

## Complex formation between glutamic acid and molybdenum (VI)

FARROKH GHARIB<sup>1\*</sup>, SAIED ABEDINI KHORRAMI<sup>2</sup> and SASAN SHARIFI<sup>2</sup>

<sup>1</sup>Chemistry Department, Shahid Beheshti University, Tehran, Evin, Iran

<sup>2</sup>Chemistry Department, Islamic Azad University, Science and Research Branch, Tehran, Iran

MS received 3 July 1996

**Abstract.** Equilibria of the reaction of molybdenum (VI) with L-glutamic acid have been studied in aqueous solution in the pH range 2.5 to 9.5, using spectrophotometric and optical rotation methods at constant ionic strength (0.15 mol dm<sup>-3</sup> sodium perchlorate) and temperature 25 ± 0.1°C. Our studies have shown that glutamic acid forms a mononuclear complex with Mo(VI) of the type MoO<sub>3</sub>L<sup>2-</sup> at pH 5.5. The stability constant of this complexation and the dissociation constants of L-glutamic acid have been determined.

**Keywords.** Molybdenum (VI) complexes; L-glutamic acid complexes.

### 1. Introduction

It is now well-established that molybdenum is an essential component of several enzymes which catalyse redox reactions (Ochiai 1987; Kendrick *et al* 1992). So the study of molecular structure of molybdenum complexes of aminoacids and their derivatives has been of interest in the field of bioinorganic chemistry. In particular, the identification of the nature of the binding sites of molybdenum and determination of the stability constants of its complexes with aminoacids have been the dominant issues in many current investigations (Butler and Carrano 1991; Butler and Rosa 1992). L-glutamic acid plays an important role in biological chemistry (Gharib *et al* 1996) and the molybdenum chelates formed with it offer an interesting system for investigation.

The equilibria of Mo(VI) in aqueous solution are complex and various polynuclear species are formed in acidic solution (pH < 4.5) (Jonson and Jons 1981). In basic solution, little or no complex is formed (Cotton and Wilkinson 1972) due to the exceedingly high stability of MoO<sub>4</sub><sup>2-</sup>.

The present paper describes the complexation of Mo (VI) with L-glutamic acid in aqueous solution of pH ranging from 2.5 to 9.5. The composition of the complex was determined by continuous variations method, using spectrophotometric and polarimetric techniques and the stability constant of the complex was measured at pH = 5.5, temperature = 25 ± 0.1°C, and ionic strength = 0.15 mol dm<sup>-3</sup> (sodium perchlorate) in aqueous solution of pH ranging from 2.5 to 9.5.

---

\*For correspondence

## 2. Experimental

*Reagents:* Sodium perchlorate, perchloric acid, sodium hydroxide, sodium molybdate and L-glutamic acid were obtained from E Merck as analytical reagent grade materials, and were used without further purification. Dilute perchloric acid solution was standardized against  $\text{KHCO}_3$ . A 50 mass % sodium hydroxide solution, free from carbonate, was prepared from analytical grade material and filtered through a G4 Jena glass filter; dilute solutions were prepared using double-distilled water with specific conductance equal to  $(1.3 \pm 0.1) \mu\Omega^{-1} \text{cm}^{-1}$ , and this stock solution was standardized against  $\text{HClO}_4$ .

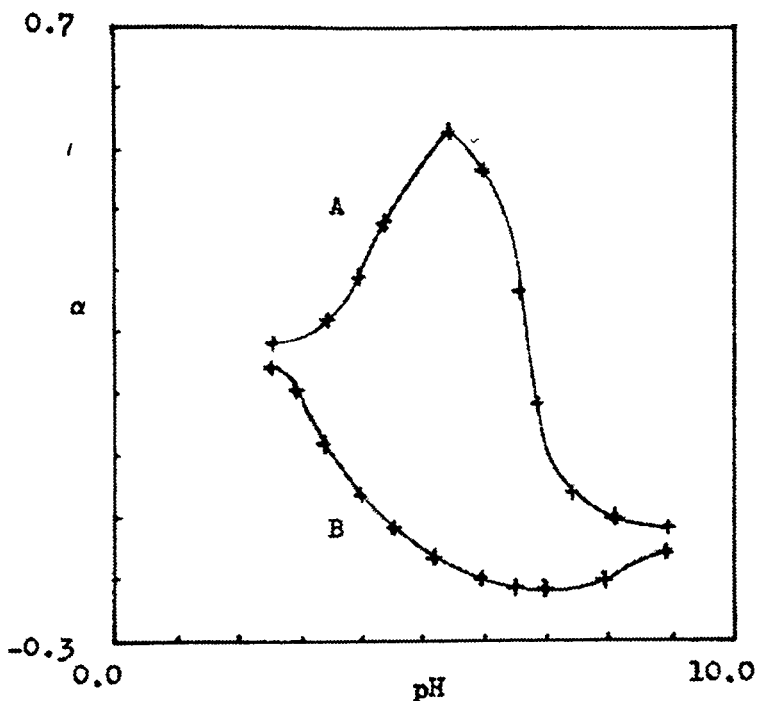
*Measurements:* All measurements were carried out at  $25 \pm 0.1^\circ\text{C}$ . The ionic strength was maintained at  $0.15 \text{ mol dm}^{-3}$  with sodium perchlorate. An Eyela pH meter, PHM 2000, was used for pH measurements. The hydrogen ion concentration was measured with an Ingold UO 3234 glass electrode and an Ingold UO 3236 calomel electrode. A  $0.01 \text{ mol dm}^{-3}$  perchloric acid solution containing  $0.14 \text{ mol dm}^{-3}$  sodium perchlorate was employed as standard pH buffer solution (Zare et al 1979; Gharib et al 1995b). Spectrophotometric measurements were performed on a UV-Vis Shimadzu 2100 spectrophotometer with a GDU20C computer and using thermostated and matched 10 mm quartz cells. The measurement cell was flow type. A Masterflex pump allowed circulation of the solution under study from the potentiometric cell to the spectrophotometric cell so that the absorbances and the pH of the solution could be measured simultaneously.

Polarimetric measurements were made at  $25 \pm 0.1^\circ\text{C}$  with an Atago model Polax-D polarimeter, equipped with a sodium lamp. A water-jacketed cell of 200 mm length and  $20 \text{ cm}^3$  total volume was used. For each experiment (for both spectrophotometric and polarimetric methods) two solutions of Mo(VI) + glutamic acid were prepared with the same concentration, but the ionic strength of the first was maintained with sodium perchlorate, and that of the second, with sodium hydroxide. The first solution was then titrated with the second one to reach the pH of interest.

## 3. Results and discussion

*Polarimetric studies:* Assuming that the optical activity of glutamic acid will change upon complexation, polarimetric measurements were made to determine if Mo(VI) is complexed by glutamic acid. Thus, we prepared different solutions of Mo(VI) + glutamic acid and L-glutamic acid alone with the same concentration but at different pH. The effect of pH on the optical rotation of such solutions is shown in figure 1. When the pH is  $< 7$ , the optical rotation of the Mo(VI) + glutamic acid solution becomes increasingly different from that of glutamic acid, indicating complexation by Mo. The difference reaches a maximum between pH 5 and pH 6 and decreases as the pH is decreased below 5, indicating that the largest amount of complex is formed in the pH range 5–6. At higher pH, the complex dissociates due to the competitive formation of  $\text{MoO}_4^{2-}$ , while at low pH values, isopolymolybdates are formed. In order to determine the composition of the complex, a continuous variation study, according to the method of Job (Beck and Nagypol 1990) was made at pH 5.5. The results are given in table 1. A rather sharp maximum at a mole fraction of Mo(VI) of 0.5 was obtained, indicating a 1:1 complex.

Butcher et al (1976) have shown, by vibrational spectroscopy, that a 1:1 Mo(VI) + glutamic acid complex isolated from neutral solution has the  $\text{MoO}_3$  core, from which we conclude that the composition of the complex indicated by the optical



**Figure 1** Effect of pH on optical rotation,  $\alpha$ , of (A) Mo(VI) (0.03M) + L-glutamic acid (0.03 M) and (B) L-glutamic acid (0.03 M)

**Table 1.** Continuous variation data for the Mo(VI)–glutamic acid system.

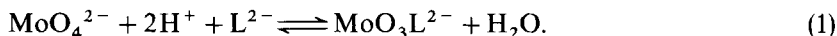
pH = 5.5;  $25 \pm 0.1^\circ\text{C}$ ; ionic strength = 0.15 M NaClO<sub>4</sub>.

Mole fraction Mo(VI)	$\alpha_{(\text{Mo} + \text{Glu})^a}$	$\alpha_{\text{Glu}}^b$	$\Delta\alpha^c$
0.0	-0.195	-0.195	0.000
0.1	-0.013	-0.175	0.162
0.2	0.077	-0.156	0.233
0.3	0.151	-0.136	0.287
0.4	0.193	-0.117	0.310
0.5	0.245	-0.098	0.343
0.6	0.218	-0.077	0.295
0.7	0.168	-0.058	0.226
0.8	0.132	-0.038	0.170
0.9	0.064	-0.019	0.083
1.0	0.000	0.000	0.000

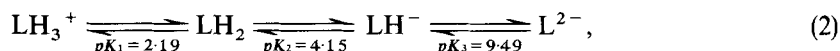
<sup>a</sup>Optical rotation of [Mo(VI)] + [glutamic acid] =  $2.50 \times 10^{-2}$  M; <sup>b</sup>Optical rotation of glutamic acid at the same concentration as in the Mo-glutamic acid solution; <sup>c</sup> $\alpha_{(\text{Mo} + \text{glutamic acid})}$

–  $\alpha_{(\text{glutamic acid})}$ .

rotation measurements is  $\text{MoO}_3\text{L}^{2-}$ , where  $\text{L}^-$  represents the fully dissociated aminoacid anion. The formation of the complex is described by (Funahashi *et al* 1981),



Glutamic acid exists in solution in one or more of four forms, depending on the solution pH (Chang 1977),



where  $\text{L}^{2-}$ ,  $\text{LH}^-$ ,  $\text{LH}_2$ , and  $\text{LH}_3^+$  refer to the different forms of glutamic acid existing at different pH values,  $pK_1$  and  $pK_2$  refer to the first and second dissociation constants of the first and second carboxyl groups respectively, and  $pK_3$  denotes the deprotonation of the amino group. The above  $pK$  values were determined by potentiometric titration under the same conditions of temperature and ionic strength as described (Gharib *et al* 1993) previously.

We have previously described (Gharib *et al* 1995a) that, at pH 5.5,  $\text{H}_2\text{L}$  and  $\text{HL}^-$  are the major species. Since the complex apparently involves the amino group, however,  $\text{L}^{2-}$  must be the species which forms the complex.

The stability constant for the 1:1 complex at pH 5.5 was estimated from a continuous variations plot of the results in table 1. The first problem is based on the fact that the total rotation,  $\alpha$ , at the maximum (mole fraction of Mo 0.5) is due to the rotation of the complex and the free glutamic acid species, neglecting the rotation of  $\text{H}_3\text{L}^+$  and  $\text{L}^{2-}$ , because their concentrations are negligible at this pH:

$$\alpha_t = \alpha_c + \alpha_{\text{H}_2\text{L}} + \alpha_{\text{HL}^-}, \quad (3)$$

since the rotation of each species in (3) is proportional to the concentration of that species, (3) may be written as,

$$\alpha_t = k_c[C] + k_{\text{H}_2\text{L}}[\text{H}_2\text{L}] + k_{\text{HL}^-}[\text{HL}^-], \quad (4)$$

where the  $k$ 's are proportionality constants and  $C$  refers to the concentration of the complex. For material balance,

$$C_L = [C] + [\text{H}_2\text{L}] + [\text{HL}^-], \quad (5)$$

where  $C_L$  is the total concentration of ligand. The values of  $k_{\text{H}_2\text{L}}$  and  $k_{\text{HL}^-}$  were obtained from the plot of  $\alpha$  versus pH for glutamic acid at the respective pH values where these forms are maximized and the other species can be neglected (pH 3.32 and 6.82). The value of  $k_c$  was obtained from a measurement of  $\alpha$  at pH 5.5 for a solution of 0.0150 M in Mo(VI) and 0.001 M in glutamic acid in which the ligand is essentially completely complexed. The values obtained were:  $k_{\text{H}_2\text{L}} = 2.83$ ,  $k_{\text{HL}^-} = -7$ , and  $k_c = 160$ . Equations (4) and (5) were then solved for  $[C]$  and  $[\text{HL}^-]$ . The concentrations of  $[\text{H}_2\text{L}]$  and  $[\text{L}^{2-}]$  were calculated from the proper equations of the dissociation constants.  $[\text{Mo(VI)}]$  was obtained from

$$C_M = [\text{Mo(VI)}] + [C], \quad (6)$$

where  $C_M$  is the total concentration of Mo(VI). The stability constant,  $K_s$ , was calculated from

$$K_s = \frac{[\text{MoO}_3\text{L}^{2-}]}{[\text{MoO}_4^{2-}][\text{H}^+]^2[\text{L}^{2-}]}. \quad (7)$$

**Table 2.** Continuous variation data for the Mo(VI) + L-glutamic acid system.pH = 5.5, (25 ± 0.1)°C, ionic strength = 0.15 M (NaClO<sub>4</sub>).

Mole fraction of Mo(VI)	A <sup>a</sup> at (nm)		
	270	275	280
0.04	0.019	0.011	0.007
0.10	0.053	0.026	0.012
0.20	0.111	0.057	0.030
0.30	0.136	0.074	0.041
0.40	0.152	0.081	0.045
0.50	0.162	0.084	0.051
0.60	0.147	0.078	0.044
0.70	0.131	0.063	0.036
0.80	0.103	0.046	0.026
0.90	0.045	0.021	0.014
0.96	0.017	0.009	0.006
1.00	0.000	0.000	0.000

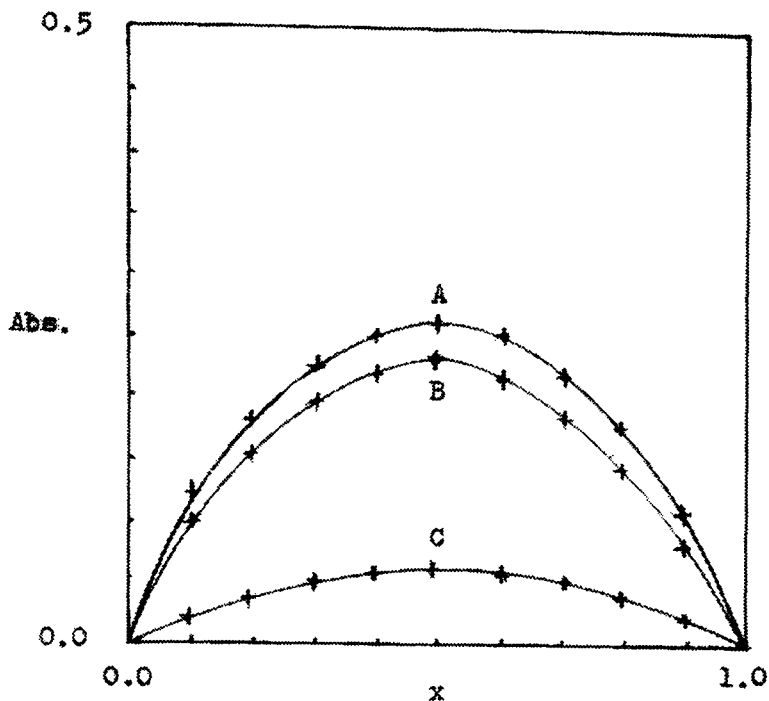
<sup>a</sup>The corrected absorbance of [Mo(VI)] + [Glutamic acid] = 1.00 × 10<sup>-2</sup> M at different wavelengths.**Table 3.** Continuous variation data for the Mo(VI) + L-glutamic acid system.pH = 5.5, (25 ± 0.1)°C, ionic strength = 0.15 M (NaClO<sub>4</sub>).

Mole fraction of Mo(VI)	A <sup>a</sup> at (nm)		
	270	275	280
0.04	0.018	0.008	0.005
0.10	0.042	0.017	0.008
0.20	0.081	0.037	0.019
0.30	0.102	0.046	0.023
0.40	0.119	0.053	0.026
0.50	0.126	0.058	0.034
0.60	0.116	0.051	0.027
0.70	0.095	0.048	0.023
0.80	0.070	0.035	0.018
0.90	0.031	0.016	0.010
0.96	0.012	0.006	0.004
1.00	0.000	0.000	0.000

<sup>a</sup>The corrected absorbance of [Mo(VI)] + [glutamic acid] = 8.00 × 10<sup>-3</sup> M at different wavelengths.

This gave the value of  $\log K_s = 16.53 \pm 0.11$ , for the total concentration of 0.0250 M of Mo(VI) and ligand.

*Spectrophotometric studies:* Using the continuous variations method, the absorbances of two different solutions of Mo(VI) and L-glutamic acid of total concentrations 0.01 and 0.008 M were determined in the UV range 265–280 nm at a constant pH of 5.5 and



**Figure 2** Continuous variation plots of absorbances of  $\text{MoO}_3\text{L}^{2-}$ , Abs., versus mole fraction of Mo(VI),  $x$ , at 265 nm for (A)  $[\text{Mo(VI)}] + [\text{glutamic acid}] = 0.01 \text{ M}$  at  $\text{pH} = 5.5$ , (B)  $[\text{Mo(VI)}] + [\text{glutamic acid}] = 0.008 \text{ M}$  at  $\text{pH} = 5.5$ , and (C)  $[\text{Mo(VI)}] + [\text{glutamic acid}] = 0.01 \text{ M}$  at  $\text{pH} = 6.2$ .

6.2. The observed absorbances were corrected for unreacted Mo(VI), from (8), and are summarized in tables 2 and 3 and plotted in figure 2,

$$A_c = A_{\text{obs}} - \varepsilon_0 [\text{Mo}], \quad (8)$$

where  $A_c$ ,  $A_{\text{obs}}$  and  $\varepsilon_0$  are the absorbance of the complex, the observed absorbance, and the molar absorptivity of Mo(VI) respectively.  $\varepsilon_0$  values, for various wavelengths, were calculated at the mole fraction of Mo(VI) equal to 1, and are shown in table 4.

In figure 2, a rather sharp maximum at a mole fraction of Mo(VI) of 0.5 was obtained, indicating a 1:1 complex. The molar absorptivity of the complex,  $\varepsilon_1$ , was calculated from the linear part of the curve at low mole fraction of Mo(VI), where essentially all the metal ions were in the form of a complex, for different wavelengths as shown in table 4. At the maximum point on the plot, the concentration of the complex is

$$[C] = A_c / \varepsilon_1. \quad (9)$$

An equation can be written for the total concentration of Mo(VI),  $C_M$ , and the total concentration of ligand,  $C_L$ , at this point:

$$C_M = [\text{Mo}] + [C], \quad (10)$$

$$C_L = [L] + [C]. \quad (11)$$

**Table 4.** Values of molar absorptivities of Mo(VI),  $\epsilon_0$ , and  $\text{MoO}_3\text{L}^{2-}$ ,  $\epsilon_1$  at different wavelengths; pH 5.5;  $(25 \pm 0.1)^\circ\text{C}$ ;  $I = 0.15 \text{ M}$  ( $\text{NaClO}_4$ ).

Molar absorptivity	Wavelength (nm)			
	280	275	270	265
$\epsilon_0$	11.6	27.9	66.3	135.2
$\epsilon_1$	14.6	24.2	47.0	74.6

Combining (9)–(11) in (7), and solving for  $K_s$ , gives the stability constant of (1). The average of  $K_s$  for different concentrations and wavelengths is  $\log K_s = 16.73 \pm 0.05$ .

The structure of sodium molybdate is tetrahedral in the solid state (Gilbert and Kustin, 1976) and is believed to be octahedral in solution with three positions to form its complexes (Honig and Kustin 1973). On the other hand, glutamic acid is a potentially tridentate ligand for metal ions with three donor sites on the terminal amino and carboxyl groups, as well as the other carboxyl group on the side chain of the molecule (Butcher *et al* 1976). Thus, Mo(VI) bonds with this tridentate ligand, and it seems unlikely that complexes of 1:2 and 1:3 stoichiometry exist in the pH range of interest (Jonson and Jons 1981).

## References.

- Beck M T and Nagypal I 1990 *Chemistry of complex equilibria* (New York: Ellis Horwood)
- Butler A and Carrano C J 1991 *Coord. Chem. Rev.* **109** 61
- Butler A and Rosa R 1992 *Inorg. Chem.* **31** 5072
- Butcher R J, Powell H K J, Wilkins C J and Yong S H 1976 *J. Chem. Soc., Dalton Trans.* 356
- Chang R 1977 *Physical chemistry with applications to biological system* (New York: Macmillan)
- Cotton F A and Wilkinson G 1972 *Advanced inorganic chemistry* 3rd edn (New York: Interscience)
- Funahashi S, Kato Y, Nakayama M and Tanaka M 1981 *Inorg. Chem.* **20** 1752
- Gharib F, Zare K and Khorrami S A 1993 *J. Chem. Eng. Data* **38** 602
- Gharib F, Zare K and Khorrami S A 1995a *J. Chem. Eng. Data* **40** 186
- Gharib F, Zare K and Khorrami S A 1995b *J. Sci. Az-Zahra Univ.* (in press)
- Gharib F, Aghai H and Ketabi S 1996 *Russ. J. Inorg. Chem.* (in press)
- Gilbert K and Kustin K 1976 *J. Am. Chem. Soc.* **98** 5502
- Honig D S and Kustin K 1973 *J. Am. Chem. Soc.* **95** 5525
- Jonson J S and Jons O 1981 *Acta Chem. Scand.* **35** 233
- Kendrick M J, May M T, Plishka M J and Robinson K D 1992 *Metals in biological systems* (New York: Ellis Horwood)
- Ochiai E I 1987 *General principles of biochemistry of the elements* (New York: Plenum)
- Zare K, Lagrange P and Lagrange J 1979 *J. Chem. Soc., Dalton Trans* 1372