Study and Calculation of Stability Constants of Molybdenum (VI) Complex with Cytosine

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Abstract
Formation equilibria of molybdate ion ($\text{MoO}_4^{2-}$) complex with cytosine in pH=5.8 has been investigated by spectrophotometric measurements in aqueous solution at 0.15 mol lit$^{-1}$ ionic strength (NaClO$_4$), and $25\pm0.1^\circ\text{C}$. In this condition the 1:1 complex has the formula $\text{MoO}_4\text{L}^{2-}$, where L represents the base of DNA. The stability constants of the complexes formed and their stoichiometries are given and interpreted. The logarithms of the cumulative stability constant $\beta_{xyz}$ of the complex: $[(\text{H})_x(\text{oxometal})(\text{cytosine})_z]$, is log $\beta_{211} = 17.321$.

Keywords: Molybdenum(VI), Cytosine, Stability constant

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Introduction

Molybdenum as a trace element plays an important role in metabolic processes. Complexes of molybdenum(V) and molybdenum(VI) with base of DNA and organic sulphur compounds are of interest as models for molybdenum-containing enzymes. These enzymes are known to catalyse a number of important biological oxo-transfer reactions where the valence of molybdenum alternates between molybdenum(VI) and molybdenum(IV) states in reactions with substrates and subsequent reactivation. There have been many studies of the complex of molybdenum (VI) with α-amino acids and some amino polycarboxylic acids (NTA, EDTA, MIDA, IDA), but to our knowledge, there is no report about the formation of complex of Mo (VI) with cytosine. This metal-ligand equilibrium was studied at 25°C with 0.15 mol lit⁻¹ sodium perchlorate as ionic medium. These thermodynamic results allow us to speculate on the structure of the complex obtained.

Experimental

Reagents- Sodium perchlorate, Sodium molybdate, Perchloric acid, Sodium hydroxide and cytosine were obtained from E. Merck as analytical reagent grade materials and were used without any purification. Dilute perchloric acid solutions were standardized against K(HCO₃).

A 50% Sodium hydroxide solution free from carbonate was prepared from the commercial, p.a.material filtered through a G4 jena Glass filter and stored in a polyethylene bottle; dilute solutions were prepared from boiled distilled water and this stock solution and were standardized titrimetrically against a standard iron (II) sulphate solution. All dilute solutions were prepared from double-distilled water, with specific conductance equal to (1.3 ± 0.1) Ω cm⁻¹ µ⁻¹.

Measurements- All measurements were carried out at 25 ± 0.1°C, the ionic strength was maintained at 0.15 mol lit⁻¹ with sodium perchlorate. A Horiba D-14 pH-meter was used for pH measurements. The pH meter has a sensitivity of 0.01 units. The hydrogen ion concentration was measured with a Horiba combination electrode, model S8720. The calibration has been done for the whole pH range (-log[H⁺]) range used. Spectrophotometric measurements were performed on a UV-vis Shimadzu 2101 spectrophotometer with an Acer Mate 486 SX/250 computer using thermostated, matched 10-mm quartz cells. The measurement cell was of the flow type. A Masterflux pump allowed circulation of the solution under study from the potentiometric cell to the spectrophotometric cell so that the absorbance and pH of the solution could be measured simultaneously.

Results and Discussion

The protonation constants of cytosine have been extensively studied in protonation constants of background electrolytes, and the results are reported in the literature. The following equilibria were studied, where L⁻ represents the fully dissociated cytosine anion: H⁺ + L⁻ ⇌ HL  

\[ K_1 = [HL]/[H^+][L^-] \] (1)
The protonation constants of the imido, $K_1$, and the second imino groups, $K_2$, of the side chain of the cytosine, base of DNA, has been determined using potentiometric techniques that shown in Figure 1, calculated using a computer program (Excel) that employs a least-squares method.

The protonation constants, expressed as log $K$, are collected in Table 1 together with the values reported in the literature, which are in good agreement with those reported before.

![Figure 1: Plot of pH of cytosine, vs the millilitres of HClO$_4$ 0.1 mol lit$^{-1}$ at 0.15 mol lit$^{-1}$ ionic Strengths of NaClO$_4$, 25 ± 0.1°C, and 50 ml of ligand of cytosine 0.005 mol lit$^{-1}$.](image)

<table>
<thead>
<tr>
<th>log $K_1$</th>
<th>log $K_2$</th>
<th>Experimental conditions</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.93 ± 0.05</td>
<td>11.85 ± 0.04</td>
<td>I=0.15M, NaClO$_4$, t= 25°C</td>
<td>This work [20]</td>
</tr>
</tbody>
</table>

Spectrophotometric results

The absorbances of the solution of Mo(VI) and cytosine at a total concentration of $10^{-4}$ mol dm$^{-3}$ in the UV range (255 to 270 nm) at a constant pH of 5.8 were determined.

The variation of absorbance of Mo[VI] and complex versus plot shown in Figure 1. It shows that the appropriate range of plot for complex formation is 5.5 to 6.5.
Figure 2:
a) Plots of absorbance of MoO$_3$L$_2^-$, Abs, vs pH. At [Mo(VI)-cyto]=3×10$^{-3}$ M at 25±0.1°C, Ionic strengths of 0.15 m NaClO$_4$, and $\lambda$ = 270nm

b) Continuous variation plot of the absorbance of complex, Abs, vs pH at [Cyto]=5×10$^{-5}$ M, [Mo(VI)]=5×10$^{-5}$ M, at 25±0.1°C, ionic strengths of 0.15M NaClO$_4$ and $\lambda$ = 260nm

The observed absorbances were corrected from eq 3-13:

$$A_{c} = A_{abs} - \varepsilon_{L} (C_{L} - [C]) - \varepsilon_{C} (C_{C} - [C])$$  \hspace{1cm} (3)

In the little concentration of Mo, $C_{M} \approx [C]$:

$$A_{c} = A_{abs} - \varepsilon_{L} (C_{L} - C_{M})$$  \hspace{1cm} (4)

$$A_{a} = \varepsilon_{C} [C]$$  \hspace{1cm} (5)

$$A_{c} = A_{abs} - \varepsilon_{L} (C_{L} - [C]) - \varepsilon_{C} (C_{C} - [C]) = \varepsilon_{C} [C]$$  \hspace{1cm} (7)

$$[C] = A_{abs} - \varepsilon_{C} C_{M} - \varepsilon_{C} C_{L}$$  \hspace{1cm} (8)

$$[M] = C_{M} - [C] = C_{M} - A_{abs} C_{M} - \varepsilon_{C} C_{L}$$  \hspace{1cm} (9)

$$M = \frac{\varepsilon_{C} C_{M} - A_{abs}}{\varepsilon_{C} - \varepsilon_{M} - \varepsilon_{L}}$$  \hspace{1cm} (10)

$$[L] = C_{L} - [C] = C_{L} - A_{abs} C_{M} - \varepsilon_{C} C_{L}$$  \hspace{1cm} (11)

$$[L] = \frac{\varepsilon_{C} C_{L} - \varepsilon_{M} C_{L} - A_{abs} + \varepsilon_{M} C_{M}}{\varepsilon_{C} - \varepsilon_{M} - \varepsilon_{L}}$$  \hspace{1cm} (12)

$$A_{C} = \frac{\varepsilon_{C} A_{abs} - \varepsilon_{M} C_{M} - \varepsilon_{C} C_{L} - \varepsilon_{L} + \varepsilon_{M} C_{L} + \varepsilon_{M} C_{M}}{\varepsilon_{C} - \varepsilon_{M} - \varepsilon_{L}}$$  \hspace{1cm} (13)

For the determination of molar Absorptivities coefficient of Mo(VI) have been prepared its solutions with different concentration, and have been measured their absorbance, the results shown in Table 2 and Figure 3.

Table 2. Absorbance values the solution of Molybdenum (VI) at different Concentration and Wavelengths pH=5.8 at 25±0.1 ºc and ionic strength of 0.15M NaClO$_4$

<table>
<thead>
<tr>
<th>$C_{M}$</th>
<th>Abs 255 nm</th>
<th>Abs 260 nm</th>
<th>Abs 265 nm</th>
<th>Abs 270 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>0.6008</td>
<td>0.3064</td>
<td>0.1528</td>
<td>0.0696</td>
</tr>
<tr>
<td>0.0004</td>
<td>0.188</td>
<td>0.104</td>
<td>0.0536</td>
<td>0.0264</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.0456</td>
<td>0.024</td>
<td>0.0112</td>
<td>0.056</td>
</tr>
</tbody>
</table>
Figure 3. Continuous Variation plots of the absorbance of Solutions of Molybdenum (VI) at different Concentration at $\lambda = 255, 260, 265$ and $270 \text{ nm}$, pH$ = 5.8$ at $25 \pm 0.1 \degree \text{C}$ and ionic strength of $0.15 \text{ M NaClO}_4$

Table 3. Molar Absorptivities of Mo (VI), $\varepsilon_M$, Cyto, $\varepsilon_L$, and MoO$_3$L$^2-$, $\varepsilon_C$, at pH$ = 5.8$, $25 \pm 0.1 \degree \text{C}$, different wavelengths, $[\text{Mo(VI)}+\text{Cyto}] = 10^{-4} \text{M}$, and ionic strength of $0.15 \text{M NaClO}_4$

<table>
<thead>
<tr>
<th>$\lambda$ (nm)</th>
<th>255</th>
<th>260</th>
<th>265</th>
<th>270</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_M$</td>
<td>450</td>
<td>240</td>
<td>112</td>
<td>56</td>
</tr>
<tr>
<td>$\varepsilon_L$</td>
<td>5304</td>
<td>6048</td>
<td>6392</td>
<td>6032</td>
</tr>
<tr>
<td>$\varepsilon_C$</td>
<td>6128</td>
<td>6736</td>
<td>6864</td>
<td>6224</td>
</tr>
</tbody>
</table>

By the known of values of the total Concentration of metal and ligand, also values of $\varepsilon_M$, $\varepsilon_L$, $\varepsilon_C$ and Absorbance were observed, we can calculate The absorbance were corrected values of the Absorbance were corrected at different wavelengths, and different mole Fraction of metal, shown in table 4.

Table 4. The absorbance Were corrected of Complex at pH $= 5.8$ and experimental conditions in each the solution of job $[\text{Mo(VI)} – \text{Cyto}] = 10^{-4} \text{M}$

<table>
<thead>
<tr>
<th>$X_\infty$</th>
<th>$X_{255}$</th>
<th>$X_{260}$</th>
<th>$X_{265}$</th>
<th>$X_{270}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{X_{255} = X_{260}}{X_{265} = X_{270}}$</td>
<td>255 = $\lambda$, 260 = $\lambda$, 265 = $\lambda$, 270 = $\lambda$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.06128</td>
<td>0.0581</td>
<td>0.0521</td>
<td>0.06224</td>
</tr>
<tr>
<td>0.2</td>
<td>0.11</td>
<td>0.092</td>
<td>0.07353</td>
<td>0.18</td>
</tr>
<tr>
<td>0.4</td>
<td>0.2167</td>
<td>0.187</td>
<td>0.168</td>
<td>0.447</td>
</tr>
<tr>
<td>0.5</td>
<td>0.2936</td>
<td>0.274</td>
<td>0.268</td>
<td>0.58</td>
</tr>
<tr>
<td>0.6</td>
<td>0.195</td>
<td>0.1743</td>
<td>0.132</td>
<td>0.368</td>
</tr>
<tr>
<td>0.8</td>
<td>0.082</td>
<td>0.066</td>
<td>0.041</td>
<td>0.132</td>
</tr>
<tr>
<td>0.9</td>
<td>0.0324</td>
<td>0.021</td>
<td>0.01632</td>
<td>0.053</td>
</tr>
</tbody>
</table>
Figure 4. Continuous variation plots of the absorbance of MoO$_3$L$_2^-$, Abs, vs the mole fraction of Mo(VI), at $25 \pm 0.1 ^\circ C$, pH=5.8, ionic strength of 0.15M NaClO$_4$, and [M]+[L]=10$^{-4}$M.

$A_c$, $A_{obs}$, and $\varepsilon_M$ are the absorbance of the complex and the observed absorbance and the molar absorptivity of Mo, respectively. $\varepsilon_M$ values were calculated at a mole fraction of Mo equal to 2 and are shown in Table 1. In figure 3, a maximum at a mole fraction of Mo equal to 0.5 was obtained, indicating a 1:1 complex.

The complex H$_x$M$_y$(cytos)$_z$(x+ny-z)$^{+}$ formed is characterized by its stoichiometry (x:y:z). The stability constant of the complexation equilibrium (14) is defined by $\beta_{xyz}$ (15):

$$xH^+ + yM^{n+} + z\text{Cytos}^- \Leftrightarrow H_xM_y(Cytos)_z(x+ny-z)^{+} \quad (14)$$

with

$$\beta_{xyz} = \frac{[H_xM_y(Cytos)_z(x+ny-z)^{+}]}{[H^+]^x[M^{n+}]^y[Cytos^-]^z} \quad (15)$$

In aqueous solution cytosine exists in its anionic form (cytos$^-$). Zwitterionic species (Hcytos$^-$) and cationic form (H$_2$cytos$^+$).

The protonation constants of cytosine have been used for computation of the stability constants have been found in the literature.$^{17}$ Result of computation is given in table 5:

Table 5. Metal complexes formation constants for cytosine in 0.15 M NaClO$_4$ at $25^\circ C$ (x,y and z are the stoichiometric coefficients of the complex who correspond respectively to the proton, metal and ligand)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Stiochiometry of complex (x:y:z)</th>
<th>log $\beta_{xyz}$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo(VI) + Cytosine</td>
<td>2:1:1</td>
<td>17.321 $\pm$ 0.015</td>
<td>this work</td>
</tr>
</tbody>
</table>

Our complexation studies of molybdenum (VI) ion with cytosine shows only the formation of MoO$_3$(cytos)$^{2-}$ complex species.
For the more consider and comparative, see the table 6. The structure of sodium molybdate is believed to be octahedral in solution \cite{24} with three positions to form its complexes with a tridentate ligand like cysteine or histidine and aspartic acid. These complexes usually have great stability constants to confirm this speculation (table 6). Values of $\log \beta$ obtained for Mo(VI) + histidine, cysteine and aspartic acid systems (table 6) are greater than that of the Mo(VI) + cytosine in the order of their stability constants: cystosine < aspartic acid < histidine < cysteine.

Assuming that these amino acids are all tridentate ligands in their molybdenum (VI) complexes \cite{5,25-26}, but cytosine is potentially a bidentate ligand toward metal ions. On the other hand, Mo(VI) will bond with this bidentate ligand, with two donor sites.

Thus, this is that same acts that we expected.

Table 6. Average values of $\log \beta$ at various wavelengths at pH=5.8

<table>
<thead>
<tr>
<th>$\log \beta$</th>
<th>experimental conditions</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.4±0.1</td>
<td>cysteine+ Mo(VI)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>I=0.1 M NaClO , 25°C</td>
<td></td>
</tr>
<tr>
<td>18.37</td>
<td>histidine+ Mo(VI)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>I=0.1 M NaClO , 25°C</td>
<td></td>
</tr>
<tr>
<td>18.2</td>
<td>aspartic acid+ Mo(VI)</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>I=0.15M NaClO , 25°C</td>
<td></td>
</tr>
<tr>
<td>17.321±0.01</td>
<td>cytosine+ Mo(VI)</td>
<td>this work</td>
</tr>
<tr>
<td></td>
<td>I=0.15M NaClO , 25°C</td>
<td></td>
</tr>
</tbody>
</table>

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References

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