A New Approach for Thermodynamic Study on the Binding of Human Serum Albumin with Cerium Chloride

G. Rezaei Behbehani,* A. Divsalar,† A. A. Saboury,‡ F. Faridbod,‡ and M. R. Ganjali‡

*Chemistry Department, Imam Khomeini International University, Qazvin, Iran. *E-mail: grb402003@yahoo.com*
†Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
‡Departments of Chemistry, University of Tehran, Tehran, Iran
Received March 27, 2009, Accepted April 26, 2009

Thermodynamics of the interaction between Cerium (III) chloride, Ce^{3+}, with Human Serum Albumin, HSA, was investigated at pH 7.0 and 27 °C in phosphate buffer by isothermal titration calorimetry. Our recently solvation model was used to reproduce the enthalpies of HSA interaction by Ce^{3+}. The solvation parameters recovered from our new model, attributed to the structural change of HSA and its biological activity. The interaction of HSA with Ce^{3+} showed a set of two binding sites with negative cooperativity. Ce^{3+} interacts with multiple sites on HSA affecting its biochemical and biophysical properties.

Key Words: Human Serum Albumin, Isothermal titration calorimetry, Cerium (III) chloride.

Introduction

Human Serum Albumin (HSA), as the most abundant protein constituent of blood plasma, has a high affinity to an extraordinarily diverse range of materials, such as drugs, metabolites, fatty acids and metal ions.[1-3] HSA can bind and carry through the bloodstream many drugs, which are poorly soluble in water and it is also responsible for the maintenance of blood pH, the drug disposition and efficacy, and the contribution of colloid osmotic blood pressure.[4-5] The unique feature of albumin is its ability to bind a wide variety of compounds, mainly because of the availability of hydrophobic pockets inside the protein network and the flexibility of the albumins to adapt its shape.[6-8] The crystallographic analysis of HSA revealed that this protein is a single-chain 66 kDa protein, which is largely α-helical, and consists of three structurally homologous domains that assemble to form a heart-shaped molecule. Each domain is a product of two subdomains, which are predominantly helical and extensively cross-linked by several disulfide bridges.[8-10]

Erbium is one of the rare chemicals that can be found in houses in equipment such as color televisions, fluorescent lamps, energy-saving lamps and glasses. All rare chemicals have comparable properties. Erbium will gradually accumulate in soils and water soils and this will eventually lead to increasing concentrations in humans, animals and soil particles. The use of erbium is still growing, due to the fact that it is suited to produce catalysers and to polish glass. Erbium is mostly dangerous in the working environment, due to the fact that damps and gasses can be inhaled with air. This can cause lung embolisms, especially during long-term exposure. Erbium can be a threat to the liver when it accumulates in the human body.[11] All erbium compounds should be regarded as highly toxic because the biological properties of the lanthanides, primarily based on their similarity to calcium, have a high affinity for Ca^{2+} sites on biological molecules and hence can act as either Ca^{2+} inhibitors or probes. Although the Lanthanide cannot gain access to intracellular organelles, they have been used as biochemical probes to study calcium transport in mitochondria and other organelles.[13] The biological properties of the lanthanides, primarily based on their similarity to calcium, have been the research basis into the potential therapeutic applications of lanthanides, since the early part of the twentieth century. The lanthanides have similar ionic radii to calcium, but by virtue of possessing a higher charge, they exhibit a high affinity for the Ca^{2+} sites on biological molecules and a stronger binding to water molecules.[12-14] One of the major physiological effects of the lanthanide (Ln^{3+}) ions is to block both the voltage operated and the receptor operated calcium channels. Ln^{3+} can block the Na^{+}/Ca^{2+} synaptic plasma membrane exchange and inhibit the skeletal, smooth and cardiac muscle contraction by blocking the Ca^{2-}-ATPase in the sarcoplasmic reticulum of the muscle. The Ln^{3-} ions themselves are unable to cross the cell membranes, but they act by blocking the exterior face of the calcium channel. Though Ln^{3-} cannot gain access to the intracellular organelles, they have been used as biochemical probes to study the calcium transport in mitochondria and the other organelles.[15-16] The lanthanides can substitute calcium in proteins, even though it should be noted that the Ln^{3+} ions can also substitute other metal ions, such as Mg^{2+}, Fe^{3-} and Mn^{2+}. The calcium dependent enzymes can either be inhibited by lanthanides, or in some cases, be activated to a similar or greater extent by calcium. It has been proposed that the stimulatory or inhibitory effect of the lanthanides may be a function of the role of calcium in the native enzyme. The interest in lanthanides regarding the biochemical reactions arises from the fact that they can be used as probes to unravel the interactions between Ca^{2+} and the biologically important molecules.[17-20]

This work represents the most comprehensive study on the interactions between Ce^{3-} cations with HSA and provides new evidence for validity of our recently introduced solvation model and more insights into the interactions of Ce^{3+} with HSA for further understanding of the effects of metal ions on the stability and the structural changes of macromolecules.

Materials

HSA was obtained from Sigma and Ce^{3+} was purchased from Merck. Protein concentrations were determined from absorbance measurements at 277 nm in 1 cm quartz cuvettes. All other materials and reagents were of analytical grade, and solutions were made in 50 mM buffer phosphate using double-distilled water.

Method

The isothermal titration calorimetric experiments were carried out on a VP-ITC ultra sensitive titration calorimeter (MicroCal, LLC, Northampton, MA). The microcalorimeter consists of a reference cell and a sample cell of 1.8 mL in volume, with both cells insulated by an adiabatic shield. All solutions were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with HSA solution (40 μM) and the reference cell contained buffer solution. The solution in the cell was stirred at 307 rpm by the syringe (equipped with micro propeller) filled with $CeCl_3$ solution (500 μM) to ensure rapid mixing. Injections were started after baseline stability had been achieved. The titration of HSA with $CeCl_3$ solution involved 30 consecutive injections of the ligand solution, the first injection was 5 μL and the remaining ones were 10 μL. In all cases, each injection was done in 6 s at 3 min intervals. To correct the thermal effects due to $CeCl_3$ dilution, control experiments were done in which identical aliquots were injected into the buffer solution with the exception of HSA. In the ITC experiments, the enthalpy changes associated with processes occurring at a constant temperature are measured.[17-20] The measurements were performed at a constant temperature of 27.0 ± 0.02 °C and the temperature was controlled using a Poly-Science water bath

Results and Discussion

We have shown previously that the enthalpies of the HSA + Ce^{3+} interactions in the aqueous solvent system can be accounted for quantitatively in terms of three factors: preferential solvation by the components of a mixed solvent, weakening or strengthening of solvent-solvent bonds by the solute and the change in the enthalpy of the solute-solvent interactions.[21-32] This treatment leads to:

$$\Delta H = \Delta H_{max}\, x'_B - \delta^{\theta}_B (x'_A L_A - x'_B L_B) - (\delta^{\theta}_B - \delta^{\theta}_A)(x'_A L_A - x'_B L_B) x'_B \quad (1)$$

The parameters $\delta^{\theta}_A = (\alpha n + \beta N)$ and $\delta^{\theta}_B = (\alpha n + \beta N)$ are the composite parameters which reflect to the net effect of Ce^{3-} cations on the HSA stability in the low and high Ce^{3+} concentrations respectively, with αn resulting from the formation of a cavity wherein "n" Ce^{3-} cations become the nearest neighbors of the HSA and βN reflecting the enthalpy change from strengthening or weakening of water + Ce^{3-} bonds of N solvent molecules ($N \geq n$) around the cavity. The positive values for $\delta^{\theta}_A = (\alpha n + \beta N)^{\theta}_A$ and $\delta^{\theta}_B = (\alpha n + \beta N)^{\theta}_B$ indicate that Ce^{3+} cations stabilized the HSA structure and vice versa. The constants α and β reflect the proportion of the total enthalpy of water + Ce^{3-} binding which is associated with the cavity formation and modification of solvent structure (water + Ce^{3+}) around the cavity, respectively. Cooperative binding requires that the macromolecule have more than one binding site, since cooperativity results from the interactions between binding sites. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity. Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, the protein exhibits negative cooperativity. If the ligand binds at each site independently, the binding is non-cooperative. $p < 1$ or $p > 1$ indicate positive or negative cooperativity of macromolecule for binding with ligand respectively; $p = 1$ indicates that the binding is non-cooperative. x'_B can be expressed as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \quad (2)$$

x_B is the fraction of the Ce^{3-} needed for saturation of the binding sites, and $x_A = 1 - x_B$ is the fraction of unbounded Ce^{3-}. Now the model is a simple mass action treatment, with metal ions replacing water molecules, at the binding sites in the present case. We can express x_B fractions, as the total Ce^{3-} concentrations divided by the maximum concentration of the Ce^{3+} upon saturation of all HSA as follows:

$$x_B = \frac{[Ce^{3+}]_T}{[Ce^{3+}]_{\max}} \quad x_A = 1 - x_B \quad (3)$$

$[Ce^{3+}]_T$ is the total concentration of Ce^{3-} and $[Ce^{3+}]_{\max}$ is the maximum concentration of the Ce^{3+} upon saturation of all HSA. In general, there will be "g" sites for binding of Ce^{3+} per HSA molecule and v is defined as the average moles of bound Ce^{3+} per mole of total HSA. L_A and L_B are the relative contributions of unbounded and bounded Ce^{3-} to the enthalpies of dilution of $CeCl_3$ with the exclusion of HSA and can be calculated from the enthalpies of dilution of $CeCl_3$ in buffer, ΔH_{dilut}, as follows:

$$L_A = \Delta H_{dilut} + x_B \left(\frac{\partial \Delta H_{dilut}}{\partial x_B} \right)$$
$$L_B = \Delta H_{dilut} - x_A \left(\frac{\partial \Delta H_{dilut}}{\partial x_B} \right) \quad (4)$$

The enthalpies of HSA + Ce^{3-} interactions, ΔH, were fitted to Eq. 1 over the whole Ce^{3-} compositions. In the procedure the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). δ^{θ}_A and δ^{θ}_B parameters have been also optimized to fit the data. The optimized δ^{θ}_A and δ^{θ}_B values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high r^2 values (0.99999) support the method. The binding parameters for HSA + Ce^{3-} interactions recovered from Eq. 1 were listed in Table 1. The agreement between the calculated

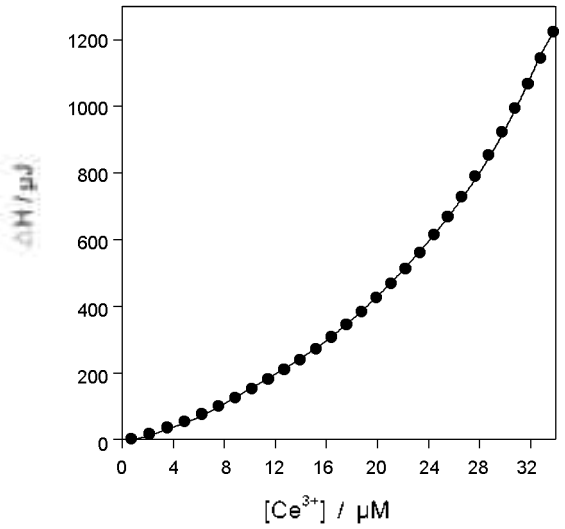

Figure 1. Comparison between the experimental enthalpies, ΔH, for HSA + Ce^{3-} interactions (•) and calculated data (lines) *via* Eq. 1.

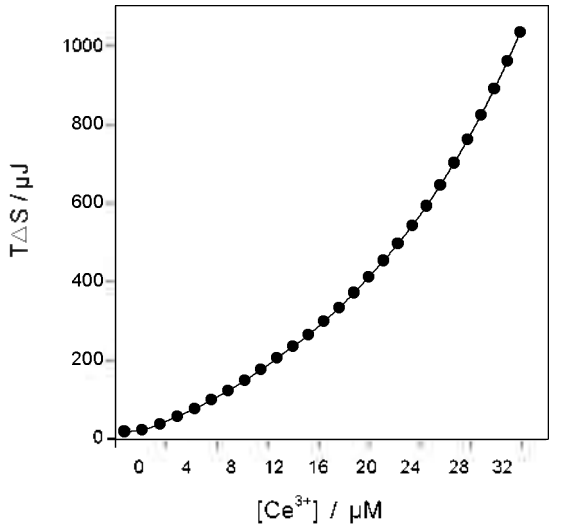

Figure 2. Comparison between the experimental entropies (•) for HSA + Ce^{3+} interactions and calculated data (lines) *via* Eq. 8.

Table 1. Enthalpies of HSA+Ce^{3+} interactions, ΔH, in Ce^{3-} solution with water at 300 K in kJ mol^{-1}. ΔH_{dilut} is the enthalpies of dilution of Ce^{3+} with water. Precision is ± 0.400 μJ or better

[Ce^{3-}] / μM	ΔH / μJ	ΔH_{dilut} / μJ	[HSA] / μM
0.7112376	2.0887042	0.9428408	44.839972
2.1186441	16.040917	3.5892824	44.523305
3.5063114	35.152086	2.8643032	44.21108
4.8746518	53.890023	2.3254594	43.903203
6.2240664	75.783316	0.7497666	43.599585
7.5549451	99.083849	-0.475433	43.300137
8.8676671	124.04309	-2.738694	43.004775
10.162602	151.10382	-5.353451	42.713415
11.440108	180.42677	-8.446066	42.425976
12.700535	209.32558	-10.22587	42.14238
13.944223	238.57877	-12.63735	41.86255
15.171504	272.07825	-15.85675	41.586412
16.3827	306.86743	-18.09911	41.313893
17.578125	344.13927	-21.22295	41.044922
18.758085	383.93124	-23.82245	40.779431
19.922879	424.74196	-26.74067	40.517352
21.072797	467.73297	-29.84252	40.258621
22.208122	512.63445	-33.51361	40.003173
23.32913	561.334	-36.85502	39.750946
24.43609	614.44173	-40.69335	39.50188
25.529265	669.22532	-44.33178	39.255915
26.608911	727.69574	-47.70793	39.012995
27.675277	788.93065	-51.4673	38.773063
28.728606	853.81211	-55.34299	38.536064
29.769137	922.02473	-59.26371	38.301944
30.797101	993.42001	-63.6224	38.070652
31.812725	1067.5611	-67.71909	37.842137
32.816229	1145.1919	-72.53487	37.616348

and experimental results (Fig. 1) is striking, and gives considerable support to the use of Eq. 1.

Φ is the fraction of HSA molecule undergoing complexation with Ce^{3+} which can be expressed as follows:

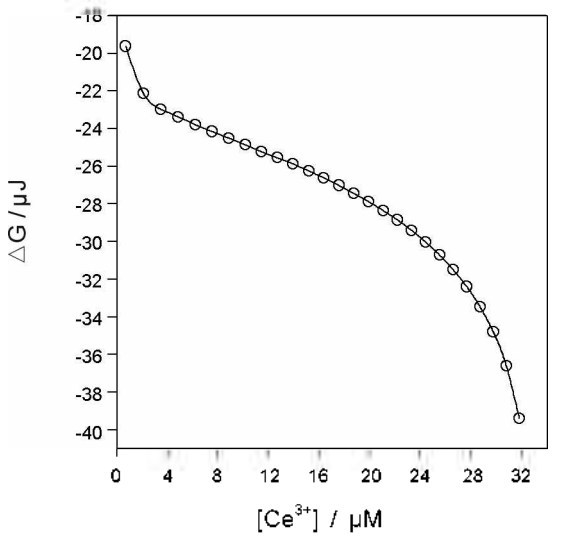

Figure 3. Comparison between the experimental free energies (o) for HSA + Ce^{3+} interactions and calculated data (lines).

$$\Phi = \frac{\Delta H}{\Delta H_{max}} \tag{5}$$

ΔH_{max} represents the heat value upon saturation of all HSA. The appearance association equilibrium constant values, K_a, as a function of Ce^{3-} free concentration, $[Ce^{3-}]_F$, can be calculated as follow:

$$K_a = \frac{\Phi}{(1-\Phi)[Ce^{3-}]_F} = \frac{\Phi}{(1-\Phi)(1-x_B)[Ce^{3+}]_T} \tag{6}$$

The association equilibrium constants for successive replacement of water molecules by Ce^{3-} cations are as follows:

$$K_a = x_A^g - \sum_{i=1}^{g} K_i \frac{x_B^i}{x_A^{i-g}} \tag{7}$$

Table 2. Thermodynamic parameters for HSA + Ce^{3+} interactions in Ce^{3-} solution with water *via* Eq. 1.

parameters	HSA + Ce^{3-}
p	0.280
δ_A^g	2.134 ± 0.075
δ_B^g	2.134 ± 0.001
K_1 / μM^{-1}	2.004 ± 0.014
K_2 / μM^{-1}	1.161 ± 0.011

K_i's are the microscopic association equilibrium constants for the equilibria:

$$HSA(H_2O)_g + i\,(Ce^{3-}) \Leftrightarrow HSA(H_2O)_{g-i}(Ce^{3+})_i \qquad \text{(E1)}$$

K_a values obtained from Eq. 6. have been fitted to Eq. 7 using a computer program for nonlinear least-square fitting. Therefore, we can approach to "g" value simply (g = 2 in this work). The Gibbs free energies as a function of Ce^{3-} concentrations can be obtained as follows:

$$\Delta G = -RTLnK_a \qquad (8)$$

Eqs. 6 and 7 allow us to have the K_a values in every concentrations of Ce^{3+} with the least standard deviations and correlation coefficients are so close to one. Gibbs energies, ΔG, calculated from Eq. 8 have shown graphically in Fig. 2. ΔS values were calculated using ΔG values and have shown in Fig. 3.

Previous reports revealed that some molecules such as different species of metal ions of mercury,[33] paclitaxel and Cu (II) complex of 5,10,15,20-tetrakis (4-*N*-benzyl-pyridyl) porphyrin bind in the two distinct sites with different affinity on HSA which is in a good agreement with our results.[34]

A nonpolar residue dissolved in water induces a solvation shell in which water molecules are highly ordered. When two nonpolar groups come together on the folding of a polypeptide chain, the surface area exposed to the solvent is reduced and part of the highly ordered water in the solvation shell is released to bulk solvent which results to an increase in the entropy. It is possible to introduce a correlation between change in δ_A^g and increase in the stability of HSA+Ce^{3+} complex. The positive values of δ_A^g and δ_B^g show that HSA is substantially stabilized against unfolding by Ce^{3+} ions. Therefore, by the definition given above, HSA can serve as a reasonable model of specific binding interactions for Ce^{3-} ions. The positive δ_A^g and δ_B^g values (Table 2) for HSA+ Ce^{3-}interaction, indicate that the HSA structure is stabilized, as a result of its interaction with Ce^{3+} ions, resulting in an increase in its biological activity. δ_A^g and δ_B^g values are equal, indicating that the most of HSA is in its native form.

p value is less than one (p = 0.28), which indicates that there is negative cooperativity in two binding sites of HSA. $K_2 < K_1$ (Table 2) would indicate that binding of the second site is inhibited (anticooperativity or negative cooperativity). This conclusion is in a good agreement with cooperativity prediction by Eq. 1 as p value recovered from this equation is 0.28.

HSA is a large globular protein with several physiological roles ranging from transport of hydrophobic metabolites, such as fatty acids and bilirubin, to the maintenance of the blood osmotic pressure and transport of metal ions like Cu^{2+}, Ni^{2-} and Zn^{2-}. Human serum albumin contains the N-terminal sequence N-Asp-Ala-His-Lys, and is able to strongly bind the metal ions (especially, transition metals such as Co, Cu and Ni) with a high affinity for nitrogen through the donor set (NH_2, N^-, N^-, N_{imid}), where NH_2 represent the terminal amino, N^- the deprotonated amide groups of residues 2 and 3 and N_{imid} the imidazole nitrogen belonging to His-3.[35,36] The characterization of the metal-transport site of HSA was carried out by Laussac and Sarkar[36] who established the participation of α-NH_2, two intervening peptide nitrogen atoms, the imidazole nitrogen atom of the histidine residue in the third position, and the side chain carboxyl group of Asp-1 in a penta-coordinate structure.[36,37] Previous studies have shown that that albumin binds one Vanadium, VO^{2+}, at the N-terminal part (the "strong" site) and several other ions via non-specific interactions with carboxylate side chains of surface amino acids ("weak" sites): the exact number of such non-specific VO^{2+} binding sites is at least five and possibly as many as twenty.[35] Therefore, it might be concluded that one of the binding site of Ce^{3+} on HSA is located at N-terminal part (the "strong" site, from K value (Table 2)) and the other weaker binding site might be located at carboxylate side chains of surface amino acids. The thermodynamic parameters obtained from the binding studies of the Ce^{3-} complex to the HSA carrier proteins may be useful in the evaluation of the structural changes induced by the ligand on the carrier protein structure.

Acknowledgments. Financial support from the Universities of Imam Khomeini (Qazvin) and Tehran are gratefully acknowledged.

References

1. Wanga, T.; Xianga, B.; Wang, Y.; Chen, C.; Dong, Y.; Fang, H.; Wang, M. *Colloids Surf. B: Biointerfaces* **2008**, *65*, 113.
2. Yue, Y.; Chen, X.; Qin, J.; Yao, X. *Dyes and Pigments* **2008**, *79*, 176.
3. Waheed, A. A.; Sridhar Rao, K.; Gupta, P. D. *Anal.* **2000**, *287*, 3.
4. Fichtl, B.; Nieciecki, A. V.; Walter, K. *Adv. Drug Res.* **1991**, *20*, 117.
5. Rieutord, A.; Bourget, P.; Troche, G.; Zazzo, J. F. *Int. J. Pharm.* **1995**, *119*, 57.
6. Xiao, Q.; Huang, S.; Liu, Y. *Biochim. Biophys. Acta (BBA) - Proteins & Proteomics* **2008**, *1784*, 1020.
7. Colmenarejo, G. *Med. Res. Rev.* **2003**, *23*, 275.
8. Zhou, Q. J.; Xiang, J. F.; Tang, Y. L.; Liao, J. P.; Yu, C. Y.; Zhang, H.; Li, L.; Yang, Y. Y.; Xu, G. Z. *Colloids Surf. B. Biointerfaces* **2008**, *61*, 75.
9. Nagaoka, T.; Fukuda, T.; Hashizume, T.; Nishiyama, T.; Tada, H.; Yamada, H.; Salomon, D. S.; Yamada, S.; Kojima, I.; Seno, M. *J. Mol. Biol.* **2008**, *380*, 83.
10. Zhang, G.; Que, Q.; Pan, J.; Guo, J. *J. Mol. Struct.* **2008**, *881*, 132.
11. Haghighi, B.; Papari, M. M.; Shahidi, D. *J. Chin. Chem. Soc.* **2008**, *55*, 245.
12. Fricker, S. P. *Chem. Soc. Rev.* **2006**, *35*, 524.
13. Wang, K.; Cheng, Y.; Yang, X.; Li, R. *Met. Ions Biol. Syst.* **2003**, *40*, 707.
14. Evans, C. H. *Trends Biochem. Sci.* **1983**, *8*, 445.

15. Tang, J.; Marcus, R. A. *J. Chin. Chem. Soc.* **2006**, *53*, 1.
16. Yeung, E. W.; Head, S. I.; Allen, D. G. *J. Physiol-London* **2003**, *552*, 449.
17. Saboury, A. A. *J. Therm. Anal. Cal.* **2003**, *72*, 93.
18. Saboury, A. A. *J. Therm. Anal. Cal.* **2004**, *77*, 997.
19. Saboury, A. A. *J. Iran. Chem. Soc.* **2006**, *3*, 1.
20. Saboury, A. A.; Moosavi-Movahedi, A. A. *Int. J. Pharmac.* **2002**, *237*, 47.
21. Rezaei Behbehani, G.; Saboury A. A. *J. Therm. Anal. Cal.* **2007**, *89*, 852.
22. Rezaei Behbehani, G.; Saboury A. A.; Bagheri A. F. *J. Therm. Anal. Cal.* **2008**, *93*, 479.
23. Rezaei Behbehani, G.; Tazikeh E.; Saboury A. A. *Bull. Korean Chem. Soc.* **2006**, *27*, 208.
24. Rezaei Behbehani, G.; Ghamamy S.; Waghorne, W. E. *Thermochim. Acta* **2006**, *448*, 37.
25. Rezaei Behbehani, G.; Saboury A. A. *Thermochim. Acta* **2007**, *452*, 76.
26. Rezaei Behbehani, G.; Saboury A. A. *J. Therm. Anal. Cal.* **2007**, *89*, 859.
27. Rezaei Behbehani, G.; Saboury A. A. *J. Colloids Surf. B: Biointerfaces* **2007**, *61*, 224.
28. Rezaei Behbehani, G. *Bull. Korean Chem. Soc.* **2005**, *26*, 238.
29. Rezaei Behbehani, G. *Acta Chimica Slov.* **2005**, *52*, 288.
30. Rezaei Behbehani, G. *J. Solution. Chem.* **2007**, *36*, 939.
31. Rezaei Behbahani, G.; Saboury, A. A.; Fallah Baghery, A. *J. Solution Chem.* **2007**, *36*, 1311.
32. Rezaei Behbahani, G.; Saboury, A. A.; Taleshi, E. *J. Solution Chem.* **2008**, *37*, 619.
33. Paal, K.; Muller, J.; Hegedus, L. *Eur. J. biochem.* **2001**, *268*, 2187.
34. Bordbar, A.; Eslami, A.; Tangestaninejad, S. *Polish J. Chem.* **2003**, *77*, 283.
35. Sanna, D.; Garribba, E.; Micera, G. *J. Inorg. Biochem.* **2009**, *103*, 648.
36. Lausaac, J. P.; Sarkar, B. *Biochemistry.* **1984**, *23*, 2832.
37. Silva, S.; Silva, R. M.; Silva, K. M. N. *J. Mol. Struct.* **2004**, *711*, 73.