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Determination of the Stability Constants (log K) for Fe-Fulvic and Fe-Humic Acid Complexes with $^{55}Fe^{3+}$

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Abstract. Stability constants of complexes formed between Fe and either fulvic or humic acids, which were isolated from podzolic soil and characterized by physical and chemical means, were determined by using a $^{55}Fe^{3+}$ radiotracer technique. Both the ion exchange-equilibrium method and the continuous variations method were applied at pH 5 and ionic strength $\mu = 0.1$.

Log K values for Fe-fulvic and Fe-humic acids complexes were 6.06 and 9.75, respectively. The stoichiometric composition x for these complexes was 1.00 for Fe-fulvic acid and 1.36 for Fe-humic acid.

A relationship appeared to exist between the stoichiometric composition of the complexes formed and the concentrations of oxygen-containing functional groups in fulvic and humic acids.

Introduction

Measurements of the metal-complexing ability of polyfunctional macromolecules in soil organic matter in terms of stability constants (log K) of metal complexes would help soil scientists to predict the behaviour of these compounds with regard to soil genesis and plant nutrition. Through its ability to form complexes with insoluble inorganic forms of metal ions, soil organic matter contributes to plant nutrition by increasing the availability of mineral nutrients [1, 2, 3, 4, 5].

In the literature there are numerous studies on the determination of stability constants of fulvic and humic complexes with Cu^{2+}, Ni^{2+}, CO^{2+}, Ca^{2+}, Al^{3+}, etc.

The objectives of this investigation were: **a)** To prepare fulvic and humic acids in the laboratory and determine some of their physical and chemical properties, and **b)** to determine stability constants of Fe-HA and Fe-FA complexes by using the radioactive $^{55}Fe^{3+}$ method which prevents the precipitation of these complexes at pH 5 or higher, by allowing very low Fe^{3+} concentration to be measured.

Materials and Methods

Preparation of Fulvic and Humic acids

The fulvic acid FA and humic acid HA were obtained from the A_1 horizon of the Roche des Podzol (Vosges-France), containing about 10% organic C. The main physico-chemical characteristics of this horizon were described by Fares [1].

Procedures for the extraction, separation and purification of FA and HA are described in Fig. 1.

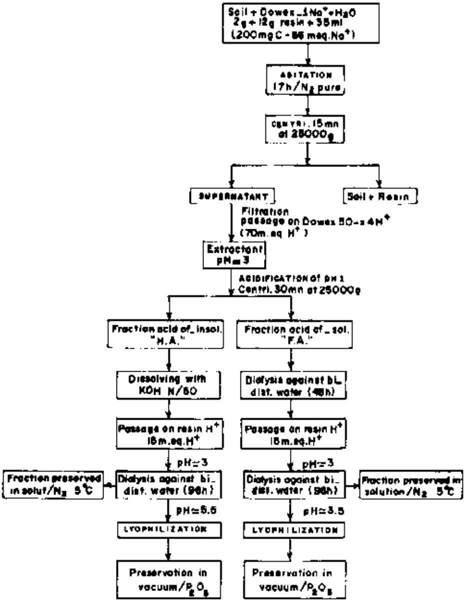

Fig. 1. Extraction & purification of F.A. & H.A.

Physical and chemical characterization of isolated FA and HA

Ultimate analysis (C,H,N and O)

C,H,N and O were determined simultaneously on the same sample of FA or HA an automatic C,H,N and O analyzer. The distribution of the major elements in the isolated HA and FA are shown in Table 1.

Table 1. Ash free composition (%) of FA and HA.

Element *Substance	C	H	N	O
HA	52.18	4.36	1.83	39.21
FA	44.91	5.98	1.24	45.82

*lyophilized and dried at 100°C for 24 h.

Elemental analysis provides useful information on the distribution of the major elements in FA and HA as shown in Table 1, the predominant elements are carbon and oxygen. Fulvic acid contains less carbon and nitrogen than humic acid; but the former contains more oxygen and hydrogen than the latter. Consequently the complexing ability of FA with metal ions should be higher than that of HA. Most of the oxygen in humic substances, specially in FA (> 90%) is present in COOH groups; phenolic-OH and $> C = O$ groups account for most of the remaining oxygen [1, 6].

Analysis of functional groups

Total acidity: Total acidity was determined by the method originally developed by Brook and Strenhell [7] and Schnitzer and Gupta [3].

Carboxyl group (–COOH): The calcium acetate method was used for determining –COOH) groups in FA and HA [3, 8].

Phenolic hydroxyl (–OH) groups: Phenolic hydroxyl groups were calculated by difference in the following manner:

$$\text{M. eq. total acidity} \quad \text{m. eq. –COOH groups} \\ \text{m. eq. phenolic –OH.} \qquad (1)$$

Carboxyl groups ($\begin{matrix}R_1\\R_2\end{matrix}\!>\!C{=}O$): The method of Fritz *et al.* [9] was used. It is based on allowing FA or HA to react with an excess of hydroxylamine in an ethanol medium.

Table 2. Oxygen-containing functional groups in FA and HA

Substance	Total acidity*	–COOH*	–OH (phenolic)	$\begin{matrix}R_1\\R_2\end{matrix}\!>C=O$ (carboxyl)
HA	6.93	4.10	2.83	4.41
FA	13.90	9.82	4.08	1.05

*Results in m eq/g. of FA or HA (dry and ash-free)

The data in Table 2 show the distribution of oxygen containing functional groups in isolated FA and HA. The total acidity of FA is higher than that of HA, while $\begin{matrix}R_1\\R_2\end{matrix}\!>C=O$ carboxyl groups are present in larger amount in HA. The acidity of exchange capacity of soil humic and fulvic substances is due mainly to the presence of ionizable hydrogen in the aromatic and aliphatic–COOH and in phenolic–OH groups. These functional groups have been shown to participate in metal-complexing. Other groups such as sulfonic acid ($-SO_2OH$), phosphoric acid ($-PO(HO)_2$), sulfhydryl–SH and amine–NH_2 exhibit a strong affinity for metal ions, but they are present only in trace amounts in humic substance [10].

Measurement of stability constants (log K)

To determine stability constants of organo metallic complexes, the method of continuous variations (CV) and the ion-exchange equilibrium method (IEE) can be used in the following manner:

Let C_1, C_2 and C_3 be the concentration of M, R and MR_2 respectively. For any mixture the following equations apply at equilibrium.

$$C_1\ (1-X)C_1^0 - C_3 \text{ and not } C_1 = (1-X) - C_3 \qquad (2)$$

$$C_2 = XC_2^0 - 2C_3 \text{ and not } C_2 = X\text{-}2C_3 \qquad (3)$$

where C_1^0 and C_2^0 are respectively the initial concentration of C_1 and C_2 in the solutions that are added together.

The complex formation or stability constant K is then:

$$K = \frac{C_3}{(X-2C_3)^2\,(1-X)-C_3} = \frac{C_3}{C_1 \,.\, C_2^2} \tag{4}$$

The application of this method leads to K determination if the concentration of the complexing agent R of (FA or HA) is known.

Ion-exchange reaction

The method of ion-exchange reaction was developed by Schubert [11] and first applied to soil organometallic complexes by Courpron [12], Fares [13] Miller and Ohlrogge [14] and Randhawa and Broadbent [3]. This method is suitable only for mononuclear complexes of the type MR_X, where M is the metal ion, R the ligand and X the number of moles of R, X must be an integer $(X\,\underline{\lambda}1)$.

According to the simplified treatment of Martell and Galvin [15] the equilibrium reaction for complex formation can be written as:

$$M + xR \rightleftarrows MRx \tag{5}$$

where M is the metal ion, R the complexing agent and x the number of moles of complexing agent which combines with one mole of metal M. The numerical value of the equilibrium constant K is:

$$K = \frac{(MR_x)}{(M)\,(R)^x} \tag{6}$$

When aplying this equation to Fe-FA or Fe-HA complexes, it was noticed that neither (R) nor X was known. The principle of ion-exchange equilibrium was used in conjunction with the resin-exchange technique to determine formation and stability constants.

Let λ_0 = distribution constant of the metal between solution and resin in the absence of a complexing agent (FA or HA), λ = the distribution constant of the metal in the presence of a complexing agent. Both λ_0 and λ should be measured under similar conditions.

$$(M) = \frac{M_r}{\lambda_0} \tag{7}$$

where M = the quantity of metal cation bound to a defined amount of a cation-exchange resin at equilibrium λ expresses the relationship between total metal cation species in solution (M) + (MR_x) and on the resin (M_r).

Thus,

$$(M) + (MR_x) = \frac{M_r}{\lambda} \tag{8}$$

or

$$\lambda = \frac{M_r}{(M) + (MR_x)}$$

Combining (7) and (8),

$$(XR_x) = \frac{M_r}{\lambda} - \frac{M_r}{\lambda_0} \tag{9}$$

Rearranging Equation (9) gives

$$(MR_x) = M_r \frac{1}{\lambda} - \frac{1}{\lambda_0} = \frac{M_r}{\lambda_0} (\frac{\lambda_0}{\lambda} - 1) \tag{10}$$

The equilibrium constant may then be written:

$$K = \frac{(\frac{\lambda_0}{\lambda} - 1)}{(R)^x} \tag{11}$$

or

$$\log (\frac{\lambda_0}{\lambda} - 1) = \log K + x \log (R) \tag{12}$$

Although, x and (R) are unknown, by measuring λ at more than one value of (R), x may be obtained directly from the slope of a plot of log [(λ_0/λ) −1] versus log of relative values of (R), since (R) unknown. Once the value of x is known and the maximum complexing capacity (MCC) of fulvic or humic acids is determined, the concentration of complexing sites (R) can be calcualted see [3, 12].

Analytical and practical limitation of log K measurement

The pH effect

The formation of organo-metallic complex (MR) involves the displacement of hydrogen ions (H^+) from FA or HA as follows:

$$M^{n+} + HmR \xrightarrow{\qquad} MR^{n-m} + mH^+$$

FA and HA contain a certain number of acidic groups and hydrogen ions are displaced into the solution. The magnitude of the pH change when metal ions are added is related to the binding tendency of the metallic complexing agent and to the stoichiometry of the complex formed. When larger amounts of metals are added to FA or HA in the course of log K or stoichiometric composition (x) measurements, portions of these metals form a complex with FA or HA, and the excess would precipitate in the form of basis salts. However, the addition of very small amounts of Fe^{3+} at relatively low pH (3.5) would lead to the precipitation of FA or HA. Therefore, measurement of log K or x of these complexes is difficult.

A radioisotope technique using $^{55}Fe^{3+}$ was found to be very useful for measurements of low concentrations of $^{55}Fe^{3+}$ using $^{55}Fe^{3+}$ with very high specific activity (S.A. = 1 m G/mg).

$^{55}Fe^{3+}$ decay by electron capture process is as follows:

$$^{56}_{26}Fe + -1^{0}e \xrightarrow{\qquad} {}^{55}_{25}Mn + hv$$

The only detectable radiation are X-rays emitted as result of orbital rearrangement.

Effect of ionic strength "μ"

The stoichimetric composition X_n(FA/Fe or HA/Fe ratio) and stability constant of any complex are affected by the ionic strength as well as by the pH of the medium. Thus the value of μ should remain constant while measuring the stability constant. It is likely that the μ of the soil solution ranges between 0.05 and 0.15 mol L^{-1} [16] so that the log K values determined at close to $\mu = 0.1$ mol L^{-1} are more relevant than those measured at other values of μ. The ionic strength can be written as:

$$\mu = \tfrac{1}{2} \sum_{i=1}^{n} (C_i Z_i^2)$$

where C_1, C_2 and C_n are concentrations in mol.L^{-1}. Z_1, Z_2 and Z_n valences of ionic species in the solution.

To measure log K and x_n, 5 cm^3 of M KCl were added to a total volume of 50 cm^3. In this case, the ionic strength value is $\mu = 0.1$.

Analytical procedure

Determination of λ and λ_0

λ_0 and λ were calculated from the following expression:

$$\lambda_0 = \frac{\alpha_0}{100 - \alpha_0} \cdot \frac{V}{Q_r}$$

also

$$\lambda = \frac{\alpha_\alpha}{100 - \alpha} \cdot \frac{V}{Q_r}$$

where α_0 and α are percent of total metal bound to exchange resin in the absence and in the presence of complexing agent (FA or HA) respectively.

V = volume of solution = 50 cm^3 in this case
Q_r = weight of exchange resin = 1 gram

Determination of the concentration of complexing sites

Once the stoichiometric composition x_n for the Fe-FA and Fe-HA complexes was established, the next step was to determine the concentration of complexing sites in order to calculate the molar concentration of FA or HA complexing sites (R) which leads finally to the calculation of the stability constant (log K).

Humic acid-Fe: In order to measure the maximum complexing capacity (MCC) of humic acid in presence of 0.1 N KC1 (μ=0.1), 10 cm^3 of $FeCl_3$ solution containing 200 ug Fe was added. The molar concentration of HA was calculated as follows:

$$(R) = \text{Mole of HA complexing sites/Liter} = \frac{X_n Q}{V.M_c}$$

where Q = the quantity in mg of Fe absorbed by HA which is equal to the difference between the added Fe "Q_0" and Fe present in the solution at equilibrium (Q_{eq})

V = the volume of reacted HA in ml.

Mc = the atomic weight of the metal cation.

x = the stoichiometric composition of the Fe-HA complex (1.362).

Results and Discussion

Composition of complexes

To determine the composition of the studies complexes, $(\frac{\lambda_0}{\lambda} - 1)$ was plotted against relative values of (R) or log-log paper. The slope of the line gives the value of Stoichiometric compositions X(FA/Fe– or HA/Fe ratios) at pH 5. These were: X=1.00 and x= 1.36 for the FA-Fe and HA-Fe complexes respectively (Figs 2 & 3). This means that one mole of fulvic acid complexing site form a complex with one mole of ferric iron (Fig. 2), and approximately four moles of humic acid complexing sites formed a complex with three moles of Fe^{3+} at the same pH (Fig. 3). The formation of molar 1:1 Fe-FA complex was also reported by Schnitzer and Khan [6].

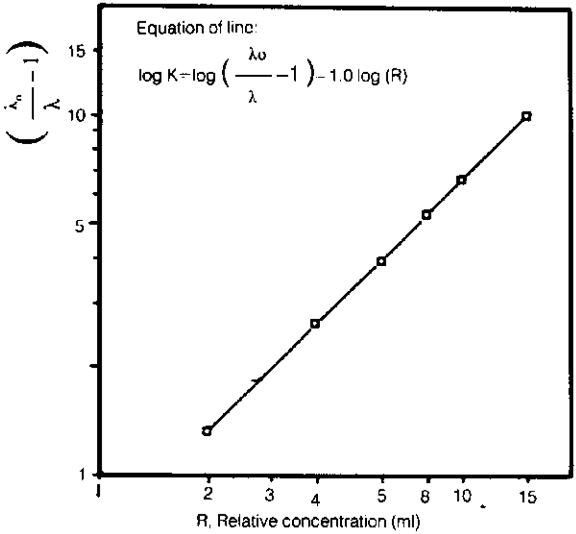

Fig. 2. Stoichiometric composition of Fe – FA complex

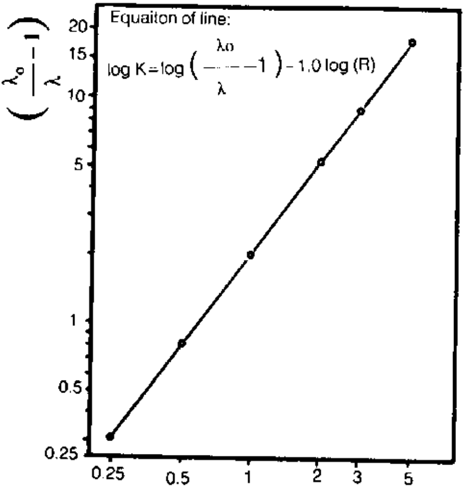

Fig. 3. Stoichiometric composition of Fe-HA complex

No values for the stoichiometric composition of the Fe-HA complex at pH 5 have so far been reported because it starts to precipitate at pH values higher than 3 when stable Fe^{3+} is used in the experiment.

Table 3. Relationship between the stoichiometric composition (x_n) of Fe-FA and Fe-HA complexes and total amounts of oxygen containing functional groups.

Stoichiometric composition "x"		Oxy.-conta.-funct. group "F.G."/mq g^{-1}	$\frac{x(FA)}{x(HA)} = A$	$\frac{F.G.(HA)}{f.g.(FA)} = B$	$\frac{B}{A}$
Fulvic acid (FA)	1.00	14.95	0.73	0.75	1.02
Humic acid (HA)	1.36	11.34			

The data shown in Table 3 indicate clearly that there is a direct relationship from only 2 data points between the total amount of these functional groups (F.G.) present in FA and HA and the stoichiometric composition (x) value of their complexes with ferric iron.

Thus

$$\frac{\text{F.A.(HA))/F.G. (FA)}}{\text{x(FA)/x(HA)}} = 11.02$$

Stability constant

Once the stoichiometric composition x of the complexes was established, the next step was to calculate the stability constant (log K). The latter was calculated for each datum point from the following equation which is derived from equation (12):

$$\log K = \log \left(\frac{\lambda_0}{\lambda} - 1\right) - x \log (R) \qquad (13)$$

Table 4. Stability constant of Fe-FA complex at pH5 and μ0.1

Concentration F.A. in Mole/ L.10^{-6}	cpm^{55}Fe^{3+} (*) in the solution	λ_0	λ	$\frac{\lambda_0}{\lambda} - 1$	α_0	log K
0.0	1876	10.56.66			95.48	
1.14	4125		453.03	1.332	90.06	6.066
2.28	6100		290.16	2.61	85.30	6.062
3.42	7950		211.00	4.000	80.84	6.066
4.57	9710		163.51	5.797	76.60	6.076
5.71	11185		135.51	6.797	73.4	6.075
8.57	14335		94.75	10.152	65.45	6.073

$\bar{X} = 6.069$

Added radioactivity = 41500 cpm/50 ml 1,87.10^{2} Ci

(*) Average of 5 replications.

The data used in these calculations are present in Table 4 and 5. The log K value for the FA complex was 6.06 while that for Fe-HA was 9.75, (both at pH 5 and ionic strength = μ0.1).

This result proves that the stability of the Fe-HA complex is higher than that of the Fe-FA complex. It would be interesting to account for this difference.

Table 5. Stability constant of Fe-HA complex at pH5 and μ0.1.

Concentration of H.A. in mole L.10^{-7}	cpm$^{55}Fe^{3+}$ (*) in the solution	α_o	λ_o	λ	$\frac{\lambda_o}{\lambda} - 1$	=	log K
0.0	2000	98.18	898.39				
0.28	2555	93.84		762.21	0.298		9.744
0.57	3475	91.62		547.12	0.808		9.767
1.15	5480	86.79		328.65	2.010		9.773
2.30	10190	75.44		153.63	5.440		9.775
3.45	13945	66.39		98.79	9.014		9.775
5.76	20565	50.44		50.89	18438	1.362	9.763
						Average value = 9.760	

Added radioactivity = 41500 cpm/50 cm^3
(*) Average of 5 replications

Finally, the calculated stability constants for Fe-FA and Fe-HA are considerably lower than those for complexing agents such as EDTA. This means that the ferric ion, when complexed by FA or HA, would be more readily available to plant roots and microorganisms than when sequestered by EDTA or similar reagents.

References

[1] Fares, F. "Contribution a l'Etude de la Dynamique du Phosphore Associe a la Matiere Organique des Sols." *These Doctorat d'Etat Institut National de Polytechnique de Lorraine,* No. Centre National de la Recherche Scientifique: A.O. 12649 (1976).

[2] Levesque, M. "Contribution of Fulvic Acid and Fulvametallic Complexes to Mineral Nutrition of Plants." *Canadian Journal of Soil Science,* 50 (1970), 385-395.

[3] Schnitzer, M. and Gupta, U.C. "Determination of Acidity in Soil Organic Matter." *Soil Science Society America Proceeding,* 99 (1965), 274-277.

[4] Schnitzer, M. and Skinner, S.M. "Organo-metallic Interactions in Soils: 7-Stability Constants of Pb^{++}, N^{i++}, Mn^{++}, Co^{++} and $Mg^{\pm+}$ Fulvic Acid Complexes." *Soil Science,* 103 (1967), 247-252.

[5] Zunino, H.; Peirano, P.; Aguilera, M. and Schalscha, E.B. "Measurement of Metal-Complexing Ability of Polyfunctional Macromolecules: A Discussion of the Relationship between the Metal-Complexing Properites of Extracted Soil Organic Matter and Soil Genesis and Plant Nutrition." *Soil Science,* 119 (1975), 210-216.

[6] Schnitzer, M. and Khan, S.U. "Humic Substances in the Environment." *Marcel-Dekker* (1972).

[7] Brook, J.D. and Sternhell, S. "Chemistry of Brown Coals, III. Pyrolytic Reactions." *Australian Journal of Applied Science,* 2 (1985), 303-320.

[8] Blom, L.; Edelhausen, L. and Krevele, D.W. "Chemical Structure and Properties of Coal." *18. Fuel* 36 (1975), 135-153.

[9] Fritz, J.S.; Yamamura, S.A. and Bradford, E.C. "Determination of Carboxyl Compounds." *Analytical Chemistry,* 31 (1959), 260-263.

[10] Flaig, W.; Beutelspacher, H. and Rietz, E. "Chemical Composition and Physical Properties of Humic Substances." In *Soil Components, Vol. I. Organic Components.* Springer-Verlag, Berlin, New York: Ed. Journal Giesking, 1975.

[11]Martell, A.E. and Calvin, M. *Chemistry of the Metal Chelate Chelate Compounds*. New York: Prentice-Hall Inc., 1952.

[12] Courpron, F. "Determination de la Constant de Stabilite des Complexes Formes enter le Fer et les Substances dans l'Extrait Equeux des Residus de Vegetaux d'Origines Divereses." *Annals of Agronomy*, 25, No. 5 (1971), 275-356.

[13] Fares, F. "Contribution à l'Etude de la Dynamique du Phosphore Organique dans les Sols." *These Docteur Ingenieur Universite de Nancy I*, No. Centre National de al Recherche Scientifique: A.O. 10681 (1974).

[14] Randhawa, N.S. and Broadbent, F.E. "Soil Organic Matter-Metal Complexes. 5. Reactions of Zinc with Model Compounds and Humic Acid." *Soil Science*, 99 (1965), 295-300.

[15] Mastuda, K. and Ito, S. "Absorption Strength of Zinc for Soil Humus. Relationship Between Stability Constants and Humification." *Soil Science. Plant Nature*, 16 (1970), 1-10.

[16] Miller, M.H. and Ohlrogge, A.J. "Water-soluble Chelating Agents in organic Materials." *Soil Science Society America Proceeding*, 22 (1958), 225-228.

تعيين درجة ثبات (log K) معقد الحديد مع الأحماض الفلفية ومعقد الحديد مع الأحماض الهيومية باستعمال الحديد الثلاثي المشع $^{55}Fe^{3+}$

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(استلم في ٢ ربيع الآخر ١٤١١هـ ، قُبل للنشر في ٢١ ذي القعدة ١٤١١هـ)

ملخص البحث. لقد عينت درجة ثبات (log K) المعقدات المتشكلة بين الأحماض الفلفية والهيومية المستخلصة من أفق A_1 لتربة بودزول ، والحديد الثلاثي ، وذلك بعد تحديد الثوابت الفيزيائية والكيميائية لهذه الأحماض ، وقد استعمل لهذا الغرض الحديد المشع $^{55}Fe^{3+}$ كما استخدمت كل من طريقتي التوازن التبادلي الأيوني ، والتغيرات المستمرة ، عند أس هيدروجيني = ٥ وقوة أيونية = ١ر٠ ميكرون.

لقد بلغت قيمة (log K) لمعقديْ الحديد مع الأحماض الفلفية والحديد مع الأحماض الهيومية ٦,٠٦ ، ٩,٧٥ على التوالي ، أما قيمة التركيب الستوكيومتري x لهذين المعقدين فقد كانت = ١ لمعقد الحديد مع الأحماض الفلفية و ١,٣٦ لمعقد الحديد مع الأحماض الهيومية ، كما تبين أن هناك علاقة مباشرة بين قيمة التركيب الستوكيومتري x لهذه المعقدات ومحتوى كل من الأحماض الفلفية والهيومية من المجموعات الوظيفية الأكسيجينية.