A SPECTROPHOTOMETRIC STUDY OF THE CHELATE OF IRON (III) WITH SORBOHYDROXAMIC ACID AND ITS ANALYTICAL APPLICATION

CHUEN-YING LIU (劉春櫻) AND PENG-JOUNG SUN (孫炳榕)
Department of Chemistry, National Taiwan University

(*Received Sept. 22, 1975*)

Iron (III) chelates of sorbohydroxamic acid have been studied spectrophotometrically in aqueous solution. Formation of sorbohydroxamic acid chelates was *p*H dependent. The "normalized absorbance" concept proposed by Likussar and Boltz has been utilized in the continuous variation plot method of determining the composition of metal chelates and the formation constants. The mole ratio of sorbohydroxamic acid to ferric ion was found to be 1 to 1 stoichiometry at *p*H lower than 2. The formation constant of this chelate has been evaluated to be 6.0×10^{11} at a constant ionic strength of $0.1\,M$ at 30°C. A spectrophotometric determination of ferric ion with sorbohydroxamic acid was proposed in conjunction with the present work.

Complexation of metal ions by hydroxamic acids is the basis of a number of analytical determinations[1]. The best known of these complexes is the one with Fe^{+3}, the beautiful purple color of which forms the basis for the sensitive qualitative and quantitative determination of carboxylic acids and their derivatives[2].

Recent progress in hydroxamic acid chemistry has been stimulated the synthesis of a number of medicinally active hydroxylamine derivatives[3].

Sorbohydroxamic acid was highly effective in controlling the growth of food fungi[4]. It was also studied as an antiseptic agent for blood preservation[5]. Selective inhibition by sorbohydroxamic acid of deoxyribonucleic acid synthesis in Ehrlich as'cites tumor cells was also reported[6]. All of these properties may be attributed partly to the presence of the conjugated double bond system in the C_6 aliphatic chain[6] and its chelate-forming properties.

Since the ferric ion is the principal inorganic constituents of the blood plasma, it is interesting to investigate the properties and behaviors of sorbohydroxamic acid toward ferric ion for the better understanding of biological importance of sorbohydroxamic acid.

EXPERIMENTAL

Reagents:

A. Synthese of reagents

The starting material, ethylsorbate, has been prepared from sorbic acid (Fluka

J. Chinese Chem. Soc., 23. 219-227 (1976)

AG. Buchs SG. made in Switzerland) and absolute ethanol using an ordinary esterification method. Ethylsorbate was purified by distillation under reduced pressure (b. p. 87-89°*C*, 18 *mmHg*). The method of Blatt[7] was used to prepare a methyl alcohol solution of the potassium salt of the desired hydroxamic acid, and the free hydroxamic acid was obtained by the method of Wise and Brandt[8]. Sorbohydroxamic acid was obtained as a white scale with m. p. of 122-123°*C* which was in accordance with literature[9].

B. Perchlorates of Fe^{+3} were obtained from the G. F. Smith Chemical Co., stock solutions of this material (about 0.1 *M* in metal ion) were standardized by EDTA titration[10] All other chemicals used in the experiment were of analytical reagent grade.

Instrument:

A Hitachi model 624 digital spectrophotometer connected with Hitachi model QD_{15} recorder and 10-*mm* quartz cells were used for all absorbance measurements. The *p*H of the solution was measured by Radiometer titrator equipped with saturated calomel (Type K 401) and glass (Type G 202B) electrodes. The electrodes were checked against Merck Buffer-Titrisol (*p*H 4.00 and 7.00).

RESULTS AND DISCUSSION

Absorption spectra of sorbohydroxamic acid

Its ultraviolet spectrum in acid solution had a single absorption maximum at 262 *nm*. In alkaline solution, the maximum absorption shifted to 255 *nm* as shown in Fig. 1.

The *p*Ka of sorbohydroxamic acid is determined according to the following equation

$$pKa = pH + \log\frac{d_I - d}{d - d_M}$$

Where d_I is the absorbance of the ion, d_M is the absorbance of the molecule, and d is the absorbance of the mixture of ion and molecule at the same analytical wavelength. The *p*Ka value was obtained by calculation from at least seven sets of experimental values of absorbance at various degrees of ionization. The analytical wavelength for determination of *p*Ka was set at 300 *nm* where ionic form of reagent has maximum absorbance and molecular form of reagent has almost no absorption, thus, at which wavelength the greatest difference in optical density between ion and molecule is observed. The reagent concentration of 4.0×10^{-5} *M* was used for the determination of *p*Ka. The ionic strength and the *p*H of the solution were adjusted with sodium perchlorate, perchloric acid and sodium hydroxide. The *p*Ka obtained is 8.80±0.01 at ionic strength of 0.1 and room temperature (30°*C*), which was in accordance with the literature[9].

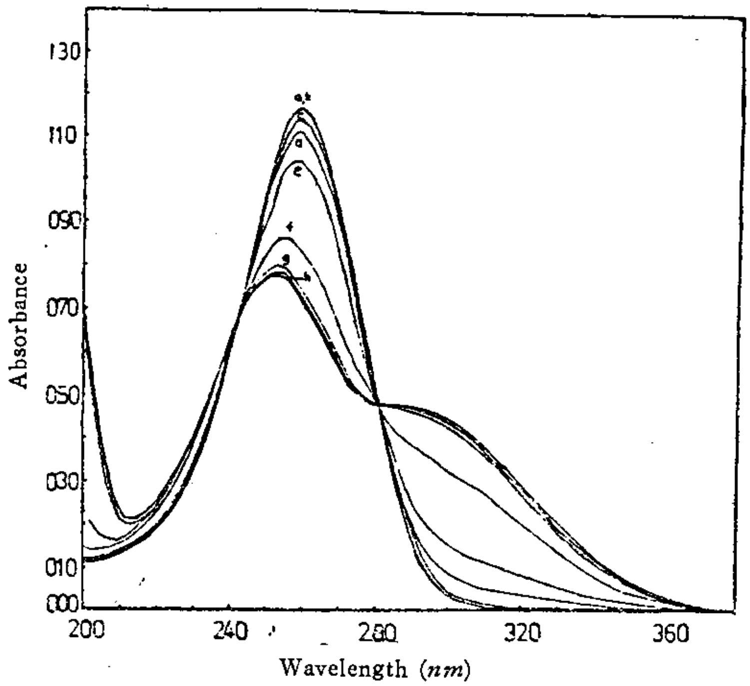

Fig. 1. Absorption spectra of sorbohydroxamic acid at different *p*H.
$C_R=4.0\times10^{-5}M$ $\mu=0.1M$
*p*H: a=2.75, b=3.25, c=6.95, d=7.91, e=8.37, f=9.22, g=10.08, h=10.49, i=11.48

Absorption spectra of the sorbohydroxamic acid chelate of iron (III)

For the formation of ferric chelate, the hydronium ion concentration plays an important role on the stability as well as the composition of these chelates.

In the case of ferric system, it was observed that the absorption band was shifted to the longer wavelength with the decreasing *p*H as illustrated in Fig. 2 and Fig. 3, which suggested the existence of chelate species of different compositions. In the solution below *p*H 2.0, there existed only one to one chelate.

Effect of sorbohydroxamic acid on the absorbance of iron (III) chelate

When the concentration of metal ions and the *p*H were kept at constant, the change in the concentration of sorbohydroxamic acid might have effect on absorption of the mixture. For iron (III) chelate, as the concentration of ligand increased to an amount more than three times that of metal ion, the absorption band shifted gradually toward shorter wavelength as shown in Fig. 4.

Composition and stability constant of ferric-sorbohydroxamic acid

I. Determination by Likussar's and Boltz's method[11]

A new theoretical and generalized approach to the method of continuous variation in which no approximations are necessary was employed for the determination of the conditional constant of metal complexes.

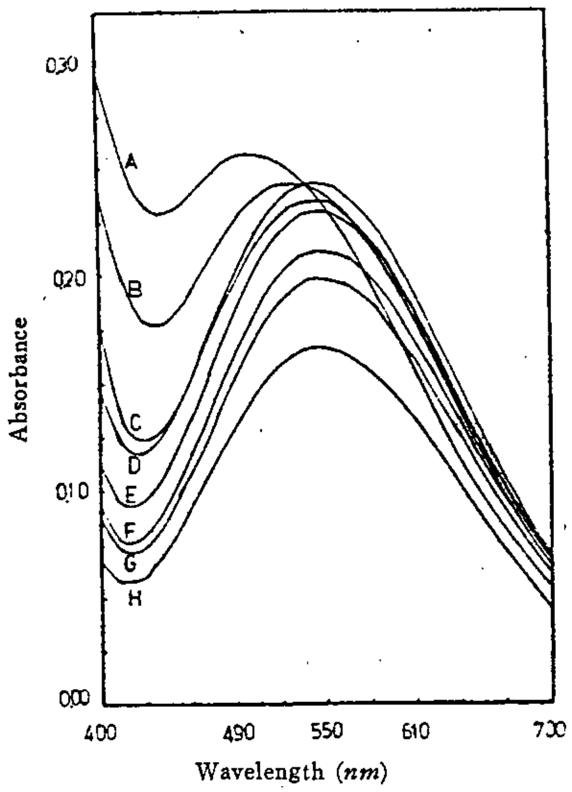

Fig. 2. Absorption spectra of ferric-sorbohydroxamic acid complex at different pH.
Composition: $C_M=4.0\times10^{-4}M$, $C_R=2.0\times10^{-4}M$
pH: A=3.78, B=3.17, C=3.00, D=2.70, E=2.38, F=2.12, G=1.98, H=1.75

The continuous variation plot for ferric-sorbohydroxamic acid complex employing a normalized absorbance scale was shown in Fig. 5. It is a 1:1 complex.

The conditional constant of the chelate was determined according to the following equation.

$$K_c=\left[\frac{(m+n)}{C_M+C_R}\right]^{(m+n-1)}\cdot Y\cdot[(m+n)x-mY]^{-m}\cdot[(m+n)(1-x)-nY]^{-n}$$

Where m, and n represent the number of moles of metal and ligand, respectively, and

$$x=\frac{C_M}{C_M+C_R},\qquad Y=\frac{[A_T-(A_T)_R]}{[(A_T)_{max}-(A_T)_R]}$$

$(A_T)_R$: the absorbance of the reference solution.

$(A_T)_{max}$: It is obtained by measuring the absorbance of a solution containing a definite excess of ligand and a metal concentration $(C_M)_{max}$. The metal concentration selected for $(C_M)_{max}$ is equivalent to that corresponding to the mole fraction of metal at the stoichiometric composition of complex—i. e., the maximum in the Y $vs.$ mole fraction plot.

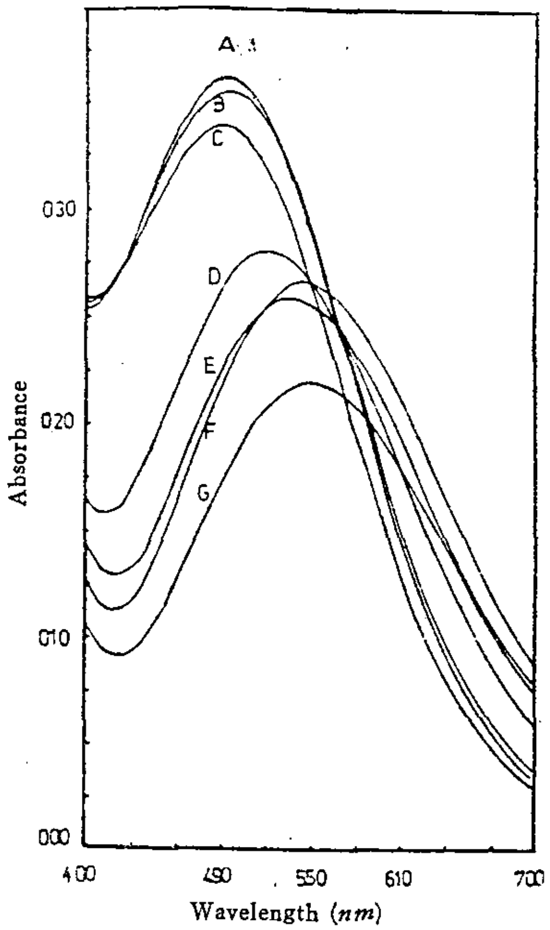

Fig. 3. Absorption spectra of ferric-sorbohydroxamic acid complex at different *p*H.
Composition: $C_M=2.0\times10^{-4}M$, $C_R=4.0\times10^{-4}M$,
*p*H: A=4.12, B=4.08, C=4.00, D=3.15, E=2.73, F=2.51, G=2.10

The total molarity of the system. C_M+C_R, was $4.0\times10^{-4}M$ at *p*H 1.8. The conditional constant obtained is 6.0×10^4. The fraction of free sorbohydroxamic acid present as anion form, L, in the experimental condition is

$$\alpha=\frac{[L]}{C_{HL}}=\frac{K_a}{[H^+]+K_a[H^+]}$$

The formation of metal-sorbohydroxamic acid chelates can be represented by $M^{3+}+L^-=ML^{2+}$, Hence, the formation constant

$$K_f=\frac{[ML^{2+}]}{[M^{3+}][L^-]}=\frac{[ML^{2+}]}{[M^{3+}]C_{HL}\cdot\alpha}=\frac{K_c}{\alpha}=6.0\times10^{11}$$

II. Determination of stability constant by log-ratio method

Since only the 1:1 complex of ferric and sorbohydroxamic acid presented in water at lower *p*H, the stability constant was determined by the log-ratio method

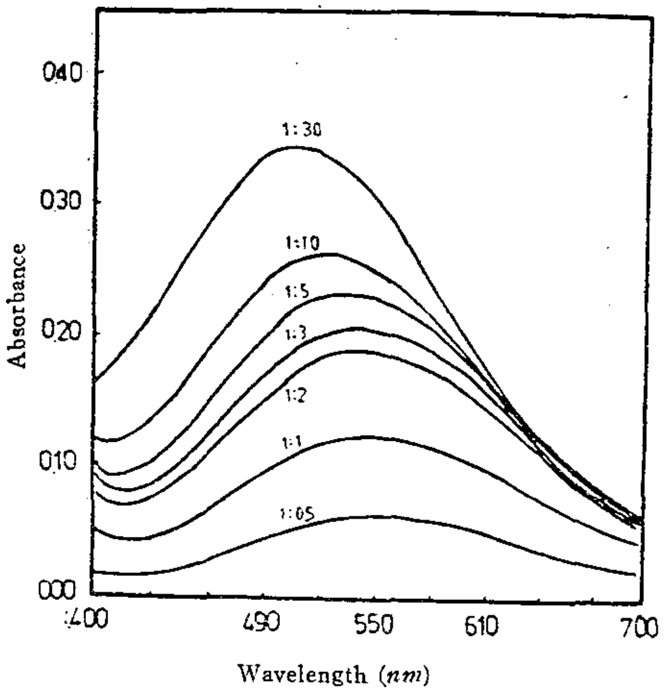

Fig. 4. Effect of sorbohydroxamic acid concentration on the absorbance of ferric-sorbohydroxamic acid chelates. $\mu=0.1M$, $p\text{H}=1.8$, $C_M=8.0\times10^{-5}M$

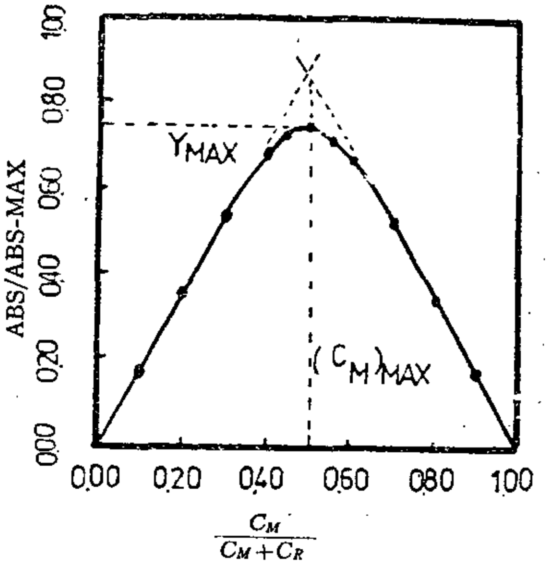

Fig. 5. Continuous variations plot for ferric-sorbohydroxamic acid complex.
($Y_{max}=0.75$), $C_M+C_R=4.0\times10^{-4}M$, $p\text{H}=1.8$, $\lambda=550\ nm$

proposed by Diehl and Lindstrom[12]. Because the determination of stability constant is carried out at $[H^+]=0.6\,M$ and the acidic nature of the ligand is very weak, so that its proton is released on chelation. The apparent stability constant of complex at a given pH's, K_h is related to the following equation in the log-ratio method, that is

$$\log\frac{[MnL]}{[HL]}=n\log[M]+\log K_h$$

The plot of $\log[MnL]/[HL]$ *vs.* $\log[M]$ gives the straight line with slope n, the combining ratio and intercept, K_h.

The experimental work was carried out with a series of the solution containing the same total concentration of $2.0\times10^{-4}\,M$ sorbohydroxamic acid and the different concentration of ferric ion at $[H^+]=0.6\,M$ at 30°*C*. The results are shown in Fig. 6. The combining ratio obtained by the log-ratio method was found to be 1.06 indicating one to one chelate is formed at the given condition. The result was in good agreement with that obtained by continuous variation plot. The apparent stability constant of one to one chelate formed by sorbohydroxamic acid and ferric ion is found to be $K_h=10^{3.20}$ at $[H^+]=0.6\,M$. The stability constant,

$$K_f=\frac{[K_h\cdot H^+]}{K_a}=6.0\times10^{11}$$

which was in accordance with the previous result.

Analytical application

The following procedure may be proposed for the spectrophotometric determination of ferric ion with sorbohydroxamic acid:

To a suitable aliquot containing 33.5-558.5 *μg* of ferric ion is added an excess of reagent (2.5 *ml* of $4.0\times10^{-3}\,M$). The pH of the solution is adjusted to 1.8 with 0.10 *N* perchloric acid solution. Total volumes of the aqueous are then made to 50 *ml* by the addition of water. The absorbance is measured at 550 *nm* and the amount of ferric ion is obtained from the calibration curve drawn under identical conditions.

Fig. 7 shows the concentration-absorbance plots at 550 *nm* at pH 1.8. The concentrations of sorbohydroxamic acid in the experiment were fixed at $2.0\times10^{-4}\,M$. The ferric-sorbohydroxamic acid system conforms to Beer's law over the concentration range 0.67-11.17 *μg/ml*.

Effect of foreign anions and cations in the determination of ferric ion has been studied by preparing synthetic solutions containing known amounts of ferric ion and varying amounts of foreign ions. Using 4.47 *ppm* of ferric ion, it was found that the following ions, present in amounts in *ppm* shown in parentheses, did not cause deviation of more than ±2% in absorbance.

Sulfate (50), chloride (200), nitrate (250), phosphate (20), fluoride (1), oxalate (0.2), Pb (II) (0.4), Zn (II) (44), Mn (II) (106), Cu (II) (1.3), Ni (II) (102), Co (II) (40).

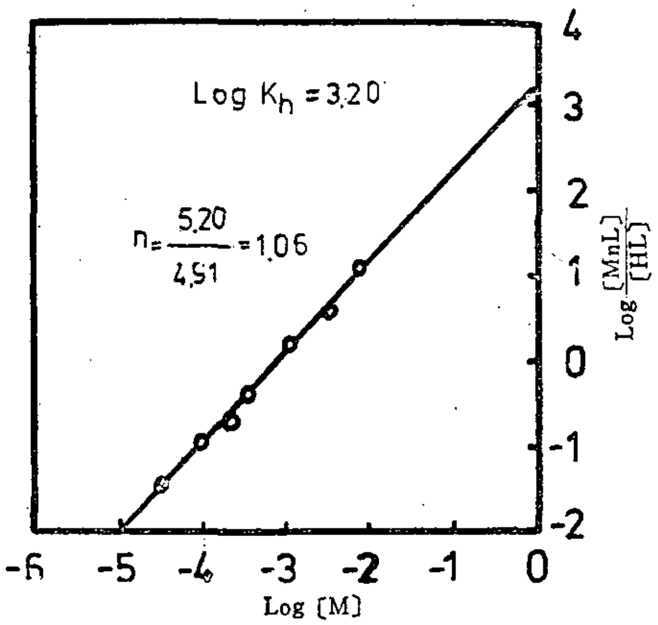

Fig. 6. Log-ratio method plot for ferric and sorbohydroxamic acid system at $[H^+]=0.6M$, $\mu=0.1M$, $\lambda=550\,nm$ and 30°C.

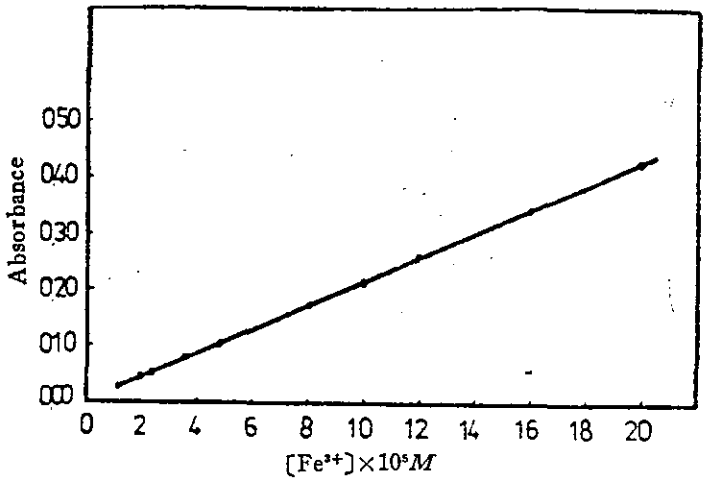

Fig. 7. Validity of Beer's law.
$\mu=0.1M$, $C_R=2.0\times10^{-4}M$, $pH=1.8$

ACKNOWLEDGEMENT

This work was supported in part by the National Science council of the Republic of China, to which thanks are due.

REFERENCES

(1) T. J. King and P. G. Harrison, *J. Chem. Soc. Chem. Comm.*, 815 (1972).
(2) D. W. Knight and G. H. Cleland, *J. Chem. Educ.*, 47, 781 (1970).
(3) R. T. Coutts, *Can. J. Pharm. Sci.*, 1, 27 (1967).

(4) **J. A. Troller and R. A. Olsen,** *J. Food Sci.*, **32**(2), 228 (1967).

(5) **A. I. Smirnova-Shitova and L. S. Shulutko,** Mater. Resp. Se'zda Gematol. Transfuziologov Belouiss., 2nd, 194 (1973); C.A., **82**, 70862z (1975).

(6) **G. R. Gale, A. B. Smith and E. M. Walker, Jr.,** *Cancer Res.*, **30**(1), 24 (1970); C.A., **72**, 109377r (1970).

(7) **A. H. Blatt,** "Organic Syntheses", Vol. II, John Wiley and Sons, Inc., New York, N.Y., p. 67 (1944).

(8) **W. M. Wise and W. W. Brandt,** *J. Am. Chem. Soc.*, **77**, 1058 (1955).

(9) Commonwealth Scientific and Industrial Research Organization, Anstralian Appl. June 13, p. 15 (1961); C.A., **60**, 413e (1964).

(10) **G. Schwarzenbach and H. Flaschka,** "Die Komplexometrische Titration", 5th Ed., Ferdinand Enke Verlag, Stuttgart, Germany (1965).

(11) **W. Likussar and D. F. Boltz,** *Anal. Chem.*, **43**, 1265 (1971).

(12) **H. Diehl and F. Lindstrom,** *Anal. Chem.*, **31**, 414 (1959).