is not hydrolyzed by water. Consequently, it seemed of interest to find whether one might observe catalysis by the salt, hydronium.chloride, which presumably would be comparable in effectiveness to the two active catalysts of this research. It is assumed our failure to observe such catalysis implies that hydrolysis did occur. Possibly experiments at a lower temperature might be more successful.

Acknowledgment.—This research has been carried out under contract AT(45-1)-244 between the U. S. Atomic Energy Commission and Oregon State College.

CORVALLIS, OREGON

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF DUKE UNIVERSITY]

A Spectrophotometric Study of the Hydrolysis of Iron(III) Ion. II. Polynuclear Species[1]

BY RONALD M. MILBURN AND W. C. VOSBURGH

RECEIVED JULY 14, 1954

The hydrolysis of iron(III) ion has been investigated at 25° over a wide range of iron(III) concentrations and ionic strengths. The results give direct evidence for the importance of polynuclear iron(III) species at total iron(III) concentrations of 10^{-3} M or greater. The most reasonable postulate consistent with the data involves dimerization of the first hydrolysis product. The first hydrolysis and dimerization equilibrium constants have been determined over the ionic strength range studied. With increasing ionic strength the first hydrolysis constant decreases while the dimerization constant increases, in qualitative accord with Debye–Hückel theory. The absorption spectrum of the pure hydrated iron(III) ion has been redetermined through the range 320–360 mμ with care to avoid interference by polynuclear species.

An earlier report described a spectrophotometric determination of the first hydrolysis constant of iron(III) ion.[2] It was believed that polynuclear species would not be important in the 10^{-4} M iron(III) solutions used. The presence of polynuclear species in more concentrated solutions was suggested as an explanation for the failure of Bjerrum's method of corresponding solutions.

We have extended the work of Siddall and Vosburgh to cover a wide range of iron(III) concentrations. In accord with previous findings, optical densities of 10^{-4} M iron(III) solutions indicate that over the greater range of acidities studied the first hydrolysis is alone important. But results for 10^{-3} and 10^{-2} M iron(III) solutions cannot be explained on the basis of mononuclear species alone. The simplest postulate in agreement with experiment involves dimerization of the first hydrolysis product. This is in agreement with Hedström,[3] who assumes existence of the dimer to explain e.m.f. data on iron(III) perchlorate solutions. Hedström has evaluated equilibrium constants for the dimerization and first and second hydrolysis reactions at 25° and the high ionic strength of 3. In the present study equilibrium constants at 25° have been evaluated for the first hydrolysis and dimerization reactions over a wide range of ionic strengths. Our results for the ionic strength of 3 are in good accord with Hedström. However, the dimerization is strongly dependent on ionic strength, the constant decreasing to approximately one-fiftieth its former value as the ionic strength is reduced from 3 to zero.

Experimental

Materials.—Water was purified by refluxing ordinary distilled water with potassium dichromate and sulfuric acid for three to four hours and redistilling in an all-glass apparatus. Standard perchloric acid and sodium perchlorate solutions, prepared as previously described,[2] were used for the adjustment of hydrogen ion concentration and ionic strength. The solutions gave negative tests for chloride and iron(II) ions.

A stock iron(III) perchlorate solution was used for the preparation of other iron(III) solutions. Reagent grade hydrated iron(III) perchlorate was recrystallized from perchloric acid solution. The resulting crystals were filtered in a sintered glass crucible and some of the excess perchloric acid was washed away with water. The remaining iron(III) perchlorate was dissolved in water and the solution was analyzed for iron(III) by the iodometric method.[4] After diluting to approximately one tenth molar, the solution was reanalyzed for iron(III). The perchloric acid content was determined by titrating with standard sodium hydroxide to a phenolphthalein end-point. Toward the end of the titration, after each addition of alkali, the precipitated iron(III) hydroxide was allowed to settle and the color of the supernatant liquid was observed. The excess perchloric acid was calculated from the total alkali required and the known iron(III) concentration. The stock solution was 0.1002 M in iron(III) and 0.0122 M in perchloric acid.

Technique.—Optical density measurements were made with a model DU Beckman spectrophotometer, provided with a thermostat to allow the temperature of solutions to be held at 25 ± 0.1°. Most measurements were made in 10-cm. Corex cells or in 1-cm. quartz cells. For some solutions with large optical densities 0.9-cm. cell spacers were placed in the 1-cm. cells to give a path length of approximately 0.1 cm. The exact path length was determined by comparing the optical densities of a solution as measured with and without the spacers. Test cells were calibrated against blanks over the wave length range used. In no case did the optical densities vary sufficiently with wave length for the recorded value to depend on the slit width.

All measurements were made after the iron(III) perchlorate solutions, prepared from the stock iron(III) solution, had been allowed to mature for about five days. No evidence was found for changes with time after dilution, except when the acidity was too low and hydroxide ultimately precipitated. In this case the results were discarded. The time for maturing was allowed in view of changes in properties reported by other workers.[5-8]

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[1] From a thesis by R. M. Milburn in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Duke University.

[2] T. H. Siddall and W. C. Vosburgh, THIS JOURNAL, **73**, 4270 (1951).

[3] B. O. A. Hedström, *Arkiv Kemi*, **6**, 1 (1953).

[4] A. I. Vogel, "Quantitative Inorganic Analysis," Longmans, Green and Co., London, second edition, 1951, p. 356.

[5] A. B. Lamb and A. G. Jacques, THIS JOURNAL, **60**, 967, 1215 (1938).

[6] A. R. Olson and T. R. Simonson, *J. Chem. Phys.*, **17**, 1322 (1949).

[7] T. V. Arden, *J. Chem. Soc.*, 350 (1951).

[8] T. Ito and N. Yui, *Sci. Rep. Tohoku Univ.*, Series I, **37**, 19 (1953).

The Absorption Spectrum of the Hydrated Iron(III) Ion.—Rabinowitch and Stockmayer[9] developed the relation

$$\bar{e} = d/C = e_{Fe^{3+}} + e_1k_1/h_0 \qquad (1)$$

where $\bar{e}$ is the average absorption coefficient, d the optical density for a 1-cm. path, C the total iron(III) concentration, $e_{Fe^{3+}}$ the absorption coefficient of the hydrated iron(III) ion, e_1 the absorption coefficient of the $FeOH^{2+}$ ion, k_1 the first hydrolysis constant, and h_0 the hydrogen ion concentration of introduced perchloric acid. They used equation 1 to calculate $e_{Fe^{3+}}$ values from the optical densities of two solutions with different compositions but the same ionic strength.

To redetermine values of $e_{Fe^{3+}}$, optical densities were measured at two ionic strengths on iron(III) solutions of varying acidities. Results are plotted in Fig. 1. Although the two series agree well,[10] the linear relation predicted by equation 1 does not hold. The curvature cannot be explained by approximations in deriving equation 1.[11] One or more further hydrolysis products, including polynuclear species, could account for the curvature. However, in the hypothetical case when $1/h_0 = 0$, none of the iron(III) can be hydrolyzed. On this basis we identify $\bar{e}$ extrapolated to $1/h_0 = 0$ with $e_{Fe^{3+}}$.

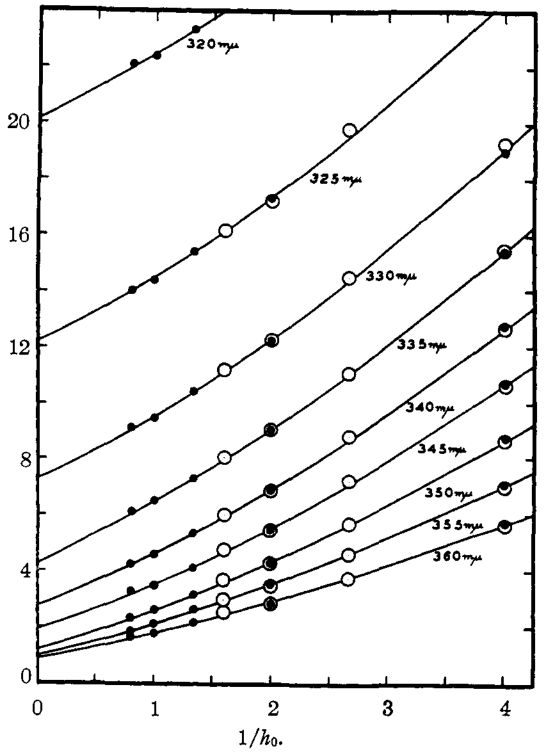

Fig. 1.—Test of equation 1 and determination of $e_{Fe^{3+}}$. All solutions 0.050 M in iron(III) perchlorate. Ionic strengths: points ○, 0.925; points ●, 1.55. Values of $e_{Fe^{3+}}$ from extrapolation of $\bar{e}$ to $1/h_0 = 0$: at 360 mμ, 0.90; 355 mμ, 1.00; 350 mμ, 1.20; 345 mμ, 1.94; 340 mμ, 2.84; 335 mμ, 4.30; 330 mμ, 7.30; 325 mμ, 20.2.

The First Hydrolysis.—Siddall and Vosburgh determined k_1 from the equation

$$1/d_h = 1/Ce_1 + [H^+]/Ce_1k_1 \qquad (2)$$

where d_h is the optical density of hydrolyzed iron(III) for a 1-cm. path. The approximations were made, $[H^+] = h_0$ and $d_h = d$. We have measured k_1 over an extended range of ionic strengths. Minor refinements account for the acid produced by hydrolysis and the contribution by the Fe^{3+} ion to the optical density.

Optical densities were measured at 340 mμ on 10^{-4} M iron(III) solutions. At each of four ionic strengths plots of $1/d$ against h_0 gave preliminary estimates of k_1. These were used to calculate α, the fraction of iron(III) as $FeOH^{2+}$ ion, from

$$\alpha^2C + \alpha(h_0 + k_1) - k_1 = 0 \qquad (3)$$

Corrections for absorption by the Fe^{3+} ion were made from

$$d_h = d - (1 - \alpha)Ce_{Fe^{3+}} \qquad (4)$$

Plots of $1/d_h$ against the corrected acidity, $[H^+] = h_0 + \alpha C$, allowed final estimates of k_1. Further successive approximation led to no change. The ionic strengths and k_1 values follow: $\mu = 0.0147$, $k_1 = 4.25 \times 10^{-3}$; $\mu = 0.040$, $k_1 = 3.64 \times 10^{-3}$; $\mu = 0.090$, $k_1 = 3.18 \times 10^{-3}$; $\mu = 1.07$, $k_1 = 1.62 \times 10^{-3}$. The first three values agree well with Siddall and Vosburgh, the fourth lies outside their range. The intercepts at $[H^+] = 0$, all identical within experimental limits, gave $e_1 = 925$. One solution at each of seven further ionic strengths was then measured, each solution being 10.45×10^{-3} M in perchloric acid. Equation 2 with $e_1 = 925$ was used to calculate k_1. The acidities and optical densities were corrected using equations 3 and 4. Results follow: $\mu = 0.101$, $k_1 = 2.89 \times 10^{-3}$; $\mu = 0.201$, $k_1 = 2.39 \times 10^{-3}$; $\mu = 0.301$, $k_1 = 2.14 \times 10^{-3}$; $\mu = 0.601$, $k_1 = 1.84 \times 10^{-3}$; $\mu = 1.00$, $k_1 = 1.60 \times 10^{-3}$; $\mu = 2.00$, $k_1 = 1.50 \times 10^{-3}$; $\mu = 3.00$, $k_1 = 1.28 \times 10^{-3}$.

In Fig. 2 we compare our results with others for 25°. Our values are summarized by

$$\log k_1 = -2.172 - 2.04\mu^{1/2}/(1 + 2.4\mu^{1/2}) - 0.01\mu$$

Except for $\mu > 1$, the term $0.01\,\mu$ may be neglected.

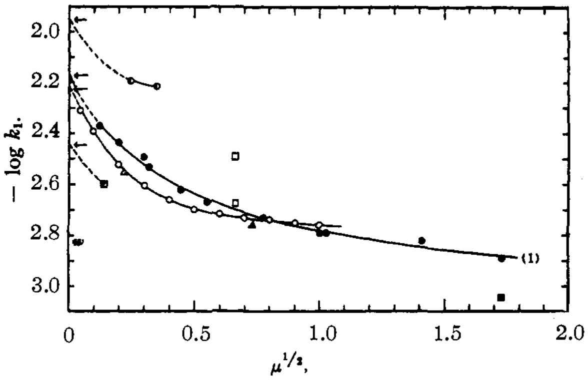

Fig. 2.—Comparison of our experimental values for the first hydrolysis constant with a curve (I) representing the semi-empirical expression $-\log k_1 = 2.172 + 2.04\,\mu^{1/2}/(1 + 2.4\mu^{1/2}) + 0.01\,\mu$ and with other reported values for 25°: present investigation, ●; Barb, Baxendale, George and Hargrave,[12] □; Bray and Hershey,[13] ○; Brosset,[14] ▲; Hedström,[3] ■; Ito and Yui,[8] ◌; Lamb and Jacques,[4] ◪; Lindstrand,[15] ◐; Olson and Simonson,[6] △.

Polynuclear Species.—Measurements at 340 mμ were extended to 10^{-3} and 10^{-2} M solutions. In Fig. 3 we plot $1/\bar{e}_h$ (equal to C/d_h) against $[H^+]$, with $\mu = 1.07$.[16] The points for the three iron(III) concentrations deviate widely from the single straight line predicted by equation 2. Mononuclear species alone would give coincident curves

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[9] E. Rabinowitch and W. H. Stockmayer, THIS JOURNAL, **64**, 335 (1942).

[10] A third series of measurements, at an ionic strength of 2.80, gave points falling slightly above the curves of Fig. 1, indicating that a perchlorate complex may be of some importance at high perchlorate ion concentrations. This is in qualitative agreement with J. Sutton, *Nature*, **169**, 71 (1952).

[11] The approximations made,[9] $[Fe^{3+}] = C$ and $[H^+] = h_0$, could only lead to curvature in the opposite direction.

[12] W. G. Barb, J. H. Baxendale, P. George and K. R. Hargrave, *Trans. Faraday Soc.*, **47**, 591 (1951).

[13] W. C. Bray and A. V. Hershey, THIS JOURNAL, **56**, 1889 (1934).

[14] C. Brosset, *Svensk Kem. Tidskr.*, **53**, 434 (1941).

[15] F. Lindstrand, *ibid.*, **56**, 251 (1944).

[16] Equation 3, with $k_1 = 1.62 \times 10^{-3}$, and equation 4 were used to calculate $[H^+]$ and $\bar{e}_h$ from h_0 and d.

for the three concentrations. Lack of coincidence is direct evidence for polynuclear species.[17]

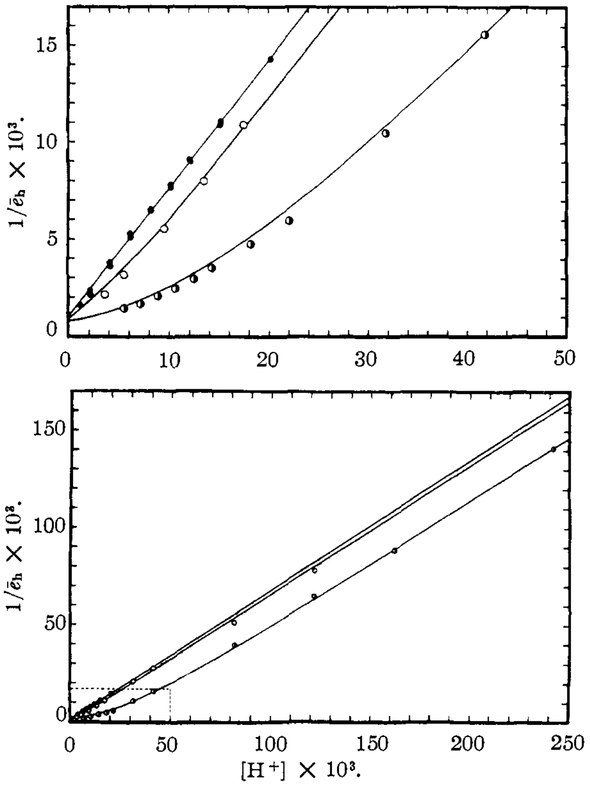

Fig. 3.—Comparison of experimental values of $1/\bar{e}_h$ for 340 mμ (indicated by points) with curves calculated using equation 10, ionic strength = 1.07, iron(III) concentrations: $C = 10^{-4}$ *M*, points ●; $C = 10^{-3}$ *M*, points ○; $C = 10^{-2}$ *M*, points ◑. The upper diagram is an enlargement of the area of the lower diagram bounded by broken lines.

The simplest assumption[18] accounting for the results is the reaction

$$2FeOH^{2+} \rightleftharpoons Fe(OH)_2Fe^{4+} \qquad (5)$$

With reaction 5 and the first hydrolysis alone important the following equations hold.

$$k_1 = [FeOH^{2+}][H^+]/[Fe^{3+}] \qquad (6)$$

$$k_d = [Fe(OH)_2Fe^{4+}]/[FeOH^{2+}]^2 \qquad (7)$$

$$C = [Fe^{3+}] + [FeOH^{2+}] + 2[Fe(OH)_2Fe^{4+}] \qquad (8)$$

$$d_h = e_1[FeOH^{2+}] + e_d[Fe(OH)_2Fe^{4+}] \qquad (9)$$

Eliminating the concentration terms $[Fe^{3+}]$, $[FeOH^{2+}]$ and $[Fe(OH)_2Fe^{4+}]$ from equations 6–9, we obtain

$$d_h^2 4k_dk_1^2 + d_h\{k_1^2(2e_1 - 4k_de_dC - e_d) + 2k_1h(e_1 - e_d) - e_dh^2\} + k_1^2C(e_de_1 - 2e_1^2 + k_de_d^2C) + k_1he_de_1C = 0 \qquad (10)$$

where h stands for $[H^+]$. With $k_1 = 1.62 \times 10^{-3}$ and $e_1 = 925$, the assumption of $e_d = 3.0 \times 10^3$ and $k_d = 7.5 \times 10^2$ leads to best agreement between calculated curves and experimental points. The comparison is shown in Fig. 3.

The disagreement at low acidities, which would be increased slightly if dimerization were considered in correcting the acidity, cannot be resolved without creating disagreement at high acidities. Small amounts of polymer from second hydrolysis product would account for the low points. Neglect of dimerization in the 10^{-4} *M* solutions used in the determination of k_1 and e_1 can be justified. As C approaches zero, equation 10 reduces to equation 2. But both these equations fit the experimental points for the 10^{-4} *M* solutions well.

There is considerable uncertainty in the values of k_d and e_d in best accord with the data, as the calculated curves are relatively insensitive to small changes in one constant if the other is suitably adjusted. We attach uncertainties of $\pm 2.5 \times 10^2$ to k_d and $\pm 1.0 \times 10^3$ to e_d.

Ionic Strength Dependence of k_d.—Measurements were made at 340 mμ on eight 10^{-2} *M* iron(III) solutions of different ionic strengths. All solutions were 41.2×10^{-3} *M* in perchloric acid. Introduction of k_1 and d_h into equation 10, with $e_1 = 925$, $e_d = 3.0 \times 10^3$, $C = 10^{-2}$ and $h = 41.7 \times 10^{-3}$,[19] allowed calculation of k_d. The values obtained were: $\mu = 0.10$, $k_d = 170$; $\mu = 0.20$, $k_d = 260$; $\mu = 0.30$, $k_d = 330$; $\mu = 0.40$, $k_d = 390$; $\mu = 0.60$, $k_d = 500$; $\mu = 1.00$, $k_d = 710$; $\mu = 2.00$, $k_d = 1160$; $\mu = 3.00$, $k_d = 1600$. The rapid decrease of k_d with decreasing ionic strength is in accord with Debye–Hückel theory. The expression

$$\log k_d = 1.46 + 4.08\mu^{1/2}/(1 + 2.1\mu^{1/2}) + 0.075\mu$$

describes k_d over the ionic strength range studied. The constant term corresponds to $k^0{}_d = 30$.

The Isosbestic Point.—It was observed by Olson and Simonson[6] and was confirmed by Siddall and Vosburgh[2] that an isobestic point exists for dilute iron(III) perchlorate solutions at about 273 mμ. We have measured optical densities in this region for 10^{-4}, 10^{-3} and 10^{-2} *M* iron(III) solutions. For each concentration the acidity was varied while the ionic strength was held at 1.07. Typical results[20] are shown in Fig. 4. The isosbestic point at 273 mμ is apparent for the 10^{-4} *M* solutions, but not for the 10^{-2} *M* solutions. This is confirmatory evidence for polynuclear species in the 10^{-2} *M* solutions.[21]

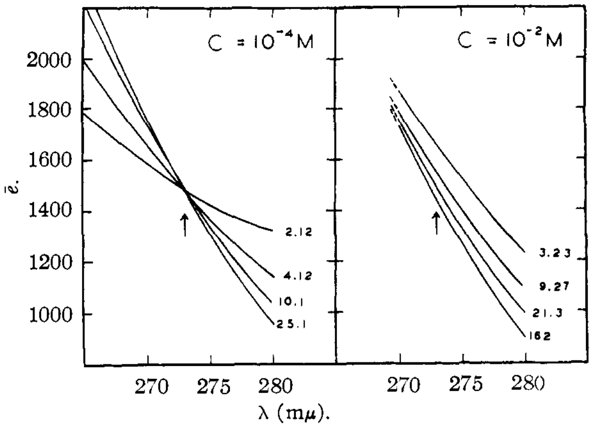

Fig. 4.—Average absorption coefficient for 10^{-4} and 10^{-2} *M* iron(III) perchlorate solutions as a function of wave length. The acidity in each series was varied while the ionic strength was held at 1.07. Acid concentrations in millimoles per liter are given beside each curve.

Conclusion

Evidence has been presented for polynuclear hydrolyzed iron(III) species in 10^{-3} and 10^{-2} *M* iron(III) solutions. The data are explained by dimerization of the first hydrolysis product according to reaction 5. This is in agreement with Hedström. The dimerization constant has been

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[17] Correction of the acidity for further hydrolysis would lead to slightly increased disagreement at low acidities.

[18] For the reaction $Fe^{3+} + FeOH^{2+} \rightleftharpoons Fe(OH)Fe^{5+}$, no values assumed for the equilibrium constant, and for the absorption coefficient of the $Fe(OH)Fe^{5+}$ ion, give calculated curves fitting the experimental points. Curvature in the wrong direction is found at high acidities.

[19] In view of the uncertainty in e_d, an average value of h for the eight solutions was used.

[20] Results for 10^{-3} *M* solutions were intermediate to those for 10^{-4} and 10^{-2} *M* solutions.

[21] For mononuclear species $\bar{e}$ would be independent of the total iron(III) concentration, although it would be a function of hydrogen ion concentration. But larger acidities were used for the 10^{-2} *M* solutions (refer to Fig. 4, noting that the additional acid produced by hydrolysis would be greater for more concentrated iron(III) solutions).

determined by Hedström at 25° for the ionic strength of 3, a value of $15(\pm 2) \times 10^2$ being obtained.[22] At this temperature and ionic strength we find k_d to be $16(\pm 5) \times 10^2$. However, we find the dimerization is strongly dependent on ionic strength, becoming much less important at lower ionic strengths.

For the determination of the first hydrolysis constant 10^{-4} M iron(III) solutions of moderately large acidities were used. Under these conditions polynuclear species can be neglected. A comparison between reported values for the first hydrolysis constant at 25° has been given in Fig. 2. The values obtained by Brosset,[12] Bray and Hershey,[13] Olson and Simonson,[6] and Hedström,[3] are in good or reasonable agreement with us. All these workers were dealing with iron(III) solutions under true equilibrium conditions, and with the exception of Hedström, who took polynuclear species into account, all used iron(III) solutions that were sufficiently dilute to avoid serious errors from polynuclear species.

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[22] In terms of the constants reported by Hedström, k_d is given by $x_{22}/(x_{11})^2$.

[CONTRIBUTION FROM THE ARMY MEDICAL SERVICE GRADUATE SCHOOL, WALTER REED ARMY MEDICAL CENTER]

Infrared Spectroscopy of Human Hemoglobins. I. The Effects of Varying *p*H and *p*D on the Spectra Obtained in the Solid and Liquid Phase

BY DANIEL L. LARSON AND JOSEPH MCLAUGHLIN, JR.

RECEIVED SEPTEMBER 2, 1954

Infrared absorption spectra have been obtained on normal adult human hemoglobin in the dried and liquid phases. Sixteen peaks have been studied over the range of 3.1 μ (3220) to 9.1 μ (1100), and compared when run under acid, neutral or alkaline conditions. In the *liquid* phase dissolved in *deuterium oxide* the peaks at 3.1, 3.4, 4.7, 6.15, 6.9 and 7.65 μ showed no change; the peaks at 5.8 and 6.5 μ increased in intensity in acid *p*D; and the peaks at 6.35 and 7.15 μ increased in intensity at alkaline *p*D. In the infrared spectrum of human hemoglobin from *dried* films cast from *water* solutions the peaks at 3.1, 3.4, 6.15, 6.5, 6.9, 7.65 and 8.6 μ showed no change; the peaks at 3.3, 5.8 and 8.1 μ increased in intensity at acid *p*H; and the peaks at 7.2 and 9.1 μ increased in intensity at alkaline *p*H. In the infrared spectrum of human hemoglobin from *dried* films cast from *deuterium oxide* solution the peaks at 3.1, 6.15, 7.65 and 8.6 μ showed no change; the peaks at 3.4, 4.15, 5.8, 6.9 and 8.1 μ showed increased intensity at acid *p*D; and the peaks at 6.5, 7.2 and 9.1 μ showed increased intensity at alkaline *p*D.

In the course of the rapid development of applied infrared spectroscopy over the last decade, many substances of biologic interest have been examined. However, relatively little information has appeared in the literature on the infrared characteristics of proteins. Technically, it has been difficult to obtain satisfactory patterns on proteins in solutions except by use of deuterium oxide as the solvent.[1] The patterns obtained on dried protein films cast from watery solutions are usually a mixture of varying degrees of denatured and undenatured proteins. And, finally, the interpretation of infrared patterns of proteins is made difficult due to the large number of infrared-sensitive groupings in the molecules.

Recent observations have established that the position and intensity of absorption peaks in the infrared analysis of proteins may be influenced by the *p*H or *p*D of the material under examination. In the study of the Raman spectra of amino-acids, Edsall, Otvos and Rich have shown[2–4] that the disappearance of the carbon double bond oxygen frequency at 5.8 μ (1745 Kaysers)[5] on ionization, and the replacement by a lower frequency near 7.12 μ (1400), is characteristic of ionized carboxyl groups.

In the examination of dried deuterated protein films, Lenormant has shown[6] that the absorption band at 6.45 μ (1550) shifts to 6.9 μ (1450), but this shift is incomplete.

Ehrlich and Sutherland noted[7] that upon the addition of base, the band at 5.87 μ (1703), characteristic of un-ionized carbonyl groups, is replaced by a band at 6.4–6.45 μ (1560–1550), and by another less prominent band at 7.1 μ (1410). They observed that in the examination of isoionic bovine plasma albumin in deuterium oxide, the band present at 6.4 μ (1560) disappears upon the addition of DCl and is replaced by a shoulder on the low wave length side of the band at 6.08 μ (1655) simultaneous with a disappearance of the shoulder at 7.13 μ (1405). Upon the addition of NaOD, however, the bands at 6.45 μ (1550) and 7.1 μ (1405) were restored. These variations in the spectra were attributed to changes in ionization of the carboxy groups.

Lenormant and Blout observed[8] the presence of the band at 6.35 μ (1575) in alkaline heavy water, and that the band disappeared in acid heavy water; however, the band could be made to reappear on bringing the *p*D back to 9 or 10. Similar, but less marked, changes occurred at the 7.15 μ (1400) region. The band at 6.45 μ (1550) remained upon acidification and disappeared in alkaline solution. This change was said to be irreversibly completed. It was also noted that when deuterium solutions of albumin, gamma globulin or ovalbumin were heated to 100° for 2 minutes, the 6.45 μ (1550) band disappeared. They suggested that this behavior could

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(1) R. C. Gore, R. B. Barnes and E. Peterson, *Anal. Chem.*, **21**, 382 (1949).

(2) J. T. Edsall, J. W. Otvos and A. Rich, THIS JOURNAL, **72**, 474 (1950).

(3) J. T. Edsall, *J. Chem. Phys.*, **4**, 1 (1936).

(4) J. T. Edsall, *ibid.*, **5**, 508 (1937).

(5) The term "Kayser" is identical with the term "reciprocal centimeter." See Joint Commission for Spectroscopy, C. J. Bakker, *J. Opt. Soc. Amer.*, **43**, 410 (1953). Throughout the remainder of the paper, the number in parentheses following the wave length refers to Kaysers or reciprocal centimeters.

(6) H. Lenormant, *Ann. chim.*, **383**, 459 (1950).

(7) G. Ehrlich and G. B. B. M. Sutherland, *Nature*, **172**, 671 (1953).

(8) H. Lenormant and E. R. Blout, *ibid.*, **172**, 770 (1953).