Metal Chelates of Tyrosine-I-Nitroso-2-Naphthol by Spectrophotometry

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Stability constants of the 1 : 2 Metal Chelates of Tyrosine-1-Nitroso-2-Naphtho with divalent metal ions: Mg(II), Fe(II), Ni(II), Cu(II), and Co(II) are reported at the room temperature. The stability increase in the sequence: Mg (II) 〈Fe(II) 〈Co(II) 〈Cu(II) 〈Ni(II), in the alkline media.

In the earlier papers, [1,2,3] Tyrosine-1-Nitroso-2-Naphthol complex and Tyramine-1-Nittroso-2-Naphthol complex have been reported as a method of the quantitative analysis of the tyramine. The work describes similar study, but this investigation is connected with some metals. These metallic chelates are studied by the optical densities decreasing, according to be used amount of metals, in other words, within the limited value the Lambert-and Beer's Law is satisfied, and also the expected wavelength appears. 1-Nitroso-2-Naphthol shows the maximum wavelenth at 380±10mu., and Nitroso-radical electron configuration tells there is at least one unpaired electron make a charge transfer band.[4,5] The charge transfer electron occures from a n (oxygen) to a π^*(nitrogen): (n——π^*)

Tyrosine-1-Nitroso-2-Naphthol complex shows the maximum peak at wavelength

(1) Sidney Udenfriend, and J.R. Cooper,: J. Biol. Chem., 196,227 (1952)
(2) Clark, C.T. and Udenfriend, S.: J. Biol. Chem., 210, 139 (1954)
(3) Gerngross, O. Voss, K. and Herfelt, T., Ber. Chem. Ges., 66, 435 (1933)
(4) Theory and Applications of Ultraviolet Spectroscopy, by H.H. Jaffe and Milton Orchin,; John Wiley and Sons, Inc. (1962), chapter 9, 18.
(5) Molecular Orbital Theory, by C.J. Ballhausen and H.B. Gary., W.A. Benaamin, Inc. chapter 8, 92-105

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420±10 mu. and the metallic chelates show different wavelength, according to what sort of of metal being used and also it is very important for PH of the ligand solution.

Experimental

Reagents: 1-Nitroso-2-Naphthol, Matheson Coleman & Bell, Norwood, Ohio; Tyrosine, Cobaltous acetate, Magnesium acetate, Nickel acetate, Nickel acetate, and Copper acetate, Fisher Scientific Company, St. Louis, Missouri; Ferrousammonium sulfate, Scientific product, Evanston, Illinois; EDTA sodium salt, Sigma Chemical company, St. Louis Missouri.

Spectrometer: Beckman Model DB-G grating spectrophothometer. For measurment of cell aqueous soutions approximately $1cm^2$ cell and 0.025mm were used as a slit of the spectrometer. The accuracy of frequency deter-mination was ±0.5% in the spectral ranges studied and also machine itself's error.

PH measurement: Beckman Model G PH meter. Calibration of the PH reading was made with standard buffer solutions which was purchased from the Fisher Scientific company, error ±0.05 at 25°C.

Reagents preparing: 1-Nitroso-2-Naphthol, 0.001M; Tyrosine, 0.001M; Mg(II) 0.00 1M; Co(II), 0.001M; Cu(II), 0.001M; Cu(II), 0.002M; Ni(II), 0.01M; and Fe (II), 0.0024M. All reagents were dissolned in distilled water that was ion-exchanged resin passed water.

Results and Discussions

I. Complex formation of Tyrosine-1-Nitroso-2-Naphthol.

It is named the Tyrosine-1-Nitroso-2-Naphol complex(TNN). 1-Nitroso-2-Naphthol is dissolved in 25% alcohol, it gives a strong band at 380 mu., and Tyrosine is dessolved in 0.01M sodium hydroxide, it gives a strong band at 430mu. Above regents were reacted in the constant temperature water bath for 30 minutes, which temperature was 37°C.

If these reagent react as an equimolary, it is possible to have the following equation.

$aN+bT \rightleftharpoons NaTb=C \cdots\cdots (1)$

where aN is the concentration of 1-Nitroso-2-Naphthol,

bT is the concentration of Tyrosine,

C is the concentration of the complex (TNN)

$K=[N]^a \times [T]^b/[C] \cdots\cdots\cdots (2)$

where K is the instability constant

$$K=\frac{(0.17316\times10^{-3})\ (0.18119\times10^{-3})}{(0.35436\times10^{-3})}=0.0886\times10^{-3}\ \text{mole/liter}$$

If an equimolar of 1-Nitroso-2-Naphthol (0.001M) and Tyrosine (0.001M) concentration (S) mixed in proportion is $X:(1-X)$

$xS=[N]+a[C] \rightleftharpoons C_N \ \cdots\cdots (3)$

$(1-X)S=[T]+b[C]=C_T \cdots\cdots (4)$

By the calculation, 1-Nitroso-2-Naphthol reacts with Tyrosine 1:1 1000:999.5 so it reacts as the 1 : 1 complex. Molar absorptivity of the complexes at the wavelength 420mu, as we see through Fig. 3.

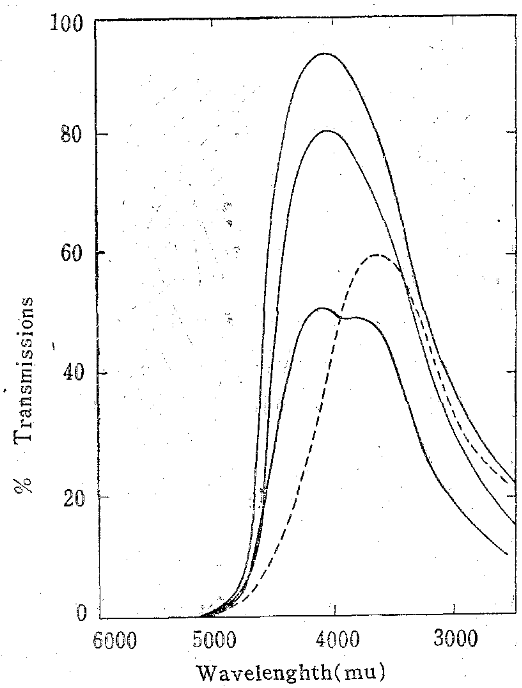


Fig 3) Spectra of 1-Nitroso-2-Naphthol(···)
Tyrosine-1-Nitroso-2-Naphthol(—)

$$eC=\frac{A}{b\times c}\times\frac{1000cm^2}{mole}\cdots\cdots\cdots(5)$$

where eC is the molar absorptivity and b is the light pass length. The C is the concentration of the complex.

Equilibrium of complex formation is the following equation;

$$aN+bT\rightleftharpoons Na-Tb\cdots\cdots\cdots(6)$$

$$K=\frac{[Na-Tb]}{[N]^a[T]^b}\cdots\cdots\cdots(7)$$

$$K=C/(C_N-C)(C_T-C)\cdots\cdots\cdots(8)$$

where C is the concentration of the complex(TNN), C_N, C_T, is the initial concentration both of Tyrosine and 1-Nitroso-2-Naphthol, and K is the equilibrium constant of TNN.

$K=11.29\times10^3$ mole/liter

Therefore, equation (5) and (8) give the following equation:

$$\frac{C_Nb}{A}=\frac{1}{C_T\ K_eC}+\frac{1}{eC}\cdots\cdots\cdots(9)$$

The above equation tells that a plot of C_Nb/A versus $1/C_T$ should be linear with Slope $1/KeC$ and intercept $1/eC$, figure 8.

$$\frac{C_Nb}{0.086}=\frac{1}{C_T\ KeC}+\frac{1}{eC}\cdots\cdots\cdots(10)$$

Absorbance	Concentratio of Tyr. M/L	eC(1×10^6)	C_N mole/liter
0.086	0.1812×10^{-3}	0.23	0.17316×10^{-3}
0.041	0.3624×10^{-3}	0.12	
0.022	0.5436×10^{-3}	0.06	

II. Metal chelates of the Mg (II)-TNN

The stability constant of the Mg(II)-TNN complex is able to figure out from the following equilibrium reaction;

$$TNN+Mg(II)\rightleftharpoons Mg(II)-TNN\cdots\cdots\cdots(11)$$

$$K=\frac{[Mg(II)-TNN]}{[TNN][Mg(II)]}\cdots\cdots\cdots(12)$$

where K is the stability constant of the Mg(II)—TNN complex, Therefore,

$K=1.18\times10^3$ mole/liter

Molar absorptivity of the Mg(II)—TNN complex at the wavelength 600±10mu., as it is shown at Fig.4 from the equation (5) is able to calculate.

$$\frac{C_{TNN}B}{A}=\frac{1}{C_{Mg(II)-TNN}}+\frac{1}{eC}\cdots\cdots\cdots(13)$$

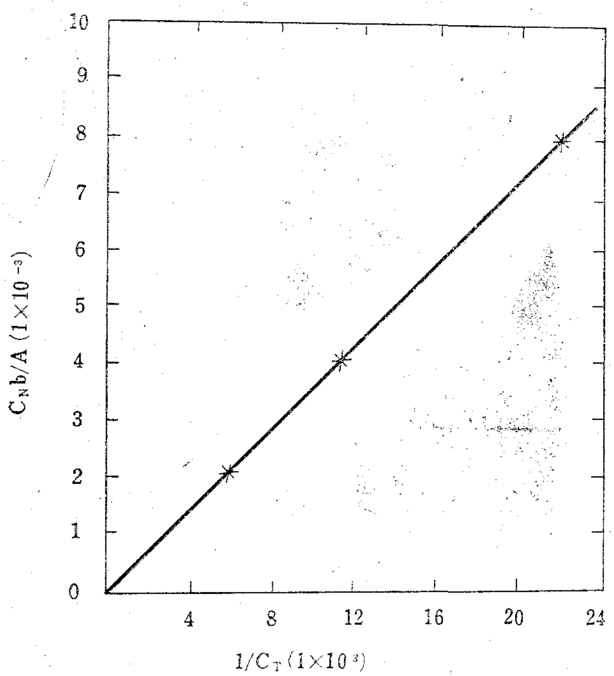


Fig. 8 $C_N b/A$ as a function of $1/C_T$ in Complex formation ofTyr.-1-Nitroso -2-Naphthol at the Wavelength 420±10 mu.

Absorbance	Concentration of Mg(II) M/L	$eC\ (1\times10^{6})$	C_{TNN} mole/liter
0.099	0.1826×10^{-3}	0.18	0.35435×10^{-3}
0.078	0.3624×10^{-3}	0.15	
0.061	0.5435×10^{-3}	0.11	

A plot of $C_{TNN}b/A$ versus $1/C_{Mg(II)}$ should be linear, it shows at Fig. 9. In this metal chelate complex with the ligand, TNN's concentration is 0.35435×10^{-3} mole/liter and the initial metal concentration is 0.1826×10^{-3} mole/liter, for molar absorptivity.

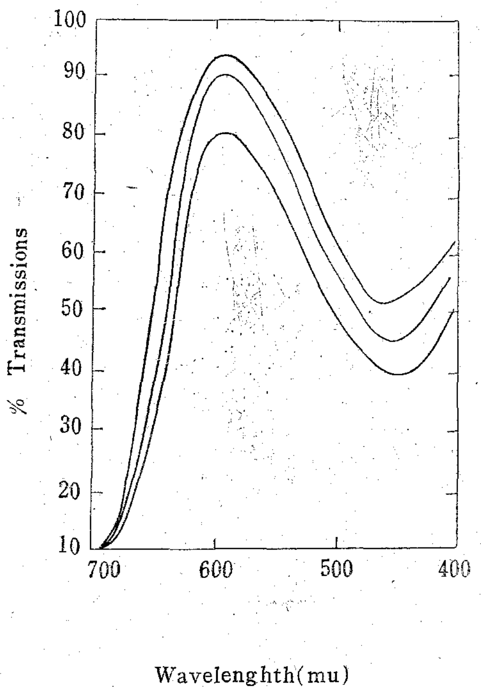


Fig. 4 Metal Mg(II) Chelate with Tyrosine-1-Nitroso-2-Naphthol Complex

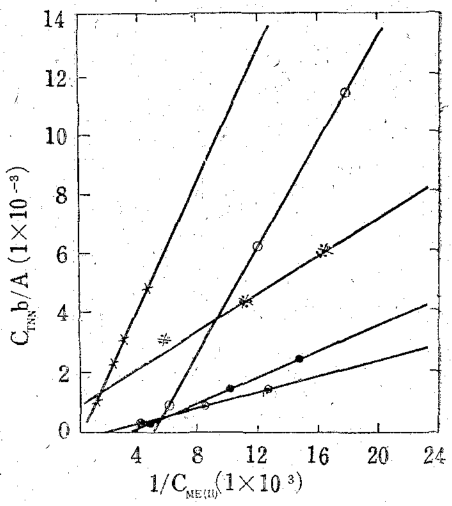


Fig. 9 $C_{TNN}b/A$ as a function of $1/C_{Me}$(II) in Complex formation of Me(II)-TNN at the wavelength Mg(II)-TNN(*) 600 mu Ni(II)-TNN(0); 390mu; Cu(II)-TNN (•) 400 mu; Co(II)-TNN(⊗) 400mu: Fe(II)-TNN (×) 600 mu.

III. Metal chelate of the Ni(II)-TNN

The stability constant of the Ni(II)—TNN complex is able to figure out from the following chemical equation;

TNN+Ni(II)$\rightleftharpoons$Ni(II)—TNN.........(14)

By the Mass Action Law, the stability constant of the Ni(II)—TNN complex is able to calculate: K=8.73$\times10^{3}$ mole/liter.

Molar absorbptivity of the Ni(II)—TNN complex at the wavelength 390$\pm$10mu. Fig.5 from the equation (5) is able to calculate. The concentration of the this metal chelate is 0.5237$\times10^{-3}$ mole/liter. From the equation (9), the following relationship is being made. A plot of $C_{TNN}b/A$ versus $1/C_{Ni(II)}$ should be linear, it shows at Fig. 9.

$$\frac{C_{TN}b_N}{A}=\frac{1}{C_{Ni(II)}KeC}+\frac{eC}{1}\cdots\cdots(16)$$

Absorbance	Concentration of Ni(Ⅱ) M/L	eC (1×10^6)	C_{TNN} mole/liter
0.387	0.1693×10^{-3}	0.73	0.35435×10^{-3}
0.056	0.3386×10^{-3}	0.11	
0.031	0.5079×10^{-3}	0.06	

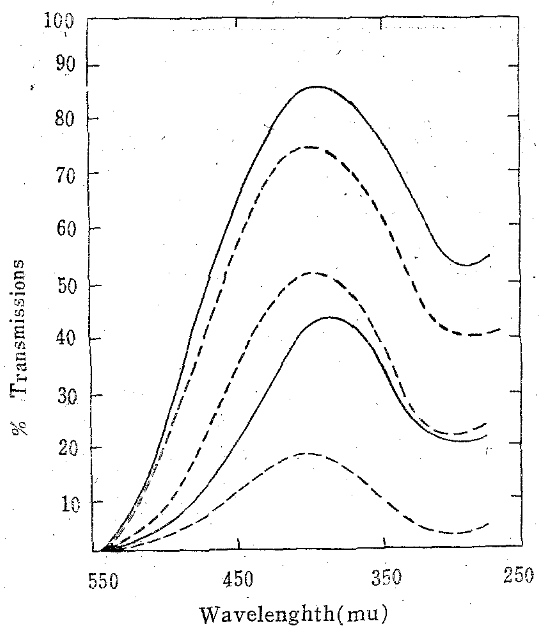


Fig 5) Spectra of Metal Chelates Ni(Ⅱ)-TNN (—); Cu(Ⅱ)-TNN(···)

IV. Metal chelate of the Cu(II)-TNN

The stability constant of the Cu(II)-TNN complex is able to figure out from the following equilibrium reaction;

$$TNN+Cu(II)\rightleftharpoons Cu(II)TNN\cdots\cdots(17)$$

The stability constant of the Cu(II)−TNN complex, $k=7.7\times10^3$ mole/liter.

From the equation (9), the following relationship is accepted for a plot of $C_{TNN}b/A$ versus $1/A$ versus $1/C_{Cu(II)}$ It gives a straight line, which is shown by the Fig. 9.

$$\frac{C_{TNN}b}{A}=\frac{1}{C_{Cu(II)}KeC}+\frac{1}{eC}\text{.........}(19)$$

Absórbance	Concentration of Cu(Ⅱ) M/L	$eC(1\times10^6)$	C_{TNN} mole/liter
0.745	0.2023×10^{-3}	1.3	0.35×10^{-3}
0.276	0.4045×10^{-3}	0.5	
0.125	0.6067×10^{-3}	0.2	

Molar absorbance coefficient of the *Cu*(II)—TNN complex at the wavelength 400 ±10mu. (Fig.5) from the equation (5) is able to get. The concentration of Cu(II)—TNN chelate is 0.5567×10^{-3} mole/liter.

V. Metal chelate of the Co(II)—TNN

The solubility constant of the Co(II)—TNN complex is able to calculate from the following equilibrium reaction;

$$\text{TNN}+\text{Co(II)}\rightleftarrows\text{Co(II)}-\text{TNN}\text{.........}(20)$$

The stability constant of the Co(II)—TNN complex, $K=6.8\times10^3$mole/liter. Molar absorptivity of the Co(II)—TNN complex at the wavelength 400± 10mu..(Fig.6) from the equation (5) is able to be get. Where the concentration of the Co (II)-TNN complex is 0.5946×10^{-3} mole/liter. From the equation (9), the following formular is able to get.

$$C_{TNN}\ b/A=1/C_{Co(II)}\ KeC+1/eC\text{.........}(22)$$

Absorbance	Concentration of Co(Ⅱ) M/L	$eC\ (1\times10^6)$	C_{TNN} mole/liter
0.770	0.2401×10^{-3}	1.3	0.35435×10^{-3}
0.469	0.4804×10^{-3}	0.9	
0.208	0.7206×10^{-3}	0.4	

A plot of $C_{TNN}b/A$ versus $1/C_{Co(II)}$ should be a linear, it shows at the Fig. 9

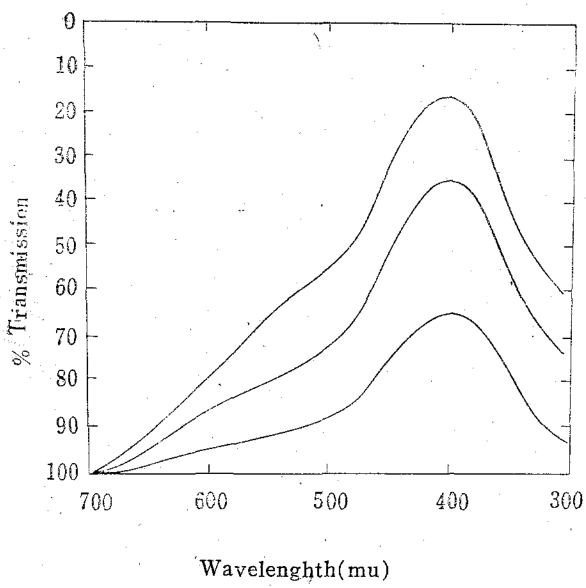


Fig. 6 Spectra of Co(Ⅱ)-TNN Complex at the bands 400mu& 500mu.

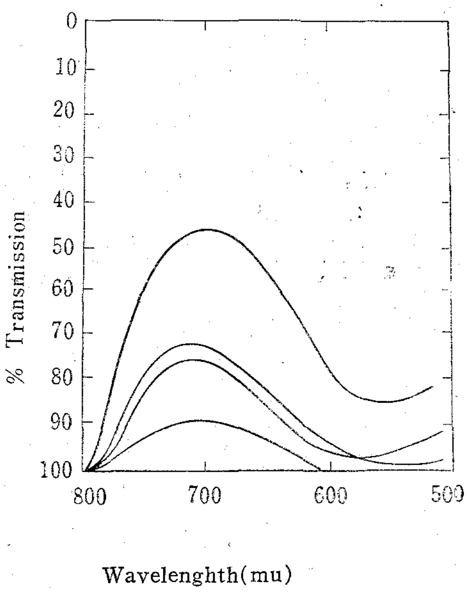


Fig. 7 Spectra of Metal Chelate Fe(Ⅱ)-TNN Complex

VI. Metal chelate of the Fe(II)—TNN

The stability constant of the Fe(II)—TNN complex is able to figure out from the ollowing reaction;

TNN+Fe(II)$\rightleftharpoons$Fe (II)—TNN.........(23)

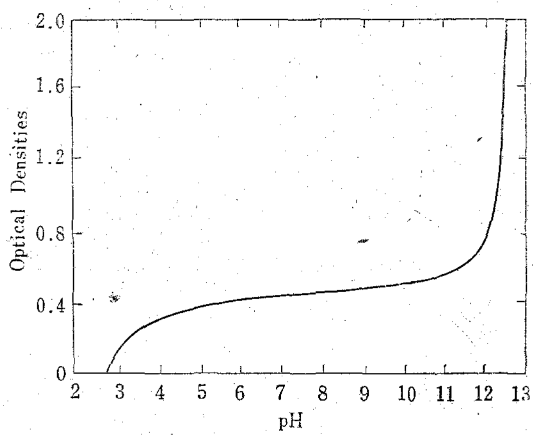
The stability constant of the fe(II)—TNN complex, $K=3.92\times10^{3}$ mole/liter Molar absorptivity of the Fe(II)—TNN complex at the wavelenghth 600$\pm$10mu. (Fig.7) from the equation (5) is able to be get. Where the concentration of the complex is 1.3172×10^{-3} mole/liter. From the equation (9)

$C_{TNN}b/A=1/C_{Fe(II)}KeC+1/eC$.........(25)

Absorbance	Concentration of Fe(Ⅱ) M/L	$eC(1\times10^{6})$	C_{TNN} mole/liter
0.264	0.96284×10^{-3}	0.2	0.35435×10^{-3}
0.146	1.9256×10^{-3}	0.1	
0.111	2.8884×10^{-3}	0.08	

A plot of $C_{TNN}b/A$ versus $1/C_{FeII)}$ should be a linear, it shows at Fig. 9

Through the above results, it is necessary to think about Molecular Orbital relationship between the central metals and the lagands. First of all, Nitroso group (—NO) has 11 valence electrons, so it has at least one unpaired elec̵tron. [6,7,8] It transfers to π-orbital position/and be a anti-bonding formed. Therefore it says that metal-to-Ligands charge transfer occure instead of Ligand-to-metals. These metal complexes are stable in π-orbital. It is able to explaine by the Crystal Field Theory and Molecular Orbital Theory. These metal ions' electron configuration are d^6,d^7, d^8, and d^9 system. Through the crystal field energy stabilzation, the stability order is Fe (II) 〈Co(II) 〈Cu(II) 〈Ni(II). All the metals react with ligands in alkaline



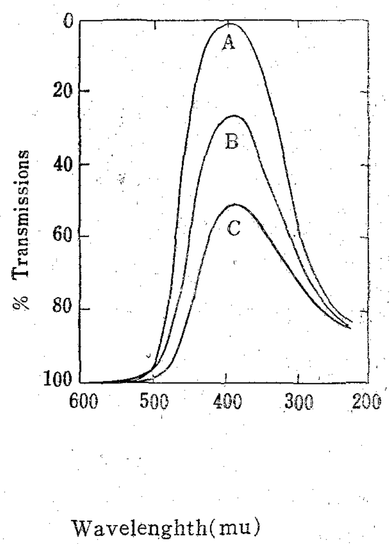


Fig. 2 Optical densities of 1-Nitroso-2-Naphthol as a function of the pH, at the waveleng hth 380 ±10mu (25°C)

Fig. 1) Spectra of 1-Nitroso-2-Naphthol at a various pH ranges
A=pH 12.6, B=pH 11.0, C=pH 3.5

6. L. Pauling, The nature of the chemical band, 2nd edition, p 266-271, Cornell University Press, Ithaca (1940)
7. H. L. Johnston and X. F. Gianque, J. Am. Chem. Soc., 51, 3194 (1929)
8. E. Lips, Helv. Phys. Acta., 8, 247 (1935)

solution: PH 8.0—12.

Through the Fig.1 and Fig. 2, the dissociation or Ionization constant is calculated.[9] The results are, $pK_1=4.05$, $pK_2=10.2$ $pKa=8.92\times10^{-5}$ $pKb=1.45\times10^{-11}$.

In the case of these metals, they are π-bonding complexes. Therefore, the color of transition metal complexes come from an excitation of an electron from a lower level to a higher. So it is able to achieved by the absorption in range of visible light. From the early reporter, Tyrosine and Phenylalanine are related with mental disease as a hereditary. Through this investigation, TNN-Metal (II) complex is stable in a body fluid like solution. Therefore, the metal complex does not divided i a body and the mental hereditary is able to prevent possibly.

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9. Theory and Applications of ultraviolet spectroscopy, H. H. Jaffe and Milton Orchin. (1964). Chapter 20, p. 560-572