COBALT COMPLEXES OF SOME SULFUR-CONTAINING LIGANDS

By

BAILIE JACK McCORMICK

Bachelor of Science
West Texas State College
Canyon, Texas
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LIGANDS

Thesis Approved:

George Goodin
Thesis Adviser

W E Harrington

Clarence M. Cunningham

Tom E Moore

Paul Arthur

Robert MacVicar
Dean of the Graduate School

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CHAPTER I

INTRODUCTION

This thesis is concerned with cobalt complexes of hexanethiol, 3-mercaptopropionic acid, and cysteine. Study of these complexes was undertaken to learn more about sulfur as a donor atom and to ascertain some of the factors involved in the reduction of molecular oxygen by cobalt(II)-cysteine complexes. This reduction reaction may be akin to those occurring in living organisms and consequently is of considerable interest.

The experimental data obtained in the past several years have made it possible to correlate the relative donor properties of many atoms. Group V and VII atoms are now fairly well characterized; but the behavior of the elements of Group VI is complex, and more data are needed to systematize the coordinating ability of atoms in this group (1).

Of the Group VI atoms, oxygen is the most thoroughly studied, and the chemical literature contains many references to coordination compounds in which oxygen is a donor. In contrast, relatively few studies have been made of ligands which form metal complexes by coordination through sulfur.

Formation of a coordinate bond involves the "donation" of a pair of electrons from a ligand atom to a metal cation. The extent of electron transfer depends mainly on two factors, the ionic potential of the cation, and the polarizability of the anion (2). As each of these

factors increases, so must the negative charge accumulating on the cation. The sulfur atom is readily polarizible, and, as a result, it forms quite stable complexes with metals having highly positive electrode potentials, i.e. metals which can accept considerable amounts of negative charge. Cobalt(III) is a good electron acceptor since its electrode potential is +0.43 volts (calculated from oxidation potentials given by Latimer (3)).

Sulfur has other properties that make it an interesting donor. Since vacant d-orbitals are present, non-bonding d-electrons from the cation may be transferred to the ligand with reduction of the excess charge on the metal. This "back coordination" can lead to stabilization of the complex and to formation of double bonds.

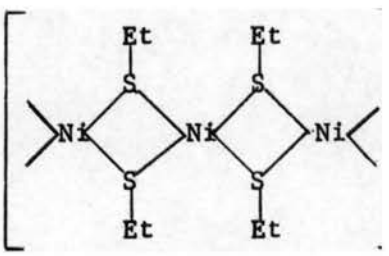
A striking example of such bonding is found in the metal carbonyls, such as $Fe(CO)_5$ (1). For these compounds, structures can be written involving many dative bonds from the ligands to the metal, but they correspond to molecules with such large accumulations of negative charge on the metal atom that they cannot be taken seriously. However, it is now generally accepted that "back coordination" of non-bonding d-electrons on the metal to π antibonding orbitals on carbon and oxygen relieves the excess charge on the metal (4). In this way, stable molecules are formed. In the case of sulfur, d_π orbitals act as acceptors.

Perhaps the most distinguishing property of sulfur as a donor is that it can, and many times does, form a "bridge" between the metal ions in poly-nuclear complexes. These bridges have been shown to be considerably more stable than halide "bridges" (5).

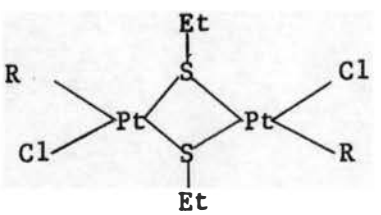
The simplest sulfur-containing ligands are the aliphatic mercaptans, such as ethanethiol; except for cysteine and 3-mercaptopropionic acid,

the literature references given here pertain exclusively to coordination compounds which involve these simple aliphatic mercaptans as donors.

That mercaptans form salts with metals has been known since their discovery by Zeise in the early 19th century (6). The early workers in this field described these materials as simple salts (6), which in some instances they probably are; but it has been shown more recently that coordination compounds also are formed. The most notable example is reported by Jensen (7), who prepared an insoluble polymeric complex from ethanethiol and nickel(II), to which the following structure was assigned [Et = C_2H_5]:



This is the only complex compound found in the literature in which a mercaptan is the sole ligand. However, some mixed-ligand complexes of the type [R = $P(CH_2CH_2CH_3)_3$, Et = C_2H_5]:



have been prepared and their isomerism studied (5,8,9).

As a ligand, 3-mercaptopropionic acid has received some attention. Fernando and Freiser (10) considered using this thio-acid as an analytical reagent for various metals. Spessard (11) prepared and measured

the visible and ultraviolet spectrum of hydrogen tris(3-mercaptopropionato)cobaltate(III).

Another sulfur-containing ligand of great interest is cysteine, $HSCH_2CH(NH_2)COOH$, which will hereafter be designated by the abbreviation, HScyH. Cysteine has one basic and two acidic groups, all of which might conceivably take part in complex formation; since steric strain makes their simultaneous coordination impossible, isomeric complexes may be formed involving O-S, N-S, or N-O chelation.

Study of chelates formed by this naturally occurring amino acid provides information concerning metal-sulfur bonds. Another interesting problem is the rapid reaction of cobalt(II) and cysteine with gaseous oxygen. The reduction of gaseous oxygen by certain compounds provides the energy used for the performance of most human activities and for the very maintenance of life processes in most organisms. These reactions usually involve complex mechanisms and occur slowly unless catalyzed. A better understanding of this biologically important reaction is desirable. Knowledge of the process of transferring electrons to oxygen may be gained by investigating the cobalt-cysteine-oxygen system.

Michaelis and co-workers (12,13,14) first investigated the cobalt-cysteine-oxygen system. Working in the pH range 7.5-8.5, they observed that: (1) cobalt(II) and cysteine reacted with oxygen to form a brown product, the amount of which apparently increased with cysteine/cobalt ratio up to a value of about 3; (2) with excess cysteine, one atom of oxygen was consumed per cobalt ion; (3) with excess cobalt (i.e. at ratios less than 3) one atom of oxygen was consumed per molecule of cysteine; and (4) chelation took place through the carboxyl and sulfhydryl groups. On this basis, it was proposed (14) that the brown product

was a cobaltous cysteine-cystine complex formed according to the equation:

$$(1)\quad 2Co^{+2} + 6HScyH + O_2 \longrightarrow 2[Co(Scy)(cyS\text{-}Scy)]^{-2} + 2H_2O + 8H^{+1}.$$

While the experimental data of Michaelis and co-workers has been confirmed by others (15), this formulation of the brown product is controverted by all subsequent evidence and is untenable in the light of present knowledge.

Kendall and Holst (15), working at pH 7.4, found that a brown cobalt(III)-cysteine complex could be formed from two moles of cysteine and one cobalt ion. In addition, some cystine was formed along with the brown complex; their oxygen consumption data were consistent with those of Michaelis and his co-workers. It is interesting to note that Kendall and Holst thought all three groups of cysteine were coordinated to cobalt. The brown product was formulated as $[Co(Scy)_2]^{-1}$, and the following oxidation scheme was proposed:

$$(2a)\quad 3[Co(Scy)_2]^{-2} + \text{oxidant} \longrightarrow 2[Co(Scy)_3]^{-3} + Co^{+2}$$

$$(2b)\quad [Co(Scy)_3]^{-3} \longrightarrow [Co(Scy)_2]^{-1} + (Scy)^{-2}$$

$$(2c)\quad 2(Scy)^{-2} + \text{oxidant} \rightarrow [cyS\text{-}Scy]^{-2}$$

$$(2d)\quad 2(Scy)^{-2} + Co^{+2} \rightarrow [Co(Scy)_2]^{-2}.$$

The relative importance of reactions 2c and 2d depended on the oxidizing agent and the conditions. For example, with indigo disulfonate, no cystine and the maximum amount of brown complex were formed, whereas with oxygen, some of both products were formed. The following equations represent the concurrent over-all reactions:

(3) $4[Co(Scy)_2]^{-2} + O_2 + 2H_2O \longrightarrow 4[Co(Scy)_2]^{-1} + 4OH^{-1}$

(4) $4HScyH + O_2 \rightarrow 2HcyS\text{-}ScyH + 2H_2O$.

This formulation of the brown complex was confirmed by Schubert (16,17), who isolated a brown complex of empirical formula $H[Co(Scy)_2]\cdot 4.5H_2O$ by acidifying the solution obtained from cysteine, cobalt, and oxygen at pH 8.0. Schubert (18) did not believe that Kendall and Holst's mechanism was correct and suggested that brown $[Co(Scy)_2]^{-1}$ was formed from the corresponding cobaltous complex, $[Co(Scy)_2]^{-2}$. It should be noted that Schubert's experiments were conducted at a much higher concentration than the earlier investigations.

In the pH range 11-12 from oxygen, cysteine, and cobalt with cysteine/cobalt ≥3, Schubert (17) obtained a green cobaltic triscysteinate which was isolated as the potassium salt, $K_3[Co(Scy)_3]\cdot 3H_2O$. This material was stable only at pH values greater than 10. At lower pH values, Schubert reported that it decomposed to a brown cobaltic biscysteinate and cysteine.

At pH 5, Schubert was able to isolate a red complex having the empirical formula $H_3[Co(Scy)_3]\cdot 4H_2O$. This material was isomeric with the green triscysteinate formed at pH 11-12.

Schubert suggested that chelation in all these compounds took place through the carboxyl and sulfhydryl groups, with the amino groups remaining uncoordinated. It is important to note that despite Schubert's thorough preparative work, he did not report the isolation of a cobaltic monocysteinate.

Neville (19) showed, in conditions similar to those employed by Schubert, that the amount of oxygen absorbed was 0.5 atom per cobalt

ion, and that the same stoichiometry also applied at pH 7.8 and lower concentrations (ca. 0.015-0.01 M cobalt) for cysteine/cobalt ratios up to 3. At higher ratios, more oxygen was consumed.

Neville and Gorin (20) demonstrated by cryoscopic techniques that the brown cobaltic biscysteinate was mononucleate. Furthermore, they investigated the complexes of cobalt with 2-aminoethanethiol, which is structurally similar to cysteine, and found that these complexes have ultraviolet and visible spectra very similar to those of cobalt and cysteine. Since only sulfhydryl-amino coordination is possible in the case of 2-aminoethanethiol, Neville and Gorin concluded that N and S were the donor atoms in the brown cobaltic biscysteinate. This conclusion is open to some criticism, but Neville (21) obtained further evidence in support of this hypothesis. Mercapto compounds not containing an amino group either did not form complexes or gave ones with greatly different spectra.

Gorin and co-workers (22) have recently reported on an investigation of some cobalt(III) complexes of cysteine. The triscysteinato-N,S-cobaltate(III) anion, hydrogen triscysteinato-O,S-cobaltate(III) and hydrogen biscysteinato-N,S-cobaltate(III) were discussed with respect to acid-base properties, spectra, and structures. The related complexes, hydrogen tris(3-mercaptopropionato)cobaltate(III) and tris(2-aminoethanethiolato)cobalt(III) were also discussed and compared to the cysteine complexes.

Spessard (11) has made a preliminary study of the ion-exchange behavior of cobalt(III)-cysteine complexes. He concluded that cis and trans diaquo isomers could be separated using ion-exchange techniques.

Since, as shall be shown, cobalt(II) complexes are involved in the

reaction of cobalt-cysteine systems with oxygen, brief reference should be made to what is known about them. Schubert (16,17) was able to prepare and isolate three complexes, containing one, two, and three molecules of cysteine per ion of cobalt(II). The green cobaltous monocysteinate was formed directly from cysteine and cobaltous ion at pH 7-8. This complex was described as a grass-green, water insoluble dimer with the formula $[Co_2(Scy)_2]\cdot 4H_2O$. In dilute base and in the absence of oxygen, half its cobalt was deposited as cobaltous hydroxide, and a cobaltous biscysteinate was formed. The green cobalt(II) biscysteinate was formed at pH 11-12 by direct reaction of cobalt(II) and cysteine. Schubert reported its formula to be $K_2[Co(Scy)_2(H_2O)_2]$. He considered the possibility that the cobalt monocysteinate was the cobalt salt of the biscysteinate. At very high pH (ca. 11-12) and a 3 to 1 or greater ratio of cysteine-cobalt, Schubert isolated a violet cobaltous triscysteinate. It was formulated as $K_4[Co(Scy)_3]\cdot 4H_2O$.

Albert (23) investigated the formation of cobalt(II)-cysteine complexes by the pH titration method of Bjerrum (24,25) and calculated values of $10^{8.8}$ and $10^{16.2}$ for the stability constants of the 1:1 and 2:1 complexes, respectively. However, Albert reported that the formation curve was of "good symmetry" only up to an $\bar{n}$ of 1.4. Hence the reported stability constants must be accepted as only approximate.

As has been stated, the research work reported in this thesis deals with cobalt complexes of hexanethiol, cysteine, and 3-mercaptopropionic acid. The first section is concerned with the red complexes which have hexanethiol, cysteine, or 3-mercaptopropionic acid as the ligand molecule. The conditions for preparation of these complexes are described, and the results of studies on some of their physical properties, such as

molecular weight, viscosity and solubility are reported. Also reported are ultraviolet, visible, and infrared spectral data. The results indicate that the complexes are polymeric and that, in the case of cysteine and 3-mercaptopropionic acid, only sulfhydryl coordination is involved. This conclusion is at variance with previous reports which describe these complexes as involving oxygen-sulfur coordination (22).

The second section describes work done on the mechanism of the reaction of cobalt(II)-cysteine systems with oxygen. Spectral data are reported for various cobaltous and cobaltic cysteinates. The complexes which exist in the absence of oxygen depend quite strongly on pH, concentration, and cysteine/cobalt ratio. The effect of each of these variables has been investigated. Low pH favors the simplest complex [Co(Scy)], while high pH favors the complex $[Co(Scy)_3]^{-4}$. The course of the air-oxidation of cobalt(II)-cysteine solutions is also quite sensitive to the above-mentioned variables. This phenomenon has been investigated and, based on the results obtained, a new mechanism is proposed for the reaction of oxygen with cobalt(II)-cysteine systems. The proposed mechanism accounts for the formation of cystine in some conditions by postulating a catalytic cycle involving cobalt. This mechanism is distinctly different from that proposed heretofore (15). The need for working in air-free conditions led to the development of numerous specialized techniques which are described.

The final section of this thesis is concerned with the nature of cobalt(III)-cysteine complexes which involve nitrogen and sulfur as donors. It has been established that the oxidation of cobalt(II)-cysteine mixtures can give rise to a mixture of products (11), but their exact nature is not known. The mixture of products may contain

geometrical isomers which would be difficult to separate. These products have been investigated using ion-exchange chromatographic techniques, and some progress has been made in identifying them. The brown cobaltic biscysteinate formed at pH 8.0 is a trans-diaquo isomer, while the decomposition product of cobaltic triscysteinate is a cis-diaquo isomer of cobaltic biscysteinate. The rate of reaction of these isomers with cysteine in various conditions of pH has been studied. Also the conversion of the cis-diaquo to the trans-diaquo isomer has been investigated. Finally, an uncharged, brown binucleate complex was formed in some conditions, and its character is reported.

CHAPTER II

PREPARATION AND PROPERTIES OF COBALT(III) COMPLEXES INVOLVING SULFHYDRYL COORDINATION

Results and Discussion

From cobalt(II), cysteine, and oxygen in concentrated solution at approximately pH 5, Schubert (16) prepared a deep-red complex having the formula $H_3[Co(Scy)_3]\cdot 4H_2O$. This complex has been named hydrogen triscysteinato-O,S-cobaltate(III) by Gorin and co-workers (22). Here, for brevity, the red complex will be called R-TRIS.[1]

It is of interest to determine what groups are coordinated to the cobalt ion in R-TRIS. Schubert thought it likely that the carboxyl and sulfhydryl groups were involved. Neville (26) prepared a complex from N-formylcysteine which had a spectrum in the region 200 to 600 mμ very similar to that of R-TRIS, and, on this basis, he proposed that chelation involved the sulfhydryl and carboxyl groups. Gorin and co-workers (22) prepared a cobalt(III) complex of 3-mercaptopropionic acid which was formulated as $[Co(SCH_2CH_2COO)_3]^{-3}$; this complex had a spectrum nearly identical to that of R-TRIS. On the other hand, Spessard (11) was unable to prepare a cobalt(III) complex from methionine($CH_3SCH_2CHNH_2COOH$). Based on these experiments, he concluded that oxygen-sulfur chelation was involved in both the 3-mercaptopropionic acid complex and R-TRIS.

[1] In this symbolism, R signifies the red color of the complex, and TRIS indicates that three ligands are bound per cobalt ion.

In all of these investigations, it has been assumed that a chelate ring is formed by coordination of two of the groups present in cysteine or 3-mercaptopropionic acid. This assumption is valid in most cases since chelates are nearly always more stable than complexes involving unidentate ligands, e.g. tris(ethylenediamine)cobalt(III) ion is far more stable than hexamminecobalt(III) ion (27). However, it is possible that only the sulfhydryl group would be coordinated.

Complexes of Cobalt(III) and Hexanethiol

In order to investigate this possibility, it was decided to study cobalt(III) complexes of ligands containing only the sulfhydryl group, and the ligand chosen for study was hexanethiol, henceforth abbreviated HShex. Although mercaptans have notoriously bad odors, hexanethiol is a fairly non-volatile liquid whose odor, while strong, is not overwhelming.

The first preparations were carried out by adding alcoholic sodium hydroxide to an alcoholic mixture of cobalt(II) chloride and hexanethiol, aerating the mixture for 3-4 hours and allowing to stand overnight. A voluminous, red precipitate was formed. If the experiment was done in the absence of air, only a green solution formed; when exposed to the air, a fine, red precipitate was rapidly deposited. This experiment indicates that the red complex involves cobalt(III).

It was then decided to try to prepare this complex from dichlorobis(ethylenediamine)cobalt(III) chloride. A 6:6:1 molar ratio of hexanethiol, base, and cobalt was used, and on adding the dichlorobis-(ethylenediamine)cobalt(III) to the mercaptan-base mixture, a complex was rapidly precipitated. This complex differed in some respects from

that prepared from the direct reaction of cobalt(II), hexanethiol, base, and air: it was more easily filtered; freshly prepared, it was reddish-brown instead of red; and it was considerably more soluble in chloroform, about 30 g./liter as opposed to 3 g./liter. On the other hand, the chloroform solutions had identical spectra (Figure 1, curve A), and the solid complexes had the same composition and appearance after drying and pressing out or recrystallizing from chloroform. The characteristic reddish-brown precipitate could be formed rapidly from dichlorobis(ethylenediamine)cobalt(III) chloride, hexanethiol, and base in the absence of oxygen.

The composition of the complex was established by elemental analysis for cobalt and carbon. These analyses indicated the empirical formula $[Co(Shex)_3]$. Based on this formula, yields of 95-100% were obtained in the synthesis of the complex. Also, based on this empirical formula, molar absorbancy coefficients of 23,400, 8,590, and 8,430 were calculated at 510, 417, and 270 mμ, respectively.

The empirical formula of this inner complex, $[Co(Shex)_3]$, poses an interesting problem. If octahedral coordination is to be maintained, and to the writer's knowledge there is no known example of a coordination number of other than six for cobalt(III), the complex must have a polymeric structure such as that shown in Figure 2. The suggested name for this complex is poly-μ-tris-(hexanethiolato)cobalt(III) (28).

All of the physical properties of the complex are in accord with a structure such as shown in Figure 2. Such highly cross-linked materials are known to be quite insoluble in most solvents and are usually chemically inert (29). Poly-μ-tris-(hexanethiolato)cobalt(III) was insoluble in: water, dioxane, 15M ammonium hydroxide, 12M hydrochloric acid, 16M

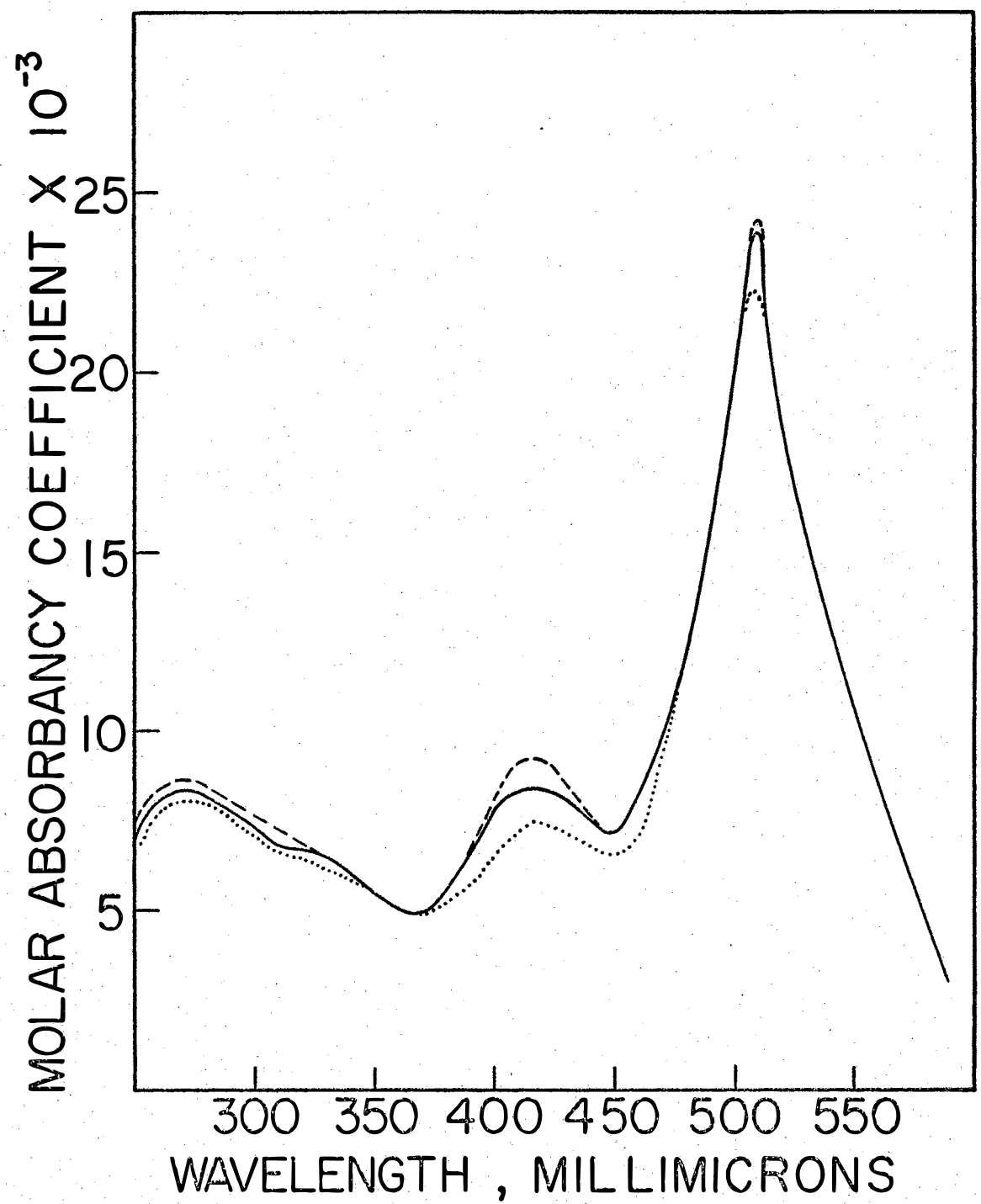


Fig. 1. Spectra of: A, $[Co(Shex)_3]$ (———);
B, $[Co(Scy)_3]^{-3}$ (- - - - -);
C, $[Co(SCH_2CH_2COO)_3]^{-3}$ (·····).

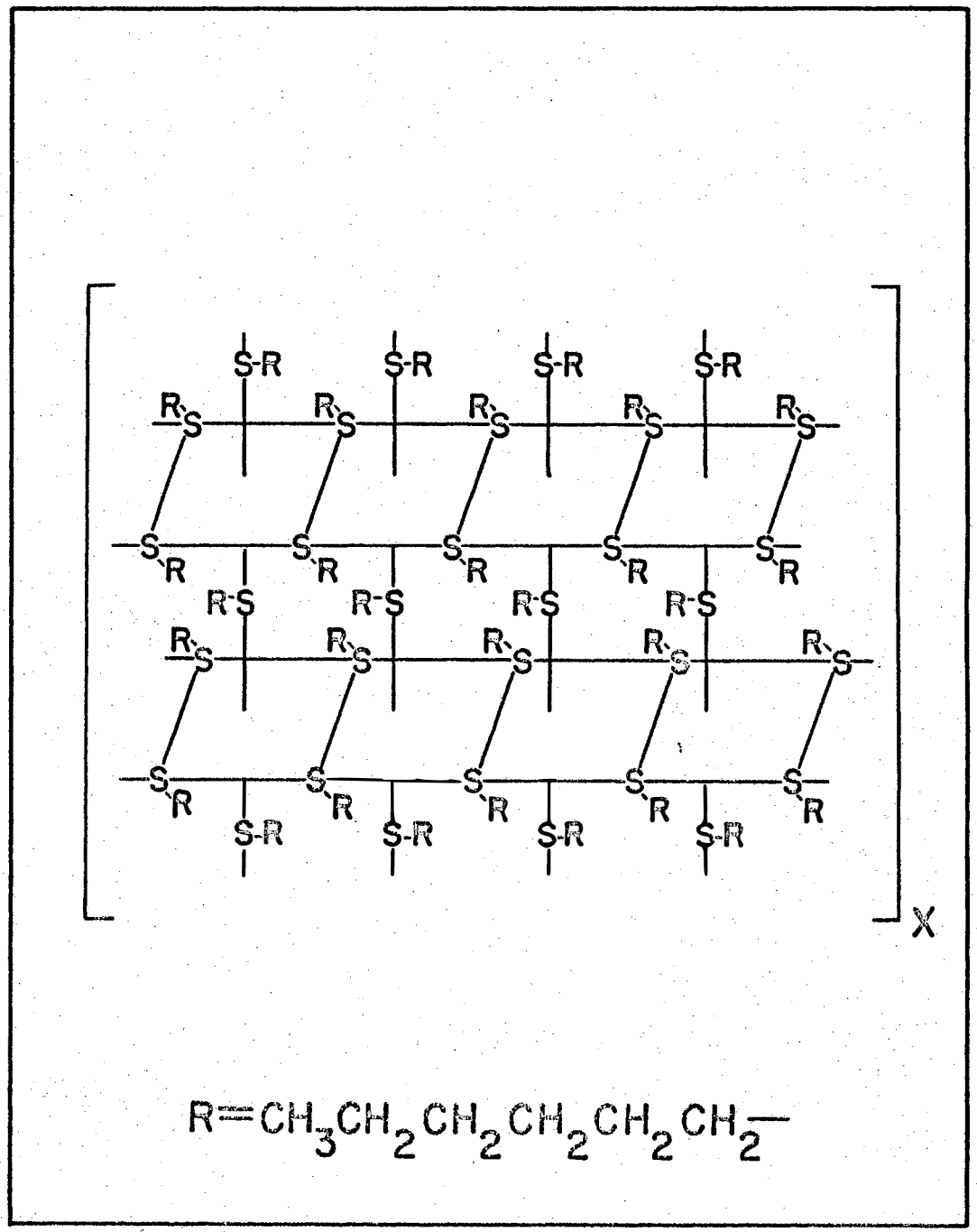


Fig. 2. Structure of Poly-μ-tris(hexanethiolato)cobalt(III).

sulfuric acid, dimethylformamide, dichloroacetic acid, and ethyl acetate; very slightly soluble[2] in benzene, acetic acid, diethylamine, carbon tetrachloride, and bromobenzene; and moderately soluble in chloroform. Even in chloroform the dissolution was slow, and a mechanical shaker was used in preparing solutions.

The complex was extremely inert. It gave no reaction with warm, concentrated sulfuric acid or sodium hydroxide. The only destructive reagent found was concentrated nitric acid. Despite this extreme chemical inertness, the complex was heat unstable, decomposing around 160° C.

When a lump of the complex was dissolved in chloroform, considerable swelling occurred before and during the slow dissolution, and the chloroform solution so formed was quite viscous. These observations are identical to the description of polymer dissolution processes given by Billmeyer (30) who says

> "Although low polymers may dissolve rapidly to fluid solutions, the solution of most polymers is slow. The system often undergoes a long preliminary swelling process during which the solvent penetrates into the solute particles. This stage is followed by the gradual breakdown and dispersion of the swollen particles to solutions of high viscosity."

The viscosity of the chloroform solutions was investigated in more detail, and the intrinsic viscosity was determined. Values of 3.2 to 3.8 were obtained. In general, it can be said that such high viscosities are indicative of a high-polymeric structure; for example, polystyrene samples which show intrinsic viscosities of 3.55 and 0.45 in toluene at 25° C. have molecular weights of 2,060,000 and 115,000, respectively (31).

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[2]By "very slightly soluble" it is meant that the complex dissolved to the extent of 0.02-0.1 g./liter.

Accurate estimates of molecular weight have been made for some polymers by applying the modified Staudinger equation:

$$\eta = KM^{\alpha}$$

where η is the intrinsic viscosity and M is the molecular weight. K and α in this equation are empirical constants, which are calculated from measurements on samples whose molecular weight has been determined by other means. These constants vary widely depending on the solvent and structure of the polymer; there are no close similes or valid theoretical principles from which one might estimate values applicable to the polymeric complex under discussion. About all that can be said is that the molecular weight would be high, and that the structure shown in Figure 2 would be quite extensive.

In addition to the viscosity experiments, attempts were made to determine the molecular weight of poly-μ-tris(hexanethiolato)cobalt(III) by thermodynamic methods. An osmometer was constructed, and the osmotic pressure of $[Co(Shex)_3]$ in chloroform was investigated. However, much to the author's chagrin, these experiments were inconclusive, as stable and reproducible pressure heads were not obtained. Similarly, ebullioscopic measurements were unsuccessful. Details of these experiments are found in the experimental section.

Complexes of Cobalt(III) with Cysteine and 3-Mercaptopropionic Acid

Hydrogen triscysteinato-O,S-cobaltate(III), first prepared by Schubert (16) and investigated by Spessard, Gorin, and co-workers (11, 22), and hydrogen tris(3-mercaptopropionato)cobaltate(III) (TMPC) prepared by the latter investigators, are very similar in appearance to

poly-μ-tris(hexanethiolato)cobalt(III). Also, the visible and ultraviolet spectra of these three complexes are nearly identical (Figure 1). The molar absorbancy coefficients are given in Table I.

TABLE I

PEAK MOLAR ABSORBANCIES OF R-TRIS AND TMPC

Wavelength in mμ	Peak Molar Absorbancy Coefficient	
	R-TRIS	TMPC
510	24,100	22,600
417	8,000	6,400
270	8,900	8,200

Since the close correspondence of these spectra is indicative of similar structures, it was decided to measure the infra-red spectra of the cysteine and 3-mercaptopropionic acid complexes and seek evidence for or against coordination of the carboxyl groups. Some general facts concerning the spectra of carboxylic acids and their complexes have been discussed by Cotton (32). In Table II are listed some pertinent infra-red absorption data for carboxylic acids, their salts and their metal complexes; also given are some relevant data concerning R-TRIS and TMPC.

Since the copper(II), nickel(II), and zinc(II) glycinates have carbonyl absorption bands at the same wavelength as potassium glycinate, Sen (33), Sweeney (34), and co-workers concluded that the carboxyl-metal bond was "essentially ionic" in these complexes. However, it was pointed out by Cotton (32) that these conclusions must be accepted with some reservations; these complexes are hydrated, and there may be

hydrogen bonding between carbonyl groups and water which might counteract the shift due to covalent complexing.

TABLE II

SELECTED INFRA-RED ABSORPTION DATA[3]

Compound	Wavelength of Absorption Band	Assignment of Absorption Band(s)
Monomeric Carboxylic Acids	1760-1770 $cm.^{-1}$	carbonyl
Dimeric Carboxylic Acids	1690-1740 $cm.^{-1}$	carbonyl (hydrogen bonded)
Esters	1737-1750 $cm.^{-1}$	carbonyl
Ethylglycinate	1750 $cm.^{-1}$	carbonyl
Carboxylate Salts	1560-1620 $cm.^{-1}$	assymetric stretching mode of CO_2^{-1} group
Potassium Glycinate	1600 $cm.^{-1}$	assymetric stretching mode of CO_2^{-1} group
Bisglycinatocopper(II) monohydrate	1600 $cm.^{-1}$	carbonyl
Bisglycinatonickel(II) dihydrate	1600 $cm.^{-1}$	carbonyl
Bisglycinatozinc(II) monohydrate	1600 $cm.^{-1}$	carbonyl
R-TRIS	1625, 1380, 1340, 1300 $cm.^{-1}$	
TMPC	1710 $cm.^{-1}$	
Potassium Salt of TMPC	1575 $cm.^{-1}$	

[3]Taken in part from Cotton (32), Sen (33), and Sweeney (34). For the assignment in R-TRIS and TMPC see below.

In the case of TMPC, which is not hydrated, this factor does not come into question. If the carboxyl group were coordinately bound to cobalt, it would not be able to participate in hydrogen bonding with an adjacent carboxyl group. As seen in Table II, TMPC does, nevertheless, have a band at 1710 $cm.^{-1}$, the frequency characteristic of dimeric carboxylic acids. This indicates that the carboxyl is not covalently bound and forms a hydrogen bond with an adjacent carboxyl group.

The potassium salt of TMPC showed a strong absorption band at 1575 $cm.^{-1}$, which is in the characteristic range of carboxylic acid salts. In carboxylic acid salts, the band around 1560-1620 $cm.^{-1}$ is the result of assymetric CO_2^{-1} stretching. This frequency is characteristic, because the CO bond orders become equal (ca. 1.5) in RCO_2^{-1} by mesomerism. Had there been a strong interaction between the cobalt and the carbonyl group, the CO bond orders in the RCO_2^{-1} group would have been dissimilar, and the characteristic frequency would have been shifted.

Based on these spectral data, it is concluded that any interaction between the carboxyl group and cobalt in TMPC must be weak. Most probably the carboxyl-cobalt bond is basically ionic.

Since several possibilities for hydrogen bonding exist in hydrated R-TRIS, its infra-red spectrum is much more difficult to interpret than that of TMPC. Due to the writer's limited knowledge of infra-red absorption spectroscopy, the absorption bands given in Table II were not assigned. In spite of this difficulty, it is thought that here, too, the carboxyl group was not covalently bound to the cobalt ion.

Since the carboxyl group is not coordinated in R-TRIS or TMPC, these complexes must have structures similar to that given in Figure 2 for poly-μ-tris(hexanethiolato)cobalt(III). Viscosity and diffusion

studies indicated that this is indeed the case. R-TRIS and TMPC dissolved in dilute sodium hydroxide had intrinsic viscosities of 0.1 and 0.23, respectively. Although these intrinsic viscosities are considerably lower than that of the hexanethiol-cobalt complex, they are still large. For comparison, it may be pointed out that polyisobutylene with a molecular weight of 20,000 has an intrinsic viscosity of 0.20 in diisobutylene at 20° C.

To obtain additional information concerning the molecular size of R-TRIS and TMPC, their rate of diffusion through a cellulose membrane was compared to that of a monomeric complex, potassium ethylenediaminetetraacetatocobaltate(III). The formula weight of this complex anion, 350, makes it an ideal standard for comparison, since R-TRIS and TMPC have empirical formula weights of 410 and 350, respectively. In a typical experiment, a solution of the complex was placed inside of a cellulose dialysis bag which was then rotated in a reservoir of pure solvent. The rate of diffusion was determined by measuring the absorbance of the reservoir solution as a function of time. The results for the ethylenediaminetetraacetatocobaltate(III) anion, $[Co(enta)]^{-1}$, are shown in Table III.

After 9.5 hours the absorbancy of the solution inside the dialysis bag was 0.770; thus, equilibrium was essentially established in about 10 hours.

The results of the diffusion of TMPC are also given in Table III. After 56 hours, the absorbancy of the solution inside of the dialysis bag was 278.

After 51 hours the reservoir solution in the diffusion of R-TRIS was only faintly pink and absorbancy measurements were not made. However,

the absorbancy of the solution inside of the bag was 180.

TABLE III

ABSORBANCY OF THE RESERVOIR SOLUTION AS A FUNCTION OF TIME IN THE DIFFUSION OF $[Co(enta)]^{-1}$ and $[Co(SCH_2CH_2COO)_3]^{-3}$

Time in Hours	$[Co(enta)]^{-1}$ Absorbancy at 535 mμ	$[Co(SCH_2CH_2COO)_3]^{-3}$ Absorbancy at 510 mμ
0.3	0.250	0
1.0	0.480	0
1.5	0.590	0
2.0	0.655	0
2.5	0.690	0
3.0	0.720	0
3.6	0.740	0
4.3	0.740	0
9.5	0.750	0
20		0.065
28		0.200
32		0.310
45		0.780
56		1.10

Obviously, after 50-60 hours equilibrium was far from being established in both experiments. These results are interpreted to mean that both R-TRIS and TMPC are much larger molecules than $[Co(enta)]^{-1}$; certainly they are not monomeric.

Experimental

1. Chemicals.--Hexanethiol was obtained from the Eastman Kodak Co., Rochester 3, New York. It was used without further purification. 3-Mercaptopropionic acid was obtained from Evans Chemetics, Inc., New York 17, New York and was purified by vacuum distillation at 2 mm. of pressure. Cysteine hydrochloride monohydrate (B grade) was a product of the California Corporation for Biochemical Research, Los Angeles 63, California. This too, was used without further purification. Dichlorobis(ethylenediamine)cobalt(III) chloride and tris(acetylacetonato)-cobalt(III) were prepared as described in "Inorganic Synthesis" (35, 36). Potassium ethylenediaminetetraacetatocobaltate(III) dihydrate was prepared by a method developed by Dwyer, Gyarfas and Mellor (37). Unless otherwise specified, all other chemicals were of analytical reagent grade.

2. Spectral Measurements.--All visible and ultraviolet spectral work was done using a Beckman Model DU Spectrophotometer and 10 mm. pyrex or silica absorption cells. In all cases, pure solvent was used as the reference solution. Molar absorbancy coefficients were calculated by dividing the absorbancy at a given wavelength by the molarity of the solution based on the empirical formula of the complex. Infra-red absorption spectra were measured with a Beckman Model IR-7 Infrared Spectrophotometer. Solid samples (0.2-0.5 mg.) were ground with 200 mg. of dry potassium bromide, and the resulting mixture was pressed into a transparent disc. As the complexes were difficult to disperse in the potassium bromide, very thorough, vigorous grinding was required. The spectrum was determined using the micro-beam condenser attachment.

3. Preparation of $[Co(Shex)_3]$ from Cobalt(II) Chloride.--In 250 ml. of denatured ethyl alcohol was dissolved 5.1 g. (4.30×10^{-2} moles) of hexanethiol. To this was added 3.85 g. (1.4×10^{-2} moles) of cobalt-(II) chloride hexahydrate in 50 ml. of ethanol. Next, 200 ml. of ethanol containing 1.8 g. (4.5×10^{-2} moles) of sodium hydroxide was added slowly to the mixture. The resulting solution was oxygenated for 3-4 hours with a stream of air and then was allowed to stand overnight. A reddish-brown precipitate formed; it was filtered in a medium-porosity, fritted-glass filter funnel, washed with alcohol and dried in a vacuum oven at 50° C. and 10 mm. of pressure. Yields were 85-95% based on the amount of cobalt taken.

If no base were added, only a small amount of a gummy, red precipitate was formed.

Purification of the complex consisted of dissolving the dried product in the minimum amount of chloroform required, filtering into an equal volume of ethanol, and then collecting the precipitate in a fritted-glass filter funnel. The precipitate was washed with ethanol and dried for 4 hours at 50° C. and 10 mm. of pressure. Calculated for $[Co(Shex)_3]$: Co 14.36%, found 14.89%; carbon 52.68%, found 51.24%.

4. Preparation of $[Co(Shex)_3]$ from Dichlorobis(ethylenediamine)-cobalt(III) chloride.--A solution of $[Co(en)_2Cl_2]Cl$, 7 g. (2.45×10^{-2} moles) in 25 ml. of distilled water, was mixed with 50 ml. of denatured ethanol, and the mixture was added slowly with stirring to 16.5 g. (14.7×10^{-2} moles) of hexanethiol in a slight excess of base (15 ml. of 10 M sodium hydroxide). A reddish-brown precipitate formed immediately. The precipitate was allowed to stand overnight in contact with the mother liquor and was then filtered on Whatman Number 42 filter

paper in a Büchner funnel. The complex was washed with 300 ml. of ethanol followed by 50 ml. of ether and then air-dried. After drying, it was a reddish-brown powder which assumed a green sheen when pressed out or smeared on a watch glass. The yield was 95-100% based on the cobalt taken. Calculated for $[Co(Shex)_3]$: Co 14.36%, found 14.43%; carbon 52.68%, found 52.73%.

In the absence of oxygen, the same reddish-brown product was formed.

The complex could be prepared from hexamminecobalt(III) chloride by the same technique as described above.

5. Preparation of TMPC and Its Potassium Salt.--To a thick slurry of 12.3 g. of sodium acetate in a small amount of water was added 3 g. of 3-mercaptopropionic acid. Ten ml. of 1 M cobalt(II) chloride was then added.

The acetate/acid mole ratio was 5, and the acid/cobalt mole ratio was 3. A fleeting green color was observed and quickly obscured by a reddish-brown precipitate.

The thick slurry was allowed to stand exposed to the air for 12 hours with occasional stirring. After this time the precipitate was filtered on paper in a Büchner funnel and pressed dry as possible.

The precipitate was dissolved in several ml. of 0.1 M sodium hydroxide, and the resulting solution was filtered. Concentrated hydrochloric acid was added to the solution until it was distinctly acid and a reddish-brown precipitate had formed. The precipitate was filtered, washed with water until the filtrate was neutral and then washed with a few ml. of alcohol. After air-drying, the compound was further dried at 80° C. in a vacuum oven for 4 hours. Yields were 75-80% based on the cobalt taken. Calculated for $H_3[Co(SCH_2CH_2COO)_3]$: carbon 28.88%,

found 28.70; hydrogen 4.04%, found 4.28. The potassium salt was prepared by dissolving 0.5 g. of $H_3[Co(SCH_2CH_2COO)_3]$ in 50 ml. of 0.1 N potassium hydroxide, filtering, and adding 15 ml. of ethanol with stirring. The precipitate so formed was filtered and washed successively with 20% alcohol, 50% alcohol and ether. It was dried at 50° C. in a vacuum oven.

6. Preparation of $H_3[Co(Scy)_3]\cdot 4H_2O$.--The preparation was carried out in acetate buffer according to the directions given by Spessard (11). Cysteine hydrochloride monohydrate and sodium acetate in a molar ratio of one to five were mixed with enough water to make a thick paste. Then 0.33 moles of cobalt(II) chloride per mole of cysteine was added. The suspension was stirred 30 minutes and allowed to stand twelve hours in air. After standing, the precipitate was treated with water and filtered. The precipitate was dissolved in base and reprecipitated in the manner described in section 5. Yields were about 90% based on the cobalt taken.

7. Solubility Experiment.--The solubility of $[Co(Shex)_3]$ was qualitatively determined by placing about 0.1 g. of the complex and approximately 10 ml. of the solvent in a test tube. The solutions were allowed to stand for several days with periodic shaking, and the color of the solutions was noted. The pink color of $[Co(Shex)_3]$ could easily be seen in concentrations as low as 0.01-0.02 g. per liter.

8. Temperature Stability Experiment.--A small quantity of $[Co(Shex)_3]$ was placed on the heating block of a Fisher-Johns Melting Point Apparatus and observed under magnification as the temperature was raised slowly. Evidence of decomposition (smoking) was observed around 160°-170° C. At 200° C. the material crumbled to a gray powder. When

placed in a flame, the complex evolved a gas that burned with a smoky yellow flame. A gray powder remained after ignition.

9. Reaction of $CoCl_2$ with Various Mercaptans and Disulfides.--A small amount (ca. 0.5 g.) of $CoCl_2 \cdot 6H_2O$ was dissolved in ethanol and added to ethanolic solutions of dodecanethiol, tertiary hexyl mercaptan and butyl disulfide. In the absence of base, no reaction was observed in any of the solutions. On addition of alcoholic sodium hydroxide, voluminous, reddish-brown precipitates formed in the mercaptan solutions, but no reaction was observed in the butyl disulfide solution.

10. Viscosity Experiments.--For viscosity determinations in chloroform a special narrow-bore Ostwald viscometer was constructed. The capillary was about 0.4 mm. in diameter and 16 cm. long, and the overall length was 32 cm. In a typical experiment, 5.0 ml. of liquid was placed in the viscometer, which was kept in a constant temperature water bath at 24.8° C. The solutions were either allowed to stand in the viscometer for temperature equilibration, or were equilibrated before placing in the viscometer. The flow time of pure, reagent grade chloroform was 99.2 ± 0.2 sec.

Flow times were measured for at least three different concentrations. The specific viscosity was calculated from the equation:

$$\beta = \frac{T_s - T_o}{T_o}$$

where T_s is the solution flow time and T_o is the flow time of pure solvent. In using this equation it was assumed that the density of pure chloroform was the same as that of the solutions. This assumption is valid unless highly accurate measurements are desired; for example,

the measured density of chloroform was 1.467 gm./ml., while that of a solution containing 0.3000 g. of $[Co(Shex)_3]$ per 100 ml. of chloroform was 1.471 gm./ml.

The intrinsic viscosity was calculated from a plot of β/c versus c, where c is the concentration of complex in grams per 100 ml. Extrapolation to zero concentration gave the intrinsic viscosity. The results for $[Co(Shex)_3]$ are given in Table IV.

TABLE IV

INTRINSIC VISCOSITY OF $[Co(Shex)_3]$ SOLUTIONS

Method of Preparation	Intrinsic Viscosity
$CoCl_2$ + HShex + O_2 + NaOH	3.3
$[Co(en)_2Cl_2]Cl$ + HShex + NaOH	3.2-3.8

Similar techniques and apparatus were used for determining the intrinsic viscosity of TMPC and R-TRIS solutions. In this case, a commercial Ostwald viscometer was used. The flow time of pure water was 126.5 $\pm$ 0.2 sec. and that of 0.1 N NaOH was 128.9 $\pm$ 0.2 sec. The samples were dissolved in 0.1 N NaOH to give concentrations between 0.08-0.90 g./100 ml. The intrinsic viscosity was 0.23 and 0.1 for TMPC and R-TRIS, respectively.

For purposes of comparison, the flow time of $[Co(en)_2Cl_2]Cl$ was measured. In this case, the flow time of a solution containing 1.0 g. of complex per 100 ml. of water was only 1 sec. greater than that for the pure solvent.

11. Osmometry Experiments.--A Zimm-Meyerson osmometer was

constructed (38) and fitted with special membranes for use in organic solvents. These were obtained from the Schleicher and Schuell Co., Keene, New Hampshire. The osmometer was filled with a chloroform solution and suspended by a wire into a reservoir of pure chloroform, which was kept at 25° C. by a water bath. The height of the pressure head developed was measured with a cathetometer.

In a typical experiment using 21.16 g. $[Co(Shex)]_3$ per liter of chloroform, the head of the chloroform solution rose to a maximum in about 8 hours, after which time it decreased to practically nothing. The decrease was observed over a period of 5 days. The reservoir solution was slightly colored after the run; however, tests showed this was not due to leakage, but that it was due to diffusion through the membrane. The only explanation that can be offered for the anamolous behavior of the osmotic measurements is that low molecular weight, slightly colored fractions diffused through the membrane causing the osmotic pressure to drop slowly. In any case, reliable measurements were not obtained, and molecular weights could not be calculated.

12. Ebullioscopic Experiments.--Attempts to measure the molecular weight of $[Co(Shex)_3]$ from the boiling point elevation in a Cottrell apparatus (39) failed because the solution was too viscous and did not percolate up and over the thermometer bulb.

13. Diffusion Experiments.--Samples were placed in short lengths (ca. 5 cm.) of 15 mm. diameter cellulose dialysis tubing that was obtained from the Visking Co., Chicago 38, Illinois. The ends of the bags were carefully sealed by tying thread tightly around the tube. To one end of the tube was tied a 1/2 inch long, Teflon-covered, magnetic stirring bar. The filled bags were then placed in a 100 ml. graduated

cylinder which contained 50 ml. of distilled water and placed on top of a magnetic stirring apparatus.

The absorbance of the reservoir solution was determined as a function of time. Unless dilutions were necessary, the aliquots taken for absorbance measurements were returned to the reservoir.

The solvent used was 0.1 N sodium hydroxide for R-TRIS and TMPC and water for $K[Co(enta)]\cdot 2H_2O$. To determine any possible effect dilute base might have had on the membrane, 0.600 g. of $K[Co(enta)]\cdot 2H_2O$ was dissolved in 100 ml. of 0.05 N sodium hydroxide, and this solution was placed in a dialysis bag in the usual fashion. Since dilute base hydrolyses $[Co(enta)]^{-1}$, the results obtained were not strictly comparable to those above, but equilibrium was established in about 8 hours.

14. Analyses.--Much difficulty was encountered in finding a suitable method for cobalt analyses. With ethylenediaminetetraacetic acid as the titrant and murexide as the indicator, reproducible end points were not obtained. However, a suitable gravimetric method was eventually developed and is given in detail below.

The sample to be analyzed was weighed into a small porcelain crucible and was then carefully ignited over a small flame. Care was taken to prevent ignition of the evolved gases. After ignition to a dull-gray powder, the samples were heated in a muffle furnace at 750-800° C. for about 4 hours. The resulting gray ash was then dissolved by adding about 3 ml. of concentrated hydrochloric acid and heating over a hot plate. The dissolution sometimes took several days, and the hydrochloric acid was replenished as needed.

The blue solution obtained was diluted with water, neutralized with ammonium hydroxide and washed into a Berzelius beaker. Cobalt was

deposited electrolytically from this solution in the prescribed manner (40); a Sargent-Slomin Electrolytic Analyzer was used. The precision was 5-10 parts per thousand.

Carbon and hydrogen determinations were performed by Dr. Alfred Bernhardt, Mulheim (Ruhr), Germany.

CHAPTER III

REACTION OF COBALT(II) AND CYSTEINE WITH OXYGEN

Results and Discussion

Kendall and Holst (15) have made the most thorough investigation of the reaction of cobalt(II) and cysteine with oxygen. Their work and the work reported in this chapter deals with complexes in which cysteine coordinates through nitrogen and sulfur. Kendall and Holst proposed an oxidation mechanism which involved $[Co(Scy)_3]^{-3}$ as an intermediate; the ultimate products of oxidation depended on the oxidant. In some cases, mainly the brown complex $[Co(Scy)_2]^{-1}$ was produced, while in others, cystine was a major product; many times the reaction produced a mixture of both.

Kendall and Holst's work is quite admirable, and they deserve credit for postulating at an early date a mechanism which recognized the formation of cystine in some cases and which might account for the catalytic effect of cobalt in the formation of this product. However, as shall be shown, their suggestion must be modified in many essential details in view of what has been subsequently ascertained about cysteine complexes in particular and complex formation in general.

According to the current theory of "stepwise" formation of complexes (25), one would expect the nature of the complexes present in an anaerobic solution of cobalt(II) and cysteine to depend quite strongly on the conditions, especially pH, cysteine/cobalt ratio, and absolute

concentration. The products of oxidation would also depend on these variables, since cobalt(II) complexes are undoubtedly their precursors. This chapter describes a study of the cobalt(II) complexes and their oxidation products.

The first system investigated was cobalt(II) and cysteine at pH 11. In this case, cobalt(II) ion at 0.01 M concentration and excess cysteine in the strict absence of oxygen formed a purple-violet complex; the color intensity was not appreciably decreased as the cysteine/cobalt ratio was reduced to three. However, further reduction of the cysteine/cobalt ratio resulted in the formation of a pink precipitate of cobaltous hydroxide, in addition to the purple complex in solution. This indicated that the complex contained three molecules of cysteine per cobalt(II) ion; it presumably is the same as the 3:1 complex isolated in solid form by Schubert (17). The "delicate pink" color observed by Michaelis and Barron on mixing cobalt(II) and excess cysteine likely was due to this complex in more dilute solution. The visible spectrum of the complex is represented in Figure 3, curve A. The ultraviolet spectrum was not measured due to the difficulty of preparing sufficiently dilute, yet unoxidized, solutions. Also, the data represented in Figure 3, curves B and C, show that no appreciable amount of this complex was formed at pH 7.8 or 5.8 and the same cysteine/cobalt ratio, although lower complexes were formed. The visible spectrum of the analogous tris(2-aminoethanethiolato)cobalt(III) complex was also measured. An absorption maximum was shown at 560 mμ. The spectral similarity of this complex to the triscysteinatocobaltate(III) ion supports Neville's hypothesis that N-S coordination is involved in the cobalt-cysteine complex.

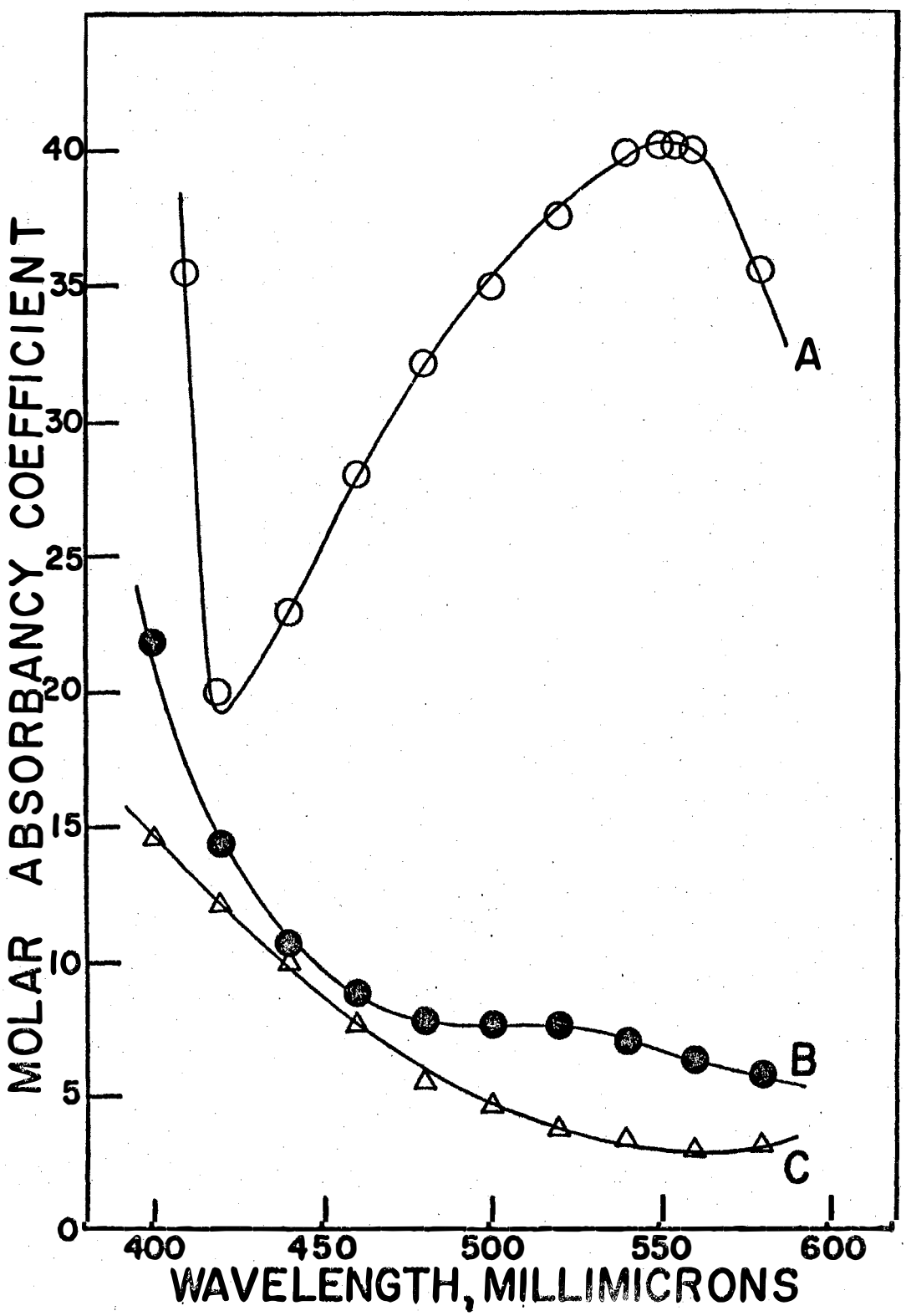

Fig. 3. Spectra of Cobaltous Complexes at Cysteine/Cobalt Ratios of 4: A, pH 11; B, pH 7.8; C, pH 5.8.

Since the cobalt(II) complexes were extremely sensitive to air oxidation, measurement of their spectra required the use of a special technique. A special absorbance cell was devised for the purpose. The apparatus and techniques are described in detail in the experimental section. A series of careful preparations and measurements gave a value of 40.8 for the molar absorbancy coefficient at 550 mμ of the triscysteinatocobaltate(III) complex, hereafter referred to as PTNS.[1]

Exposure of PTNS to gaseous oxygen resulted in the formation of a green product. The oxidation was quite rapid. When a 10^{-2} M solution of PTNS was oxidized with a stream of air and the absorbance measured as a function of time, the absorbance at 585 mμ had reached a constant value by the time of the first reading, in about 6 minutes. The absorbance remained constant for 100 minutes, after which time the experiment was terminated.

The spectrum of this green product, shown in Figure 4, was the same as that obtained in pH 11 buffer from limited oxygen, cobalt(II), and cysteine (22). That this product was identical to the green $K_3[Co(Scy)_3]\cdot 3H_2O$ complex prepared by Schubert was demonstrated by repeating Schubert's preparation and determining its spectrum. At 585 mμ the molar absorbancy coefficient was about 280.

To show that the conversion by oxygen of PTNS to triscysteinatocobaltate(III), hereafter referred to as GTNS,[1] was quantitative, a

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[1] In this type of abbreviation, the first letter signifies the color of the complex (B, brown; G, green; P, purple); the second letter indicates the number of ligands bound per cobalt ion (B, bis; T, tris); and the next two letters indicate the atoms coordinated to the cobalt ion.

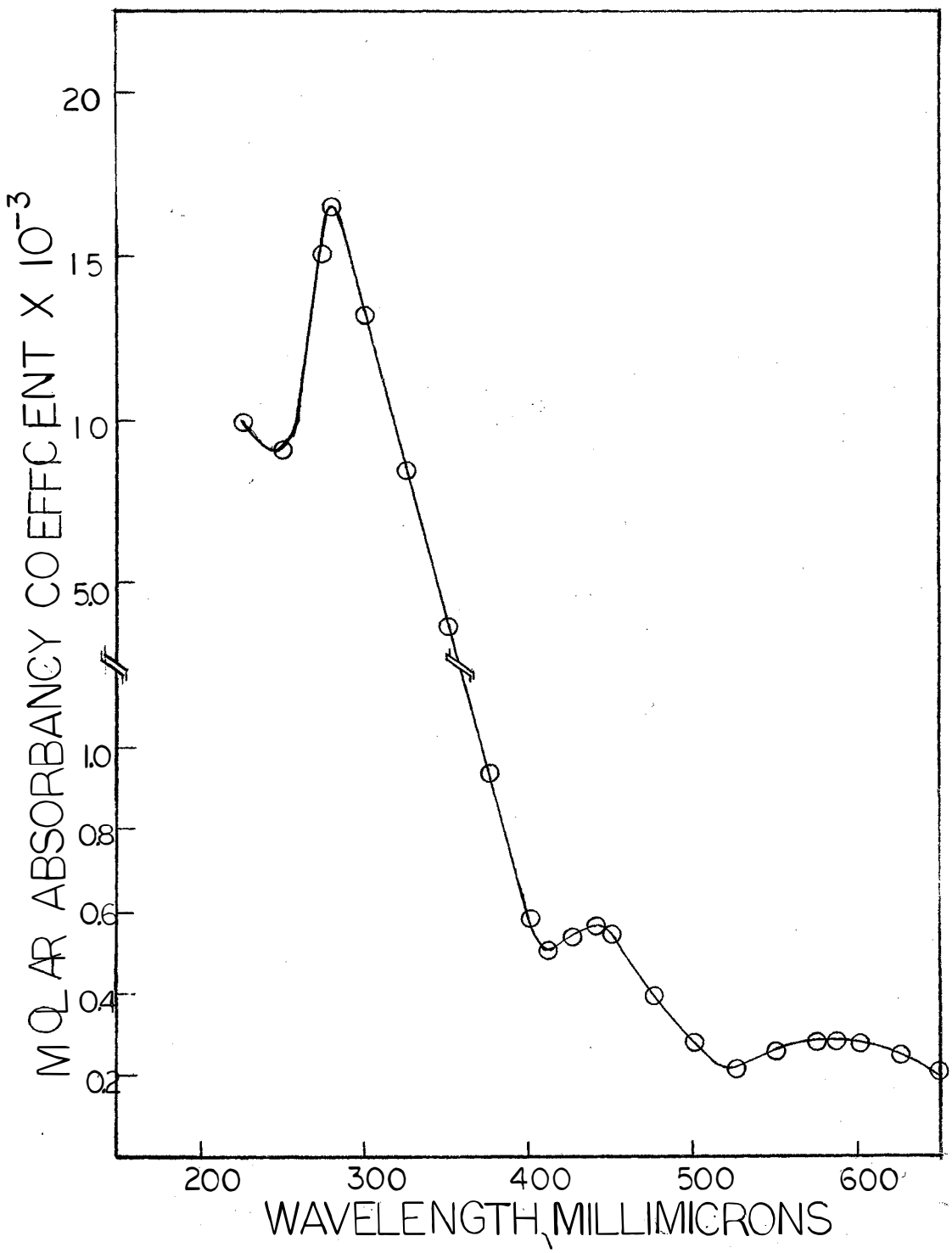


Fig. 4. Spectrum of GTNS.

10^{-2} M solution of PTNS was oxygenated for several minutes and the absorbance measured at 585 mμ. Based on a molar absorbancy coefficient of 280 for GTNS at 585 mμ, the reaction was found to be essentially quantitative.

From these experiments one can conclude that the reaction of oxygen with cobalt(II) and excess cysteine at pH 11 involves a simple oxidation of PTNS to GTNS according to the equation:

$$(1)\quad 4[Co(Scy)_3]^{-4} + O_2 + 2H_2O \longrightarrow 4[Co(Scy)_3]^{-3} + 4OH^{-1}.$$

Since the reaction involves the transfer of four electrons, it indubitably proceeds by way of some intermediate steps, but the nature of these steps cannot be inferred from the evidence now available.

An effort to reduce GTNS to PTNS was made. In basic solution sodium hypophosphite is an excellent reducing agent, having a potential of 1.65 v. Nevertheless, addition of $NaH_2PO_2 \cdot H_2O$ to a solution of GTNS under nitrogen did not effect reduction of the cobalt(III) complex. Thus, as far as can now be determined, reaction (1) is irreversible.

Some effort was directed toward developing an analytical procedure for dissolved oxygen using PTNS as a reagent. This should work quite well since the oxidation of PTNS produces a well-defined product in a quantitative fashion. The results obtained indicated that the method is feasible. In a typical experiment, 0.007 g. of oxygen was found per liter of distilled water. This compares favorably with the accepted value of 0.0068 g./liter under the same conditions (41). Although this method showed promise, it was not pursued further since it holds no advantage over some previously developed analytical procedures (41).

The green complex, GTNS, was fairly stable toward further oxidation. However, upon prolonged exposure to oxygen, the complex reacted

further to give brown-colored products, the spectrum of which is shown in Figure 5, curve A. This brown material is discussed further in Chapter IV.

The next system investigated was that of cobalt, cysteine, and oxygen at pH 7.8. Exposure of cobalt(II)-cysteine mixtures to oxygen at this pH produced a brown-colored product, in accordance with previous reports (12,13,14,15). In a representative experiment, 0.01M cobalt-(II) and 0.03M cysteine were mixed in buffer at pH 7.8 under air-free conditions, and an aliquot portion of the mixture was diluted with 100 volumes of air-containing buffer. The brown color developed quickly, a constant absorbance having been attained by time the first measurement was taken, after seven minutes. The spectrum in the region between 260 and 460 mμ is shown in Figure 5, curve B. This spectrum is very similar to, but not identical with, that of the brown biscysteinatocobaltate(III) complex prepared and isolated by Schubert (16). The molar absorbancy coefficients were 8,400, 5,800, 2,050 at 280, 350, and 442 mμ, respectively.

A series of experiments was then conducted in a similar manner, with cysteine/cobalt ratios varying between 1 and 5; the results are plotted in Figure 6. The data are not very precise, as indicated roughly by the size of the experimental points, but it can be seen clearly that a break occurs at a ratio of about 2. This indicates that the product of the reaction is a bis complex. As five stereoisomeric forms are possible for biscysteinatocobaltate(III) complexes, it seems likely, indeed it should be expected, that the product in question is a mixture containing somewhat different proportions of the possible isomers. This proposition is discussed further in Chapter IV of this thesis.

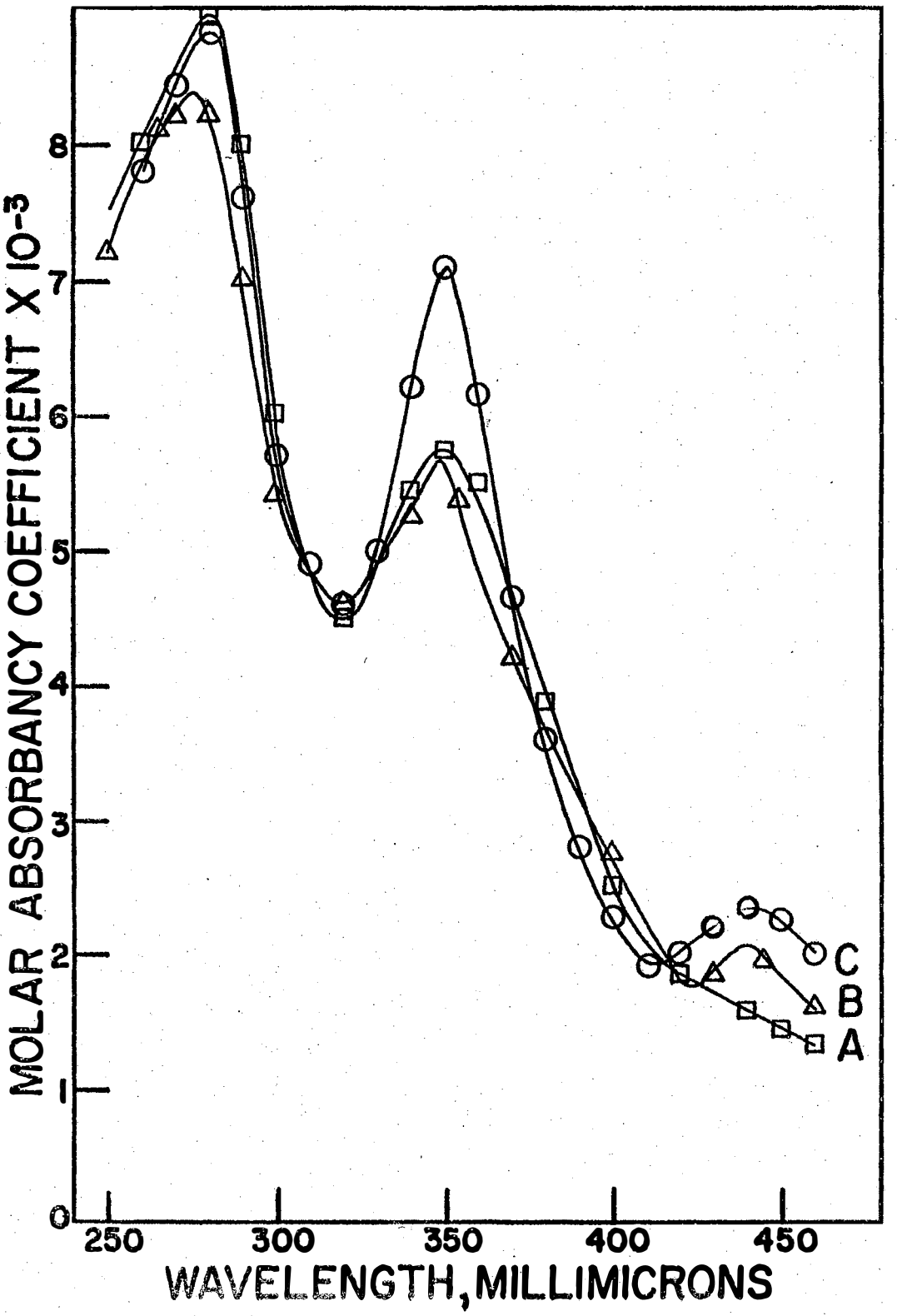


Fig. 5. Spectra of Cobaltic Complexes: A, Brown pH 7.8 Hydrolysis Product of Green $[Co(Scy)_3]^{-3}$; B, Brown pH 7.8 Oxidation Product; C, Brown pH 5.8 Oxidation Product.

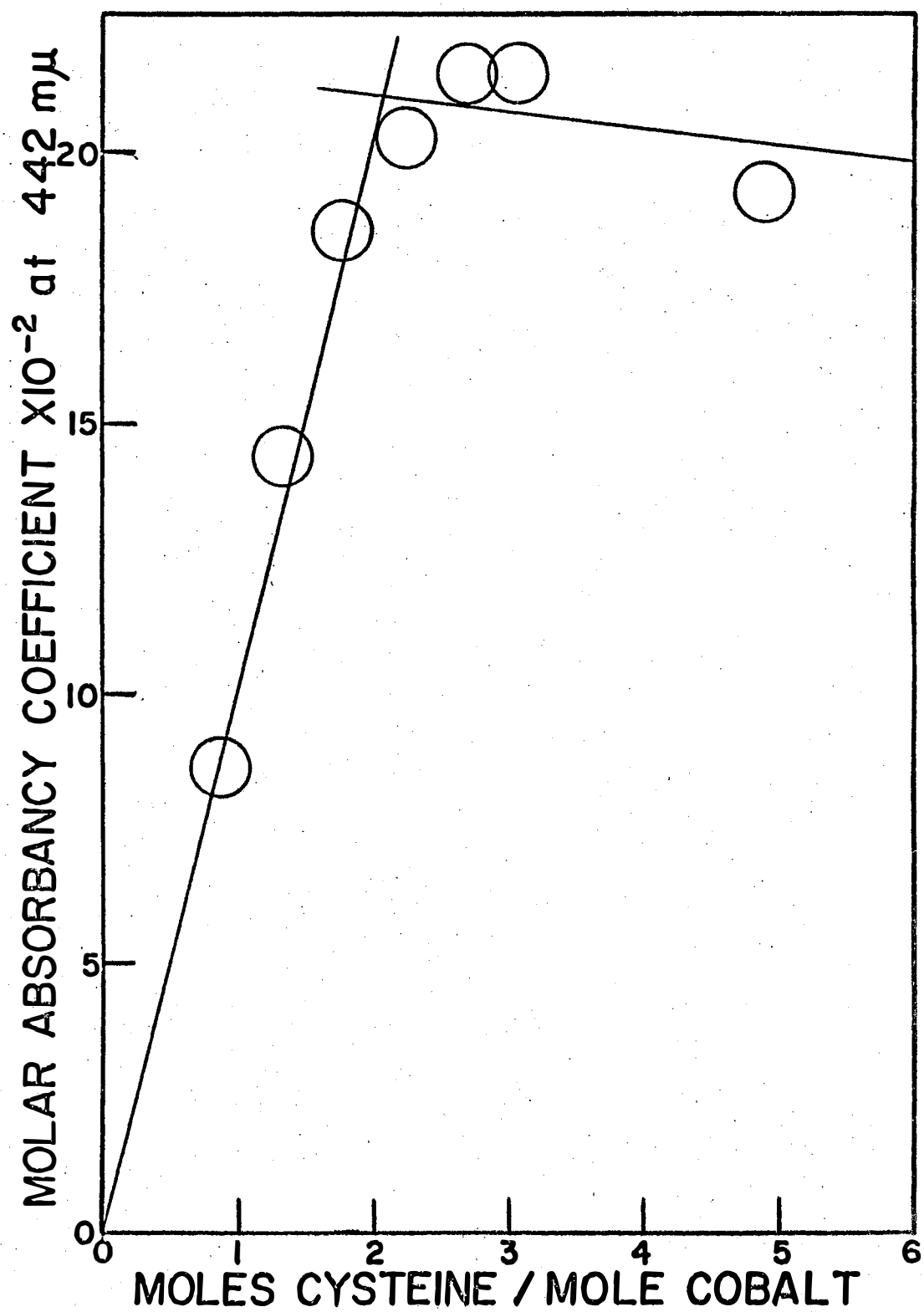


Fig. 6. Relation of Brown Color Development to Cysteine/ Cobalt Ratio at pH 7.8.

It was shown in Figure 3, curves B and C, that complexes are present in anaerobic pH 7.8 and 5.8 mixtures of cobalt and cysteine, and these complexes may be the precursors of the products formed on oxidation. Additional knowledge concerning these cobaltous complexes is desirable. To ascertain the nature of the cobalt(II)-cysteine complexes in the pH range 5-8, the Bjerrum titration technique was employed (25). This technique involves the titration of a mixture of cation and ligand with base and measurement of the pH after each increment of base. These data make possible the estimation of $\bar{n}$, the average number of ligands bound per cation at a given pH, and of the stability constants. A plot of $\bar{n}$ versus the negative logarithm of the free ligand concentration is called the formation curve. Although the Bjerrum method is not inordinately difficult, the associated derivations and calculations are algebraically involved. These details are given in Appendix A.

Selected results of these experiments and brobdingnagian calculations for 10^{-2} M cobalt are given in Table V. In a typical experiment, about three times this many data were gathered.

The $\bar{n}$ values are essentially constant in the pH range 6.5-7.3. This uniformity is generally indicative of binucleate complex formation. To test this point, the experiment was repeated with 10^{-3} M cobalt. If binucleate complexes were indeed formed, the formation curve should vary with metal concentration (42). Shown in Table VI are the results obtained with 10^{-3} M cobalt. Formation constants were not calculated.

As expected with 10^{-3} M cobalt, the $\bar{n}$ values were not constant in the pH region 6.5-7.3. This gives confirmation of the existence of a binucleate complex in 10^{-2} M cobalt in this pH region. It is interesting to consider the structure of this binucleate complex. A possibility

TABLE V

RELATION OF $\bar{n}$ AND pH IN 10^{-2} M COBALT(II) - CYSTEINE SOLUTIONS

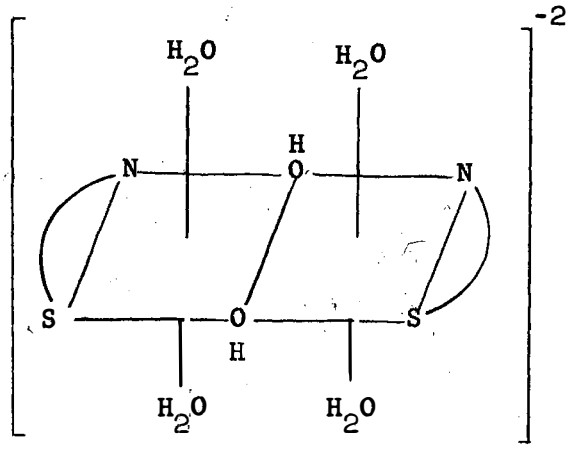
mls. 1.032 M NaOH	pH	-log [Scy^{-2}]	$\bar{n}$
0.45	5.35	9.35	0.23
1.07	5.50	9.10	0.56
2.33	5.95	8.00	1.18
2.73	6.51	7.22	1.37
2.83	6.70	6.85	1.44
2.97	6.92	6.42	1.44
3.01	7.00	5.99	1.44
3.28	7.30	5.38	1.49
3.76	7.56	5.27	1.78
4.45	7.98	4.58	2.00
4.69	8.11	4.39	2.08
5.47	8.60	3.75	2.33
5.95	9.10	3.20	2.47

TABLE VI

RELATION OF $\bar{n}$ AND pH IN 10^{-3} M COBALT(II) - CYSTEINE SOLUTIONS

mls. 1.023 M NaOH	pH	-log [Scy^{-2}]	$\bar{n}$
0.05	6.01	8.97	0.25
0.11	6.18	8.67	0.55
0.21	6.47	8.16	1.20
0.29	7.10	6.98	1.41
0.35	7.42	6.40	1.66
0.41	7.79	5.75	1.84
0.47	8.11	5.24	2.00
0.53	8.41	4.80	2.14
0.57	8.71	4.43	2.21

is:



However, this is pure speculation, and no further evidence supporting this structural assignment is presently available.

The $\bar{n}$ value at pH 7.8 with 10^{-2} M cobalt(II) is of direct pertinence to the course of the oxidation reaction at this pH. Seen in Table V, $\bar{n}$ is very nearly 2. As has already been shown, the brown oxidation product formed at this pH has two molecules of cysteine per cobalt. It is therefore logical to assume that the reaction with oxygen involves the conversion of the cobaltous biscysteinate to the cobaltic biscysteinate.

The next series of experiments was designed to determine the oxygen uptake of cobalt-cysteine mixtures under various conditions. Most of the work was done using a Warburg apparatus; however, a few experiments were done using a specially constructed absorption apparatus. These experiments did not furnish a great deal of useful information. In order to obtain significant volume changes in the gas burette of the apparatus, concentrated solutions (ca. 0.1 M) were required; the

reduction of an equivalent amount of oxygen produced sufficient hydroxyl ions to cause considerable variation of the pH during the course of a reaction. Even 0.3 M phosphate buffer did not suffice to hold the pH constant. Since the mechanism of the oxidation reaction depended critically on pH, the oxygen absorption measurements could not be used to determine reaction stoichiometry. However, the oxygen uptake of 0.1 M cysteine at pH 5.8 in the absence of cobalt was successfully investigated, with the result that cysteine absorbed no oxygen over a period of five hours.

Kendall and Holst reported that the oxidation at pH 7.8 produced varying amounts of cystine. To investigate this, a series of experiments was run, in which the cobalt(II) concentration was made 0.01, 0.001, and 0.0001 M and the cysteine/cobalt ratio kept constant at 2.8. The solutions were robustly buffered to insure constancy of the pH during oxidation. The molar absorbancy (calculated on the basis of the cobalt taken) which developed on exposure to air was 1,950, 2,050, and 1,650, respectively. Moreover, no cystine could be recovered by direct isolation from the most concentrated reaction mixture (up to 30% cysteine could, of course, be converted to cystine without reducing the final yield of complex). Direct isolation of cystine was not attempted at 0.001 and 0.0001 M concentration, but based on the assumption that the cysteine not bound in the brown complex was converted to cystine, the yield of cystine estimated from the absorbancy coefficients is 0 and 40%, respectively.

What Kendall and Holst proposed as a general mechanism appears to be applicable to the reaction only at pH 11; the purple triscysteinatocobaltate(II) complex reacts with oxygen to give the green

triscysteinatocobaltate(III). However, formation of the brown biscysteinatocobaltate(III) complex takes place very rapidly in conditions, such as pH 7.8 and cysteine/cobalt ratio of 2, in which the purple triscysteinatocobaltate(II) complex cannot be present in appreciable amounts. Schubert (18) suggested that the precursor of brown biscysteinatocobaltate(III) is the corresponding cobalt(II) complex. This suggestion is more logical and at least qualitatively consistent with what is known about this complex. The proposed reaction at pH 7.8 is:

$$(2)\quad 4[Co(Scy)_2]^{-2} + O_2 + 2H_2O \longrightarrow 4[Co(Scy)_2]^{-1} + 4OH^{-1}.$$

Since, in carefully controlled conditions, no cystine was produced at pH 7.8, it was deemed necessary to find circumstances where cystine is produced and to investigate the oxidation mechanism under these conditions. Next, it seemed of interest to investigate the reaction at still lower pH values. As shown in Table V, $\bar{n}$ reaches a value of 1 at about pH 5.8, and this pH was chosen for investigation.

Since a 1:1 cobalt(II)-cysteine complex would be uncharged, it was thought that some knowledge of its properties might be gained by extracting it into an organic solvent. A solution of cobalt(II) and cysteine hydrochloride was titrated under nitrogen while being vigorously stirred in the presence of petroleum ether, chloroform, benzene or carbon tetrachloride. No evidence was obtained for the extraction of [Co(Scy)] since the organic layer remained colorless in all cases. On exposing the pH 5.8 reaction mixture to oxygen, a brown complex was formed, with a spectrum similar to, but not identical with, the other brown products so far discussed (Figure 5, curve C). The rate of oxidation was much slower, about 5 hours being required to attain a constant

absorbance in conditions similar to those employed at pH 7.8.

Most important, substantial amounts of cystine were formed. At 0.01 M cobalt(II) and a cysteine/cobalt ratio of 3, the absorbancy coefficient of the product attained a value of 1,940, and an amount of cystine corresponding to 20% of the cysteine originally taken could be isolated from the reaction mixture. At 0.001 and 0.0001 M concentration, direct isolation of the cystine was not attempted, but the molar absorbancy coefficients became constant at values of 1,350 and 550, respectively. The yield of cystine can be estimated from the absorbancy coefficients, if it is again assumed that the cysteine not bound in the brown complex was converted to cystine. Such a calculation indicates the conversion of 55 and 82% of the original cysteine to cystine at 0.001 and 0.0001 M cobalt(II) concentration, respectively.

It will be recalled that, at pH 7.8, no cystine was recovered from experiments with 0.01 M cobalt, and the maximum amount of complex was formed even at 0.001 M cobalt concentration; at 0.0001 M cobalt concentration, however, the amount of complex was diminished, and the percentage of cysteine converted to cystine in the conditions was estimated to be 40%. These estimates must be considered approximate owing to the limited precision of the data and to the way in which they must be used; nevertheless, it can clearly be seen that cystine is produced in varying amounts, depending on the conditions. Low pH and low absolute concentration of reagents favor the formation of cystine.

The occurrence of this reaction can explain the puzzling stoichiometric relationship observed by Neville at pH 8.0 (19). He found in experiments done with cysteine/cobalt ratios of 3 to 8.8, that the consumption of oxygen was 0.75 atom per cobalt ion; he also reported that

"appreciable amounts of crystalline cystine were formed". The results can be taken to indicate that, in the conditions, 0.5 mole of cystine was formed per mole of complex.

The formation of cystine increases as the pH is lowered and the absolute concentration is decreased. Since these conditions favor the simplest complex, it is suggested as a speculation, that the mechanism of cystine formation at pH 5.8 involves the oxidation of [Co(Scy)] to $[Co(Scy)]^{+1}$, followed by disproportionation to cobalt(II) and cystine; thus the catalyst is regenerated. Such a mechanism is consistent with what is known in general about the properties of cobalt(III) ion.

An attempt was made to substantiate this mechanism by preparing a solution of cobaltic sulfate and adding it to a solution of cysteine at pH 5.8. When $Co_2(SO_4)_3$ was added to water, its green color only slowly reverted to the characteristic pink of hydrated cobalt(II). However, when added to a solution of cysteine at pH 5.8, the green color rapidly changed to pink, indicating the oxidation of cysteine and the simultaneous reduction of cobalt(III). This is in accord with the mechanism proposed above.

Experimental

1. Materials.--All reagents were of analytical reagent grade, except as otherwise specified. L-Cysteine hydrochloride monohydrate (B grade) was obtained from the California Corporation for Biochemical Research, Los Angeles 63, California; it was analyzed for -SH content by a ferricyanide oxidation method. In 100 ml. of pH 7.0, 0.1 M phosphate buffer was dissolved a 30-mg. sample of cysteine. A 1.0 ml. aliquot of this solution was mixed with 1.0 ml. of 0.00386 M potassium

ferricyanide and the mixture diluted to 10.0 ml. with buffer. The difference in absorbance at 410 mμ between this solution and a blank composed of 1.0 ml. 0.00386 M potassium ferricyanide and 9.0 ml. of buffer was then measured. The -SH content was calculated from this difference using 990 for the molar absorbancy coefficient of ferricyanide. The cysteine used in this work was 90% pure.

Nitrogen of commercial grade was deoxygenated by passing it through acid vanadous solutions (43). Air-free water was prepared by boiling deionized water for 30 minutes; it was allowed to cool and stored under nitrogen.

2. Apparatus and Special Procedures.--A Beckman Model GS pH meter equipped with glass and calomel electrodes was used for pH measurements. Spectrophotometric measurements were made in 1-cm. cells with a Beckman Model DU spectrophotometer.

A simple anaerobic absorption cell which allowed the determination of solution spectra in the virtual absence of oxygen was constructed in the following way. A narrow-mouthed, glass-stoppered cell was used which, in this work, was the 10-mm. rectangular type (Beckman 2100-10-73); however, a cylindrical cell would also be appropriate. The neck of the cell was fitted snuggly with a cap made by trimming a rubber vaccine bottle stopper (Sargent S-9075); a 6-mm. long cylinder was cut from the open end, and two parallel slices were taken from the sides so the cap was no wider than the cell holder.

Two No. 20 hypodermic needles, bent at right angles about 2.5 cm. from the tip, were inserted through the diaphragm of the stopper, and one of them was connected to an inert gas supply by a short piece of rubber tubing, 3/16 inch in inside diameter, and glass tubing if

necessary. The cell was flushed with gas for 10 minutes, and the solution to be examined was then introduced into the cell with a hypodermic syringe. The syringe was then removed, followed by the outlet needle, and finally the inlet needle; this sequence ensured that a slight positive pressure was maintained in the cell until the last needle was removed and protected against the inward leakage of air. This was done quickly, and the gas flow was adjusted at this stage so that only a slight pressure was built up.

Preparations and measurements to be conducted in the absence of air were executed in a 180 or 300-ml. lipless beaker fitted with a rubber stopper which had holes drilled in it to accommodate glass and calomel electrodes, the tip of a 10-ml. micro-burette, a gas inlet tube, and an exhaust port. The beaker was first flushed with oxygen-free nitrogen, then air-free water and reagents were added as desired, with nitrogen flowing through the vessel at a brisk rate. Samples for spectral determinations were withdrawn and handled as described.

3. Test of Cell.--Using this technique, the cell was tested with PTNS. A dilute solution of PTNS 6.6×10^{-3} M, was prepared and transferred to the cell with a hypodermic syringe. The cell was then allowed to stand at room temperature, and the absorbance was measured at various time intervals. The results are given in Table VII.

PTNS has a molar absorptivity of 24.4 at 445 mμ while its oxidation product, GTNS, has an absorptivity of 514--i.e., complete oxidation would result in a twenty-fold increase in absorbance. Thus, the absorbancy after 30 minutes corresponds to 1% oxidation, or the absorption of 0.05 μ mole of oxygen in that interval. Although the cell is not absolutely air-tight, it serves sufficiently for many purposes, since

most spectra can easily be determined in 30 minutes.

TABLE VII

ABSORBANCY OF PTNS IN THE ANAEROBIC CELL

Time, Minutes	Absorbancy at 445 mμ
0	0.161
5	0.166
10	0.172
15	0.176
20	0.183
25	0.191
30	0.194

4. Observations on the Cobalt(II)-Cysteine System at pH 11-12.--To an air-free solution of cobalt(II) chloride at pH 11-12, cysteine hydrochloride monohydrate in varying amounts was added with stirring. The following results were recorded:

(1) with a cysteine/cobalt ratio of 1, a faint purple solution and a pink precipitate of $Co(OH)_2$ was formed,

(2) with a cysteine/cobalt ratio of 2, the purple color of the solution deepened, but the solution remained turbid due to suspended $Co(OH)_2$, and

(3) with a cysteine/cobalt ratio of 3, the solution turned to a clear, distinct purple, and all $Co(OH)_2$ was dissolved.

5. Preparation and Oxidation of Purple $[Co(Scy)_3]^{-4}$.--The preparation and spectral measurements were done in the absence of air. Cysteine

hydrochloride monohydrate was added to 100 ml. of air-free 10^{-2} M cobalt(II) chloride to give a cysteine cobalt ratio of 4. Air-free 1 M sodium hydroxide was then added to give a pH of 11. At this pH the solution had a purple-violet color. To oxidize $[Co(Scy)_3]^{-4}$, air was passed through the solution and the absorbancy determined at 585 mμ after 1:10 dilution with water. The results of this experiment are shown in Table VIII.

TABLE VIII

OXIDATION OF PTNS AS A FUNCTION OF TIME

Time of Oxidation in Minutes	Absorbancy
6.25	0.301
14.50	0.295
22.10	0.298
50.55	0.302
97.10	0.300

From these data one can show that the conversion of PTNS to GTNS is quantitative. Based on 280 as the molar absorbancy coefficient for GTNS at 585 mμ, the concentration of GTNS after the oxidation was calculated to be 0.0107 M, which is essentially the same as the concentration of PTNS before oxidation.

6. Preparation of a Cobalt(II)-2-Aminoethanethiol Complex at pH 11-12.--To a 10^{-2} M solution of $CoCl_2$ was added 2-amino-ethanethiol and base to give ligand/cobalt ratio of 3.5 and a pH of 11-12. A deep blue complex which showed spectral properties very similar to $[Co(Scy)_3]^{-4}$

quickly formed. A maximum absorbance was shown at 560 mμ with a molar absorbancy coefficient of about 45.

7. Attempted Reduction of GTNS to PTNS.--To a basic, anaerobic solution of approximately 10^{-2} M $[Co(Scy)_3]^{-3}$ was added small portions of NaH_2PO_2. After each addition the solution was vigorously stirred. No evidence for the reduction of GTNS was observed after adding an excess of NaH_2PO_2 and letting the solution stand for some time.

8. Rate of Color Formation Upon Oxidation of Cobalt-Cysteine Mixtures.--One ml. of air-free 1.00 M cobalt(II) chloride was mixed with 100 ml. of air-free phosphate buffer, pH 7.8. Air-free 1 M cysteine hydrochloride was then added to give the desired cysteine/cobalt ratio, and air-free 1.0 M sodium hydroxide was added to restore the pH to 7.8. The spectrum of an aliquot portion of this solution was then determined without dilution. All of these operations were conducted in the absence of air. One ml. of the solution was then added to 100 ml. of air-saturated phosphate buffer and the absorbance at 442 mμ determined as a function of time. The oxidation was complete before the first measurement was taken, after seven minutes.

A similar experiment was done in phthalate buffer at pH 5.8; in this case, the absorbance became constant only after several hours.

9. Cysteine/Cobalt Ratio and Brown Complex Formation.--Six two-ounce polyethylene bottles were fitted with stoppers having a nitrogen inlet and exhaust port. The bottles were deaerated by passing nitrogen through them for several minutes. One ml. of air-free 0.2500 M cobalt chloride, air-free 0.116 M cysteine and pH 7.8, 1 M phosphate buffer were mixed in the bottles to give the desired cysteine/cobalt ratio and a total volume of 25 ml. The cysteine solution was prepared by

dissolving 2.1954 g. of cysteine hydrochloride monohydrate in 50 ml. of pH 7.8 buffer, adding 10 M NaOH to adjust the pH back to 7.8, and diluting the resulting solution to 100 ml. with buffer. During the addition of reagent, nitrogen was passed rapidly through the containers. The solutions were then carefully shaken for several minutes. Then the stoppers were removed, 1 ml. aliquots withdrawn and transferred to 100 ml. volumetric flasks. These aliquots were allowed to stand exposed to the air for 45 minutes, then diluted to 100 ml. with water, and the absorbance was measured at 442 mμ.

10. Bjerrum Titration of Cobalt(II)-Cysteine Systems.--To 89.0 ml. of air-free distilled water in the titration vessel mentioned above was added 1.0 ml. of air-free 1.003 M cobaltous chloride and 10.0 ml. of air-free 0.417 M cysteine hydrochloride. This gave a cysteine/cobalt ratio of 4. The addition of these reagents was made with nitrogen rapidly passing through the solution. The mixture was then titrated with air and carbon dioxide-free 1.032 M sodium hydroxide, and, after each increment of base, the pH was accurately measured. In a typical experiment, 10 ml. of base was added in about 60 increments; this gave pH changes of a few tenths of a unit. During the experiment oxygen was rigorously excluded.

A similar experiment was conducted with 10^{-3} M cobalt(II), 0.0417 M cysteine hydrochloride, and 1.003 M sodium hydroxide. In this case, 1.28 ml. of base was added in about twenty increments. In both experiments, the solution was rapidly stirred with a magnetic stirrer and Teflon-covered stirring bar.

11. Oxygen Uptake of Cysteine Solutions.--The rate of oxygen uptake of cysteine and cobalt(II)-cysteine solutions at pH 5.8 was

determined with a constant pressure apparatus consisting of a thermostated two-compartment flask connected to a gas burette. In the first experiment, 1.0 ml. of approximately 0.1 M cysteine in pH 5.9 buffer was placed in one compartment of the flask and the rate of oxygen uptake measured with the gas burette; no appreciable amount of oxygen was absorbed over a period of five hours. The second experiment was conducted in the same fashion except that 1.0 ml. of a 0.1 M solution of cobalt(II) chloride was placed in the other compartment of the flask; to initiate the experiment, the cobalt was poured into the cysteine-containing compartment. In this case, oxygen was quite rapidly absorbed by the solution. In both experiments, the solution was vigorously stirred with a magnetic stirrer and Teflon-covered stirring bar.

12. Attempted Extraction of [Co(Scy)].--To an approximately 10^{-2} M solution of cobaltous chloride was added enough cysteine hydrochloride to give a cysteine/cobalt ratio of 4. The initial pH was 2-3. About 50 ml. of an organic solvent, either petroleum ether, benzene, chloroform, or carbon tetrachloride was then added, and the solution was carefully deaerated with nitrogen for 30 minutes. To the air-free solution was added air-free 1.0 N sodium hydroxide to give the desired pH of 5.8. The mixture was then vigorously stirred to mix the aqueous and organic layers. In no case was any of the yellow-green complex present in the aqueous solution extracted by the organic solvent.

13. Cystine Formation.--To 100 ml. of air-free pH 5.9 buffer was added 1.0 ml. of 1.00 M cobalt(II) chloride and enough cysteine hydrochloride monohydrate to make the cysteine/cobalt ratio 3. The solution was then oxygenated by blowing a stream of air through it; 1.0 ml. aliquots were withdrawn at various times, diluted with water, and the

absorbance was measured at 442 mμ. After the absorbance reached a constant or nearly constant value, the solution was filtered under suction through a weighed, 2.5-ml., medium-porosity, fritted-glass filter crucible; the crystalline cystine obtained was washed with a small amount of water and dried. The amount of cystine was then determined by weighing the crucible; as a check, the cystine was dissolved with dilute HCl and the crucible again dried and weighed.

A similar experiment was conducted at pH 7.8 using the same procedure as above. At both pH 5.8 and 7.8, cystine is quite insoluble (44) and no correction for solubility was made.

14. Reaction of Co(III) and Cysteine at pH 5.8.--Cobaltic sulfate was prepared according to the directions given by Palmer (31). The preparation was carried out using a Sargent-Slomin Electroanalytical Analyzer and platinum electrodes. To 12 g. of powdered $Co(SO_4)\cdot 6H_2O$ in a Berzelius beaker was added 100 ml. of 10 N H_2SO_4. The cobalt salt was then dissolved by rapidly stirring with the rotating anode of the Analyzer. After all of the cobalt(II) sulfate was dissolved, the Berzelius beaker was surrounded with an ice bath, and the solution was cooled to and kept at 10° C. throughout the preparation. The oxidation of Co^{+2} to Co^{+3} was then carried out electrolytically.

The color of the electrolyte changed from pink through violet to a greenish-blue. Although it is possible by continued oxidation to obtain crystals of $Co_2(SO_4)_3$, in this case the oxidation was stopped at the point where the solution was greenish-blue in color.

Small aliquots of this solution were then added to water and to a pH 5.8 buffered 0.1 M cysteine solution. When added to water, the greenish-blue color was only slowly discharged; however, when added to

the cysteine solution, the color immediately turned from greenish-blue to the characteristic pink of hydrated Co(II). This indicated a rapid reduction of Co^{+3} by cysteine.

CHAPTER IV

ISOMERISM OF THE BISCYSTEINATO-N,S-COBALTATE(III) COMPLEXES

Results and Discussion

Schubert (16,17) prepared and isolated in the solid state both the biscysteinato-N,S-cobaltate(III) and triscysteinato-N,S-cobaltate(III) ions, the former as a brown insoluble acid, $H[Co(Scy)_2(H_2O)_2]\cdot 3H_2O$ (BBNS), and the latter as a green potassium salt, $K_3[Co(Scy)_3]\cdot 3H_2O$ (GTNS, see Chapter III). The brown insoluble acid was obtained by reacting a mixture of cobalt(II) and cysteine with oxygen at pH 7-9, followed by precipitation with a mineral acid. The green complex was formed from cobalt(II), excess cysteine, and oxygen at pH values above 10. Schubert reported that the potassium salt was stable; however, the product obtained by Spessard (11), following Schubert's directions, was blue-gray in color and unstable. In these complexes chelation involves both nitrogen and sulfur.

Schubert (17) observed that solutions of GTNS were decomposed by lowering the pH below 10, and he presumed that the brown product formed was BBNS. Spessard (11) investigated this problem further and found that the brown decomposition product was similar, but not identical, to the brown product formed directly from cobalt(II), cysteine, and oxygen at pH 8-9. His conclusions were based mainly on ultraviolet and visible spectral evidence; however, he also performed ion-exchange chromatography on the two products and obtained different elution patterns. Spessard

(11) demonstrated that GTNS solutions were stabilized by the addition of cysteine, especially at high pH values. This fact was verified in the work for this thesis.

There are five possible stereoisomeric forms for biscysteinatocobaltate(III) complexes, with the structures represented schematically in Figure 7. The reaction of GTNS as the pH is lowered might be represented by the equation:

$$(1)\ [Co(Scy)_3]^{-3} + H^{+1} \rightleftharpoons [Co(Scy)_2]^{-1} + HScy^{-1}.$$

The product of this reaction would necessarily be a cis-diaquo isomer, and it seemed likely that the slight differences between this product and BBNS might be due to the fact that the latter complex is a trans-diaquo isomer. The decomposition of GTNS by acid stands in marked contrast to the stability of the biscysteinatocobaltate(III) complexes, which can be dissolved in concentrated hydrochloric or sulfuric acid with little change in spectrum. The binding constant for the third ligand must be many orders of magnitude smaller than the constants for the first two.

It is a well-established fact that bidentate ligands such as cysteine do not span trans positions. Thus, the rate of reaction of BBNS and the decomposition product of GTNS with cysteine might give an indication of their geometrical configuration. The reaction was carried out in the pH range 10-11. At 585 mμ, BBNS and GTNS have molar absorbancy coefficients of 750 and 280, respectively; thus, the reactions can be easily followed by measuring the decrease in absorbance at this wavelength. Cysteine and sodium hydroxide were added to a 0.1M solution of BBNS to give a cysteine/BBNS mole ratio of 2 and a pH of 10.5; the

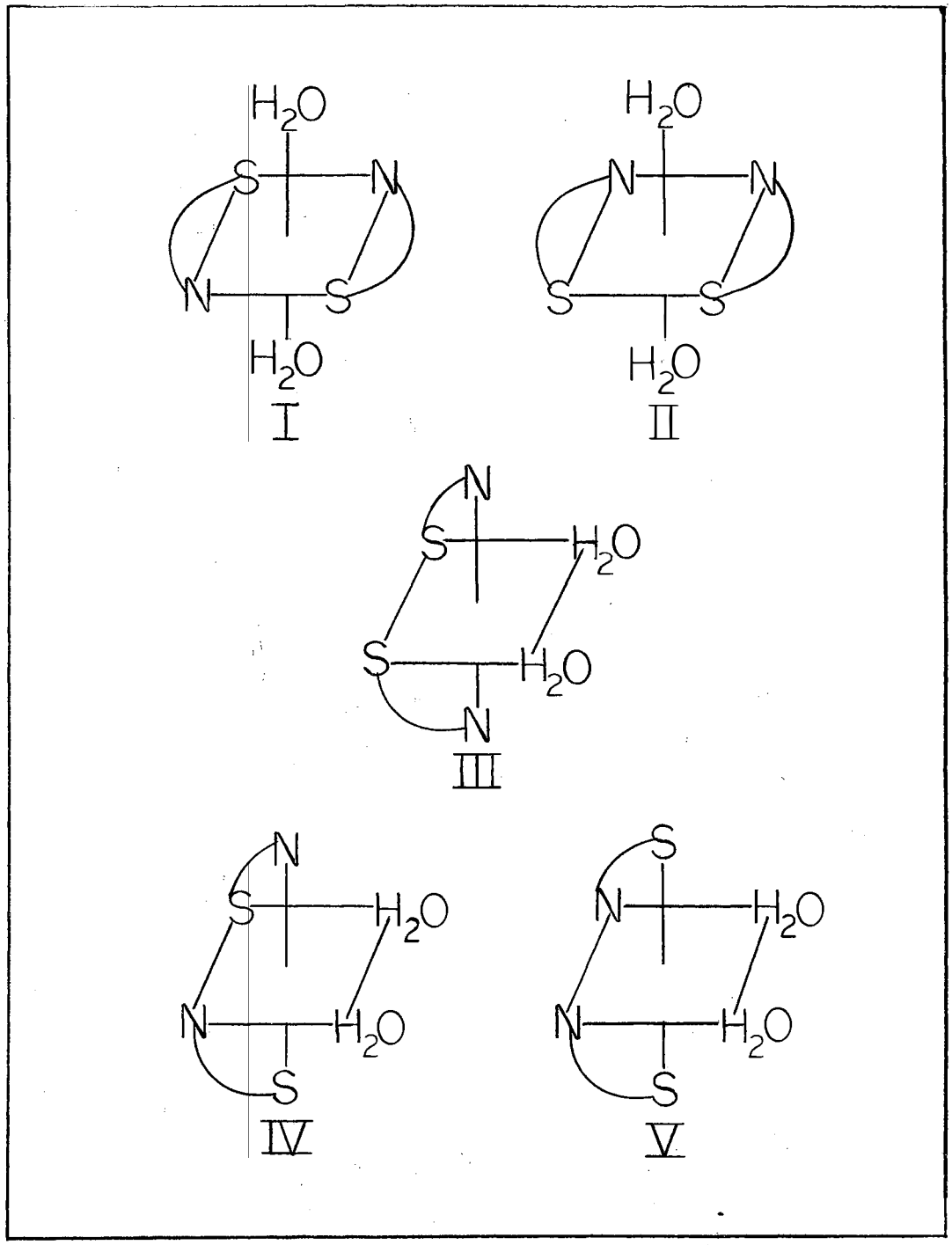


Fig. 7. Isomers of the Biscysteinato-N,S-cobaltate(III) Ion.

absorbance of the resulting solution was measured as a function of time. As shown in Figure 8, about 55 minutes were required for the reaction to reach completion. For BBNS which had been in a strongly acid environment for 4 hours, the rate of reaction with cysteine was faster, about 30 minutes being required for complete conversion to GTNS. Due to the lack of knowledge concerning the exact nature of BBNS, this difference in reaction rate cannot be precisely explained.

In a parallel experiment, GTNS was decomposed by adding an equimolar amount of hydrochloric acid and letting the solution stand for 2 minutes. Base was then added until pH 11 was reached; no cysteine was added since that formed in the decomposition of GTNS should still be present. As seen in Figure 8, the GTNS was completely regenerated in 5-10 minutes.

These results can be easily rationalized. Since cysteine cannot span trans positions, the initial product formed from GTNS is a cis-diaquo isomer (structures III, IV, V) which reacts quite readily to re-form GTNS. On the other hand, BBNS reacts more slowly, and this is interpreted to mean that it is a trans-diaquo isomer which must undergo a relatively slow, rate-determining rearrangement before a cysteine molecule can add to form GTNS. Although these experiments established the relative positions of the water molecules in the biscysteinato-cobalt(III) complexes, they do not suffice to distinguish between structures I and II or III, IV and V.

In order to investigate the interconversion of the cis and trans isomers, GTNS was again decomposed with an equimolar amount of acid, and the solutions were allowed to stand 1.5 and 4 hours before raising the pH to 10-11. Also, cysteine was added to replace that lost by oxidation.

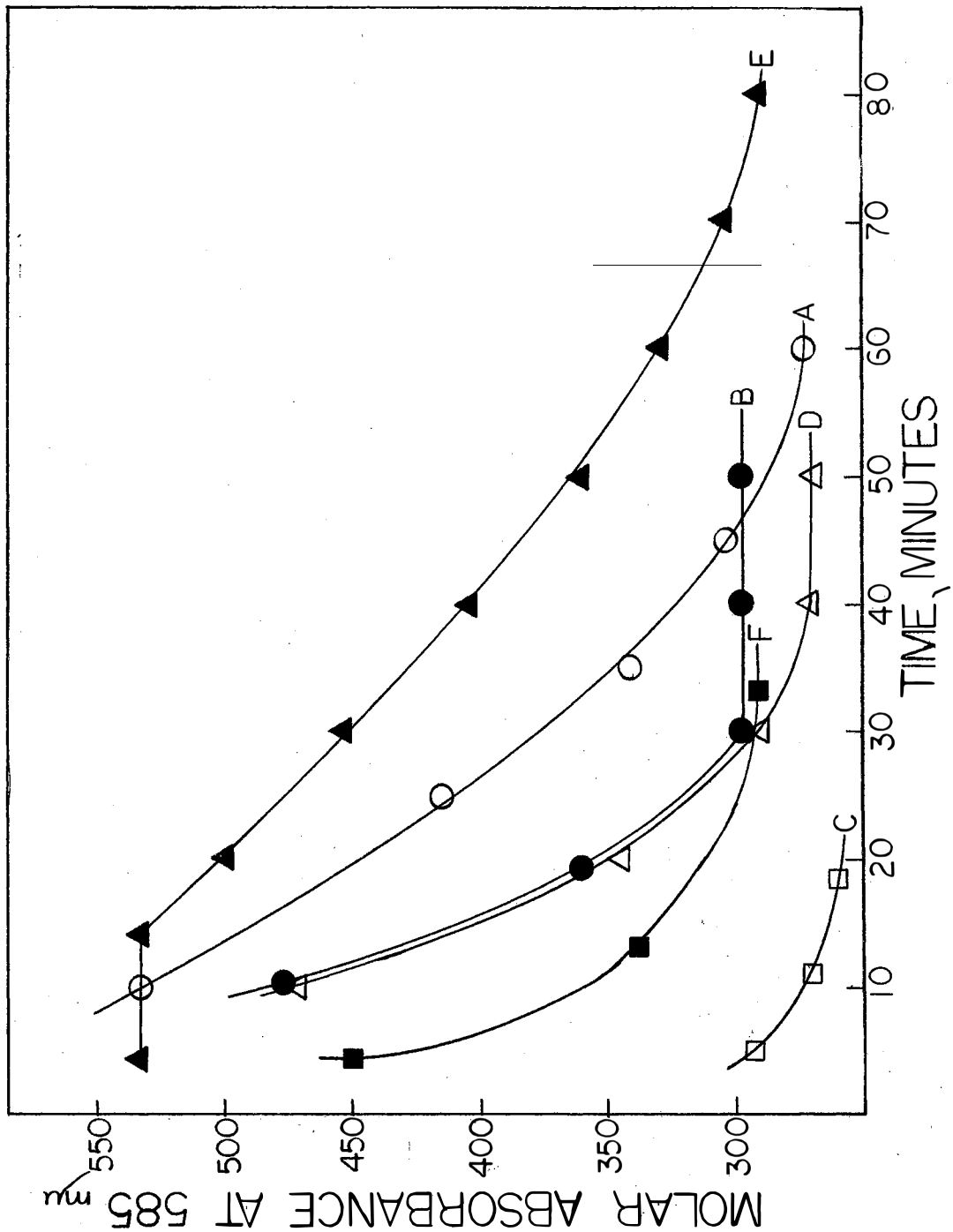


Fig. 8. .Reaction of Cysteine with: A, BBNS; B, BBNS after Acid Treatment for 4 Hours; and with GTNS after Acid Treatment for: C, 2 Minutes; D, 1.5 Hours; E, 4 Hours; F, 2 Minutes and then 2 Hours at pH 8.

The results are shown in Figure 8. The solution which was acidified for 1.5 hours required 30 minutes to form GTNS; the rate of conversion is intermediate between that of trans-BBNS and cis-BBNS. Thus, some of the cis isomer had undergone a rearrangement to the trans isomer during the period of acidification. In the case of the solution that was acid for 4 hours, 80-90 minutes were required for the brown complex to revert to the green tris complex. At first it was thought that the only reaction occurring in the highly acid conditions was a conversion of the cis to the trans isomer; however, since this solution reacted with cysteine even slower than BBNS, the situation must be more complex. Another reaction possibly takes place, converting the cis isomer to a binucleate complex, which does not react readily with cysteine. It was thought that the rearrangement of the cis isomer might be halted at pH 8. Accordingly, GTNS was acidified for 2 minutes, then the pH was raised to 8, and the solution was allowed to stand 2 hours. As shown in Figure 8, the cis complex still rearranged, albeit much slower than in highly acid conditions.

These results made it clear that it would be difficult to separate the isomers shown in Figure 7 by ion-exchange chromatography, since cis complexes would rearrange in the course of the separation. Nevertheless, some ion-exchange work was done. Spessard (11) reported that the elution of BBNS produced a small amount of brown material which was easily removed from an anion exchange resin with 0.1M chloride; he also obtained several other fractions, all of which were brown. Spessard's work was repeated using gradient elution techniques. Shown in Figure 9 is the elution pattern obtained upon chromatographing the solution obtained from cobalt(II), cysteine, and oxygen at pH 7.6-8.0. A product which

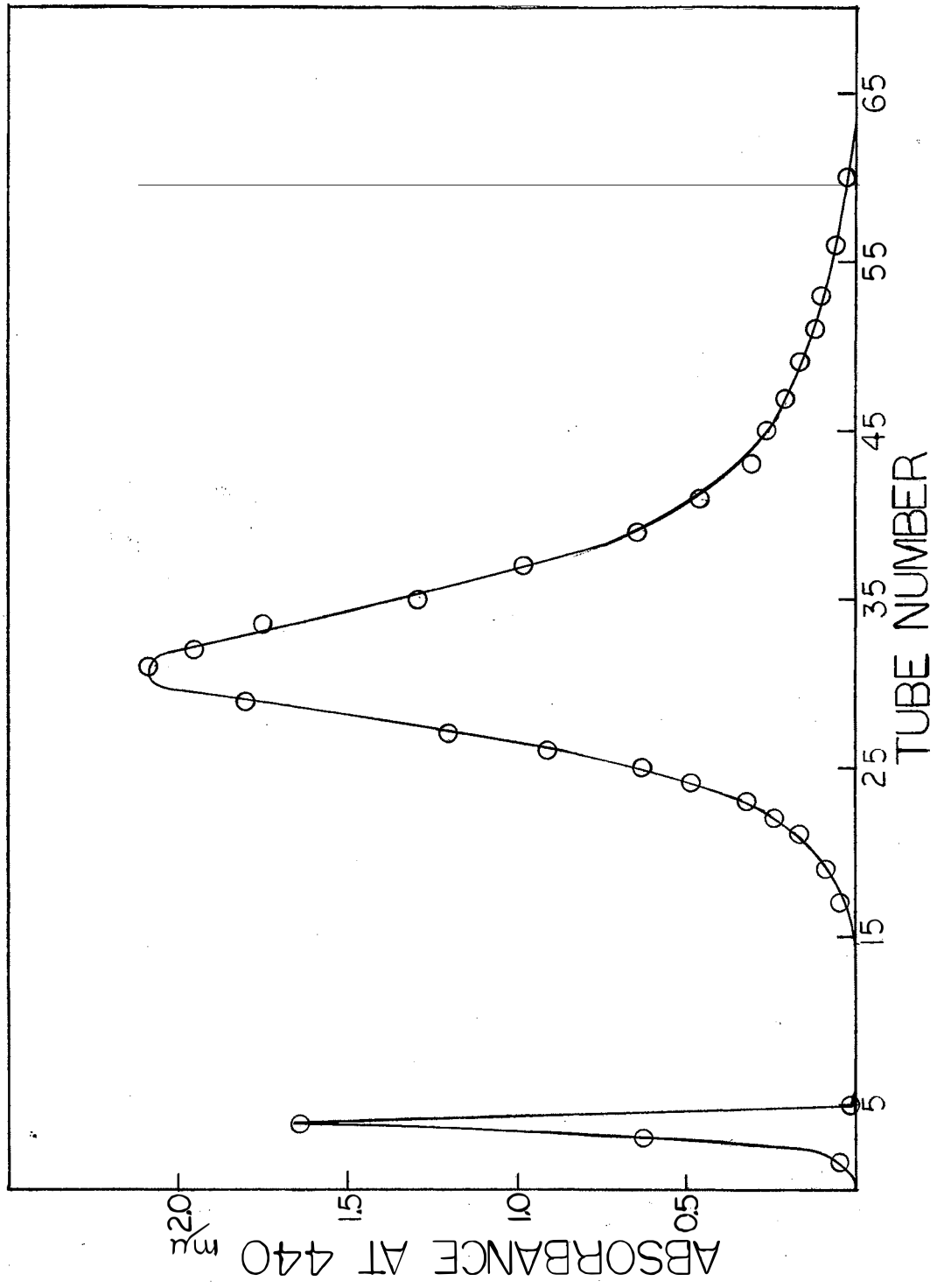
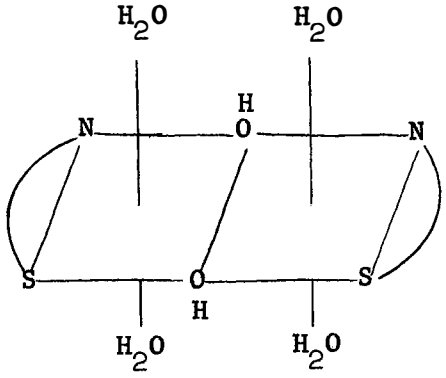


Fig. 9. Elution Pattern of the Solution Obtained from Cobalt(II), Cysteine, and Oxygen at pH 7.6-8.0.

showed absorption maxima at 270, 350, and 440 mμ was easily eluted from the column by the first few ml. of eluent. Since the complex showed no affinity for the resin, it must not be anionic. Based on the assumption that both the products eluted have the same molar absorbancy, it can be shown that the first product eluted comprised 7-9% of the total. The complex also was eluted from a cation exchange resin with water. The complex is not charged, and the following structure is proposed:



Similar ammonia complexes are known (46).

It was thought that the hydroxo bridges might be broken by acid hydrolysis. However, no evidence for or against this could easily be obtained, since the spectra of the binucleate complex and its hydrolysis product(s) are probably very nearly the same. In contrast to BBNS, this uncharged product did not react with cysteine at pH 11, but this evidence alone does not suffice for a definite structural assignment.

The second peak in the elution curve is due to the trans biscysteinatocobaltato(III) complex. The material appeared to be homogeneous, and only one component was eluted; thus, the ion-exchange system used in this work did not serve to distinguish between structures I and II. BBNS was prepared by acidifying the original solution obtained from cobalt(II), cysteine, and oxygen, filtering the precipitate and thoroughly washing with water. When the complex prepared in this fashion was

dissolved in base at pH 8 and chromatographed, none of the above-mentioned, easily-eluted material was obtained; thus, the binucleate complex was not precipitated by acid, and by using careful techniques, pure BBNS was obtained. The filtrate obtained was also chromatographed, and, in this case, a relatively large amount of the binucleate complex was eluted, in addition to a small amount of BBNS.

Experimental

1. Preparation of BBNS.--To 5 g. (2.57×10^{-2} moles) of cysteine hydrochloride monohydrate in 50 ml. of air-free water was added 12.5 ml. of 1.03M cobalt(II) chloride (1.28×10^{-3} moles). This solution was thoroughly stirred, deaerated with nitrogen and adjusted to pH 7.8 with 7 ml. of 10N sodium hydroxide. Air then was bubbled through the solution for 45 minutes. During the aeration, the pH was maintained in the range 7.5-8.0 by the addition of hydrochloric acid as needed. After aeration, the brown solution was allowed to stand for 2 hours, after which time 12.5 ml. of 1.0N hydrochloric acid (1.25×10^{-2} moles) was added to precipitate the complex; the final pH was about 2. The solution and precipitate was stirred 15 minutes, and the precipitate was collected by filtration, washed with water, alcohol, and ether. The yield was 80%.

The exact color of the complex seemed to vary with the conditions of preparation. In some cases, an almost black product was obtained, while at other times, a distinctly brown product was formed. It has been assumed that BBNS is stable in the solid state; however, upon standing for several weeks, the BBNS prepared in this work developed a distinct odor of hydrogen sulfide.

2. Preparation of GTNS.--The method used was essentially that described by Schubert (17). In 10.4 ml. of 1M cobaltous chloride was dissolved 5.6 g. of cysteine hydrochloride monohydrate. The solution was cooled in an ice bath to 5°C. and then was added to 26 ml. of cold, 5.1M potassium hydroxide. The cold mixture was aerated for 2.5 hours. Then 40 ml. of ethanol were added slowly, and the mixture was allowed to stand for 45 minutes. After filtering the mixture through a Büchner funnel and sucking the precipitate dry as possible, the precipitate was dissolved in 30 ml. of water, and the resulting solution was filtered. To the filtrate was added 30 ml. of ethanol. The green precipitate that formed was collected in a Büchner funnel and washed with alcohol and ether. The complex was air-dried, and the yield based on the cobalt taken was 82%.

When Schubert's directions were very carefully followed, especially with regard to the amount of alcohol added in the precipitation steps, a green potassium salt was obtained. If a greater amount of alcohol were added, slightly more precipitate was obtained, but the color of the product was gray instead of green. Upon standing, GTNS decomposed to a gray-brown powder, and an odor characteristic of organic amines developed.

3. Reaction of BBNS and the Decomposition Products of GTNS with Cysteine at pH 10-11.--BBNS, 0.388 g. (1×10^{-3} moles), was dissolved in 10 ml. of 0.1N sodium hydroxide. A 1.0 ml. aliquot was withdrawn and diluted to 50.0 ml. for absorbancy measurements. The molar absorbancy was 750 at 585 mμ. To the remaining 9.0 ml. of BBNS was added 2 ml. of 1.0N sodium hydroxide followed by 0.218 g. (2×10^{-3} moles) of cysteine. An additional ml. of 1.0N sodium hydroxide was required to

raise the pH to 10.5. The absorbance of the solution, after a 1:50 dilution with water, was measured as a function of time. Another experiment with BBNS was carried out in a similar manner, except that the BBNS was dissolved in 10 ml. of 0.1N hydrochloric acid for 4 hours before cysteine and base were added.

GTNS was decomposed by adding 0.587 g. (1×10^{-3} moles) to 10 ml. of water; to this solution was added 1.0 ml. of 1.0N hydrochloric acid, and the solution was then allowed to stand for an appropriate length of time (2 minutes, 1.5 or 4 hours). After this time, 3.0 ml. of 1.0M sodium hydroxide and 0.218 g. (2×10^{-3} moles) of cysteine were added. The pH was 10.3. The absorbancy of the solution was measured as a function of time, after a 1:50 dilution.

Essentially the same technique was used to determine the rate of conversion of the cis to trans isomers at pH 8. In this case, the GTNS was decomposed with acid for 2 minutes and then readjusted to pH 8 with 0.23 ml. of 1.0M sodium hydroxide. After standing for 2 hours, 2.77 ml. of 1.0M sodium hydroxide was added, followed by 0.218 g. (2×10^{-3} moles) of cysteine. The pH was 10. Absorbancy was again measured as a function of time.

4. Ion-Exchange Experiments.--An ion-exchange column was constructed from a 50 cm. length of 19 mm. o.d. glass tubing. At the bottom of the column was an extra-coarse fritted disc and a Teflon stopcock.

The resin was prepared by adding Dowex 1 Anion Exchange Resin (200-400 mesh, chloride form) to a beaker of water, stirring, allowing the resin to settle and decanting any suspended material. The resin was washed into the column with water and then back-washed by attaching

a 50 cm. length of equal-diameter glass tubing to the top of the column and running distilled water in at the bottom. The resin bed thus was expanded to about twice its original volume, and all of the resin beads were set into vigorous motion. The resin was allowed to settle, and the back washing was repeated once again. A layer of glass wool was installed on top of the resin bed to prevent agitation of the beads by incoming liquids, which entered through a 0.5 mm. capillary tube held snugly in place by a rubber stopper.

The column was connected by Tygon tubing to a gradient-elution apparatus. This consisted of two, 250 ml. separatory funnels connected by a Tygon tube running from the outlet of one funnel (solution chamber) to a 15 cm. length of 0.5 mm. capillary tubing, which was inserted through a rubber stopper into the mouth of the second funnel (mixing chamber). The solution chamber was mounted about 12 inches above the mixing chamber and about 50 inches above the outlet of the column. A magnetic stirrer was mounted to stir the contents of the mixing chamber.

In a typical experiment, 200 ml. of 1.0M sodium chloride was placed in the solution chamber, and 200 ml. of water was placed in the mixing chamber. The sample then was put on the column, and all stoppers were snugly seated. The run was initiated by opening the stopcocks on the two funnels and the column. If all the stoppers were air-tight, the sodium chloride solution ran into the mixing chamber at nearly the same rate as the solution flowing through the column. In this way, a gradual and continuous increase in eluent concentration was obtained.

Forty-drop (3.2 ml.) fractions were collected in 13 x 100 mm. test tubes which were automatically changed by a fraction collector (Packard Instrument Co., La Grange, Ill.). The rate of elution was 1 drop/15 sec.,

and a typical run required 8-10 hours.

To investigate the products formed at pH 8 from cysteine, cobalt-(II) and oxygen, 0.3 ml. of the solution obtained before the addition of the acid as described in section 1 was placed on the column and eluted. Quite quickly two brown bands separated, the first moving quite rapidly down the column. All of this material was collected in the third and fourth fractions. The second band moved more slowly down the column and started coming off when the eluent was 0.1-0.2M. The final eluent concentration was 0.6M. The absorbancy of the fractions was measured with a Beckman model DU Spectrophotometer and 10 mm. pyrex cells. All other ion-exchange experiments were conducted in a similar manner. Before each run, the column was washed with 100 ml. of distilled water.

The area under the elution curves was determined with an Ott Compensating Polar Planimeter (Fredrick Post Co., Chicago, Ill.).

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APPENDIX A

DERIVATION OF THE EQUATIONS NECESSARY TO DETERMINE $\bar{n}$ AS A FUNCTION OF pH

The quantity $\bar{n}$ is defined as the average number of donor groups bound per metal ion present, and experimentally, a method is used whereby the ligand concentration [A] is directly determined or calculated from the data (24). The value of $\bar{n}$ at a particular pH may be determined by the use of the equations derived below.

The following is for the particular case of cobalt and cysteine and the following abbreviations are used:

$[Scy]_t$ = total concentration of cysteine,

$[Co^{+2}]_t$ = total concentration of cobalt,

$[Scy^{-2}]$ = concentration of free ligand and

$[Co(Scy)_n^{(2-2n)}]$ = concentration of a particular complex.

It is evident that

$$(1)\quad [Scy]_t = [Scy^{-2}] + [HScy^{-1}] + [H_2Scy] + [H_3Scy^{+1}] + [Co(Scy)] + 2[Co(Scy)_2^{-2}] + 3[Co(Scy)_3^{-4}].$$

By definition,

$$(2)\quad \bar{n} = \frac{[Co(Scy)] + 2[Co(Scy)_2^{-2}] + 3[Co(Scy)_3^{-4}]}{[Co^{+2}]_t}$$

and

$$(3)\quad [Co^{+2}]_t\,\bar{n} = [Co(Scy)] + 2[Co(Scy)_2^{-2}] + 3[Co(Scy)_3^{-4}].$$

Now from (1) and (3),

$$(4)\quad [Scy]_t = [Scy^{-2}] + [HScy^{-1}] + [H_2Scy] + [H_3Scy^{+1}] + [Co^{+2}]_t\bar{n}.$$

By definition the following equations are true:

$$(5)\quad K_1' = \frac{[H^{+1}]\,[H_2Scy]}{[H_3Scy^{+1}]},$$

$$(6)\quad K_2' = \frac{[H^{+1}]\,[HScy^{-1}]}{[H_2Scy]}, \text{ and}$$

$$(7)\quad K_3' = \frac{[H^{+1}]\,[Scy^{-2}]}{[HScy^{-1}]}.$$

These K' values are the reciprocals of the usual ionization constants, and in this work, they were taken from Albert (23). From equations (5), (6), and (7) it can be shown that:

$$(8)\quad [H_3Scy^{+1}] = \frac{[H^{+1}]^3\,[Scy^{-2}]}{K_1'\,K_2'\,K_3'},$$

$$(9)\quad [H_2Scy] = \frac{[H^{+1}]^2\,[Scy^{-2}]}{K_2'\,K_3'}, \text{ and}$$

$$(10)\quad [HScy^{-1}] = \frac{[H^{+1}]\,[Scy^{-2}]}{K_3'}.$$

Now combining (4), (8), (9), and (10) and rearranging gives:

$$(11)\quad \bar{n} = \frac{[Scy]_t - [Scy^{-2}]\left\{1 + \frac{[H^{+1}]}{K_3'} + \frac{[H^{+1}]^2}{K_2'\,K_3'} + \frac{[H^{+1}]^3}{K_1'K_2'K_3'}\right\}}{[Co^{+2}]_t}.$$

All quantities in this equation are obtainable from experimental data. If $[Scy^{-2}]$ can be measured directly, equation (11) suffices to determine $\bar{n}$. However, in the work done for this thesis, $[Scy^{-2}]$ was determined from the experimental data according to the equation derived below. $[H^{+1}]$ was determined with a pH meter.

(12) $[H^{+1}]_t = [H^{+1}] + [HScy^{-1}] + 2[H_2Scy] + 3[H_3Scy^{+1}]$.

(13) $[H^{+1}]_t = 2[Scy]_t - [NaOH]$, where $[NaOH]$ is the concentration of the added NaOH.

Now from (12) and (13),

(14) $[H^{+1}] + [HScy^{-1}] + 2[H_2Scy] + 3[H_3Scy^{+1}] = 2[Scy]_t - [NaOH]$.

Now when (8), (9), and (10) are substituted into (14) and the resulting equation is rearranged, the following equation is obtained.

$$(15)\quad [Scy^{-2}] = \frac{2[Scy]_t - [H^{+1}] - [NaOH]}{\frac{[H^{+1}]}{K_3'} + \frac{2[H^{+1}]^2}{K_2'K_3'} + \frac{3[H^{+1}]^3}{K_1'K_2'K_3'}}.$$

It should be noted that, in this equation, the sodium hydroxide concentration is that required to titrate H_2Scy, not H_3Scy^+; thus, if cysteine hydrochloride is added initially, the volume of NaOH added must be corrected for the amount required to neutralize the hydrochloride, before applying equation (15).

VITA

Bailie Jack McCormick

Candidate for the Degree of

Doctor of Philosophy

Thesis: COBALT COMPLEXES OF SOME SULFUR-CONTAINING LIGANDS

Major Field: Chemistry

Biographical:

Personal Data: Born in Amarillo, Texas, August 20, 1937, the son of Bailie E. and Donabel Bushong McCormick. Married Theresa Ethelda Mickey, November 28, 1958. One child, Michelle Lee McCormick, born October 21, 1961.

Education: Attended primary and secondary school in Canyon, Texas; graduated from Canyon Public High School in 1955; received the Bachelor of Science degree from West Texas State College, with a major in chemistry, in May, 1959. Entered the Graduate School of Oklahoma State University in September, 1959, and completed the requirements for the Doctor of Philosophy degree, with a major in Physical Chemistry, in August, 1962.

Professional Experience: Du Pont Fellow in Chemistry at West Texas State College, 1957-1959; participated in the Student Trainee Program at the Amarillo Helium Plant from June, 1958 to September, 1958; Graduate Research Assistant, Oklahoma State University, 1959-1960; Cooperative Graduate Fellow, National Science Foundation, Oklahoma State University, 1960-1962; Dow Fellow, Oklahoma State University, summer, 1962.

Membership in Professional Societies: Phi Lambda Upsilon, American Chemical Society, Associate Member of Sigma Xi.