the corresponding dimethylamine compounds would be stable only at lower temperatures than the methylamine compounds.

Compounds having a fractional composition are also known in the case of the ammoniates of the rare earth halides. With samarium chloride, the compound, $SmCl_3 \cdot 2.66NH_3$ was shown to exist.[5b] The tendency to form this type of complex seems to be greater in the methylamine complexes. These fractional compounds are stable phases as they are reproducible on different samples and can be obtained reversibly either from the lower or from the higher compound. The decomposition curve of the neodymium chloride–methylamine system at 305 mm. pressure gave the same compounds as at 700 mm., but with somewhat lower decomposition temperatures.

IOWA CITY, IOWA

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUQUESNE UNIVERSITY]

# Association of Imidazole with Nickel(II) and Alkaline Earth Ions[1,2]

BY NORMAN C. LI, TING LI CHU, CHARLES T. FUJII AND JAMES M. WHITE

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The interaction of imidazole with nickel(II) ion was determined by means of polarographic and *p*H measurements at ionic strength 0.15. The coördination number of nickel ion for imidazole is 6. The successive logarithmic formation constants at 25° are: 3.27, 2.68, 2.15, 1.65, 1.12 and 0.52. The structure of the complex is that of an octahedron and the bonds are essentially ionic. From the absorption spectra of nickel imidazole complexes, the molar extinction coefficient of $NiIm^{++}$ is calculated to be approximately 3.3 l. mole$^{-1}$ cm.$^{-1}$ at 650 m$\mu$, the same as the corresponding value for $NiNH_3^{++}$. Barium and calcium ions have slight tendency to enter into complex formation with imidazole at 25 and 35°.

## Introduction

As part of a general program on studies of complex formation between different metallic ions and the imidazoles,[3] this paper presents the results on the nickel(II) complexes of imidazole, using the polarographic, potentiometric, magnetic and spectrophotometric methods. Nickel(II) was chosen because the configuration of the complex formed can be determined from the coördination number and magnetic moment. Thus if the complex is paramagnetic then the bonds are essentially ionic and the complex has a tetrahedral or octahedral configuration, depending on whether the coördination number is four or six, respectively.

Cohn, *et al.*,[4] have shown that barium and calcium ions appear not to form association complexes with human serum albumin. In view of the accumulating evidence that in the combination of some other metals, namely, zinc, cadmium and copper, with serum albumin the principal binding sites are the imidazole groups of the histidine residues of the protein molecule, it was thought worthwhile to determine whether these alkaline earth ions enter into complex formation with imidazole or not. The results obtained at 25 and 35° are included in this paper.

## Experimental

**Material.**—Imidazole purchased from Eastman Kodak Co. was treated and analyzed as previously described.[3] Stock solutions of $NiCl_2$ and $Ni(NO_3)_2$ were prepared and analyzed by precipitation wih dimethylglyoxime. All other compounds were of reagent grade and were used without further purification or analysis.

**Magnetic Susceptibility Measurements.**—The magnetic susceptibilities of a series of nickel(II) chloride solutions containing varying amounts of imidazole were measured at room temperature (approx. 18°) by the Gouy method. The apparatus has been described elsewhere.[5] It was calibrated with distilled water and with a standard $NiCl_2$ solution. Densities required for calculation of the magnetic susceptibilities were determined by the usual pycnometer method.

**Other Measurements.**—The polarographic and potentiometric *p*H measurements were as previously described.[3] Spectrophotometric measurements were made with a Beckman quartz spectrophotometer, Model DU.

## Results

Table IA and B summarize the polarographic results obtained for imidazole solutions containing nickel(II) and barium ions, respectively, at 25° in aqueous medium. A plot of $-E_{1/2}$ *vs.* log $C_{Im}$ for the nickel(II) complexes yields a line of slope 0.18. Since the theoretical slope corresponding to $p = 6$, where $p$ is the number of groups coördinated to each metal ion (see equation 1, ref. 3), is 0.178, the complex is $NiIm_6^{++}$.

TABLE I

(A) POLAROGRAPHIC RESULT FOR SOLUTIONS CONTAINING $5 \times 10^{-4}$ *M* $Ni(NO_3)_2$, 0.05% GELATIN, 0.15 *M* $KNO_3$ AND VARYING CONCENTRATIONS OF IMIDAZOLE

| $C_{Im}$ | log $C_{Im}$ | $-E_{1/2}$ | $E_{3/4} - E_{1/4}$ |
|---|---|---|---|
| 1.2363 | 0 09213 | 1.053 | 0.038 |
| 1.1288 | .05262 | 1.046 | .037 |
| 1.0384 | .0164 | 1.038 | .038 |
| 1.0326[a] | .01393 | 1.037 | .037 |
| 0.9616 | −0.01701 | 1.033 | |
| .0000 | | 1.106 | .091 ($i_d = 2.18$ $\mu$a.) |
| .000[a] | | 1.101 | .11 ($i_d = 2.64$ $\mu$a.) |

[a] 0.022% gelatin.

(B) POLAROGRAPHIC RESULT FOR SOLUTIONS CONTAINING $5 \times 10^{-4}$ *M* $Ba(NO_3)_2$, 0.15 *M* $(C_2H_5)_4NBr$ AND VARYING CONCENTRATIONS OF IMIDAZOLE

| $C_{Im}$ | $-E_{1/2}$ | $E_{3/4} - E_{1/4}$ |
|---|---|---|
| 0.9856 | 1.925 | 0.030 |
| .7885 | 1.922 | .030 |
| .0000 | 1.924 | .028 |

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[1] Taken in part from the M.S. theses of C. T. Fujii and J. M. White, Duquesne University.

[2] This investigation was supported in part by a research grant from the National Science Foundation, Grant No. G510.

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

[4] E. J. Cohn, D. M. Surgenor, K. Schmid, W. H. Batchelor, H. C. Isliker and E. H. Alameri, *Faraday Soc. Disc.*, 176 (1953).

[5] T. L. Chu and S. C. Yu, THIS JOURNAL, **76**, 3367 (1954).

Potentiometric $p$H titrations of nickel(II) ion with imidazole were carried out at 25.0° and the results are recorded in Table II.

TABLE II

TITRATION OF NICKEL(II) CHLORIDE–IMIDAZOLE MIXTURES

Initial solution: 30.00 ml. of solution containing 0.10$M$ total imidazolium nitrate, 0.02$M$ $KNO_3$, 0.01$M$ $NiCl_2$. Titrated with 1.0536$M$ KOH.

| KOH, ml. | $p$H | log (A)<br>(A) ≡ (imidazole) | $n$ |
|---|---|---|---|
| 0 10 | 4.35 | 3.78 | 0.33 |
| .20 | 4.82 | 3.33 | .65 |
| .30 | 5.13 | 3.04 | .95 |
| .40 | 5.36 | 2.83 | 1.26 |
| .50 | 5.56 | 2.65 | 1.54 |
| .60 | 5.72 | 2.51 | 1.80 |
| .70 | 5.87 | 2.38 | 2.03 |
| .90 | 6.13 | 2.17 | 2.46 |
| 1.00 | 6.25 | 2.07 | 2.63 |
| 1.20 | 6.46 | 1.91 | 2.95 |
| 1.30 | 6.55 | 1.85 | 3.11 |
| 1.70 | 6.88 | 1.66 | 3.65 |

In order to evaluate the individual formation constants from the data of Table II, we have used the modified Scatchard method.[3] In this case we have defined $Q = \bar{n}/(6 - \bar{n})$ (A), where $\bar{n}$ is the average number of ligand molecules bound per metallic ion present and (A) is the concentration of free ligand molecules, and have plotted log $Q$ *vs.* $\bar{n}$, obtaining a straight line. Because of the greater error in the extreme high and low $p$H regions, some of the points in the extreme ends of the curve are disregarded in drawing the graph. The values of log $Q$ at $\bar{n} = 0$ and $\bar{n} = 6$, obtained from extrapolations of the line, are the values of log $\chi_1$ and log $\chi_6$ respectively, where $\chi_1$ and $\chi_6$ are the first and sixth "intrinsic constants." The formation constants are then derived from the equations

$$k_1 = 6\chi_1;\ k_6 = \chi_6/6$$

The extrapolations of log $Q$ give 2.49 and 1.30 at $\bar{n} = 0$ and $\bar{n} = 6$, respectively, from which the following values are obtained: log $k_1$ = 3.27; log $k_6$ = 0.52. The extrapolations of log $Q$ are attended by some uncertainty, but the values of log $k_1$ and log $k_6$ are probably accurate to ±0.05. The intermediate formation constants are obtained in the way suggested by ref. 3. From the straight line obtained in plotting log $Q$ *vs.* $\bar{n}$, the values of log $Q$ at $\bar{n}$ = 1.5, 2.5, 3.5 and 4.5 are easily obtained. From these, and from the definition of $Q$, we have calculated the values of $p$A at $\bar{n}$ = 1.5, 2.5, 3.5 and 4.5, which are taken to be the values of log $k_2$, log $k_3$, log $k_4$ and log $k_5$, respectively.

The successive logarithmic formation constants at 25°, $\mu = 0.15$, for nickel(II) complexes of imidazole are given in Table III. The values of nickel(II) ammonia complexes at 30°[6] (2 $N$ $NH_4NO_3$) are also given for comparison.

TABLE III

SUCCESSIVE LOGARITHMIC FORMATION CONSTANTS OF IMIDAZOLE AND AMMONIA WITH NICKEL(II) ION

| | Imidazole | Ammonia |
|---|---|---|
| log $k_1$ | 3.27 | 2.80 |
| log $k_2$ | 2.68 | 2.24 |
| log $k_3$ | 2.15 | 1.73 |
| log $k_4$ | 1.65 | 1.19 |
| log $k_5$ | 1.12 | 0.75 |
| log $k_6$ | 0.52 | 0.03 |

The potentiometric $p$H titrations of imidazole with barium nitrate and calcium nitrate are too numerous to include here. We have performed some 20 titrations on these complexes, the ratio of metal ion to total imidazolium nitrate or imidazolium perchlorate in the initial solutions varying from 10 to 0.1, the total ionic strength in each case being constant at 0.15. Because of the small formation constants of the alkaline earth complexes, high precision is not easily obtained. However, for the purpose of magnitude only, the value of log $k_1$ for the barium and calcium complexes of imidazole may be taken to be identical and less than 0.2. The $pK'$ of imidazole at 35° is determined to be 6.92 and the formation constants of the barium and calcium complexes at 35° are the same as at 25°. The $pK'$ and log $k_1$ values depend on the total ionic strength only, and are independent of whether perchlorate or nitrate is used to maintain ionic strength constant.

The absorption spectra for varying ratios of imidazole to total nickel are shown in Fig. 1. All values of extinction coefficient are given for a one-cm. path length, in terms of the total nickel concentration in moles per liter of solution.

Magnetic susceptibility measurements were made on solutions containing 0.05 $M$ $NiCl_2$ and the following concentrations of imidazole: (a) 0, (b) 0.05 $M$, (c) 0.10 $M$, (d) 0.15 $M$, (e) 0.20 $M$, (f) 0.25 $M$. The magnetic moment for all these six solutions is the same, the value being 3.24 ± 0.05.

## Discussion

The concentrations of imidazole shown in Table IA are very high relative to the metal ion concentration ($5 \times 10^{-4}$ $M$), so that we may assume only the highest order complex exists in these solutions. The formula of this complex is, as we have seen, $NiIm_6^{++}$. It will be noticed that the half-wave potential of the nickel complexes is more positive than that of the hexaquo nickel ion. The reduction of the latter is irreversible, as shown by the abnormally large value of $(E_{3/4} - E_{1/4})$, but the complexes are reduced much more reversibly. This type of behavior has been cited by Kolthoff and Lingane[7] for the nickel complexes of thiocyanate and pyridine. Since the reduction of the hexaquo nickel ion is not reversible, we have not proceeded to calculate the over-all formation constant of the nickel complex from the polarographic data.

The individual formation constants of nickel complexes of imidazole are obtained by the $p$H method and are listed in Table III. There is almost a constant difference between the corresponding formation constants of the imidazole and ammonia complexes of nickel. It may be mentioned here that these $p$H measurements were made before the polarographic measurements. Since it is not possible to tell from the $p$H data what the highest

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(6) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

(7) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd. ed., Interscience Publishers, New York, N. Y., 1952, p. 486.

order complex is, at first we assumed it to be $NiIm_4^{++}$ and proceeded to use the Bjerrum method[6] of successive approximations in order to obtain the final values of $k_i$. After five successive approximations, the formation "constants" were still not constant and $k_4$ became higher than $k_3$. It was only after the coördination number is known from the polarographic data, that we were able to use the modified Scatchard method and obtain a reasonable set of formation constants.

It is seen in Fig. 1 that as the ratio of imidazole to total nickel is increased, the values of $\lambda_{max}$ become smaller and $\epsilon_{max}$ become larger. This shift toward shorter wave lengths as coördination number is increased is also observed by Klotz, *et al.*,[8] for copper(II) complexes. The absorption shown in Fig. 1, curve 2, where the molar ratio of imidazole to nickel ion is 0.2 is almost wholly due to $Ni^{++}$ and to the complex $NiIm^{++}$. From the formation constants given in Table III, it may be calculated that approximately 0.2 of the total nickel is in the form of this complex and 0.8 in the form of free nickel ion, the proportion of higher complexes present being negligible. At 650 mμ, which is approximately the absorption maximum, the extinction coefficient is 2.10. From Fig. 1, curve 1, $\epsilon = 1.81$ at 650 mμ for $Ni^{++}$. From these values of extinction coefficients we calculate the molar extinction coefficient for $NiIm^{++}$ as approximately 3.3 l. $mole^{-1}$ $cm.^{-1}$. It is interesting to note that the corresponding quantity for the $NiNH_3^{++}$ complex is 3.31 at the same wave length.

Magnetic susceptibility measurements show that no decrease in paramagnetism occurs on adding imidazole to nickel. This indicates that the bonds are essentially ionic and, since the coördination number is six, the configuration of the complex is octahedral.

Both the polarographic data in Table IB and the *p*H data indicate that the barium and calcium ions have only slight tendency to enter into complex formation with imidazole. This is in line with Bjerrum's findings[6] that ammine formation is negligible and that log $k_1$ for the calcium complex of ammonia is −0.2, and is also in line with the statement of Cohn, *et al.*,[4] that barium and calcium ions do not appear to form complexes with human serum albumin. Katz and Klotz[9] report binding of calcium ion by bovine serum albumin, and they state that the binding sites are the carboxyl groups, rather than the imidazole groups, of the protein molecule. Their conclusion of the nature of the binding site was drawn on the basis that calcium forms much more stable complexes with simple molecules containing carboxyl ions than with ammonia. The tacit assumption was made by them, therefore, that imidazole binding to calcium was approximately equivalent to ammonia binding, an assumption now shown to be quite accurate.

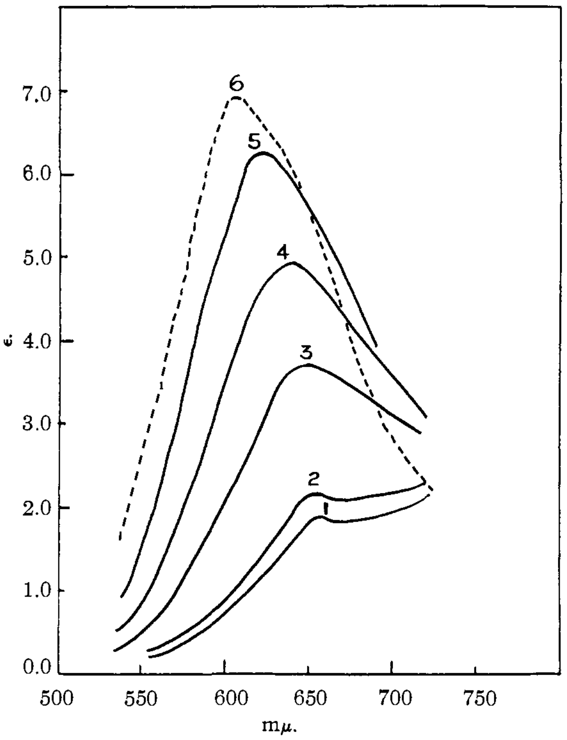

Fig. 1.—Absorption spectra of nickel imidazole complexes. In the following the first number indicates the molar ratio of imidazole to total nickel; where there is a second number, it is the value of −log (Im): curve (1), 0; curve (2), 0.2; curve (3), 1.3,2.62; curve (4), 2.2,2.14; curve (5), 3.6,1.67; curve (6), 7.8,1.19.

Schubert[10] reports that in the temperature range 3–40° the formation constant of alkaline earth–organic acid complexes is, within experimental error, nearly unchanged. The negligible temperature effect on formation constants of the alkaline earth complexes is in line with our finding that the complexing tendency of barium and calcium with imidazole is essentially the same at 25 as at 35°.

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PITTSBURGH, PA.

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(8) I. M. Klotz, I. L. Faller and J. M. Urquhart, *J. Phys. Colloid Chem.*, **54**, 18 (1950).

(9) S. Katz and I. M. Klotz, *Arch. Biochem. Biophys.*, **44**, 351 (1953).

(10) J. Schubert, *J. Phys. Chem.*, **56**, 113 (1952).