Reactions of $\text{Cr}^{3+}$ with aspartic acid within a wide pH range

Yahia Z. Hamada*, Nabil Bayakly, Mohammed Shafi, Sherry Painter, Vanessa Taylor, Jasmine Greene and Khalid Rosli

Division of Natural and Mathematical Sciences, LeMoyne-Owen College, Memphis, TN, USA

Formation of the metal complexes of aspartic acid (Asp) with the chromium metal ion ($\text{Cr}^{3+}$) in solutions using potentiometric titrations is presented within a wide pH range ($\sim$3.5 to $\sim$10.5) at 25°C and $I = 0.10 \text{ M NaNO}_3$. Concentration distribution diagrams revealed that the main complex formed within this pH range is the bis $\text{Cr}^{3+}$ complex. Literature stability constant values for the $\text{Cr}$–Asp complexes were used to construct concentration distribution diagrams. Complexes taken into consideration were the simple one-to-one complex, the bis-complex, and the bis-mono-protonated complex, namely, $\text{Cr}$–Asp, $\text{Cr(Asp)}_2$, and $\text{Cr(Asp)}_2\text{H}$. The corresponding Log $\beta$ values of these complexes were 12.46, 21.86, and 24.30, respectively. UV–Vis spectra demonstrate $\text{Cr}^{3+}$–Asp binding. The UV–Vis spectra were collected from a system that reached a high level of equilibrium state (50 days’ equilibrium time).

Keywords: Aspartic acid; Bis $\text{Cr}^{3+}$ complex; Distribution diagrams; UV–Vis

Cite: Complex Met. 2014, 1, 46–51
Received 18 November 2013 Accepted 9 January 2014

1. Introduction

Unlike di- and hexa-valent chromium, trivalent chromium ($\text{Cr}^{3+}$) is considered to be an essential metal that forms what is known as “the low-molecular-mass chromium-binding complex” (LMMCr) [1]. LMMCr has been suggested to be a complex that contains four $\text{Cr}^{3+}$ centers and an oligopeptide composed of glycine, aspartic acid, glutamic acid, and cysteine, with varying degrees of amino acid ratios [2]. For example, the ratio of glycine to aspartic acid to glutamic acid to cysteine is 3.22 to 1.98 to 3.91 to 1.75 for the LMMCr complex isolated from rabbit liver [3]. Although there are many studies regarding essential $\text{Cr}^{3+}$ [1–21], the isolation and characterization of this LMMCr complex has not been achieved, and thus its precise structure is still unknown [5,6,9].

In mammals, Asp is a known neurotransmitter [22]. Many biochemistry textbooks state that both aspartic acid and glutamic acid are co-substrates of one another in the so-called aspartate transaminase [1,22–24]. The presence of transaminases in muscle and liver cells makes them useful markers of tissue damage. A detailed literature survey indicated that very few papers reported the stabilities of $\text{Cr}^{3+}$ with Asp [8,25,26]. In previous works different researchers worked out the $\text{Cr}^{3+}$–Asp reaction within a very narrow pH range (from 1.7 to 4.0). Although the stability constants of $\text{Cr}^{3+}$ with Asp complexes have been reported in these references, the identities of the species at the neutral and the alkaline pH values were missing [25,26]. Herein, we are reacting both Asp and $\text{Cr}^{3+}$ within a wide pH range ($\sim$3.5 to $\sim$10.5). The simple
bis-complex was identified as the main metal complex at the physiological and alkaline pH values.

2. Experimental section

2.1. Materials and equipments

Analytical-reagent-grade L-(-)+aspartic acid 98%, C₄H₇NO₄, FW 133.10 g mol⁻¹ and chromium nitrate nonahydrate, Cr(NO₃)₃·9H₂O 99%, FW 400.15 g mol⁻¹ (Fisher Scientific) were used as received. The pH values of all solutions were adjusted using standardized sodium hydroxide NaOH solution. The pH values were measured using Orion pH electrode and a model 720A+ Orion pH meter. The ionic strength of all solutions was adjusted to 0.1 M by using 10% v/v 1.0 M NaNO₃ solution. Strong cation exchange Dowex 50X8-100 resin was purchased from ACROS. Doubly deionized (DI) water was used to prepare all solutions.

2.2. Standardization of the pH electrode and the stock solutions

All stock Cr³⁺ solutions were standardized by eluting a known volume (typically one mL) through the Dowex 50X8-100 resin packed in a seven-inch long by one-inch diameter glass column and titrating the eluant with a standard NaOH solution. The stock Cr³⁺ ion concentrations were in the range of 0.0501–0.0503 M. The average of seven runs was used as the final Cr³⁺ solution concentration. The 720A+ Orion pH meter has the precision of one-thousandth of the pH value. The Orion pH electrode was calibrated before each titration with two buffer points, buffer pH four and buffer pH seven at 25°C.

2.3. Potentiometric titrations and distribution diagrams

In all Cr³⁺-Asp potentiometric titrations, the NaOH solution was always the titrant. The methods used to prepare, standardize, and prevent the contamination of the titrant with atmospheric CO₂ have been described elsewhere [5,6,9,27–29]. The interaction of Cr³⁺ with Asp was monitored in different Cr³⁺ to Asp molar ratios typically 0 : 1, 1 : 0, 1 : 1, 1 : 2, and 1 : 3 molar ratios. In a typical titration system, Asp solution was added; then the equivalent of the Cr³⁺ ion solution followed by dilution to 100 mL total volume with DI water. The total concentration of metal ions was set to 1.0 × 10⁻³ M, which is typical for potentiometric titrations [5,6,8,9,23–27]. Before each titration, the metal–Asp mixtures were allowed to stir for 15–20 min to reach a state of equilibrium. The NaOH solution was added in 100 µL increments using an Eppendorf micropipette with continuous stirring. The time intervals between the additions of the NaOH solution were set to 3 min, which was sufficient to get each of the pH values stabilized and reach a state of equilibrium. Each potentiometric titration has been repeated at least three times. Distribution diagrams were generated using the Hyperquad simulation and speciation (HySS) program [30] using pKw value of 13.78 taken from Sweeton et al. [31].

2.4. UV–Vis spectrophotometer measurements

A sample of 50 mL of 0.05 M Cr³⁺ and 100 mL of 0.05 M Asp were placed in a 250 mL beaker that was sealed and left to stabilize for 1200 h (50 days). The initial pH after complete stabilization was 1.55. Freshly prepared ~0.1 M NaOH was added to deprotonate the carboxylate(s) and the amine groups in order to enhance the metal binding. All UV–Vis data were collected from this solution. All UV–Vis spectroscopy were conducted using an HP 8452 A, single beam, diode array spectrophotometer. Samples were prepared in DI water at 25°C. The entire UV–Vis spectrum to the near infrared (IR) range was scanned from 200 nm to 1100 nm using quartz cuvette with optical path length of 1 cm. Reference cuvettes were used in each run and they were filled with equal volume of DI water.

3. Results and discussion

3.1. System standardization

A standard phosphoric acid solution (H₃PO₄) was titrated to calibrate the whole potentiometric titration system (pH electrode, pH meter, and the working solutions) before gathering the actual potentiometric titrations for either the free Asp titrations, the free Cr³⁺ titrations, or the Cr³⁺: Asp reaction system in different molar ratios. Figure S1 in the supplementary material shows the calibration curves of H₃PO₄. In potentiometric titrations, the equivalent of added base is defined as the number of moles of NaOH per number of moles of the sample under determination (phosphoric acid in this case). The definition of equivalent has been used before [5,6,9,25–29].

3.2. Free Asp potentiometric titration system

Figure 1 shows the potentiometric titration graphs of free Asp. Sharp inflections separate the ammonium proton from the β-carboxylic acid proton. The insets of Figure 1 show the correlation of the volume of added titrant and

Complex Met. © 2014 The Author(s). Published by Taylor & Francis. 47
the observed pH versus the potential in millivolts. The titrations of free aspartic acid and its distribution diagram showed that the ligand is a di-protic acid in which two protons were released into the aqueous solution as expected. Figure S2 gives more details about the two pKa values of aspartic acid 3.71 and 9.66 from Martell et al. [8].

### 3.3. Cr$^{3+}$: Asp potentiometric titration system

The Cr$^{3+}$: Asp has been studied potentiometrically in various Cr$^{3+}$ to Asp ratios, namely 0 : 1, 1 : 0, 1 : 1, 1 : 2, and 1 : 3 (Table 1). No visible precipitates were observed for 0 : 1, 1 : 1, 1 : 2, and 1 : 3 of the Cr$^{3+}$: Asp titration systems. However, the seven titrations of the free Cr$^{3+}$ (i.e. the 1 : 0 titrations) showed a faint precipitate around pH 6. It is worth mentioning that the 1 : 0, 1 : 1, 1 : 2, and 1 : 3 showed very similar graph shapes in which there were minor and major inflection points. Figure 2 is a representative titration graph in triplicate for the 1 : 2 titration system.

Figure 3 shows the distribution diagram using the HySS program [30] for the Cr$^{3+}$: Asp in 1 : 2 molar ratio under identical conditions set forth for the potentiometric titrations shown in both Figures 1 and 2. The species used to generate this graph were the simple one-to-one complex, the bis-complex, and the bis-mono-protonated complex.

![Figure 1](image1.png)

**Figure 1.** (a) Four potentiometric titrations of free Asp in 0.1 M NaNO$_3$, 25°C. Sharp inflections separate the ammonium proton from the β-carboxylic acid proton. The insets are (b) correlation of the volume of added titrant in mL vs. potential in mV, and (c) is the correlation of measured pH values (pH$_{obs}$) vs. potential in mV.

![Figure 2](image2.png)

**Figure 2.** Representative graph of potentiometric titrations of Cr$^{3+}$: Asp in 1 : 2 ratio in 0.1 M NaNO$_3$, 25°C. The 1 : 1 and the 1 : 3 ratios show similar titration patterns.

---

**Table 1.** The exact locations of the inflection points for Cr$^{3+}$: Asp potentiometric titrations in different molar ratios at 25°C and 0.1 M NaNO$_3$.  

| Titration system | Minor inflection point (Eq.$^a$ of NaOH) | Major inflection point (Eq. of NaOH) |
|------------------|----------------------------------------|-------------------------------------|
|                  | Average | SD | Average | SD |
| Cr$^{3+}$: Asp   |         |    |         |    |
| 0 : 1(4)$^b$     | n/a     |    | 0.96    | 0.03 |
| 1 : 0(7)         | 0.90    | 0.02 | 2.97    | 0.07 |
| 1 : 1(4)         | 1.89    | 0.08 | 3.34    | 0.08 |
| 1 : 2(4)         | 2.83    | 0.11 | 4.18    | 0.12 |
| 1 : 3(4)         | 3.99    | 0.18 | 5.67    | 0.18 |

$^a$Eq. is defined as the number of moles of NaOH per number of moles of the metal ion.

$^b$Number of runs.
namely, Cr-Asp, Cr(Asp)$_2$, and Cr(Asp)$_2$H. The corresponding Log $\beta$ values of these complexes were 12.46, 21.86, and 24.30, respectively [25]. The stability constants reported by Maslowska were 12.15, 21.13, and 24.07, respectively [26]. Using either set of stability constants did not change the profile shown in Figure 3.

3.4. UV–Vis absorption spectra

Figure 4 is the UV–Vis absorption spectra of the Cr$^{3+}$: Asp system in 1 : 2 molar ratio after 50 days to reach a high state of equilibrium. No precipitate formation was observed as the pH was increased from 1.55 to 4.10. The solution had a bluish color in this pH range. The characteristic peaks of the Cr$^{3+}$ ion have been observed. The chromium absorption peaks have been observed before [5,9], but to the best of the researcher’s knowledge, they have not been reported for the Cr$^{3+}$: Asp system. The peak that appeared at 405 nm was assigned to the $4A_2g$ to $4T_1g$ electronic transition. The peak that appeared at 550 nm was assigned to the $4A_2g$ to $4T_2g$ electronic transition. The peak that appeared at 300 nm was assigned to the nitrate ion in the solution [9]. These peaks’ assignments are in excellent agreement with Tanabe–Sugano diagrams [32]. The electrostatic energy calculated from equation (1), $-71.1$ kJ mol$^{-1}$, is within the correct range of typical electrostatic interactions of similar compounds in aqueous solutions [8,32]. Based on the values of stability constants found in the literature, the proposed binding mode of Asp must be tridentate and coordinated facially via the NH$_2$ group and the (O$\alpha$) and the (O$\beta$) as shown in scheme 1 [33]. This conclusion was reached because a mono-dentate or a bi-dentate binding of the Asp ligand to Cr$^{3+}$ with such high stability could be envisioned. (The simple one-to-one complex has a Log $K$ of $\sim 12.46$ [25].)

A detailed literature survey indicated that the proposed binding mode of Asp to Cr$^{3+}$ is supported by the literature
data \[8,33,35–38\]. The bis-complex has the proposed structure shown in scheme 2 due to the following reasons: (1) The coordination sphere of the chromium ion is satisfied with six-donor atoms (MN2O4), which is well documented in the literature \[34–36\], (2) the stability constants for both the simple one-to-one and the bis-complex have such high values indicating neither a mono- nor a bi-dentate \[8,25,26\], and (3) such structures have been seen with other metal ions, Cu2+ and Co3+.

Distribution diagrams clearly showed that the bis-complex is the dominant species at the physiological pH value. Based on the values of the stability constants and the change in intensity of the UV–Vis absorption spectra with pH, and the change in the IR bands, it can be explicitly shown that the main species present in solution are those presented in equations (2) and (3).

\[
\text{Cr}^{3+} + \text{H}_2\text{Asp} \rightarrow [\text{Cr(Asp}^{2-})]^+ + 2\text{H}^+, \quad (2)
\]

\[
\text{Cr(Asp}^{2-})^+ + \text{H}_2\text{Asp} \rightarrow [\text{Cr(Asp}^{2-})_2]^- + 2\text{H}^+. \quad (3)
\]

It is hoped that this report will stir strong research and discussion among the science community about the ever-expanding branch of inorganic biochemistry “metal speciation in health and medicine” for which a significant forum was presented very recently in Inorganic Chemistry \[39,40\] in addition to many previous references \[41–43\].

### Acknowledgement

Special thanks go to Dr Ted Burkey of the University of Memphis for helpful comments.

### References

[1] N.W. Tietz. In *Textbook of Clinical Chemistry*, C.A. Burtis, E.R. Ashwood (Eds), 2nd edn, p. 19106, Saunders, Philadelphia, PA (1994).

[2] J.B. Vincent. *J. Am. Coll. Nutr.*, **18**, 6 (1999).

[3] A. Yamamoto, O. Wada, T. Ono. *Eur. J. Biochem.*, **165**, 627 (1987).

[4] J.B. Vincent. *Polyhedron*, **20**, 1 (2001).

[5] Y.Z. Hamada, B. Carlson, J. Dangberg. *Synth. React. Inorg., Met.-Org., Nano-Met. Chem.*, **35**, 515 (2005).

[6] Y.Z. Hamada, N. Bayakly, A. Peipho, B. Carlson. *Synth. React. Inorg., Met.-Org., Nano-Met. Chem.*, **36**, 469 (2006).

[7] S. Bohdan, J.R. Perumareddi. *Inorg. Chim. Acta*, **358**, 4571 (2005).

[8] A.E. Martell, R.M. Smith, R.J. Motekaits. *Critical Stability Constants Database, Version 6.0*, NIST, Texas A & M University, College Station, TX, USA (2001).

[9] Y.Z. Hamada, B.L. Carlson, J.T. Shank. *Synth. React. Inorg. Met.-Org. Chem.*, **33**, 1425 (2003).

[10] J.B. Vincent. *Acc. Chem. Res.*, **33**, 503 (2000).

[11] J.B. Vincent, Y.S.J. Ramirez, S.A. Wolski. *J. Bioinorg. Chem.*, **5**, 129 (2000).

[12] B.J. Clodfelder, J. Emmanuelle, D.D. Hepburn, N.E. Chakov, H.S. Nettles, J.B. Vincent. *J. Biol. Inorg. Chem.*, **6**, 608 (2001).

[13] M. Thompson, R. Connick. *Inorg. Chem.*, **20**, 2279 (1981).

[14] J.E. Finholt, M. Thompson, R. Connick. *Inorg. Chem.*, **20**, 4151 (1981).

[15] H. Stunzi, W. Marty. *Inorg. Chem.*, **22**, 2145 (1983).

[16] A. Levina, P.A. Lay, N.E. Dixon. *Chem. Res. Toxicol.*, **14**, 946 (2001).

[17] J.C. Chang, L.E. Gerdorn, N.C. Baenziger, H.M. Gooff. *Inorg. Chem.*, **22**, 1739 (1983).

[18] C.A. Green, R.J. Bianchini, J.J. Legg. *Inorg. Chem.*, **23**, 2713 (1984).

[19] W.E. Broderic, J.J. Legg. *Inorg. Chem.*, **24**, 3724 (1985).

[20] G. Abbay, T.W. Gilbert. *Polyhedron*, **5**, 1839 (1986).

[21] J.A. Cowan. In *Inorganic Biochemistry/An Introduction*, Wiley-VCH Inc., Hoboken, NJ (1997).

[22] R.H. Garrett, C.M. Grisham. *Biochemistry, Instructor*, 3rd Edn, Thomson Brooks/Cole, Belmont, CA (2005).

[23] A.L. Lehninger. In *Principles of Biochemistry*, D.L. Nelson, M.M. Cox (Eds), 3rd Edn, WORTH, New York (2000).

[24] D. Voet, J.G. Voet, C.W. Pratt. *Fundamentals of Biochemistry – Life at the Molecular Level*, 2nd Edn, John Wiley and Sons, Inc., Hoboken, NJ (2006).

[25] V. Kora, S.M. Nair, R. Duraivasamy, S. Muchi. *J. Chem. Soc. Dalton Trans.*, **2**, 291 (1982).

[26] J. Maslowska, L. Chruscinski. *Polyhedron*, **5**, 1131 (1986).

[27] Y.Z. Hamada, W. Zhepeng, W.R. Harris. *Inorg. Chem.*, **42**, 3262 (2003).

### Funding

This work was supported by the NSF [grant number HRD-1332459] and the American Chemical Society SEED program.

### Supplemental data

Supplemental data for this article can be accessed at doi:10.1080/2164232X.2014.883291.
[28] W.R. Harris, B. Yang, S. Abdollahi, Y. Hamada. *J. Inorg. Biochem.*, 76, 231 (1999).

[29] Y.Z. Hamada, W.R. Harris. *Inorg. Chim. Acta*, 359, 1135 (2006).

[30] L. Alderighi, P. Gans, A. Ienco, D. Perters, A. Sabatini, A. Vacca. *Coord. Chem. Rev.*, 184, 311 (1999).

[31] F.H. Sweeton, R.E. Mesmer, C.F. Baes, Jr. *J. Sol. Chem.*, 3, 191 (1974).

[32] S.F.A. Kettle. *Physical Inorganic Chemistry. A Coordination Chemistry Approach*, Spektrum, University Science Book, Sausalito, CA (1996).

[33] V. Subramaniam, P.E. Hoggard. *J. Inorg. Biochem.*, 54, 49 (1994).

[34] C. Kallay, K. Varnagy, G. Micera, D. Sanna, I. Sovago. *J. Inorg. Biochem.*, 99, 1514 (2005).

[35] N.M. Shuaib, H.M. Marafie, O. Al-Fulaij, M.S. El-Ezaby. *J. Chem. Eng. Data*, 44, 1348 (1999).

[36] M. Watabe, H. Yano, Y. Odaka, H. Kobayashi. *Inorg. Chem.*, 20, 3623 (1981).

[37] J.A. Weyh, R.E. Hamm. *Inorg. Chem.*, 7, 2431 (1968).

[38] J. Huheey, E. Keiter, R. Keiter. *Inorganic Chemistry: Principles of Structure and Reactivity*, 4th Edn, Harper Collins College Publishers, New York (1993), and references therein.

[39] D.C. Crans, T.J. Meade. *Inorg. Chem.*, 52, 12181 (2013).

[40] D.C. Crans, K.A. Woll, K. Prusinkas, M.D. Johnson, E. Norkus. *Inorg. Chem.*, 52, 12262 (2013).

[41] Y.Z. Hamada, N. Bayakly, D. George, T. Greer. *Synthesis and Reactivity of Inorganic and Metal-Organic and Nano-Metal Chemistry*, 38, 664 (2008).

[42] Y.Z. Hamada, H. Holyfield, K. Rosli, T. Burkey. *J. Coord. Chem.*, 62, 721 (2009).

[43] K. Elvingson, A.D. Keramidas, D.C. Crans, L. Pettersson. *Inorg. Chem.*, 37, 6153 (1998).