the usual statistical relations[4,8]

$$\mathbf{k}_1 = 4\kappa_1;\ \mathbf{k}_2 = 3\kappa_2/2;\ \mathbf{k}_3 = 2\kappa_3/3;\ \mathbf{k}_4 = \kappa_4/4$$

the log κ values have been converted to the log **k** values listed in Table IV. Included for comparison are log **k** values interpolated from those computed previously.[14]

TABLE IV

SUCCESSIVE ASSOCIATION CONSTANTS FOR Cu(II) AND Zn(II) WITH IMIDAZOLE

Log **k** values for ionic strength 0.16 and 25°.

	Cu(II) complexes		Zn(II) complexes	
	Former[a]	Present	Former[a]	Present
log $\mathbf{k}_1$	4.33	4.20	2.57	2.52
log $\mathbf{k}_2$	3.54	3.42	2.36	2.32
log $\mathbf{k}_3$	2.82	2.88	2.22	2.32
log $\mathbf{k}_4$	2.03	2.05	2.01	2.05

[a] Y. Nozaki, F. R. N. Gurd, R. F. Chen and J. T. Edsall, THIS JOURNAL, **79**, 2123 (1957).

The method of Scatchard for estimating successive constants was chosen in preference to that of Bjerrum because it yields a value of $\mathbf{k}_1$ that is probably more precise. If this type of study is to be useful as a model for the reactivity of imidazole groups in proteins it is usually the value of $\mathbf{k}_1$ that must be estimated most accurately.[4] The present values for $\mathbf{k}_1$ are probably more accurate than those previously reported, but the differences are not marked. Comment has been made previously on the errors involved in arriving at the other successive constants.[8,14] In the present study the higher values of $\bar{\nu}$ have not been explored because the technique that we have used does not allow accurate measurements of the kinetics when the half-life approaches one or two minutes. The value of $\mathbf{k}_4$ is therefore rather uncertain. Nevertheless, the logarithm of the product of the constants for the Cu(II)-system agrees well with the value of 12.6 determined polarographically by Li, White and Doody.[18]

Discussion

The foregoing results show that the kinetic and equilibrium methods of measuring the concentration of basic imidazole are mutually compatible. The presence of Cu(II) or Zn(II) ions does not affect the kinetic measurements, and the presence of NPA or its hydrolysis products does not affect the equilibrium measurements detectably. Studies to be reported later have shown a similar compatibility of the measurements when the imidazole radical is incorporated in the histidyl residue of a peptide.

In terms of their potential role in assessing the reactivity of imidazole groups in peptides and proteins, the two methods show an interesting contrast in characteristics. The kinetic method depends for its usefulness on the fact that it does not alter a condition of equilibrium, whereas the equilibrium method depends on the alteration of such a condition of equilibrium and on our ability to describe the new multiple equilibrium quantitatively in terms of the mass law.

The nucleophilic properties of the basic imidazole group resemble those of classes of groups in proteins, such as amino and phenoxyl groups,[19,20] and probably others. It is to be anticipated, therefore, that other classes of groups in proteins in addition to imidazole groups may react with NPA. Whether or not the products of the reaction are stable or unstable, the combined approach described here should be directly applicable whenever relatively minute quantities of NPA are employed.

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NEW YORK, N. Y.

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[18] N. C. Li, J. M. White and E. Doody, THIS JOURNAL, **76**, 6219 (1954).

[19] F. W. Putnam, in "The Proteins," (H. Neurath and K. Bailey, eds.), Vol. IB, Academic Press, New York, N. Y., 1953, p. 893.

[20] W. L. Koltun, THIS JOURNAL, **79**, 5681 (1957).

[CONTRIBUTION FROM DEPARTAMENTO DE QUÍMICA, FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DA UNIVERSIDADE DE SÃO PAULO]

Spectrophotometric Investigation in the Near Ultraviolet of the Cobalt(II) Monothiocyanato Complex

BY PASCHOAL SENISE AND MADELEINE PERRIER

RECEIVED MARCH 19, 1958

Use has been made of the absorption band in the near ultraviolet of the $CoSCN^+$ ion to determine the formation constant of this ion in acid aqueous solutions of unit ionic strength, by two independent methods. At *p*H 3.0 and 25 ± 1 the average value of that constant obtained by the method of McConnell and Davidson was found to be 10.09 ± 0.12 and by the method of corresponding solutions of Bjerrum 10.38 ± 0.07.

Introduction

The nature of aqueous solutions containing cobaltous and thiocyanate ions has been investigated spectrophotometrically by several authors.[1-4] Consecutive formation of complexes was found to occur, the first species formed being the cation $CoSCN^+$. The value of the formation constant of this ion reported by Lehné[2] is very uncertain—as stated by the author—since its calculation was based on the rather small difference of the molar extinction coefficients of the complex and cobalt(II) ions in the visible range of the spectrum. A dif-

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(1) A. v. Kiss and P. Csokán *Z. physik. Chem.*, **A186**, 239 (1940).

(2) M. Lehné, *Bull. soc. chem. France*, 76 (1951).

(3) A. K. Babko and O. F. Drako, *Zhur. Obshchei Khim.*, **20**, 228 (1950); *C. A.*, **44**, 5684 (1950).

(4) P. W. West and G. F. Vries, *Anal. Chem.*, **23**, 334 (1951).

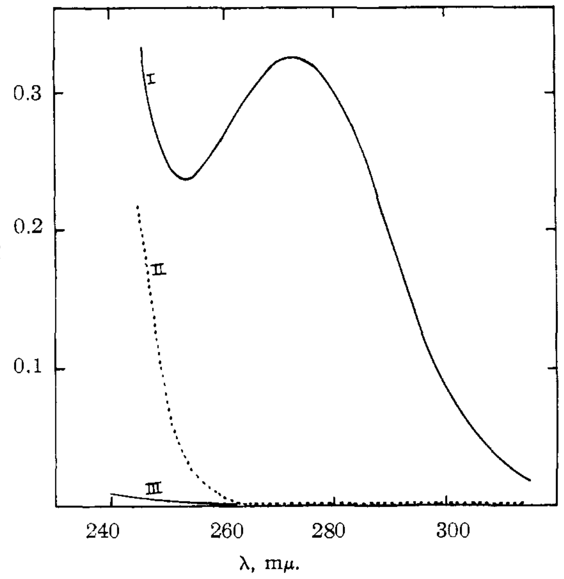

Fig. 1.—Ultraviolet absorption spectra of: 0.08 *M* cobaltous perchlorate and 0.002 *M* sodium thiocyanate (curve I); 0.002 *M* sodium thiocyanate (curve II); 0.08 *M* cobaltous perchlorate (curve III); $I = 1.0$; *p*H 3.0; $t = 25°$.

ferent value, determined by an indirect method, is given by Saini and Sapetti.[5]

No use seems to have been made for this purpose of the well defined absorption band in the near ultraviolet which is observed when cobalt(II) solutions are treated with small amounts of thiocyanate. Since in this particular region, cobaltous ions do not absorb (Fig. 1), it was believed that a more reliable value of the formation constant could be obtained.

Experimental

Apparatus and Materials.—A Beckman DU spectrophotometer and 1 cm. silica cells were used throughout the study. Determinations of *p*H were made with the help of a Beckman model G *p*H meter.

Cobaltous perchlorate stock solution was prepared from C.P. cobaltous carbonate and C.P. 20% perchloric acid, a slight excess of the former being employed. Standardization was carried out gravimetrically by precipitation with α-nitroso-β-naphthol.[6] C.P. sodium thiocyanate was freed from traces of iron by repeated batch extractions of concentrated aqueous solutions with ethyl ether. Anhydrous sodium perchlorate was prepared from C.P. sodium carbonate and perchloric acid. Acidity was adjusted by means of C.P. perchloric acid.

Measurements were made in a room maintained at 25°. The temperature of the solutions during the determinations showed variation always less than one degree.

Both methods of McConnell and Davidson[7] and of the corresponding solutions of Bjerrum[8] were employed. Readings were taken in every case at 265, 268, 270, 271, 272, 273, 274, 275, 278 and 280 mμ, although for the sake of convenience, results obtained at five different wave lengths were selected to be reported below.

Results and Discussion

Influence of *p*H.—Preliminary experiments showed that variation of acidity had no significant effect on the absorbance values of solutions provided the *p*H was maintained below 3.

Continuous Variation Study.—The formation of the $CoSCN^+$ complex was confirmed by employing the method of continuous variation[9,10] in the ultraviolet (Fig. 2).

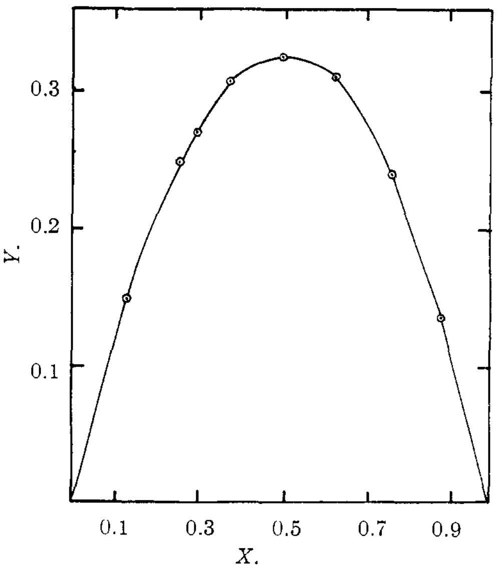

Fig. 2.—Continuous variation study: cobaltous perchlorate and sodium thiocyanate mixtures at total concentration of 0.02 *M*; *p*H 3.0; $I = 1.0$; $t = 25°$; $\lambda = 273$ mμ.

McConnell–Davidson Method.—In all series of experiments sodium thiocyanate was kept 0.002 molar and the concentration of cobalt(II) perchlorate varied between 0.02 and 0.14 molar, the total ionic strength of 1.0 being attained by the addition of sodium perchlorate. Measurements were taken against reference solutions of the same composition as the samples, except that no thiocyanate was present. Results obtained with a series of solutions of *p*H 3.0 are reported in Table I. The absorbance of the thiocyanate ion was not taken into consideration, since it was found to be 0.001 for all wave lengths considered. The constant $K = (CoSCN^+)/(Co^{2+})(SCN^-)$ was not evaluated graphically but through the application of the method of averages to the experimental data.

Determinations made with another series of solutions of the same composition but with *p*H adjusted at 2.0, led to the following average value of the formation constant: av. $K = 9.98 \pm 0.19$.

Bjerrum Method of Corresponding Solutions.—Two series of solutions being necessary to provide data for calculations, the first one was kept 0.05 and the second 0.01 molar in cobaltous perchlorate while the concentration of sodium thiocyanate was varied between 0.001 and 0.004 molar in both series. Sodium perchlorate was added to adjust the total ionic strength to unity. Readings were

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[5] G. Saini and C. Sapetti, *Atti accad. sci. Torino*, **86**, 246 (1951–1952).

[6] C. Mayr and F. Feigl, *Z. anal. Chem.*, **90**, 15 (1932); *cf.* F. Welcher, "Organic Analytical Reagents," Vol. III, D. Van Nostrand Co., New York, N. Y., 1949, p. 303.

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[9] P. Job, *Ann. chim. (Paris)*, [10] **9**, 113 (1928).

[10] W. C. Vosburgh and G. R. Cooper, THIS JOURNAL, **63**, 437 (1941).

TABLE I

FORMATION CONSTANT OF THE $CoSCN^+$ ION—MCCONNELL–DAVIDSON METHOD

I = 1.0; pH 3.0; t = 25°. a = molarity of cobalt(II) perchlorate; b = molarity of sodium thiocyanate, b = 0.002; A = absorbance of solution of unit ionic strength a molar in cobaltous perchlorate and b molar in sodium thiocyanate; A' = absorbance of a solution of unit ionic strength a molar in cobaltous perchlorate.

a	268 mμ $A - A'$	270 mμ $A - A'$	273 mμ $A - A'$	275 mμ $A - A'$	278 mμ $A - A'$
0.020	0.120	0.123	0.125	0.122	0.119
.040	.197	.201	.206	.202	.196
.050	.235	.240	.244	.242	.235
.060	.255	.262	.268	.266	.258
.080	.310	.319	.323	.321	.310
.100	.350	.359	.367	.360	.347
.120	.377	.387	.396	.392	.377
.140	.401	.411	.420	.418	.404
K, l./mole	10.21	10.08	10.11	9.87	10.20

Av. K[a] = 10.09 ± 0.12

[a] All constants given in this paper were calculated by using data obtained at ten wave lengths as mentioned above.

TABLE II

FORMATION CONSTANT OF THE $CoSCN^+$ ION—BJERRUM METHOD

I = 1.0; pH 3.0; t = 25°. c_0 = molarity of cobaltous perchlorate; b = molarity of sodium thiocyanate; A = absorbance of a solution of unit ionic strength c_0 molar in cobaltous perchlorate and b molar in sodium thiocyanate read against a solution of unit ionic strength c_0 molar in cobaltous perchlorate.

b	268 mμ A/c_0	270 mμ A/c_0	273 mμ A/c_0	275 mμ A/c_0	278 mμ A/c_0
			c_0 = 0.05		
0.0010	2.40	2.46	2.50	2.48	2.38
.0015	3.62	3.70	3.76	3.72	3.62
.0020	4.76	4.88	4.98	4.92	4.74
.0025	5.88	6.02	6.14	6.12	5.86
.0030	6.96	7.16	7.32	7.30	7.04
.0040	8.90	9.10	9.32	9.28	8.94
			c_0 = 0.01		
0.0010	3.20	3.20	3.40	3.40	3.20
.0020	6.60	6.80	6.90	6.90	6.60
.0025	8.10	8.30	8.60	8.50	8.20
.0030	9.80	10.0	10.2	10.2	9.80
.0035	11.0	11.3	11.4	11.4	11.0
.0040	12.2	12.5	12.8	12.6	12.3
K, l./mole	10.41	10.30	10.41	10.41	10.41

Av. K = 10.38 ± 0.07

taken against reference solutions of the same composition except that thiocyanate was absent. In Table II a typical set of results is reported. The absorbance of thiocyanate ion being only 0.001 in the range studied, it was disregarded in the calculations. The method of averages was used in order to correlate data for the calculation of the formation constant.

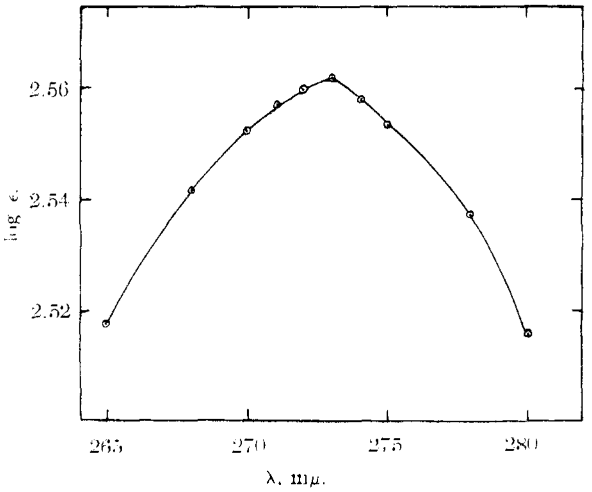

Fig. 3.—Molar extinction coefficient of $CoSCN^+$: I = 1.0; pH 3.0; t = 25°.

Determinations also were carried out with solutions of identical compositions having pH 2.0 and 1.2. The average values of the formation constants were av. K = 10.31 ± 0.11 and av. K = 10.37 ± 0.14, respectively. These values can be considered to agree satisfactorily with those obtained by the method of McConnell and Davidson inasmuch as mathematical approximations are involved in both methods.

The molar extinction coefficient, ϵ, of $CoSCN^+$ for each of the wave lengths considered was calculated using the value of K = 10.23, which is the average of the five values given above. In Fig. 3 values of log ϵ are plotted against wave length.

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