buffers alkaline to the isoelectric point consistently presented a single boundary. At *p*H 6.5, no heterogeneity was observed in acetate or phosphate buffer at ionic strength 0.1 after electrophoresis for three or five hours. The curves relating mobility with *p*H for β_1- and normal β-lactoglobulin intersect at about *p*H 6.6 (Fig. 3), so that if heterogeneity were to be observed at an alkaline *p*H it would seem least likely to appear at *p*H 6.5 and most likely to appear at *p*H 5.5 or 8.4.

The nature of the heterogeneity presented by β-lactoglobulin has been the subject of much conjecture.[18] It apparently is not the result of the isolation procedure. Crystals obtained by alcohol fractionation, as well as by salt fractionation of whey; crystals from the milk of a single animal, as well as mixed commercial milk; and even crystals from normal pasteurized milk, showed the same consistent electrophoretic heterogeneity at *p*H 4.8 in acetate buffer at ionic strength 0.1. The only exceptions to this highly reproducible pattern were found in the crystalline lactoglobulin isolated from the first-day colostrum of one cow and from the milk of a cow infected with brucellosis. In both cases, the β_1-lactoglobulin predominated, amounting to 80% in the brucellosis β-lactoglobulin and 100% in the colostrum. The possibility of aggregation or dissociation of the molecule is discredited by finding similar molecular weights for the normal and β_1-lactoglobulins. The isolation of one of the components in pure crystalline form that migrates with the velocity of the β-lactoglobulin in alkaline buffer, although it has a mobility similar to that of the immune globulins of whey at *p*H 4.8, refutes the postulate by Lundgren and Ward[18] that the heterogeneity of β-lactoglobulin is a result of the contamination with one or more of the immune globulins characterized by Smith.[19] In view of the high molecular weight (about 180,000) and the presence of carbohydrate and phosphorus in these immune globulins, it is impossible for an immune globulin to account for any component that constitutes the electrophoretic heterogeneity reported here.

The heterogeneity of β-lactoglobulin manifest at *p*H values acid to the isolectric point can be ascribed primarily to a basic difference in the net charge.

This may be attributed to a fundamental variation in the structure of the protein molecule or to the conjugation of the same molecule with another component, with resultant change in the dissociation of ionizable groups and hence difference in the net charge.

Acknowledgment.—The authors are greatly indebted to C. A. Zittle for the many helpful discussions and criticisms of this paper, and to E. S. DellaMonica for assistance in preparing the normal crystalline β-lactoglobulin.

Summary

Crystalline β-lactoglobulin, presenting a single electrophoretic boundary in buffers of ionic strength 0.1, alkaline to the isoelectric point, has been found heterogeneous in buffers acid to the isoelectric point. By means of alcohol fractionation and differential solubility at *p*H 4.8 and 5.3, one of the two components resolved by electrophoresis in acetate buffer at *p*H 4.8 has been isolated as a crystalline protein showing a single electrophoretic boundary over the conventional *p*H range in buffers of ionic strength 0.1. The per cent. nitrogen, molecular weight, optical rotation, optical density (280/250 mμ), biuret color density, solubility in water and salt, electrophoretic mobility and isoelectric point of the single-boundaried component and of the normal complex are compared.

PHILADELPHIA 18, PENNSYLVANIA

RECEIVED FEBRUARY 3, 1950

(18) Lundgren and Ward, *Ann. Rev. Biochem.*, **18**, 121 (1949).

(19) Smith, *J. Biol. Chem.*, **166**, 665 (1946).

[CONTRIBUTION FROM THE RICHARDSON CHEMICAL LABORATORIES OF THE TULANE UNIVERSITY OF LOUISIANA]

Inorganic Complex Compounds Containing Polydentate Groups. IV. Formation Constants of Diethylenetriamine–Nickel(II) and –Copper(II) Complexes

BY HANS B. JONASSEN, R. BRUCE LEBLANC AND RUTH M. ROGAN

If a metal ion can coördinate two or more groups in the formation of complex ions, it will do so stepwise, and intermediate equilibria will be present. If M represents the metal ion and A the coördinating group the equilibria are

$$M + A \rightleftarrows MA;\ k_1 = [MA]/[M][A]$$
$$MA + A \rightleftarrows MA_2;\ k_2 = [MA_2]/[MA][A]$$
$$MA_{n-1} + A \rightleftarrows MA_n;\ k_n = [MA_n]/[MA_{n-1}][A]$$

Each of these equilibria can be represented by a constant, and the product of all the constants for the individual steps is the over-all complexity constant for the equilibrium

$$M + NA \rightleftarrows MA_N;\ k_N = [MA_N]/[M][A]^N$$

where N is the maximum number of groups coördinated.

Bjerrum[1] devised a method for measuring these step equilibria constants (or formation constants) for basic coördinating groups by measuring the *p*H of solutions which contain known amounts

[1] J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

of the complexing agent and the metal ion. This method holds for polydentate groups only if the coördinating group is completely bound by chelation to the metal ion and exerts no basic function.

This paper reports the study of the formation of complexes between diethylenetriamine (abbreviated dien) and the copper(II) and nickel(II) ions.

Calculation of Constants

The following symbols will be used

C_{HNO_3} = total concn. of HNO_3 in the solution
C_{Me} = total concn. of metal ion in the solution
C_{dien} = total concn. of dien in the solution
C'_{dien} = total concn. of dien not complexed by the metal ion in the solution
C_s = total concn. of hydrogen ions bound to the uncomplexed dien
$C_s = C_{HNO_3} - [H^+] + [OH^-] = [dienH^+] + 2[dienH_2^{+2}] + 3[dienH_3^{+3}]$

$$\bar{n}_{dien} = \frac{C_s}{C'_{dien}} = \frac{k_1k_2[H^+] + 2k_1[H^+]^2 + 3[H^+]^3}{k_1k_2k_3 + k_1k_2[H^+] + k_1[H^+]^2 + [H^+]^3} \quad (1)$$

where $\bar{n}_{dien}$ is the mean number of hydrogen ions bound by each dien molecule and k_1, k_2 and k_3 refer to the stepwise dissociation constants of $dienH_3^{+3}$.[2]

$$\alpha_{dien} = [dien]/C'_{dien} \quad (2)$$

where α_{dien} refers to the fraction of the dien in the acid–base system which exists as the free dien. The following expression can be derived from (2) and the equations for the acid–base constants of dien

$$\alpha_{dien} = \frac{k_1k_2k_3}{k_1k_2k_3 + k_1k_2[H^+] + k_1[H^+]^2 + [H^+]^3} \quad (3)$$

Defining $\bar{n}$ as the mean number of dien molecules attached to each metal ion, the following equation is obtained

$$\bar{n} = (C_{dien} - C'_{dien})/C_{Me}$$

A combination of (1) and (2) gives

$$[dien] = \alpha_{dien}C_s/\bar{n}_{dien} \quad (4)$$

or

$$p[dien] = \log(\bar{n}_{dien}/\alpha_{dien}) - \log C_s \quad (5)$$

If the acid–base constants of dien,[2] the concentration of the various components of the solution, and the *p*H of the solution are known, $\bar{n}$ and *p*[dien] can be calculated from equations (1), (2), (3), (4) and (5).

Experimental

Diethylenetriamine was purified by the method previously described.[2] This consisted of vacuum distillation and precipitation of its monohydrochloride from alcohol. A stock solution, approximately 1 molar in concentration, was exactly neutralized with potassium hydroxide and standardized potentiometrically against standard acid. The neutralization with potassium hydroxide produced an equimolar concentration of potassium chloride in the stock solution of dien.

The concentration of chloride ions in the solutions from which the log *k* values were determined did not exceed 0.2 *M*. It has been shown[3] that in these concentration ranges of chloride ions no appreciable amounts of chloro-complexes are formed.

Individual solutions of approximate constant ionic strength were prepared for each measurement. These solutions were 0.1 *M* in metal ion, 0.1 *M* in HNO_3, and of varying concentration in dien. The sum of the concentration of potassium chloride and potassium nitrate was 1.00 *M*.

The *p*H values of the solutions were measured with a Beckman Model G *p*H meter standardized with Beckman buffers, *p*H of 4, 7 and 10. The solutions were maintained at 30 and 40° in constant temperature baths.

Discussion

The authors[2] previously measured the acid–base *pK* of dien at 30° and 40° at the same salt concentration as above

	30°	40°
pk_1	4.78	4.59
pk_2	9.23	8.94
pk_3	9.94	9.68

With the use of these acid–base constants, the quantities $\bar{n}$ and *p*[dien] were calculated from equations (4) and (5).

Table I contains data on the copper(II) ion and dien at 30°.

TABLE I

*p*H MEASUREMENTS OF COPPER(II) ION SOLUTIONS CONTAINING DIETHYLENETRIAMINE AT 30°

$C_{Cu(II)} = 0.1055$, $C_{KNO_3} + C_{KCl} = 1.00$ *M*

C_{dien}	*p*H	$\log \frac{\bar{n}_{dien}}{\alpha_{dien}}$	$\bar{n}_{dien}$	C_s	C'_{dien}	$\bar{n}$	*p*[dien]
0.0350	2.53	16.84	3.00	0.0970	0.0324	0.025	17.85
.0401	2.70	16.33	2.99	.0982	.0328	.069	17.34
.0452	2.79	16.06	2.99	.0986	.0330	.116	17.07
.0500	2.84	15.91	2.99	.0986	.0330	.161	16.92
.0648	2.98	15.49	2.99	.0988	.0331	.300	16.50
.0749	3.05	15.28	2.98	.0989	.0332	.395	16.28
.0850	3.10	15.13	2.98	.0992	.0333	.490	16.13
.0900	3.13	15.03	2.98	.0994	.0334	.536	16.03
.1000	3.19	14.86	2.98	.0994	.0334	.631	15.86
.1054	3.21	14.80	2.97	.0997	.0335	.682	15.80
.1199	3.37	14.33	2.96	.0993	.0335	.819	15.33
.1403	4.19	11.92	2.80	.1005	.0359	.990	12.92
.1599	4.96	9.85	2.40	.1002	.0418	1.044	10.85
.1799	6.89	5.70	2.00	.1000	.0499	1.232	6.70
.2001	7.56	4.36	1.98	.1004	.0508	1.416	5.36
.2198	8.38	2.74	1.87	.0999	.0535	1.579	3.74
.2399	8.82	1.91	1.69	.1000	.0593	1.711	2.91
.2600	9.17	1.29	1.42	.1006	.0708	1.793	2.29
.2800	9.40	0.91	1.20	.1005	.0838	1.860	1.91

Similar data, obtained at 40°, are omitted. After $\bar{n}$ exceeds 1, the values of *p*[dien] decrease very rapidly as $\bar{n}$ increases. This gives a flattening of the curve as can be seen in Fig. 1, which shows the formation curves at 30 and 40°. The curve does not approach an $\bar{n}$ value of 2 asymptotically, as does the curve of a typical 6-coördinated metal ion. This indicates that the copper (II) ion does not exhibit a stable coördination number of six in aqueous solution at this concentration. Obviously, a second dien molecule is complexed by the copper(II) ion, but it is not coördinated through all three of its amino groups.

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[2] H. B. Jonassen, R. B. LeBlanc, A. W. Meibohm and R. M. Rogan, THIS JOURNAL, **72**, 2430 (1950).

[3] G. A. Carlson, J. P. McReynolds and Frank H. Verhoek, *ibid.*, **67**, 1334 (1945).

A coördination number less than six for the copper(II) ion is indicated.

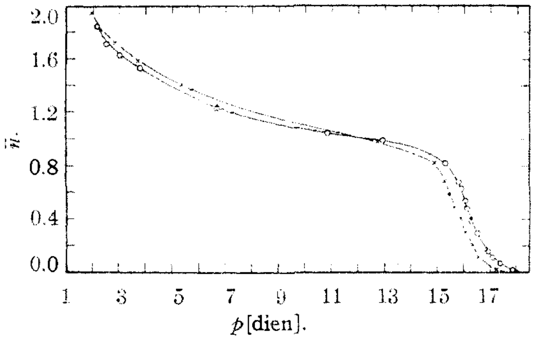

Fig. 1.—Formation curve of the copper(II) ion and diethylenetriamine at 30 and 40°: O, 30°; ×, 40°.

These data are not at variance with the observations on the copper(II) ethylenediamine system reported by Bjerrum,[4] who found a maximum coördination number of 5 for the copper(II) ethylenediamine complexes.

The polarographic investigations on the copper (II) diethylene triamine system[5] also indicate that, in the presence of a large excess of dien, two molecules of dien are coördinated to the copper(II) ion. No calculations of individual formation constants of the complex can be made from polarographic data.

Because of the assumption in deriving the equations, the coördination of the second dien through only one or two of its amino groups makes the equations inapplicable for calculation of the second formation constant of the copper(II) ion. The logarithm of the first formation constant can be calculated from

$$\log k_1 = p[\text{dien}] + \log (\bar{n}/(1 - \bar{n})) \tag{6}$$

TABLE II

FORMATION CONSTANTS OF THE COPPER(II) ION AND DIETHYLENETRIAMINE AT 30 AND 40°

$\bar{n}$	p[dien]	$\log \bar{n}/(1 - \bar{n})$	$\log k_1$
	$t = 30°$		
0.300	16.50	−0.37	16.13
.395	16.28	− .18	16.10
.490	16.13	− .02	16.11
.536	16.03	+ .06	16.09
.631	15.86	+ .23	16.09
.682	15.80	+ .33	16.13
		Av.	16.11
	$t = 40°$		
0.300	16.03	−0.37	15.66
.490	15.66	− .02	15.64
.536	15.55	+ .06	15.61
.584	15.46	+ .15	15.61
.631	15.43	+ .23	15.66
.681	15.28	+ .33	15.61
		Av.	15.63

Experimental points on the curve between $\bar{n}$ values of 0.3 and 0.7 yield the values of log k_1, listed in Table II, for the copper(II) ion at 30° and 40°.

From the data in Table II the heat of binding of $[\text{Cu(dien)}]^{+2}$ is calculated from

$$\frac{d \ln K}{dT} = \frac{\Delta H}{RT^2} \tag{7}$$

and found to be −21 kcal. The data for the formation of the nickel(II) complex ions and dien at 30° and 40° were obtained in a manner similar to that for the copper(II) ions. A plot of these data is shown in Fig. 2.

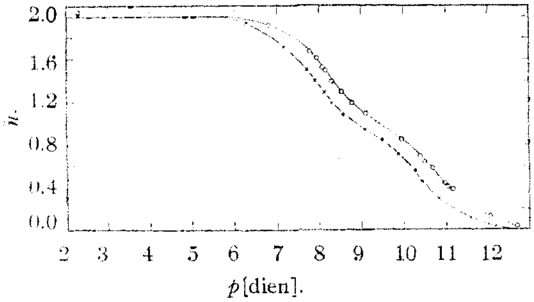

Fig. 2.—Formation curve of the nickel(II) ion and diethylenetriamine at 30 and 40°: O, 30°; ×, 40°.

It can be seen here that the curve for the nickel(II) ion is different from that of the copper (II) ion in that the former approaches asymptotically a maximum $\bar{n}$ value of 2. The curve rises rather abruptly unlike the copper(II) ion curve for $\bar{n}$ values greater than 1. There is a slight leveling off in the nickel(II) ion curve at the $\bar{n}$ value of 1 indicating the two complexes $[\text{Ni(dien)}]^{+2}$ and $[\text{Ni(dien)}_2]^{+2}$ have a rather large difference in stability. The two formation constants for these complexes can be calculated,

TABLE III

FORMATION CONSTANTS OF THE NICKEL(II) ION AND DIETHYLENETRIAMINE AT 30 AND 40°

$\bar{n}$	p[dien]	$\log \bar{n}/(1 - \bar{n})$	$\log k_1$
	$t = 30°$		
0.376	11.10	−0.22	10.88
.406	11.05	− .17	10.88
.443	10.97	− .10	10.87
.582	10.68	+ .14	10.82
.639	10.51	+ .25	10.76
.689	10.39	+ .35	10.74
		Av.	10.81
		$\log (\bar{n} - 1)/(2 - \bar{n})$	$\log k_2$
1.295	8.55	−0.38	8.17
1.399	8.32	− .18	8.14
1.502	8.16	.00	8.16
1.532	8.09	+ .06	8.15
1.604	7.93	+ .18	8.11
1.679	7.78	+ .33	8.11
		Av.	8.14

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(4) J. Bjerrum and E. J. Nielsen, *Act. Chem. Scand.*, **2**, 297 (1948).

(5) H. A. Laitinen, E. I. Onstott, J. C. Bailar, Jr., and Sherlock Swann, Jr., THIS JOURNAL, **71**, 1550 (1949).

TABLE III (*Continued*)

$t = 40°$

$\bar{n}$	p[dien]	$\log \bar{n}/(1 - \bar{n})$	$\log k_1$
0.304	10.96	−0.36	10.60
.371	10.83	− .23	10.60
.507	10.55	+ .01	10.56
.544	10.46	+ .08	10.54
.581	10.36	+ .14	10.50
.686	10.12	+ .34	10.46
		Av.	10.54
		$\log (\bar{n} - 1)/(2 - \bar{n})$	$\log k_2$
1.292	8.15	−0.38	7.77
1.463	7.92	− .06	7.86
1.530	7.79	+ .06	7.85
1.603	7.66	+ .18	7.84
1.678	7.50	+ .32	7.82
		Av.	7.83

respectively, from (6) and from

$$\log k_2 = p[\text{dien}] + \log (\bar{n} - 1)/(2 - \bar{n}) \qquad (8)$$

The values of $\log k_1$ and $\log k_2$ at 30 and 40° are obtained from $\bar{n}$ values between 0.3 and 0.7 and between 1.3 and 1.7, respectively, and are shown in Table III.

The values of $\log k_1$ at both 30 and 40° decrease slightly with increasing concentration of dien. At first it was thought that a change of structure might be the explanation for this anomalous behavior, and the magnetic susceptibilities of the solutions were investigated. The measurements showed that all solutions had paramagnetic susceptibilities of the same magnitude indicating that no shift in structure occurred.

No explanation is offered for the trend in the values of $\log k_1$ for the nickel(II) ion. The values at the $\bar{n}$ value of 0.5 are taken as the most probable values. This value at 30° is 10.85, and at 40° is 10.56.

The heat of binding of each dien molecule by the nickel(II) ion is calculated with equation (7) to be −13 kcal. This is considerably less than the −21 kcal. calculated for the binding of dien by the copper(II) ion.

Summary

I. Formation curves and constants of the copper(II) and the nickel(II) ions with diethylenetriamine are determined at 30 and 40°.

II. Heats of binding of diethylenetriamine by the copper(II) ion and the nickel(II) ion are calculated from the formation constants of the respective complexes.

NEW ORLEANS 15, LOUISIANA RECEIVED MARCH 6, 1950

[CONTRIBUTION OF THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Crystal Structure of Potassium Chlorochromate, $KCrO_3Cl$[1]

BY L. HELMHOLZ AND W. R. FOSTER[2]

Introduction

Potassium chlorochromate shows a slight but unmistakable dichroism which may be attributed to the polarized absorption of light by the chlorochromate ion itself. We have investigated the crystal structure of this substance for the purpose of determining the configuration of CrO_3Cl^- and in order to correlate this structure with the observed spectra. A comparison of the chromium–oxygen interatomic distances in the series chromate, chlorochromate, chromyl chloride is also of importance in a discussion of the electronic structure of these molecules.

Experimental

Procedure.—Potassium chlorochromate was prepared by heating a solution of potassium dichromate in concentrated hydrochloric acid to 70° and then allowing the resulting solution to stand for some hours. The salt deposits from the solution in the form of long lath-shaped crystals. Laue photographs with the X-ray beam perpendicular to the prism axis showed that most of the crystals were twinned on the largest face (100). Several untwinned samples were obtained and used for Laue and oscillation exposures. Both Cu Kα and Mo Kα radiations were used, the first for a determination of the unit cell dimensions; the second for intensity measurements in order to minimize the effect of absorption. The $hk0$ data were obtained using the method of visual estimation and the multiple film technique. The intensities of the $h0l$ reflections were determined using a Geiger counter spectrometer. A comparison of the relative intensities of the $h00$ reflections as determined by the different methods showed that the ratio of intensities of strong to weak reflections was greater for the Geiger counter technique than for the visual estimation method.

In the case of the $h0l$ spectra, the cross section of the crystal was such that absorption effects were of importance. The observed data were corrected for the effect by an integration of the function $e^{-\mu d}$ over the rectangular cross section of the crystal, d being expressed as a function of the angles between the crystal axes and the directions of the incident and diffracted beams.

Unit Cell and Space Group.—The Laue exposures showed a symmetry C_{2h} with a monoclinic angle close to 90° in agreement with the crystallographic data.[3] The twofold axis was found to lie in the plane of the large developed face perpendicular to the long axis of the crystals which was chosen to be the c-axis. The a-axis was then very nearly perpendicular to the large face.

The dimensions of the unit cell and the angle β are given in Table I with the results of Gawrych,[4] who measured the size of the unit cell but carried through no structure determination.

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[1] For detailed data supplementary to this article order Document 2836 from American Documentation Institute, 1719 N Street, Washington 6, D. C., remitting \$0.50 for microfilm (images 1 inch high on standard 35 mm. motion picture film) or \$0.50 for photocopies (6 × 8 inches) readable without optical aid.

[2] Du Pont Fellow, Washington University.

[3] Groth, "Chemische Krystallographie," Teil II, Leipzig, 1908.

[4] S. Gawrych, *Rocsniki Chem.*, **19**, 413 (1939).