Scientia Iranica, Vol. 12, No. 3, pp 311 31

SCIENTIA IRANICA

Research Note

Comparative Study of Thermodynamic Parameters of Vanadium (IV) and (V) Acetohydroxamate Complexes

K. Ali[1], N. Fatima[*] and Z.T. Maqsood[1]

Acetohydroxamic acid, $CH_3CONHOH$, forms highly stable complexes with vanadium (V) and vanadium (IV) in 1:1, 1:2 and 1:3 mole ratios. The stability of these complexes can be determined in terms of thermodynamic parameters; $\Delta G, \Delta H$ and ΔS. The preliminary data, obtained through pH titration at various temperatures, was processed and analyzed by the computer program BEST for the refinement of graphically calculated log β values. Graphs of $\ln \beta$ versus $1/T$, gave a straight line, with a slope $-\Delta H/R$ and intercept $\Delta S/R$. Enthalpy and free energy changes for V(V) complexes were found in the order of $ML > ML_2 > ML_3$ with a negative sign. Whereas entropy change was found to be in the same order but positive, for vanadium (IV) acetohydroxamic acid complexes, the order of $\Delta G, \Delta H$ and ΔS was $ML > ML_3 > ML_2$. The ΔS is most positive for a 1:1 complex, while ΔG and ΔH are more negative for the same.

INTRODUCTION

The chemistry of siderophores has attracted significant attention in recent years, due to their metal acquisition role in microorganisms and plants and their potential application in the treatment of metal overload disease [1]. Among numerous siderophore structures, the hydroxamates are of interest, due to their ability to form stable transition metal complexes through the formation of a five-membered chelate ring [2]. Hydroxamic acids have been used as therapeutic agents in chelation therapy [3].

Most mammalian cells contain vanadium at a concentration of about 20 nM, the bulk of which is probably in the reduced vanadyl (+4) form [4]. Like Molybdenum, vanadium assumes an exceptional position among the biometals because its anionic and cationic forms can participate in biological processes [5-7]. Different aspects of the coordination chemistry of vanadium (IV) and vanadium (V) relevant to bioinorganic chemistry, have been extensively discussed. Some of the investigated complexes are good models for different aspects of the metabolism and detoxification of vanadium or for a better characterization and understanding of the structural and electronic peculiarities of the coordination sphere of VO^{2+} and VO_2^+ in biomolecules [8].

Several organically chelated compounds were found more potent and less toxic than vanadium salts in vivo. For example, L-Glu(γ)HXM ([H_2N-CH(CH_2CH_2CONHOH)-COOH]) is highly active in potentiating the capability of free vanadium ions to activate glucose uptake and glucose metabolism in vivo and in vitro [9].

Dietary vanadium probably occurs mainly as $H_2VO_4 \downarrow$ and may enter cells through the phosphate transport mechanism. The standard potential of the vanadate $(H_2VO_4)^-$ to vanadyl $(VO)^{2+}$ is 1.31 V [10]. Most or all of the ingested V(V) undergoes a one-electron reduction generating VO^{2+} by biological reductants, such as glutathion, ascorbate and NADH, in the gastrointestinal tract and vanadyl undergoes autooxidation to vanadate in the presence of oxygen. This facile change between V(V) and V(IV) has an interesting feature in the biological significance of vanadium. Endogenous reducing agents and dissolved oxygen ensures that both V(V) and V(IV) species are present in serum [11-13]. The reduction kinetics of vanadate complexes with some bio-reducing agents, such as

1. *Department of Chemistry, University of Karachi, Karachi, Pakistan.*
*. *Corresponding Author, Department of Chemistry, University of Karachi, Karachi, Pakistan.*

glutathione and ascorbic acid, are currently reported. The reaction chemistry of the potent insulin-mimetic agent, bis(maltolato) oxo-vanadium (IV) has gained significant attention during the last few years [13]. The reduction rate of $[VO_2(ma)_2]\downarrow$ with glutathione is about 2000 times slower than that with ascorbic acid. An acid dependent mechanism can also be used to explain the results for the reduction with glutathione [14]. Although cis-$[VO_2(ma)_2]\downarrow$ is not a good insulin enhancing agent [15], it may be reduced in vivo to form $VO(ma)_2$, which is active. On the other hand, there is a precedent for V(V) compounds having insulin-enhancing activity. Vanadate and some peroxovanadate complexes also have potency [16].

Not a common component of enzymes, vanadium, as the vanadate ion, is an essential prosthetic group of some haloperoxidase enzymes [17]. These are the enzymes currently being elucidated in great detail [18].

Although information about the metabolism of physiological amounts of vanadium in higher forms of life is scarce, during the last few years some general aspects related to the absorption, transport, biological transformations, toxicity and excretion of vanadium, as well as its presence and activity in biological systems, could be understood [19-22].

The thermodynamic stability of the species is a measure of the extent to which these species will form, or be transferred, into other species under a set of conditions, once the system has reached equilibrium. In the language of thermodynamics, the equilibrium constant of a reaction is the measure of the heat released in the reaction and the entropy change during the reaction [23]. Entropy change values play a significant role in determining complex stability.

The entropy of a system is a measure of the amount of disorder. The greater the amount of disorder in the products of a reaction relative to the reactants, the greater will be the increase in entropy during the reaction and the greater the stability of the products.

Determination of the thermodynamic stability of the vanadium ions in +4 and +5 will help to elucidate possible mechanistic pathways which would increase its potency, as well as decrease the toxicity. The most successful complexes contained organic ligands that are reasonably soluble in both organic and aqueous environments and which are compatible with human metabolism [24]. It has recently been found that hydroxamic acid conjugated to the tripyrrolepeptide distamycin induced highly specific DNA cleavage in the presence of vanadyl ions [25]. Thus, the hydroxamate ligand seems to be the most suitable for the study of the complexation and stability of vanadium complexes. Acetohydroxamic acid is the simplest ligand, it has only two coordination sites, so, the probability of any polynuclear complex is obscured and the complexes with 1:1, 1:2 and 1:3 L/M ratios can be formed.

The thermodynamic parameters of vanadium (IV) and vanadium (V) complexes were studied with aceto-hydroxamic acid, which is the simplest and most basic hydroxamate analog.

The theoretical β values were calculated for all species from the titration curves and then the data file "FOR004.DAT" was written for each titration. Data obtained by these titrations were subjected to the computer calculation based program "BEST", for the determination of equilibrium constants. Calculations were carried out with an algorithm which calculates pH directly and minimizes the sum of the weighted squares of -log[H^+] residuals [26].

The computer program "BEST" first hypothesizes all the different species possible in equilibrium, then, guesses equilibrium constants for these species, which were initially calculated by rough manual calculation by a titration curve. Based on these guessed species values, "BEST" calculates the pH at all equilibrium points solving a set of simultaneous mass balance equations for total metal ion, total ligand and total proton ion concentration, i.e.,

$$T_M = [M] + K_{ML}[M][L] + K_{ML}K_{ML2}[M][L]^2 + --,$$

$$T_L = [L] + K_{HL}[H][L] + K_{H2L}[H]^2[L] + -- + K_{ML}[M][L] + 2K_{ML2}[M][L]^2 + --,$$

$$T_H = [H] + K_{HL}[H][L] + 2K_{H2L}[H]^2 + --.$$

The set of simultaneous equations is solved for each component, then, the weighted sum of the squares of the deviations in pH is calculated:

$$U = \Sigma w(p[H]_{\text{obs}} - p[H]_{\text{calc}})^2,$$

where $w = \frac{1}{(p[H]_{i+1} - p[H]_{i-1})^2}$ as a weighted factor, which serves to lessen the influence of less accurate pH values on the calculation. After that, the program adjusts the unknown stability constants and repeats the calculations till no further minimization of U is possible and, thus, provides the final results.

The standard deviation in pH units is obtained by the use of the following equation:

$$\sigma_{\text{fit}} = (U/N)^{\frac{1}{2}}.$$

The program, PKAS, is used to calculate the protonation constants of the ligands. It is simpler than BEST because, here, only two components (L&H) are involved. The distinguishing differences in the refinement of the protonation constants by the use of PKAS rather than BEST includes the initial advantage of selecting reasonably close approximations of the protonation constants by seeing the difference between the observed and computed average pHs.

The data file for this program requires knowledge about the:

1. Total volume of the solution,
2. Molarity of the base used for pH titration,
3. Change in pH after each step,
4. Number of millimoles of metal ions present in the solution,
5. Number of millimoles of ligand present in the solution.

RESULTS AND DISCUSSION

Recent efforts have focused on identifying vanadium compounds with increased efficacy and decreased toxicity [27-29]. The extent to which a metal will complex with a ligand is characterized by the stability constant. Large stability constants indicate that the concentration of a complex is much greater than the concentration of components. The electrostatic influence of the charge and size of a ligand is also important in determining the stability of the complex.

Potentiometry is an informative and convenient technique that has been reported for determination of the formation constant of the successive complexes formed by the metal ion and a ligand molecule or ion. When metal and ligand solutions are mixed, many species of different ML_n formula may form at the same time. As the pH varies, the relative concentration of these species changes, e.g, at low pH 1:1(ML) is the dominating species. Concentration of ML_2 increases with further increase of pH, whereas, above pH 5, ML_3 is in the highest concentration (Figures 1 and 2).

It is found that for V(IV)AHA, after pH 3, at least all the metal was complexed, where 60% was 1:3 bidentate species with no axial oxygen, while 20% was 1:2 bidentate. At pH 10, 80% of 1:2 with axial oxygen, a water molecule and 20% 1:3 were observed (Figure 3) [30].

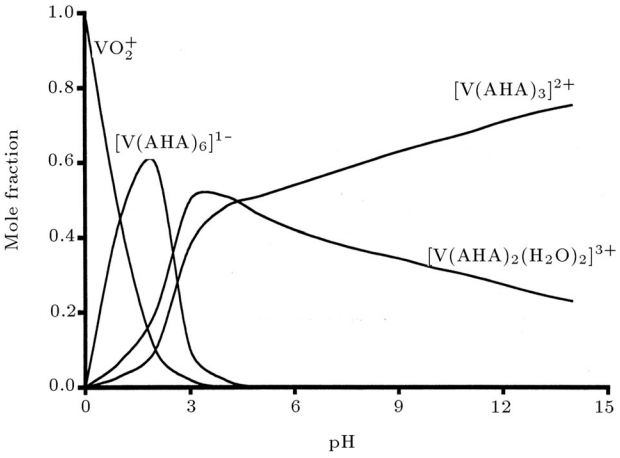

Figure 1. Potentiometric titration of V(IV)-AHA complexes $VOSO_4.5H_2O$ = $CH_3CONHOH$ = 0.250 mM and NaOH=0.20 M.

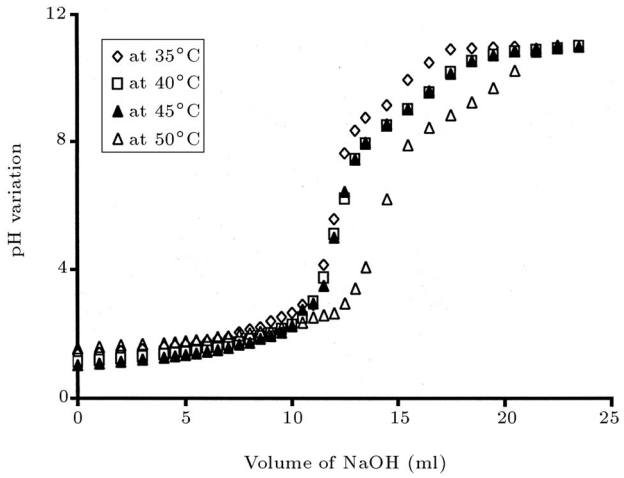

Figure 2. Potentiometric titration of V(V)-AHA complexes VCl_5 = $CH_3CONHOH$ = 0.250 mM and NaOH=0.20 M.

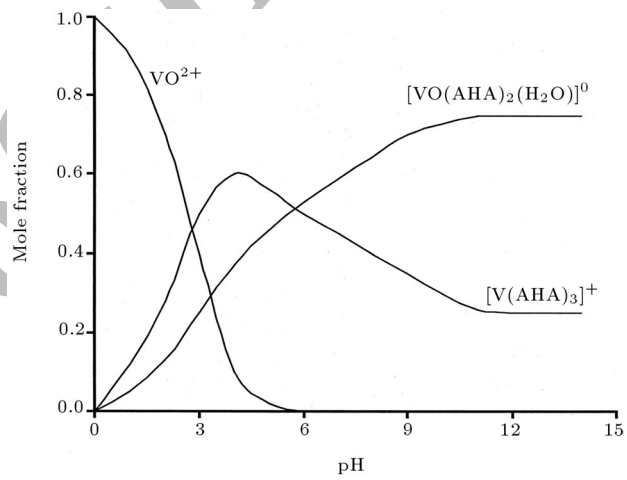

Figure 3. Species distribution graph for V(IV)-AHA complexes.

Figure 4 shows that for V(V)AHA above pH 3 nearly all metal is found to be complexed, in which 60% may be 1:6 monodentate species while 15-20% 1:2 bidentate and an almost equal percentage of 1:3 bidentate was found. For the same system at pH 10, 70% 1:3 and 30% 1:2 were observed with no monodentate species [31].

Acetohydroxamic acids behave as a mono as well as bidentate ligand. Their stability constant (K) values were determined by potentiometric titrations at different temperatures. In the case of vanadium (V), a slight change in the titration curve, with respect to temperature, was observed. In this case, the length of the depression of the curve was found to be sensitive to the temperature and was found to increase as the temperature increased (Figure 2).

In the case of vanadium (IV), some changes were also observed in the titration curve at different temperatures but were different as compared to vanadium

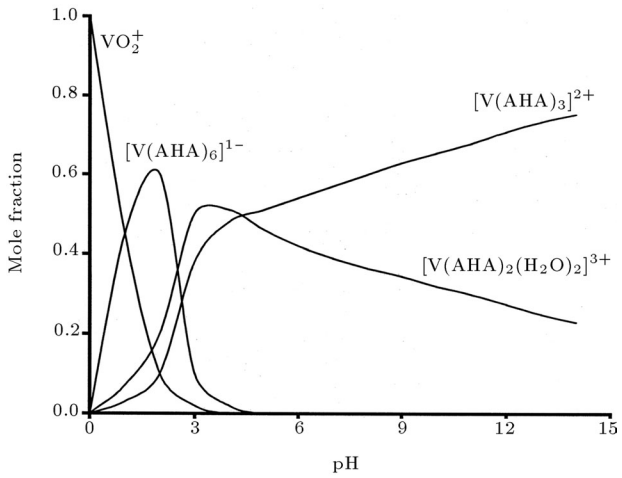

Figure 4. Species distribution graph for V(V)-AHA complexes.

(V). These curves had less depression but more twists, which showed the low stability constant values with more species present at a time. The stability of these species present at lower pH was found to be more sensitive to temperature (Figure 1). The computer program "BEST" was used for refining log β values. These refined β values with least sigma fit were selected for each species (Tables 1 and 2).

Since, at equilibrium $\Delta G = -RT \ln K$:

$$\ln K = \frac{-\Delta G}{RT} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}.$$

Since, $\ln K = \ln \beta$, therefore:

$$\ln \beta = \frac{-\Delta H}{R}.\frac{1}{T} + \frac{\Delta S}{R},$$

where β is the overall formation constant [32] and

$$\beta_n = K_1.K_2.K_3 \cdots K_n,$$

Table 1. $\ln \beta$ values for V(IV)-AHA complexes at different temperatures.

S. #	Complexes	35°C	40°C	45°C	50°C
1	$[VO(AHA)(H_2O)_3]^+$	7.139	7.599	8.061	8.521
2	$[VO(AHA)_2(H_2O)]^0$	12.21	12.67	13.13	13.59
3	$[V(AHA)_3]^+$	17.04	17.50	17.96	18.42

Table 2. $\ln \beta$ values for V(V)-AHA complexes at different temperatures.

S. #	Complexes	35°C	40°C	45°C	50°C
1	$[V(AHA)(H_2O)_4]^{4+}$	7.021	8.011	9.202	11.10
2	$[V(AHA)_2(H_2O)_2]^{3+}$	10.11	11.05	12.31	13.21
3	$[V(AHA)_3]^{2+}$	11.30	12.21	13.12	14.33

or:

$$\beta_n = \frac{[ML_n]}{[M][L]^n}.$$

Therefore, the parameter for β can be $(\text{mol/L})^{-n}$.

The graphs were plotted against $\ln \beta$ and $1/T$ to calculate $\Delta H, \Delta S$ and ΔG values (Figures 5 and 6). The slope of the graph gave -$\Delta H/R$ values and the intercept was found to be equal to $\Delta S/R$. Using $\Delta G = \Delta H - T\Delta S.\Delta G$ for each species of vanadium (IV) and vanadium (V), acetohydroxamate complexes were also obtained (Table 3). All ΔH and ΔG values calculated for vanadium (IV) and vanadium (V) acetohydroxamic acid complexes were found to be negative showing high thermal stability. In case of vanadium (IV), 1:1 complex showed a greater negative enthalpy value (-166 $kJmol^{-1}$), approximately double to that of 1:2 and 1:3 (-83 $kJmol^{-1}$ and -86 $kJmol^{-1}$, respectively), showing a high change in internal energy during formation of the complex from aqua metal ions. Free energy change,

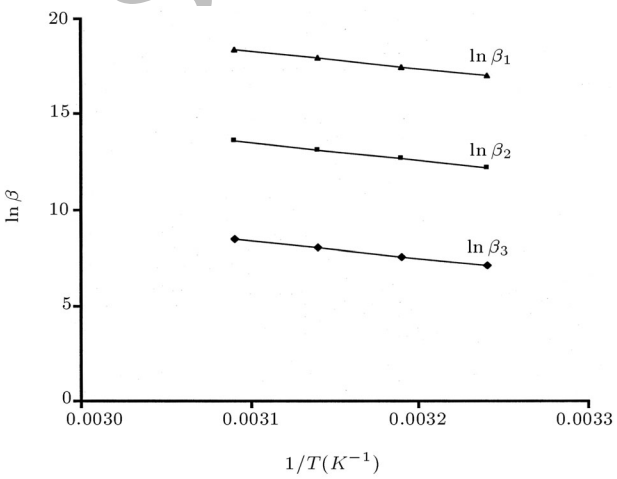

Figure 5. Heat energies of V(IV)-AHA complexes.

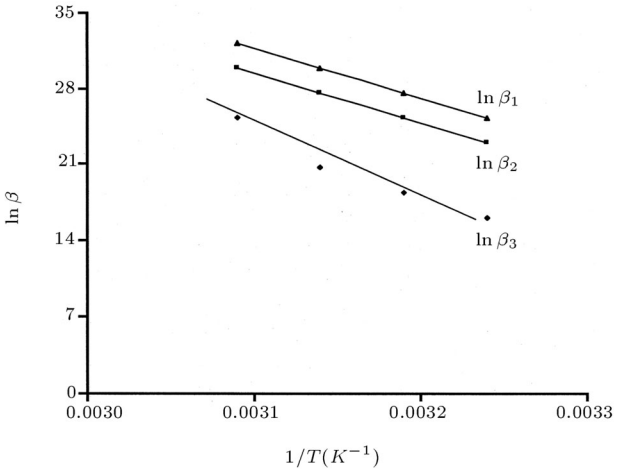

Figure 6. Heat energies of V(V)-AHA complexes.

Table 3. Thermodynamic parameters for V(IV)and V(V) acetohydroxamate complexes.

S. #	Complexes	ΔH kJ/mole ±14	ΔS J/mole/deg ±05	ΔG kJ/mole ±08
1	$[VO(AHA)(H_2O)_3]^+$	-166.0	672.3	-372.3
2	$[VO(AHA)_2(H_2O)]^0$	-83.0	475.3	-230.5
3	$[V(AHA)_3]^+$	-86.0	567.1	-261.8
4	$[V(AHA)(H_2O)_4]^{4+}$	-224.3	783.3	-457.7
5	$[V(AHA)_2(H_2O)_2]^{3+}$	-175.4	652.1	-367.8
6	$[V(AHA)_3]^{2+}$	-164.6	625.8	-351.1

in cases of each type of complex in vanadium (IV)-acetohydroxamate, were slightly different from each other, but in the order 1:1>1:3>1:2, the ML_3 type complex showed greater negative ΔG (-261.8 kJmol^{-1}) value than ML_2 (-230.5 kJmol^{-1}). It may be due to the removal of vanadyl oxygen during formation of the hexadentate complex with the bidentate ligand. The ΔG is -372.2 kj/mol for 1:1 colmplex, the highest value among all mentioned VO^{+2} complexes. Change in entropy values were also observed high and positive in all cases and in the same order as discussed in the free energy case (672.3 Jmol^{-1}, 475.38 Jmol^{-1} and 567.12 Jmol^{-1}, respectively). This can be easily proved from the following equations:

$$[VO(H_2O)_5]^{2+}+HL\rightleftharpoons[VO(H_2O)_3L]^{+}+H^{+}+2H_2O, \quad (1)$$

$$[VO(H_2O)_3L]^{+}+HL\rightleftharpoons[VO(H_2O)L_2]+H^{+}+2H_2O, \quad (2)$$

or:

$$[VL_3]^{+}+OH^{-}+H_2O\rightleftharpoons[VO(H_2O)L_2]+HL, \quad (3)$$

$$[VO(H_2O)_5]^{2+}+3HL\rightleftharpoons[VL_3]^{+}+H^{+}+6H_2O. \quad (4)$$

Equations 1 and 2 show that, in the cases of 1:1 and 1:3, the entropy should be the same. In the case of 1:2, there may be two types of mechanism, as shown in Equations 2 and 3. In case of Equation 2, the entropy should be equal to 1:1 and 1:2, but, in the case of the second mechanism, as it is in Equation 3, the change in entropy should come in as a negative value. Results showed that formation of the ML_2 species may go through both types of mechanism occurring simultaneously, therefore, the value is in between these two.

In the case of vanadium (V)-acetohydroxamate complexes, again, ΔH values are very high and negative, showing a very large change in total internal energy during the complex formation towards stability. The highest $-\Delta H$ value is found for 1:1 (-224 kJmol^{-1}) complexes, next for 1:2 (-175 kJmol^{-1}) and, then for 1:3 (-164 kJmol^{-1}). ΔG were also obtained at high negative values and in the same order (-457 kJmol^{-1}, -370 kJmol^{-1} and -351 kJmol^{-1}, respectively). Similarly, a decreasing trend in ΔS values was also observed for these species of vanadium (V), but with positive signs (783 Jmol^{-1}deg^{-1}, 652 Jmol^{-1} deg^{-1} and 625 Jmol^{-1} deg^{-1}, respectively). The following equations can justify these values:

$$[V(H_2O)_6]^{5+}+HL\rightleftharpoons[V(H_2O)_4L]^{4+}+H^{+}+2H_2O, \quad (5)$$

$$[V(H_2O)_4L]^{4+}+HL\rightleftharpoons[V(H_2O)_2L_2]^{3+}+H^{+}+2H_2O, \quad (6)$$

or:

$$[V(H_2O)_4L]^{4+}+L^{-}\rightleftharpoons[V(H_2O)_2L_2]^{3+}+2H_2O, \quad (7)$$

$$[V(H_2O)_2L_2]^{3+}+HL\rightleftharpoons[VL_3]^{2+}+H^{+}+2H_2O, \quad (8)$$

or:

$$[V(H_2O)_2L_2]^{3+}+L^{-}\rightleftharpoons[VL_3]^{2+}+2H_2O. \quad (9)$$

The results related to ΔS values of the species showed that if the mechanism shown in Equations 5, 6 and 8 is valid, then, the entropy change should be almost equal for all species. As it changes and decreases for 1:2 and 1:3, it can be concluded that, for 1:2, both of the mechanisms shown in Equations 6 and 7 may simultaneously occur while, for 1:3, the mechanism shown in Equation 9 is more favorable than in Equation 8.

When $\Delta H, \Delta S$ and ΔG values of vanadium (IV) and vanadium (V) were compared with each other, it was observed that these values were much higher in the case of vanadium (V). For example, the change in enthalpy and free energy was four to five times higher, while the change in entropy values was two, to three times greater in the higher oxidation state. This may be due to the very low positive charge of the free aqua vanadyl ion $[VO(H_2O)_5]^{2+}$, in the case of vanadium (IV), as compared to the highly charged free aqua vanadium ion $[V(H_2O)_6]^{5+}$, in the case of vanadium (V) as a starting material.

EXPERIMENTAL

In the present research work, all reagents used were of A. R. grade supplied by different sources, such as Merck, Sigma and Aldrich Riedel-de-Haen, and were employed without further purification. Doubly distilled and deionized water was used in the preparation of all stock/standard solutions.

For all pH titration, CO_2 free water was prepared by boiling redistilled and deionized water for 10 minutes and, then, cooling it in an air tight flask [33]. A 0.050 M solution of potassium hydrogen phthalate, which has the pH value of 4.01 at 25°C, was used to calibrate the pH meter.

The titration was carried for V(IV) and V(V) with acetohydroxamic acid at variable temperatures. The temperature ranges selected were 35-50°C. The same volumes of equimolar acetohydroxamic acid and vanadium(V) solutions were mixed, taken in a double walled titration cell kept on a magnetic stirrer. The rubber stopper on the cell had holes for the addition of a standard base thermometer and for a glass electrode. The temperature was controlled by circulating thermostated water through the jacket.

The temperature of the reaction mixture was maintained throughout the experiment. Aliquotes of standard 0.20 M NaOH were added with the help of a micropipette and the pH was measured after each addition with a combination glass electrode attached to a pH meter, Orion S.A model 720 having a resolution of $\pm$ 0.001 pH units.

CONCLUSIONS

From the above results, it is observed that 1:1 complexes of both complexes have high $-\Delta H$ values. This may be due to a greater residual positive charge on these complexes. In V(IV) for 1:2, when the complex is neutral, less negative ΔH was observed (-83 kJ/mole), which was about $\frac{1}{2}$ of the 1:1(-166 kJ/mole). For V(V), a high positive charge on the aqua complex produces greater penetration for the ligand and, therefore, a very high negative ΔH value (-224 kJ/mole), which shows a stable complexation. Formation of such a highly stable complex ion of V^{5+} may help to decrease its toxicity.

Replacement of monodentate ligands by bidentate also show very high ΔS values. In the case of vanadyl ion (VO^{2+}) for 1:3 complex replacement of oxygen by ligand also shows greater ΔS (567.12 kJ/mole) than 1:2 complex (475.38 kJ/mole). The highest degree of freedom is for V^{5+} 1:1 acetohydroxamate complex (783 J/mol/deg) and, therefore, ΔG is -457 kJ/mole. All values showed a most stable complexation. All V^{5+} complexes are much more favorable then V^{+4} complexes.

The above study may be very important for the removal of V^{5+} from the biological system. V^{4+} is found to be valuable for diabetes treatment and, therefore, is not toxic. It also showed less affinity towards hydroxamate as compared to V^{5+} and, hence, it can be concluded that hydroxamate may play an effective role in detoxification of the V^{+5} from the biological system.

REFERENCES

1. Sigel, H. and Sigel, A., Eds., *Metal Ions in Biological Systems*, **35**, M. Dekker, Inc. New York, USA (1998).
2. Kuzrak, B., Kozlowski, H. and Farkas, E. *Coord. Chem. Rev.*, **114**, p 169 (1992).
3. Bergeron, R.J. and Brittenham, G.M., Eds., CRC Press, Boca Raton, FL, USA (1994).
4. Goldwaser, I., Gefel, D., Gershonov, E., Fridkin, M. and Shechter, Y., *Inorg. J. Biochem.*, **80**, p 21 (2000).
5. Rehder, D., *Angew. Chem., Int. Ed. Engl.*, **30**, p 148 (1991).
6. Rehder, D., *Biometals*, **5**, p 3 (1992).
7. Rehder, D., *Coord. Chem. Rev.*, **182**, p 297 (1999).
8. Baran, E.J., *Inorg. J. Biochem.*, **80**, pp 1-10 (2000).
9. Goldwaser, I., Li, J., Gershonov, E., Armoni, M., Karneili, E., Fridkin, M. and Shechter, Y., *J. Biol. Chem.*, **274**, p 26617 (1999).
10. Black, D. and Hartshorn, A., *J. Coord. Chem. Rev.*, **9**, p 219 (1972).
11. Baran, E.J., *Bol. Soc. Chil. Quim.*, **42**, p 247 (1997).
12. Harris, W.R., Friedman, S.B. and Silberman, D., *J. Inorg. Biochem.*, **20**, p 157 (1984).
13. Chasteen, N.D., Grady, J. and Holloway, K. *Inorg. C. E. Chem.*, **25**, p 2754 (1986).
14. Song, B., Aebischer, N. and Orvig, C., *Inorg. Chem.*, **20**, p 14 (2001).
15. Yuen, V.G., Caravan, P., Gelmini, L., Glover, N., McNeill, J.H. Setyawati, I.A. Zhou, Y. and Orvig, C., *J. Inorg. Biochem.*, **68**, p 109 (1997).
16. Crans, D.C., Yang, L., Jakusch, T. and Kiss, T., *Inorg. Chem.*, **39**, p 4409 (2000).
17. Shigeki, H., Takahiro, I. and Yushin, N., *Chem. Pharm. Bull.*, **48**(5), pp 603-609 (2000).
18. Dekker, M. "Vanadium and its role in life", *Biological Systems*, H. Sigel, A. Sigel, Eds., **31**, New York, USA (1995).
19. Slebodnick, C., Hamstra, B.J. and Pecoraro, V.L., *Struct. Bonding*, **89**, p 51 (1997).
20. Crans, D.C., *Comments Inorg. Chem.*, **16**, p 1 (1994).
21. Baran, E.J., *An Acad. Nac. Ciene. Exactas Fis Nat.*, **46**, p 35 (1994).
22. Butler, A. and Walker, J.V., *Chem. Rev.*, **93**, p 1937 (1993).
23. Barnett, P., Hemrika, W., Dekker, L.H., Muijsers, A.O., Renirie, R. and Wever, I.R., *J. Biol. Chem.*, **273**, p 23381 (1998).
24. Cotton, F.A., Wilkinson, G., *Advanced Inorganic Chemistry*, 5th Ed., Wiley InterScience Publication, p 112 (1988).
25. Shaver, A., Ng, J.B., Hall, D.A., Lum, B.S. and Posner, B.I., *Mol. Cell. Biochem.*, **153**, p 5 (1995).

26. Martell, A.E. and Motekaitis, A.J. *The Determination & Use of Stability Constants*, 1st Ed., VCH (1988).
27. Kawabe, K., Tadokoro, M., Kojima, Y., Fujisawa, Y. and Sakurai, H., *Chem. Lett.*, **9** (1998).
28. Li, J., Elberg, G., Crans, D.C. and Shechter, Y., *Biochemistry*, **35**, p 8314 (1996).
29. Reul, B.A., Amin, S.S., Buchet, J.P., Ongemba, L.N., Crans, D.C. and Brichard, S.M., *Br. J. Pharm.*, **126**, p 467 (1999).
30. Ali, K., Fatima, N. and Maqsood, Z.T., *J. Iran Chem. Soc.*, **1**(1), pp 65-70 (2004).
31. Ali, K., Fatima, N. and Maqsood, Z.T., *J. Saudi Chem. Soc.*, **7**(3), pp 359-355 (2003).
32. Jordan, R.B., *Reaction Mechanisms of Inorganic and Organometallic Systems*, Ed. 1st, Oxford Univ. Press Inc. (1991).
33. Maqsood, Z.T. "Formation and activity of Fe(III) complexes with gallic acid", Ph.D Thesis, Department of Chemistry, Univ. of Karachi, Pakistan (1992).