	29.76	1.4	..	...
2×10^{-2}	10^{-3}	1.55	27.13	1.4	..	...
2×10^{-2}	10^{-3}	0.71	25.56	1.1	..	...
5×10^{-2}	10^{-3}	0.83	26.45	1.8	..	...
6×10^{-2}	2×10^{-3}	0.87	26.37	1.8	..	...
			Av. K:	1.5	5.3	4.0

TABLE III

APPARENT DISSOCIATION CONSTANTS OF THE THREE CHARGE TYPES OF MERCURIC MERCAPTO GLUTATHIONATE, Hg(GS)$_2$ AT 25°

GSH added, M	Hg(II) added, M	pH	p-[Hg^{++}]	$K_I \times 10^{41}$	$K_{II} \times 10^{42}$	$K_{III} \times 10^{42}$
1.5×10^{-2}	5×10^{-4}	>13	41.07	...	...	3.4
2×10^{-2}	5×10^{-4}	>13	41.41	...	...	2.8
2×10^{-2}	5×10^{-4}	>13	41.48	...	...	2.4
4×10^{-2}	5×10^{-4}	>13	42.35	...	...	1.4
2×10^{-2}	10^{-3}	>13	41.00	...	...	3.2
2×10^{-2}	10^{-3}	>13	41.11	...	...	2.5
4×10^{-2}	10^{-3}	>13	42.08	...	...	1.2
2×10^{-2}	2×10^{-3}	>13	40.55	...	...	3.6
4×10^{-2}	2×10^{-3}	>13	41.50	...	...	2.1
2×10^{-2}	10^{-3}	12.80	41.03	...	...	3.0
2×10^{-2}	10^{-3}	11.37	40.98	...	...	3.3
2×10^{-2}	10^{-3}	9.50	40.90	...	...	1.9
2×10^{-2}	10^{-3}	9.18	40.72	...	1.3	...
2×10^{-2}	10^{-3}	8.32	39.46	...	1.0	...
2×10^{-2}	10^{-3}	7.49	37.51	1.0	...	...
2×10^{-2}	10^{-3}	6.53	35.54	1.3	...	...
2×10^{-2}	10^{-3}	5.19	32.82	1.4	...	...
2×10^{-2}	10^{-3}	4.43	31.36	1.2	...	...
2×10^{-2}	10^{-3}	4.41	31.28	1.4	...	...
2×10^{-2}	10^{-3}	3.41	29.39	1.1	...	...
2×10^{-2}	10^{-3}	2.80	28.17	1.0	...	...
2×10^{-2}	10^{-3}	2.47	27.49	1.1	...	...
8×10^{-2}	2×10^{-3}	2.30	28.10	1.1	...	...
2×10^{-2}	10^{-3}	1.87	26.26	1.2	...	...
4×10^{-2}	10^{-3}	0.87	24.96	1.0	...	...
3×10^{-2}	5×10^{-4}	0.83	24.96	1.0	...	...
2×10^{-2}	10^{-3}	0.83	24.22	1.1	...	...
			Av. K:	1.1	1.2	2.6

TABLE IV

APPARENT DISSOCIATION CONSTANTS OF THE THREE CHARGE TYPES OF MERCURIC MERCAPTO GLUTATHIONATE AT 12°

GSH added, M	Hg(II) added, M	pH	p-[Hg^{++}]	$K_I \times 10^{43}$	$K_{II} \times 10^{44}$	$K_{III} \times 10^{44}$
2×10^{-2}	5×10^{-4}	13.2	43.24	...	...	4.1
4×10^{-2}	5×10^{-4}	13.0	44.27	...	...	1.7
3×10^{-2}	7.5×10^{-4}	13.1	43.61	...	...	2.7
2×10^{-2}	10^{-3}	13.2	42.84	...	...	4.6
4×10^{-2}	10^{-3}	13.0	43.93	...	...	1.7
4×10^{-2}	2×10^{-3}	13.2	43.50	...	...	2.0
4×10^{-2}	10^{-3}	12.8	43.77	...	...	2.5
2×10^{-2}	10^{-3}	12.7	42.78	...	...	5.4
2×10^{-2}	10^{-3}	11.90	42.77	...	...	5.4
4×10^{-2}	10^{-3}	11.28	43.89	...	...	1.8
2×10^{-2}	5×10^{-4}	10.87	43.23	...	...	4.1
2×10^{-2}	10^{-3}	10.86	42.82	...	...	4.6
2×10^{-2}	10^{-3}	9.70	42.73	...	3.9	...
2×10^{-2}	10^{-3}	9.30	42.49	...	2.7	...
2×10^{-2}	10^{-3}	8.48	41.24	...	2.2	...
2×10^{-2}	10^{-3}	7.54	39.01	4.2	...	...
2×10^{-2}	10^{-3}	6.53	36.85	6.3	...	...
2×10^{-2}	10^{-3}	6.00	35.76	6.7	...	...
2×10^{-2}	10^{-3}	5.13	34.09	5.8	...	...
2×10^{-2}	10^{-3}	4.32	32.62	4.1	...	...
2×10^{-2}	10^{-3}	3.37	30.62	5.2	...	...
2×10^{-2}	10^{-3}	2.73	29.46	3.9	...	...
3×10^{-2}	2×10^{-3}	2.74	29.51	3.9	...	...
4×10^{-2}	5×10^{-4}	1.80	28.58	4.1	...	...
2×10^{-2}	10^{-3}	1.60	27.06	5.5	...	...
4×10^{-2}	5×10^{-4}	0.88	26.60	5.3	...	...
2×10^{-2}	10^{-3}	0.88	25.56	6.1	...	...
			Av. K:	5.1	2.9	3.4

glutathione are denoted as K'_3 and K'_4). The full lines in Fig. 5 were constructed from pHg values calculated from equation 20 for $A = 2 \times 10^{-2}$ and $B = 10^{-3}$ at varying [H$^+$]. The values of K_I, K_{II} and K_{III} were taken from Tables I and II. The experimental points fit the calculated lines satisfactorily over the entire pH range. It is readily seen that at low pH the first two terms at the left side of equation 20 are negligibly small as

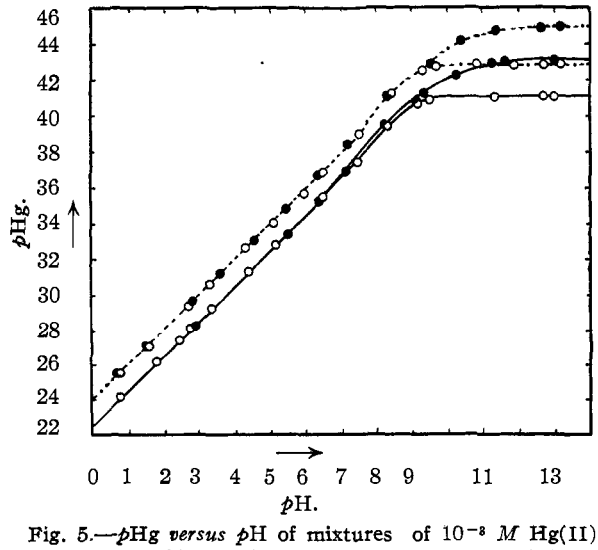


Fig. 5.—pHg *versus* pH of mixtures of 10^{-3} M Hg(II) with 2×10^{-2} M RSH (●) and 2×10^{-2} M GSH (○), respectively, at 12° (- - - -) and 25° (—).

compared to the third term and on the right side the last two terms in the bracket can be neglected as compared to the first term. Thus for low pH values, equation 20 can be written in the simplified form

$$[H^+]^2 = [Hg^{++}] \frac{(A - 2B)^2}{B} \frac{(K'_2K'_3)^2}{(K'_C)^2K_1} \quad (21)$$

or

$$2p\mathrm{H} = p\mathrm{Hg} - \log\left\{\frac{(A - 2B)^2}{B} \frac{(K'_2K'_3)^2}{(K'_C)^2K_1}\right\} \quad (22)$$

At low pH the relation between pH and pHg is thus presented by a straight line of slope 2. This relation is actually observed in Fig. 5 at 12 and 25°. The values of the three constants for cysteine and glutathione are not affected by the composition of the buffers used. K_{III} and K_{II} are practically equal and smaller than K_I for both GSH and RSH, the difference being more pronounced for cysteine. Thus at 12° the ratios of $K_I:K_{III}$ are 3700 and 15 for the mercaptides of cysteine and glutathione, respectively.

The relation of the dissociation constants K_I and K_{III} of the mercaptides to the hydrogen ion dissociation constants K'_A and K'_D of the corresponding species of the free amino acid and peptide is given in Table V.

TABLE V

RATIOS (K_{III}/K'_D) AND (K_I/K'_A) FOR CYSTEINE AND GLUTATHIONE

	K_{III}/K'_D		K_I/K'_A	
	12°	25°	12°	25°
RSH:	1.3×10^{-35}	0.9×10^{-33}	6.8×10^{-34}	2.5×10^{-32}
GSH:	2.2×10^{-35}	1.7×10^{-33}	4.6×10^{-34}	10^{-32}

The ratio K_{III}/K'_D is slightly larger and K_I/K'_A is slightly smaller for glutathione than for cysteine. The value of K_I for cysteine is of the same order of magnitude (about 5 times larger) as that of GSH. The same order of magnitude was expected, because the stability of the mercaptide is determined almost entirely by the strength of the mercury–sulfur bonds. The amino groups are in the charged form and no coördination of mercury with the nitrogen can occur under these conditions. As a matter of fact because of its positive charge the NH_3^+ group should decrease the stability. Evidently, the contribution to stability of the mercaptide by coördination between mercury and the uncharged amino groups is very small, because K_{II} and K_{III} are identical. The large ratio of K_I to K_{III} for cysteine as compared to that for glutathione indicates that coördination with the amino groups in cysteine is more favorable than in glutathione. This is structurally to be expected, but the over-all contribution to the stability is small.

From the values of K_I, K_{II} and K_{III} the values of K_E and K_F can be calculated from equations 18 and 19. From the equality of K_{II} and K_{III} it follows that K_F is of the same order of magnitude as K'_C.

The heat of formation of the various species of the mercaptides was calculated from the corresponding dissociation constants at 12 and 25° and the entropies of formation were found from the relation $\Delta S = (\Delta H - \Delta F)/T$. The pK values of the five constants, the free energies (RT ln K), heats and entropies of formation of the various species of the mercaptides are summarized in Table VI. It is seen that the mercaptides of the amino acid and the peptide have values of ΔH and ΔS of comparable magnitude.

TABLE VI

APPARENT DISSOCIATION CONSTANTS (pK VALUES), FREE ENERGIES, HEATS AND ENTROPIES OF FORMATION OF MERCURIC MERCAPTO CYSTEINATE AND GLUTATHIONATE

	$Hg(RS)_2$		$Hg(GS)_2$	
	12°	25°	12°	25°
pK_1	41.82	40.25	42.29	40.96
pK_{II}	45.27	43.60	43.54	41.92
pK_{III}	45.40	43.57	43.47	41.58
pK_E	7.39	7.10	7.97	7.85
pK_F	10.72	10.48	9.28	9.15
ΔF_1, kcal.	−54.5	−54.8	−55.2	−55.9
ΔF_{II}, kcal.	−59.1	−59.5	−56.8	−57.1
ΔF_{III}, kcal.	−59.1	−59.3	−56.6	−56.6
ΔF_E, kcal.	−9.63	−9.68	−10.4	−10.7
ΔF_F, kcal.	−14.0	−14.3	−12.1	−12.5
ΔH_1, kcal.	−46.9		−39.7	
ΔH_{II}, kcal.	−49.9		−48.2	
ΔH_{III}, kcal.	−54.5		−56.1	
ΔH_E, kcal.	−8.7		−3.6	
ΔH_F, kcal.	−7.2		−3.9	
ΔS_1, cal./deg.	+27		+54	
ΔS_{II}, cal./deg.	+32		+30	
ΔS_{III}, cal./deg.	+16		+2	
ΔS_E, cal./deg.	+4		+24	
ΔS_F, cal./deg.	+24		+29	

The heat of formation increases from the I- to III-species for cysteine as well as for glutathione. The values of ΔH indicate that the bond energy between metal and complexing agent is strongest in the species with the uncharged amino groups, as also evidenced by the large pK_{III} values. The values of ΔH_{II} are smaller than ΔH_{III} indicating that the mercury in complex III is bound more firmly than in complex II, a fact which is not reflected by the pK_{II} and pK_{III} values which are practically equal.

It is interesting to note that the entropy of formation is larger for the I- and II-species than for the III-species for both cysteine and glutathione.

The values of ΔH and ΔS for the dissociation of the amino group in the mercaptides are of the same order of magnitude as the corresponding values for free cysteine and glutathione, respectively.

In conclusion the equilibrium constants of the following reactions

$$\mathrm{Hg}\left(\begin{matrix}e_G\\ \mathrm{GS}\\ |\\ \mathrm{NH_3^+}\end{matrix}\right)_2 + 2\begin{matrix}d_R\\ \mathrm{RS^-}\\ |\\ \mathrm{NH_3^+}\end{matrix} \rightleftharpoons \mathrm{Hg}\left(\begin{matrix}e_R\\ \mathrm{RS}\\ |\\ \mathrm{NH_3^+}\end{matrix}\right)_2 + 2\begin{matrix}d_G\\ \mathrm{GS^-}\\ |\\ \mathrm{NH_3^+}\end{matrix} \quad (23)$$

$$\mathrm{Hg}\left(\begin{matrix}f_G\\ \mathrm{GS}\\ |\\ \mathrm{NH_2}\end{matrix}\right)_2 + 2\begin{matrix}a_R\\ \mathrm{RS^-}\\ |\\ \mathrm{NH_2}\end{matrix} \rightleftharpoons \mathrm{Hg}\left(\begin{matrix}f_R\\ \mathrm{RS}\\ |\\ \mathrm{NH_2}\end{matrix}\right)_2 + 2\begin{matrix}a_G\\ \mathrm{GS^-}\\ |\\ \mathrm{NH_2}\end{matrix} \quad (24)$$

can be expressed as

$$K_1 = \frac{e_R \times d_G^2}{e_G \times d_R^2} = \frac{K_{IG}}{K_{IR}} = 0.2 \text{ at } 25° \text{ and } 0.3 \text{ at } 12°$$

$$K_2 = \frac{f_R \times a_G^2}{f_G \times a_R^2} = \frac{K_{IIIG}}{K_{IIIR}} = 96 \text{ at } 25° \text{ and } 85 \text{ at } 12°$$

where the corresponding species of cysteine and

glutathione are denoted by the indices R and G, respectively.

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MINNEAPOLIS, MINNESOTA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CORNELL UNIVERSITY]

Phase Equilibria in Polymer–Solvent Systems. III. Three-component Systems[1]

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Phase equilibria data for three ternary solvent–solvent–polystyrene systems have been obtained and interpreted in terms of the statistical-thermodynamic theory of polymer solutions. Thermodynamic interaction parameters calculated from the observed plait points are consistent with those calculated for solvent–solvent and solvent–polymer pairs from binary system liquid–vapor and liquid–liquid phase equilibria data.

Introduction

Investigations on liquid–liquid phase equilibria in binary systems consisting of a polymer and a single solvent have yielded results which on the whole agree rather well with the predictions of the statistical-thermodynamic theory of polymer solutions.[2] Although the theory succeeds only qualitatively in reproducing the observed binodials, the critical miscibility temperatures T_c vary with molecular weight in the manner predicted by the theory.[3] The polymer–solvent interaction parameters calculated from this dependence of T_c on the molecular weights of polymer fractions are remarkably similar to those of chemically analogous small molecule liquid pairs.[4] This correlation of the thermodynamic functions of large and small molecules is important in providing a common thermodynamic basis for comparing polymers with possibly better characterized small molecule liquids. It may thus be possible to study with greater facility the properties of polymers which are actually peculiar to their great size and chain structure.

The practical use of solvent–non-solvent mixtures to effect separation of polymers into less heterogeneous fractions has been reviewed by Cragg and Hammerschlag.[5] Powers[6] has made several phase studies on polystyrene–mixed solvent systems. Polymer solution theory has been extended to ternary and more complex systems to explain and predict various phenomena dependent upon the free energies of the components.[7–9]

It is the purpose of this paper to interpret phase equilibria data for three ternary solvent (1)–solvent (2)–polystyrene (3) systems in the light of present statistical-thermodynamic theory. Methyl ethyl ketone (1)–methanol (2)–polystyrene (3) (system I) was studied because of its frequent use in fractionation. The interaction parameters for each pair of components in this system are unknown. Consequently, although the data may be used to determine the plait point for the infinite molecular weight polymer species, very little further information is gained in this case. System II, carbon tetrachloride (1)–cyclohexane (2)–polystyrene (3), on the other hand, is a convenient choice for checking phase equilibria theory. Two of the three interaction parameters are known from vapor pressure and liquid–liquid equilibria measurements.[4] The "co-solvent"–polymer system, ethylcyclohexane (1)–cyclohexanol (2)–polystyrene (3) (system III), offered the peculiar advantage that all three pair interaction parameters were theoretically obtainable from liquid–liquid phase equilibria measurements.

Experimental

Materials.—The parent polymers were prepared by low conversion polymerizations of styrene monomer in bulk with thermal (for the highest molecular weight polymers) or benzoyl peroxide initiation. Polystyrene fractions were obtained by fractional precipitation of the polymer from dilute solutions in butanone by successive additions of methanol.[10] Table I lists the fractions, their intrinsic viscosities in benzene solution at 30°, molecular weights calculated by the relation[11]

$$\log M = 4.013 + 0.74 \log [\eta]$$

and their percentages of the parent polymers from which they were derived. The intrinsic viscosities were measured in Ubbelohde suspended-level viscometers. Kinetic energy corrections were applied throughout, but rate of shear corrections were unnecessary (rate of shear approximately 2000–2500 sec.$^{-1}$).

Reagent purity solvents were dried and distilled at least once through a 40-cm. glass helices-packed column.

Experimental Procedure.—Phase boundary curves were determined with the aid of a titration apparatus. This apparatus consisted of two Kimble Exax 5-ml. burets, graduated to 0.01 ml., with tips sealed into the head of a solution cell. The tops of the burets were fastened by rubber tube connectors, immersed in mercury wells, to vertical glass tubes, each having a three-way silicone-lubricated stopcock in the top bend. These vertical tubes were connected by approximately 0.8 meter lengths of rubber tubing to adjacent vertical tubes which could be raised and lowered. The lengths of rubber tubing and vertical glass tubes contained columns of mercury sufficient to prevent contact of vapor with the rubber on the buret side during all manipu-

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