glycolate). The shift is in the expected direction according to the relative positions of these groups in the spectrochemical series.

The mercaptide ion exhibits an intense band in the ultraviolet which has been identified by Orgel[22] as a charge-transfer transition. In the divalent thioglycolate ion the maximum of this band occurs at 245 mμ (see Fig. 3). In the complexes, CoRS and NiRS, this band is seen to become broadened and shifted to longer wave lengths. With FeRS the shift is not as great but even here a slight overlap into the visible region occurs. The FeRS spectrum also shows a d-level transition in the region 975–950 mμ with an extinction coefficient equal to six.

The spectrum of MnRS shows no absorption in the visible regions under the present experimental conditions. The beginning of a band is observed in the same region in which the free divalent thioglycolate ion absorbs, however. Because of the appreciable dissociation of the complex an interpretation of the results is difficult.

The spectrum of ZnRS shows no absorption until about 250 mμ where the beginning of a band having a maximum at shorter wave lengths is observed. The excess RSH^- ion present in the solution, also, contributes greatly to the absorption in this same region. No attempt was made to resolve the spectrum into that of its components because the resulting spectrum of ZnRS would be obtained from the difference of large numbers.

The spectral changes of the mercaptide ion absorption band in the series of thioglycolato complexes run somewhat parallel to those observed by Katzin[23] for the iodide ion. The charge-transfer bands of the iodide ion are shifted to longer wave lengths on complexing with cobalt(II) and nickel(II), but in the lower complexes of zinc(II) a shift to shorter wave lengths occurs.

The oxidation of the metal ion brings about a shift of the charge-transfer band of the thioglycolate to even longer wave lengths. With $Fe(III)OH(RS)_2^{=2}$ the maximum occurs at 535 mμ with a shoulder at 410 mμ, and with Co(III)[24] the maximum occurs at 362 mμ with a shoulder at 450 mμ. This shift to longer wave lengths upon oxidizing the metal ion is a reflection of the greater ease with which these trivalent metal ions can accept an electron from the coördinating thioglycolate ion than can the corresponding divalent metal ions. In the latter case the transfer of electrons to the metal ions occurs less readily because highly unstable monovalent metal ions are formed.

The stability and spectra data show that the mercaptide ion exerts a strong influence upon the central metal ion of a complex. The influence can be expressed quantitatively by the value of the term *Dq* in the Orgel diagram.[19] The lack of symmetry in the present complexes precludes the accurate evaluation of *Dq* for the mercaptide ion, but it can be stated that its value is fairly large.

Whether the strong effect of the mercaptide is primarily an electrostatic one or a covalent one is not evident at the present. In the spectrochemical series the increase in *Dq* is in the order in which increased covalent bonding is expected.[25] The thioglycolate ion, on the other hand, is able to exert a strong electrostatic field. Most likely, both effects play large roles in bringing about the stability of the complexes.

[22] L. E. Orgel, *Quart. Revs.*, **8**, 422 (1954).

[23] L. I. Katzin, *J. Chem. Phys.*, **20**, 1165 (1952); **23**, 2055 (1955).

[24] Prepared by the air oxidation of the CoRS solution used in the ultraviolet studies.

[25] L. E. Orgel, *J. Chem. Phys.*, **23**, 1819 (1955).

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WEST VIRGINIA UNIVERSITY]

Metal Ion Complexes of 2-(2-Aminoethylamino)-ethanol. Reaction of the Copper(II) Complexes with Sodium Hydroxide[1,2]

BY JAMES L. HALL AND WARREN E. DEAN

RECEIVED JANUARY 2, 1958

In solution 2-(2-aminoethylamino)-ethanol, $H_2N(CH_2)_2NH(CH_2)_2OH$, (hydroxyethyl-ethylenediamine, abbreviated hn or hen-OH) forms complexes with copper(II) ion in the ratio Cu(II):hn of 1:1 and 1:2. The reaction of these complexes with further amine and with sodium hydroxide in solution has been studied by spectrophotometric, conductometric and potentiometric methods. The 1:1 complex, $Cu(hn)^{+2}$, reacts with one equivalent of sodium hydroxide to form $Cu(hen\text{-}O^-)^{+1}$ and with two equivalents of sodium hydroxide to form $[Cu(hen\text{-}O^-)(OH)]^0$ which is quite stable in solution. The 1:2 complex reacts with hydroxyl radical from excess amine or from sodium hydroxide to form $[Cu(hn)(hen\text{-}O^-)]^{+1}$, assuming the formation of a five-coördinate copper(II) ion. The 1:2 complex also reacts with excess amine to form $Cu(hn)_3^{+2}$ for which five-coördinate copper(II) ion is again assumed. In strongly basic solution both of these latter complexes lose amine molecules to form $[Cu(hen\text{-}O^-)(OH)]^0$.

Introduction

Breckenridge[3] prepared the crystalline compounds $Cu(hn)_2Cl_2$, $Cu(hn)_2Br_2$ and $[Cu(hn)Cl]Cl\cdot{}^1/_2H_2O$ by reaction in 95% ethanol of the appropriate copper salt and 2-(2-aminoethylamino)-ethanol, $H_2N(CH_2)_2NH(CH_2)_2OH$, (hydroxyethyl-ethylenediamine, abbreviated hn or hen-OH). Harvey, Tewksbury and Haendler[4] made a spectrophotometric study of copper(II) acetate and hydroxyethyl-ethylenediamine mixtures. Analysis by the method of continuous variations[5] indi-

[1] Sponsored by the Office of Ordnance Research, U. S. Army.

[2] Presented before the Southeastern Regional Meeting of the American Chemical Society, Durham, N. C., Nov. 14 to 16, 1957.

[3] J. G. Breckenridge, *Can. J. Research*, **26B**, 11 (1948).

[4] J. L. Harvey, C. I. Tewksbury and H. M. Haendler, THIS JOURNAL, **71**, 3641 (1949).

[5] W. C. Vosburgh and G. R. Cooper, *ibid.*, **63**, 437 (1941).

cated complexes in the ratio Cu(II):hn of 1:1, 1:2 and 1:4. These authors concluded that the hydroxyl radical of the amine was not involved in the coördination compounds which they identified. Martell, Chaberek, Courtney, Westerback and Hyytiainen[6] titrated the $Cu(hn)^{+2}$ complex ion with strong base and reported the stability constant of this complex (log $K_c = 10.0$ at 25° in 0.1 M KNO_3) and the constant for the reaction of the complex with hydroxyl ion ($pK_a = 7.3$). The further reaction of this complex with more amine or base was not mentioned.

Preliminary studies in this Laboratory indicated that the hydroxyethyl-ethylenediamine molecule, like the Quadrol molecule previously studied,[7] formed coördination compounds involving the hydroxyl radical. For the study of the reactions and chelate compounds involving the hydroxyl radical the hydroxyethyl-ethylenediamine molecule has advantages over the Quadrol molecule in that it forms both 1:1 and 1:2 complexes with copper(II) ion and in that it has only one hydroxyl group. The present paper presents the results of a spectrophotometric study and of conductometric and potentiometric titrations involving the reactions of the 1:1 and 1:2 complexes of copper(II) ion and this amine with sodium hydroxide and of the 1:2 complex with excess amine.

Experimental

A. **Reagents.**—Copper(II) perchlorate hexahydrate was prepared by reaction of perchloric acid on excess reagent-grade copper(II) carbonate. The solution was filtered and evaporated to yield the crystalline product which was recrystallized from water and was dried and stored over calcium chloride. Solutions were standardized by the method of Hall, Gibson, Wilkinson and Phillips.[8] Research samples of 2-(2-aminoethylamino)-ethanol were obtained from the Carbide and Carbon Chemicals Corp. as Hydroxyethyl Ethylenediamine. These samples were found to be satisfactory for the present use without further purification. Solutions were standardized by potentiometric titration with hydrochloric acid. Standard hydrochloric acid and carbonate free sodium hydroxide solutions were prepared by conventional methods.

B. **Spectrophotometric Studies.**—Absorption measurements were made with a Beckman Model DU spectrophotometer using one cm. Corex or silica cells. Unless otherwise stated, and except where prevented by high sodium hydroxide concentration, a constant ionic strength of 0.5 was maintained by addition of appropriate amounts of 1 M KNO_3 solution. Studies were made for which the amine and sodium hydroxide concentrations varied over the range zero to 0.5 M.

C. **Conductometric Titrations.**—Conductometric titrations were performed with the precise bridge built around the Leeds and Northrup Co. No. 1553 ratio box, as described previously.[8] The temperature was maintained constant at 25.0 ± 0.02° during titrations.

D. **Potentiometric Titrations.**—Potentiometric titrations were performed with a Beckman Model G pH meter. Details of this work are being presented in another report.[9]

Results and Discussion

A. **Preliminary Studies.**—The work of Harvey, Tewksbury and Haendler[4] was repeated and extended except that copper(II) perchlorate was used in place of copper(II) acetate, and the solutions were made to constant ionic strength of 0.5 with potassium nitrate. In general their work was confirmed, showing distinctly different spectra for ratios of 1:0, 1:1 and 1:2 with the spectra for ratios 1:3 and 1:4 being only slightly different from that for 1:2. The present work shows, however, that as excess amine is added there is a gradual but continuous shift in the spectra. Figure 1 shows the effect of adding excess amine. Spectra for ratios between 1:4 and 1:20 are intermediate between these two, and likewise spectra for ratios between 1:20 and 1:100 are intermediate between these two. There is little difference between the spectra for ratios 1:60 and 1:100. Harvey, Tewksbury and Haendler[4] postulated as a result of studies by the method of continuous variations[5] that their solutions contained complexes in the ratio Cu(II):hn of 1:1, 1:2 and 1:4. Their indication for the 1:4 ratio, obtained at 840 mμ, was vague. The present work favors a 1:3 ratio at this wave length. These results are based on small differences between large numbers and should be taken only as an indication of further reaction of the amine with the 1:2 complex. As will be shown later, the third amine may serve as base in this reaction and may not be coördinated.

The addition of hydroxyethyl-ethylenediamine to copper(II) perchlorate also was followed conductometrically. As 0.1135 M amine solution was added to 0.00231 M $Cu(ClO_4)_2$, there was a regular decrease in conductance to mole ratio 1:1, followed by a further regular and relatively greater decrease to mole ratio 1:2. This was followed by a gradually increasing conductance with no further definite end-point. The spectral and conductometric data both show that there is a third step in the reaction of amine with copper(II) ion which does not take place quantitatively as the amine is added and which does not go to completion with a moderate excess of amine.

B. **Action of Sodium Hydroxide on the 1:1 Complex.**—The addition of sodium hydroxide to solutions containing copper(II) ion and hydroxyethylethylenediamine in various ratios was found to have a very profound influence on the spectra of the resulting solutions. The experiments described below were intended to clarify the roles of excess amine and excess sodium hydroxide in such solutions. In particular it was desired to determine what part of the effect due to excess amine was due to coördination of nitrogen atoms and what part was due to action of the amine as base.

The effect of excess base on the spectrum of the $Cu(hn)^{+2}$ complex is shown in Fig. 2. The curve for ratio 1:1:20, Cu(II):hn:OH^- is almost identical with the one shown for 1:1:100 and others from 1:1:4 to 1:1:20 are nearly the same. Attempts to make solutions containing Cu(II):hn:OH^- ratios of 1:1:1, 1:1:2 and 1:1:3 at ionic strength 0.5 by addition of potassium nitrate resulted in turbid solutions or solutions from which small amounts of precipitate settled. Solutions at these ratios which remained clear for a few hours could be prepared without the added potassium nitrate. For these the spectra for the 1:1:1 solu-

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(6) A. E. Martell, S. Chaberek, Jr., R. C. Courtney, S. Westerback and H. Hyytiainen, THIS JOURNAL, **79**, 3036 (1957).

(7) J. L. Hall, F. R. Jones, C. E. Delchamps and C. W. McWilliams, *ibid.*, **79**, 3361 (1957).

(8) J. L. Hall, J. A. Gibson, Jr., P. R. Wilkinson and H. O. Phillips, *Anal. Chem.*, **26**, 1484 (1954).

(9) J. L. Hall, W. E. Dean and E. A. Pacofsky, forthcoming publication.

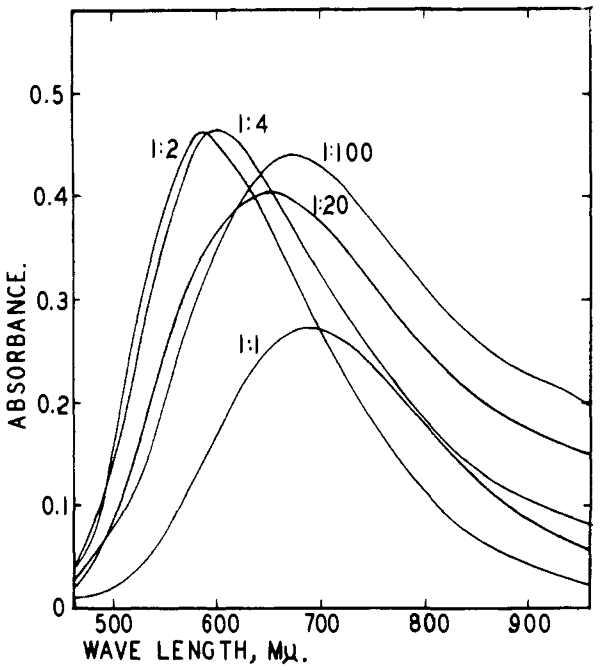

Fig. 1.—Spectra of solutions 0.005 *M* in $Cu(Cl_4)_2$ with various ratios of hn. The ionic strength is 0.5 by addition of KNO_3.

tion was intermediate between the ones for 1:1:0 and 1:1:2 but was nearer to the 1:1:2, while the curve for the 1:1:3 ratio was near to that for 1:1:2.

Potentiometric titrations in which sodium hydroxide was added to an acidified solution containing copper(II) ion and the amine in 1:1 ratio showed that after the formation of the 1:1 complex, two additional equivalents of hydroxyl ion per mole of copper(II) ion reacted in two different steps. A conductometric titration, shown as curve B of Fig. 5, also clearly shows the reaction of two equivalents of sodium hydroxide with the 1:1 complex ion but does not differentiate the two steps as does the potentiometric titration. It is assumed that these results are accounted for by the successive formation in the solutions of $Cu(hn)^{+2}$, $Cu(hen\text{-}O^-)^{+1}$ and $[Cu(hen\text{-}O^-)(OH)]^0$. In these formulas hen-OH is substituted for hn, and hen-O^- denotes removal of the proton from the hydroxyl radical. While the present results do not permit distinction between the removal of this proton and the substitution of a hydroxyl radical for a coördinated water molecule, the results previously obtained with Quadrol and copper(II) ion[7] lead us to the conclusion that the proton is removed from the hydroxyl radical of the alcohol group and there results an additional five-membered chelate ring including coördination at this oxygen atom. The $Cu(hen\text{-}O^-)^{+1}$ ion evidently undergoes dismutation to a slight extent to form $Cu(hn)_2{}^{+2}$ and $Cu(OH)_2$. Jonassen and Dexter[10] have shown that such action is complete upon the addition of sodium hydroxide to the 1:1 complex of copper(II) ion and ethylenediamine, $Cu(en)^{+2}$. This difference in the behavior of the ethylenediamine and hydroxyethyl-ethylenediamine complexes is accepted as further evidence that the oxygen atom of the hydroxyethyl-ethylenediamine complex is involved in coördination and contributes to the stability of the 1:1 complex of this amine. The near identity of the spectra of the 1:1:100 and 1:2:100 solutions shown in Fig. 2 suggests that the final result of adding sodium hydroxide to the 1:2 complex is the same as for the 1:1 complex.

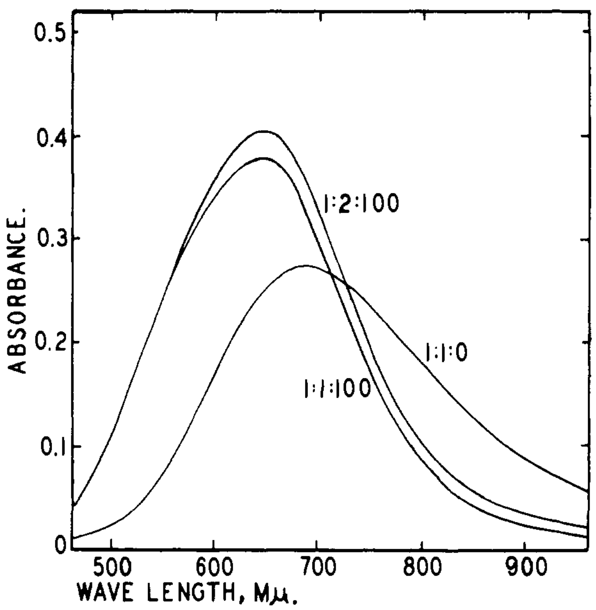

Fig. 2.—Spectra of solutions 0.005 *M* in $Cu(ClO_4)_2$ showing the effect of excess NaOH on the 1:1, Cu(II):hn, complex and the near identity of the 1:1 and 1:2 solutions in excess base.

C. Action of Sodium Hydroxide on the 1:2 Complex.—The effect, on the spectra, of addition of sodium hydroxide to the 1:2:0 solution is shown in Fig. 3. The lack of identity between any of the

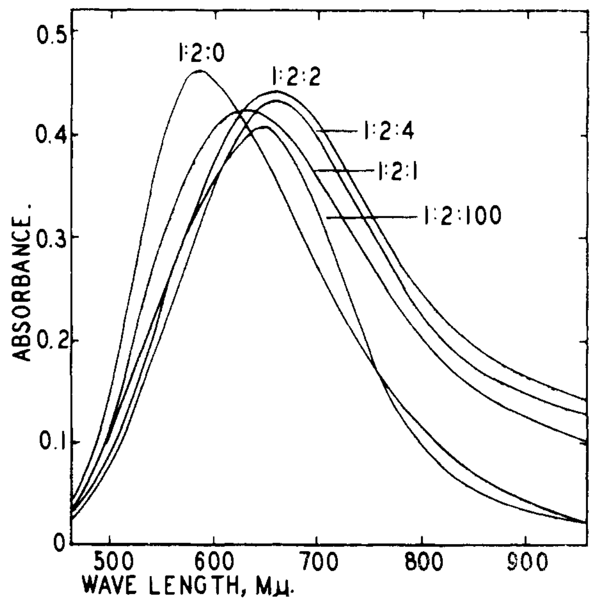

Fig. 3.—Effect of the addition of strong base on the spectra of the $Cu(hn)_2{}^{+2}$ complex. The ratios are Cu(II): hn:OH^-.

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(10) H. G. Jonassen and T. H. Dexter, THIS JOURNAL, 71, 1553 (1949).

curves of this figure (except 1:2) and those of Fig. 1 shows that in the reaction of the $Cu(hn)_2^{+2}$ complex with excess amine or strong base, the amine or strong base may not totally replace one another at any stage. The action of strong base on the $Cu(hn)_2^{+2}$ complex evidently proceeds in at least three steps. The changes in spectra for the first step are shown in more detail in Fig. 4.

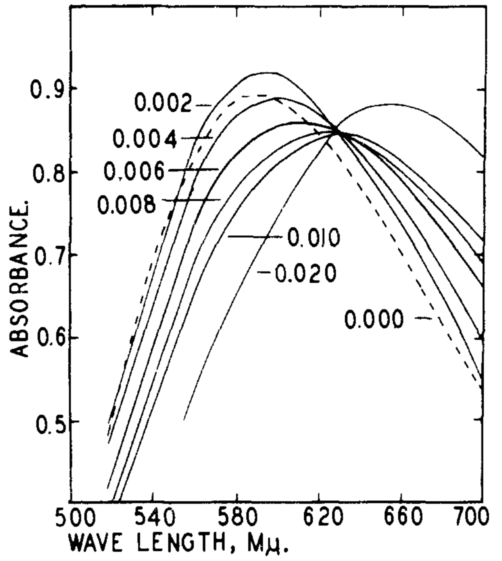

Fig. 4.—Effect of small increments of NaOH on the spectrum of the $Cu(hn)_2^{+2}$ complex. All solutions are 0.01 *M* in $Cu(ClO_4)_2$, 0.02 *M* in hn and with NaOH concentrations as shown.

Figure 4 shows the spectra of solutions 0.01 *M* in copper perchlorate and 0.02 *M* in hydroxyethylethylenediamine with sodium hydroxide added in various amounts to 0.04 *M*. These spectra show an isosbestic point at 630 mμ. The curve for the 1:2:0 ratio does not cross this point. This solution evidently undergoes some hydrolysis as its *p*H value is 6.99 as compared to *p*H values from 9.24 for the 1:1:0.2 solution to 10.69 for the 1:2:1 solution. The curve for ratio 1:2:2 varies slightly from the isosbestic point while curves for solutions containing greater amounts of sodium hydroxide vary widely as shown in Fig. 3. The spectral pattern shown in Fig. 4 resembles very closely the one shown by Jonassen, Reeves and Segal[11] for addition of sodium hydroxide to the bis-ethylenediamine–copper(II) complex. The changes in the spectral pattern which required 0.6 *M* NaOH for the ethylenediamine complex[11] require only 0.02 *M* NaOH for the corresponding hydroxyethylethylenediamine complex. For the $Cu(en)_2^{+2}$ complex it was proposed[11] that the spectral pattern obtained was the result of addition of hydroxyl ion to a fifth coördination position of the copper(II) ion. For this reaction Jonassen, Reeves and Segal[11] report an average equilibrium constant of log K = 0.7301. In the present case a reasonable assumption is that for the $Cu(hn)_2^{+2}$ complex, as NaOH is added, an additional five-membered chelate ring is formed including a fifth coördination position of the copper(II) ion and the hydroxyl oxygen atom from one of the amine molecules from which a proton is removed as the reaction proceeds. The relatively greater effect of low concentrations of sodium hydroxide on the $Cu(hn)_2^{+2}$ complex as compared with the $Cu(en)_2^{+2}$ complex may be explained by the expected greater stability of the proposed oxygen-containing five-membered chelate ring as compared with the link to the hydroxyl ion alone at the fifth coördination position. For the reaction

$$Cu(hn)_2^{+2} + OH^- \rightleftharpoons [Cu(hn)(hen\text{-}O^-)]^{+1} + H_2O$$

the stability constant calculated from the data shown in Fig. 4 is of the order of 850 or log K = 2.9, approximately.

D. Effect of Excess Amine and of Excess Sodium Hydroxide on the 1:2 Complex.—Spectral curves such as those shown in Figs. 1, 2 and 3 give considerable information about the interaction of excess amine and sodium hydroxide on solutions containing copper(II) ion. These may be noted by reference to the branch of these curves to the right of the maximum of absorption. For any solution containing copper(II) ion and hydroxyethyl-ethylenediamine in which the concentration of amine is two or more times the concentration of the copper(II) ion, addition of more amine causes this right branch of the curve to increase in absorbance and causes the maximum of the curve to shift to longer wave lengths. The limit of this shift is approximately at the position of the 1:100:0 curve shown in Fig. 1. Addition of sodium hydroxide to solutions containing amine and copper(II) ion in ratio 2:1 or greater at first causes this right branch of the curve to rise, then with more base, to lower. The maximum position is again approximately the same as for the 1:100:0 curve and the final position with great excess of base approaches the position of the 1:1:100 curve shown in Fig. 2. Thus if base is added to the Cu(II):hn mixture of ratio 1:100 the right branch of the spectral curve is lowered. If more amine is added to the 1:4:100 mixture, this part of the spectral curve is raised. The 1:4:2 curve is very close to the one for 1:100:0.

Equilibria involving at least three complexes are indicated here. The spectra for all solutions containing a great excess of sodium hydroxide approach that for the 1:1:100 ratio indicating that the end-product with such excess base contains Cu(II) ion and hn in 1:1 ratio.

The addition of excess amine to the $Cu(hn)_2^{+2}$ complex appears to result in a mixture of two or more products. First the general similarity of position of the 1:20 curve of Fig. 1 to the 1:2:1 curve of Fig. 3 indicates that at least a portion of the excess amine acts as base to remove a proton from one of the coördinated amine molecules and produce the additional five-membered chelate ring involving the oxygen atom, just as with a much smaller concentration of added sodium hydroxide.

Addition of a considerable excess of amine causes the maximum of absorbance to shift to longer wave lengths and to increase in intensity. This shift is in the same direction but is not nearly so extreme as that which Bjerrum and Nielson[12]

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[11] H. B. Jonassen, R. E. Reeves and L. Segal, This Journal, **77**, 2748 (1955).

[12] J. Bjerrum and E. J. Nielson, *Acta Chem. Scand.*, **2**, 297 (1948).

report for the addition of excess ethylenediamine to the $Cu(en)_2^{+2}$ complex in solution. This shift was assumed to be due to addition of a third ethylenediamine molecule to a fifth coördination position of the copper(II) ion. The present situation is sufficiently parallel to that of Bjerrum and Nielson[12] that we may assume that in a great excess of hydroxyethyl-ethylenediamine, a portion of the $[Cu(hn)(hen\text{-}O^-)]^{+1}$ ions and $Cu(hn)_2^{+2}$ ions are converted to $Cu(hn)_3^{+2}$ ions. Although in the same direction, the difference in spectra between the 1:2 and 1:100 ratios, Cu(II)-hn, of the present work is much less than the corresponding difference in the spectra of Bjerrum and Nielson[12] for the copper(II) ion and ethylenediamine system. It is concluded that in the present system where a competing reaction occurs, a relatively smaller amount of the $Cu(hn)_3^{+2}$ complex is formed. The fact that the maximum in absorbance is attained by addition of sodium hydroxide to the Cu(II):hn mixture of ratio 1:4 bears out the view that this maximum is due to a mixture of the proposed products. Spectra for the 1:4:2 and 1:4:4 solutions are nearly identical; more base causes the downward shift of the right branch of the spectral curve. Addition of a large excess of sodium hydroxide appears to cause a displacement of all but one of the coördinated amine molecules with the formation of $[Cu(hen\text{-}O^-)(OH)]^0$.

D. Additional Conductometric Titrations.—Two additional types of conductometric titrations were especially helpful in supplementing the results of the spectrophotometric work.

Copper(II) perchlorate was added to solutions containing hydroxyethyl-ethylenediamine with sodium hydroxide in ten- and twenty-fold excess. At the beginning of the titration and as long as the concentration of the amine was more than twice the concentration of the copper(II) ion, the slope of the conductance curve indicated reaction of less than two hydroxyl ions per mole of added copper(II) ion. As the titration proceeded and the Cu(II):hn ratio approached 1:1, the greater downward slope of the curve indicated a reaction of more than two moles of hydroxyl ion per mole of added copper(II) ion. These results may be accounted for if the following reactions are assumed

$$Cu^{+2} + 2hn + OH^- \longrightarrow [Cu(hn)(hen\text{-}O^-)]^{+1} + H_2O$$

$$[Cu(hn)(hen\text{-}O^-)]^{+1} + Cu^{+2} + 3OH^- \longrightarrow 2[Cu(hen\text{-}O^-)(OH)]^0 + H_2O$$

In this titration, after the amount of copper(II) ion added to the solution exceeds the amount of amine in the solution, copper(II) hydroxide precipitates. The slope of the line after the start of precipitation is determined by the reaction of two equivalents of hydroxyl ion per mole of copper(II) ion. If this line is extrapolated back to zero concentration of copper(II) ion it intersects the initial conductance of the sodium hydroxide–amine mixture. Thus the over-all reaction of two moles of hydroxyl ion per mole of copper(II) ion is established, in agreement with the above equations.

Figure 5 shows the results of a comparison of the addition of copper(II) ion, $Cu(hn)^{+2}$, $Cu(hn)_2^{+2}$, $Cu(en)_2^{+2}$ and an equimolar mixture of $Cu(en)_2^{+2}$ and $Cu(hn)_2^{+2}$, all as 0.05 *M* perchlorates, to 0.005 *M* sodium hydroxide solution. For the addition of copper(II) perchlorate, curve A, the initial regular decrease in conductance is accompanied by precipitation of copper(II) hydroxide. The following rise in conductance is due to the accumulation of excess copper(II) perchlorate in the solution. For

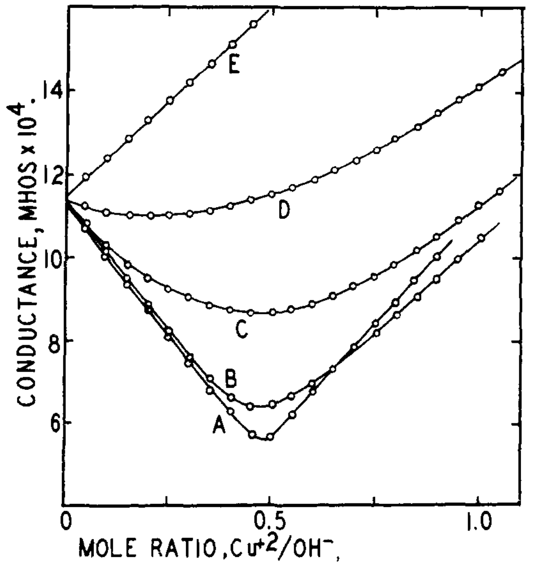

Fig. 5.—Conductometric titrations: each of the following 0.05 *M* solutions as perchlorates, added to 0.005 *M* NaOH: (A) Cu(II); (B) $Cu(hn)^{+2}$; (C) $Cu(hn)_2^{+2}$; (D) equimolar mixture of $Cu(hn)_2^{+2}$ and $Cu(en)_2^{+2}$; (E) $Cu(en)_2^{+2}$.

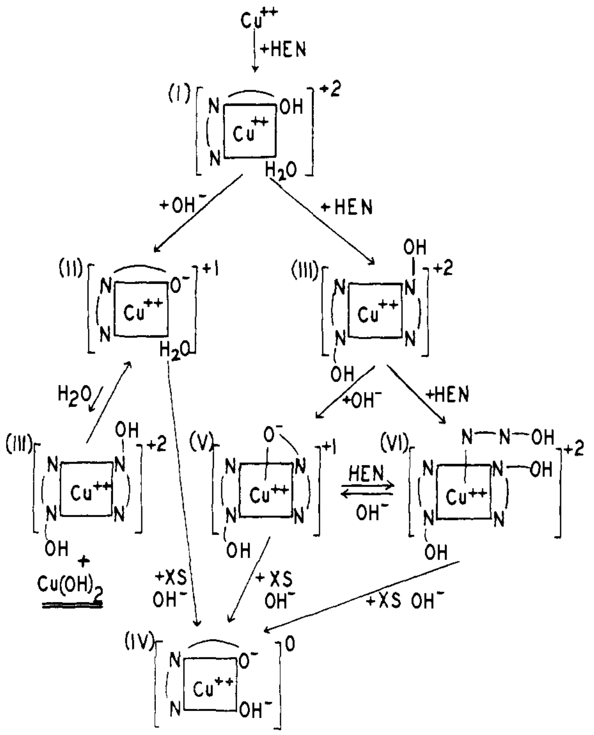

Fig. 6.—Reactions of copper(II) ion with hydroxyethyl–ethylenediamine and sodium hydroxide: (I) $Cu(hn)^{+2}$; (II) $[Cu(hen\text{-}O^-)]^{+1}$; (III) $Cu(hn)_2^{+2}$; (IV) $[Cu(hen\text{-}O^-)(OH)]^0$; (V) $[Cu(hn)(hen\text{-}O^-)]^{+1}$; (VI) $Cu(hn)_3^{+2}$.

the addition of $Cu(hn)^{+2}$, curve B, the titration is nearly identical with the one for copper(II) ion except that no precipitate is formed. The curvature in the vicinity of the end-point of curve B indicates incomplete reaction at the stoichiometric end-point. In this reaction the complex $[Cu(hen\text{-}O^-)(OH)]^0$ is evidently formed in one step. The reaction between $Cu(hn)_2^{+2}$ and hydroxyl ion, curve C, shows no definite end-point and a comparison of this curve with curves A and B indicates a total reaction of only one mole of hydroxyl ion per mole of $Cu(hn)_2^{+2}$. The initial slope, however, indicates that in the large excess of base two equivalents of hydroxyl ion per mole of $Cu(hn)_2^{+2}$ are used. This is additional confirmation that in great excess of strong base, one mole of amine is displaced from the 1:2 complex. The curve E for addition of $Cu(en)_2^{+2}$ indicates negligible reaction of this complex with the hydroxyl ion at this concentration. The curve D for the addition of the mixed complexes is evidently an average of curves C and E.

E. Potentiometric Titrations.—Titrations in which base was added to acidified solutions of the amine, $Cu(hn)^{+2}$ and $Cu(hn)_2^{+2}$ were used to calculate the acid dissociation constants of the amine salt, the stability constants of the complexes and the acid dissociation constants of the complexes. These are being reported in detail in a further publication.[9] For the formation of $Cu(hn)^{+2}$, the value of log K_1 is 10.11. For the formation of $Cu(hn)_2^{+2}$ from $Cu(hn)^{+2}$ and hn, the value of log K_2 is 7.51. For the dissociation of the proton from $Cu(hn)^{+2}$, pK_a is 7.21. These results are all in 0.5 M potassium nitrate.

Summary.—The chart shown in Fig. 6 gives a scheme which is consistent with all of the data presented here. The formulas designated V and VI are drawn to include the assumption of five-coördinate copper(II) ion but are not intended to be indicative of the structure of this ion.

Acknowledgment.—The spectra reported here were determined by Linda S. Gallo.

MORGANTOWN, W. VA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE AND THE BUREAU OF MEDICAL RESEARCH, EQUITABLE LIFE ASSURANCE SOCIETY OF THE UNITED STATES]

Coördination Complexes and Catalytic Properties of Proteins and Related Substances. I. Effect of Cupric and Zinc Ions on the Hydrolysis of *p*-Nitrophenyl Acetate by Imidazole[1]

BY WALTER L. KOLTUN, RICHARD N. DEXTER,[2] RICHARD E. CLARK[3] AND FRANK R. N. GURD

RECEIVED FEBRUARY 8, 1958

The advantages of combining different methods, particularly kinetic and equilibrium techniques, for assessing the reactivity of polar groups in proteins are described. The model system containing cupric and zinc chlorides, imidazole and imidazolium chloride has been studied simultaneously by two independent techniques that measure the concentration of free basic imidazole quantitatively. The first technique depends on the catalysis by imidazole of the hydrolysis of *p*-nitrophenyl acetate (NPA); the second technique involves measurement of the *p*H of the solution. Over a wide range of conditions the two techniques of measurement do not interfere with one another. The *p*-nitrophenolate ion released on hydrolysis of NPA combines with zinc and cupric ions about 100 times less strongly than does imidazole. Previous observations on the rate of decomposition of N-acetylimidazole are confirmed, and zinc ions are shown to have no detectable effect on the rate of decomposition. The method of Scatchard is applied to the computation of the successive association constants in the Cu(II)- and Zn(II)-imidazole systems, with results that differ only slightly from those reported previously.

Detailed knowledge of the reactivity of the polar side-chain and terminal groups of proteins is necessary for an understanding of their structure and function. At least three general approaches may be followed for assessing the reactivity of a group or class of groups: (1) the preparation of stable derivatives under defined conditions; (2) the measurement of equilibria in which the group in question takes part, for example, equilibria with cations; (3) the measurement of the catalytic activity of the group in question.

The third approach has been pursued least vigorously, except for studies specifically dealing with the catalytic properties of the highly specialized "active centers" in enzymes. We have undertaken an extensive investigation of the applicability of the catalytic method for the general assessment of the reactivity of groups in proteins. Knowledge of the catalytic potentialities of a series of model compounds containing these groups may lead also to a better understanding of enzymatic activity.

The catalytic activities of proteins are not necessarily limited to well-recognized enzymic functions. We may expect that the side-chain and terminal groups in proteins may be able to catalyze reactions according to the characteristics of comparable groups in small molecules. Presumably these catalytic effects may be assignable to the action of individual groups or of clusters of groups acting together.

The catalytic approach cannot be expected to circumvent the problem of the overlapping reactivities of the various classes of groups in proteins; for example, many of these types of groups are potentially active in general basic catalysis. Therefore, to be exploited most effectively the catalytic approach should be used in conjunction with the

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[1] This investigation was supported by research grant No. H-2739 from the National Heart Institute, U. S. Public Health Service.

[2] Lederle Medical Student Research Fellow, Cornell University Medical College, 1957.

[3] Holder of a Summer Research Fellowship under a grant from the U. S. Public Health Service to Cornell University Medical College, 1957.