# Articles

## An Isothermal Titration Microcalorimetric Study on the Interaction of Three Water-Soluble Porphyrins with Histone $H_2B$

**A. K. Bordbar,* A. R. Ghaderi, E. Safaei, S. Tangestaninejad, A. Eslami,† A. A. Saboury,‡ and A. A. Moosavi-Movahedi‡**

*Department of Chemistry, Isfahan University, Isfahan, 81746-73441, Iran*
*†Department of Chemistry, University of Mazandaran, P.O. Box 47416-1467, Babolsar, Iran*
*‡Institute of Biochemistry and Biophysics, University of Tehran, P.O. Box 13145-1348, Tehran, Iran*
*Received December 24, 2002*

In the present work, the interaction of three water soluble porphyrins, tetra(*p*-trimethyle) ammonium phenyl porphyrin iodide (TAPP) as a cationic porphyrin, tetra sodium meso-tetrakis (*p*-sulphonato phenyle) porphyrin (TSPP) as an anionic porphyrin and manganese tetrakis (*p*-sulphonato phenyl) porphinato acetate (MnTSPP) as a metal porphyrin, with histone $H_2B$ have been studied by isothermal titration microcalorimetry at 8 mM phosphate buffer, pH 6.8 and 27 °C. The values of binding constant, entropy, enthalpy and Gibbs free energy changes for binding of the first MnTSPP, and first and second TSPP and TAPP molecules were estimated from microcalorimetric data analysis. The results represent that the process is both entropy and enthalpy driven and histone induces self-aggregation of the porphyrins. The results indicate that both columbic and hydrophobic interactions act as self-aggregation driving forces for the formation of aggregates around histone.

**Key Words :** Porphyrin, Histone ($H_2B$), Isothermal titration microcalorimetry, Aggregation

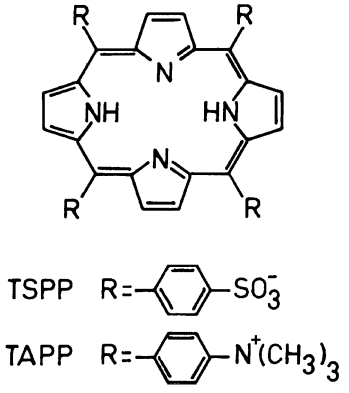
### Introduction

Interaction of porphyrins and their derivatives with proteins and DNA is one of the considerable interests since they are widely used as probes for structure and dynamics of nucleic acid and have possible medical applications.[1-3] It is known that the photochemical and photophysical properties of porphyrins and phtalocyanines are modified when they bind to proteins and DNA. Therefore, binding studies of these photosensitizers with proteins and DNA is of interest.[4,5] It would be expected that during the interaction of porphyrins with DNA, its interaction with nucleoproteins histones are also involved.

Nucleoproteins histones play a key role in the compaction of DNA. DNA is wound around a core of eight histone unites forming a DNA-protein complex called chromatin.[6,7] The five types of histones range in mass from 11 to 21 kDa with respect to their basic character; *i.e.*, high content of either Lys or Arg residue with positively charged side chains. Histons are supposed to be more resistant to oxidative attack of singlet oxygen produced in situ than albumins, because of absence or deficiency of the readily oxidizable amino acid residues such as Trp, Cys, Met and His.[8,9]

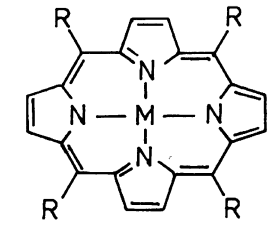
A prerequisite for a deeper insight into the molecular basis of histone-porphyrin interaction is a thorough characterization of the energetic governing complex formation. This can be done on the level of Gibbs energy, $\Delta G$, enthalpy, $\Delta H$, and entropy, $\Delta S$, changes.[10] In biochemical binding studies it is usually advantageous to determine the change in enthalpy values by direct microcalorimetric techniques. In many instances it is also possible to determine K values for binding reactions by calorimetric titration experiments.[11-18] Values for entropy changes, $\Delta S$, in solution systems are normally obtained from the difference between Gibbs energy and enthalpy changes, $\Delta G - \Delta H - T\Delta S$.

Previous studies on the organization and distribution of porphyrins showed that these molecules non-covalently bound to histone, which considerably affect sensitizing ability of porphyrins via induced aggregation, decreasing of quantum yields, $\phi_f$, and consequently, decreasing the




**Scheme 1**

*Corresponding author. Phone: 198-311-7932710; Fax: 198-311-6689732; E-mail: bordbar@sci.ui.ac.ir




**Scheme 2**

quantum yield of singlet oxygen formation.[19] Since there are no reports on the microcalorimetry and thermodynamic study on the binding of porphyrins to histone, we were interested to investigate the interaction of three water soluble porphyrins (scheme 1 and 2), tetra (*p*-trimethyle) ammonium phenyl porphyrin iodide (TAPP) as a cationic porphyrin, tetra sodium meso-tetrakis (*p*-sulphonato phenyle) porphyrin (TSPP) as an anionic porphyrin and manganese tetrakis (*p*-sulphonato phenyl) porphinato acetate (MnTSPP) as a metal porphyrin, with histone $H_2B$ by isothermal titration microcalorimetry. The thermodynamic parameters of binding have been estimated from heat values of a stepwise calorimetric titration experiment by a modified simple graphical method which has been previously used for data analysis of simple systems.[20] The estimated thermodynamic parameters have been interpreted on the basis of molecular forces.

## Experimental Section

**Materials**. Histone $H_2B$ from calf thymus was obtained from Fluca Chemical Co. TSPP and TAPP were prepared by the methods described previously.[21,22] TSPP was metallated according to the literature method.[23] These complexes were characterized by Uv/Vis spectroscopy and elemental analysis. The spectral characteristics of the isolated materials were compared to the literature values and found to be in excellent agreement. All of the chemicals, which have been used for these syntheses, were of analytical grade and purchased from Sigma Chemical Co. All solutions were prepared using double-distilled water. Porphyrin stock solutions were made by dissolving the solid porphyrin in buffer solution. Phosphate buffer solution of 8 mM concentration, pH 6.8, was used. Porphyrin stock and working solutions were stored at room temperature in the dark to avoid undesired photochemical reactions. Uv/Vis measurements were performed on a Shimadzuo 160 spectrophotometer using 1 cm quartz cuvettes.

**Methods**

**Isothermal titration microcalorimetry**: Enthalpy measurements were performed at 27.0 ± 0.005 °C using a four-chanel commercial micro-calorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The microcalorimeter was interfaced with an IBM PS/2 model PIII computer, "Thermometric Digitam 3" was the software program used. A 1000 μL injection syringe was employed throughout. The enthalpy of interaction between porphyrin and $H_2B$ was measured by transferring the protein solution to a 5 mL titration cell. The volume of $H_2B$ solution in the measuring cell was 2.5 mL. The concentration of porphyrin inside the syringe was 1 mM and the concentration of $H_2B$ in the titration cell was 0.1%(W/V). The volume of porphyrin solution injected in each step was 20 μL for TSPP and TAPP and 40 μL for MnTSPP. The enthalpy of dilution of the porphrin due to the injection was corrected by measuring as described above except excluding $H_2B$. The heat of dilution of $H_2B$ is negligible. The micro-calorimeter was frequently calibrated electrically during the course of the study.

## Results and Discussion

The data obtained from isothermal titration microcalorimetry of $H_2B$ interaction with porphyrins are shown in Figures 1. Figure 1a shows the total evolutes heat versus total concentration of porphyrin for TSPP and TAPP and Figure 1b represents the corresponding results for MnTSPP. Previous study shows that histone promotes aggregation of the bound monomeric porphyrin.[19] Hence, for analyzing the enthalpy curves for obtaining thermodynamic parameters of binding, we have to consider the following multiple equilibrium between histone (H) and porphyrin (P):

$$H + P \Leftrightarrow HP$$

$$HP + P \Leftrightarrow HP_2$$

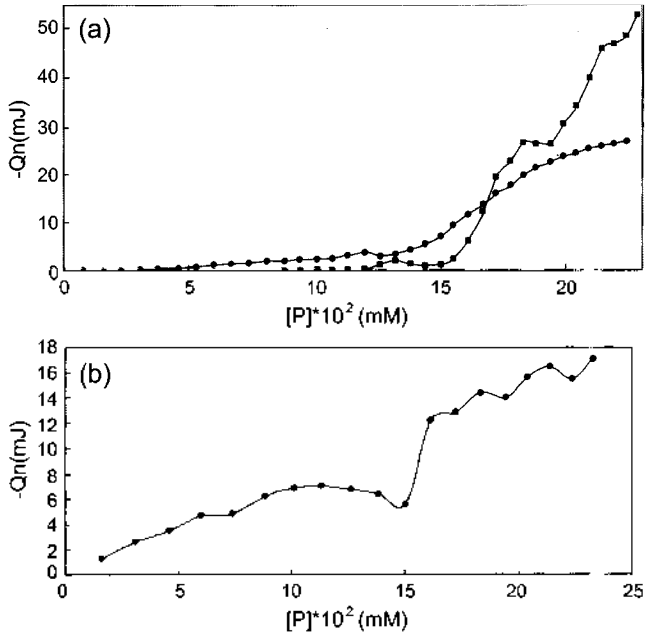



**Figure 1.** (a) Experimental heat quantities ($Q_n$) plotted versus total concentration of porphyrin ([P]). ( ■ ) TAPP. ( ● ) TSPP. (b) Experimental heat quantities ($Q_n$) plotted versus total concentration of MnTSPP ([P]).

$$\vdots$$

$$HP_{i-1} + P \Leftrightarrow HP_i$$

$$\vdots$$

A stepwise macroscopic association binding constant (equation 1), $K_i$, and enthalpy change, $\Delta H_i$, can be related to any distinguished equilibria step.

$$K_i = \frac{[HP_i]}{[HP_{i-1}][P]} \quad (1)$$

The sum of heat evolution following the nth titration step ($Q_n$) can be expressed as:

$$Q_n = Q_n^{(1)} + Q_n^{(2)} + \cdots + Q_n^{(i)} + \cdots \quad (2)$$

where $Q_n^{(i)}$ is the sum of heat evolutions following the nth titration step, due to the formation of $HP_i$ complex from $HP_{i-1}$.

The value of $Q_n^{(i)}$ relates on the amount of $HP_i$ complex formed, which can be expressed as:

$$Q_n^{(i)} = \Delta H_i \cdot V_n [HP_i]_n \quad (3)$$

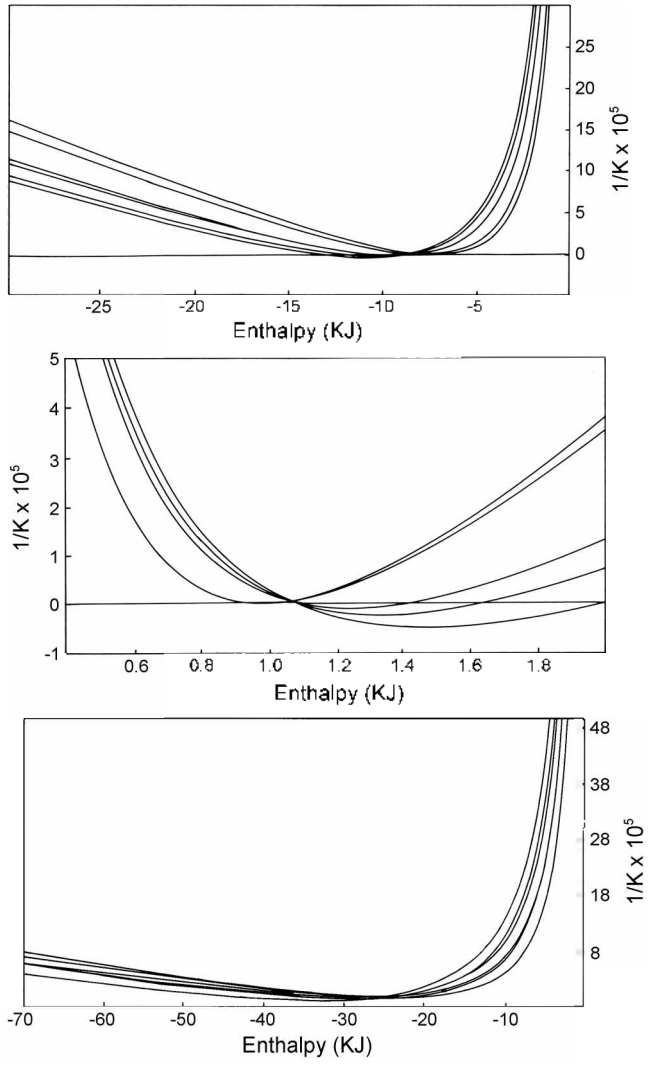



**Figure 2**. (a) Evaluation of $K_1$ and $\Delta H_1$ for interaction of TSPP with $H_2B$. The total concentration of TSPP lies between 0 to 0.074 mM. The injections of 1 to 10 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (b). Evaluation of $K_1$ and $\Delta H_1$ for interaction of TAPP with $H_2B$. The total concentration of TAPP lies between 0 to 0.088 mM. The injections of 1 to 12 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (c) Evaluation of $K_1$ and $\Delta H_1$ for interaction of MnTSPP with $H_2B$. The total concentration of MnTSPP lies between 0 to 0.194 mM. The injections of 1 to 15 are correspond to this set. Some of the curves have been deleted for better presentation of figure.

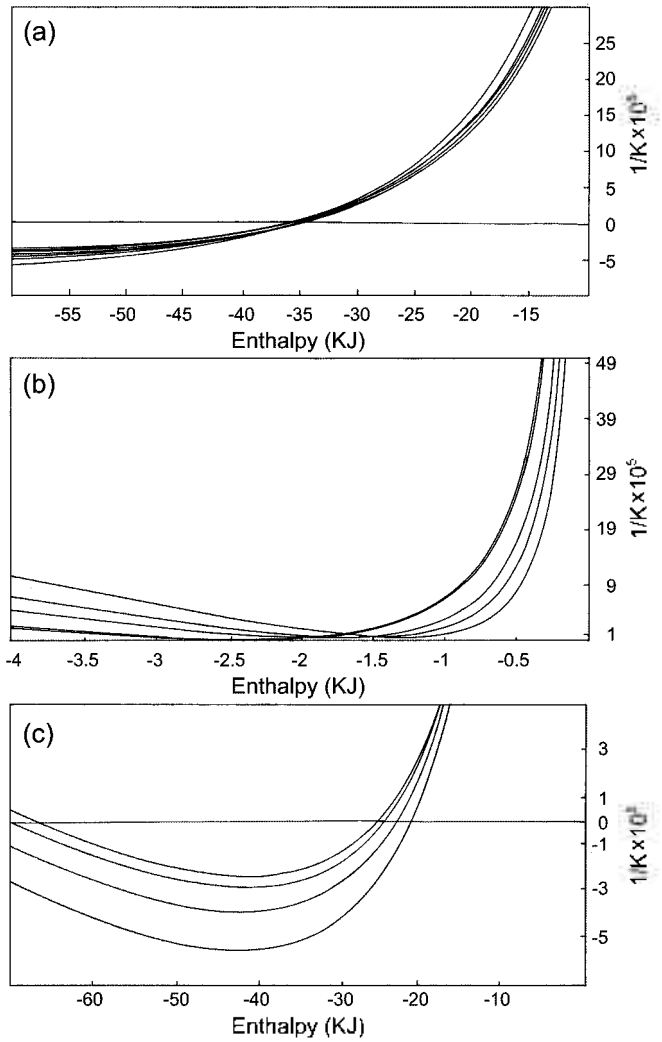



**Figure 3**. (a) Evaluation of $K_2$ and $\Delta H_2$ for interaction of TSPP with $H_2B$. The total concentration of TSPP lies between 0.088 to 0.144 mM. The injections of 11 to 21 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (b) Evaluation of $K_2$ and $\Delta H_2$ for interaction of TAPP with $H_2B$. The total concentration of TAPP lies between 0.094 to 0.126 mM. The injections of 13 to 18 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (c) Evaluation of $K_2$ and $\Delta H_2$ for interaction of MnTSPP with $H_2B$. The curves in this figure do not intersect in one point and represents the fail of our assumption. The total concentration of MnTSPP is more than 0.204 mM. The injections of 16 to 40 are correspond to this set. Some of the curves have been deleted for better presentation of figure.

where $V_n$ and $[HP_i]_n$ the volume of the reaction solution and concentration of $HP_i$ in the nth titration step, respectively.

In special case that:

$$K_1 >> K_2 >> K_3 \cdots K_{i-1} >> K_i \cdots$$

it can be assume that $HP_i$ complex is not formed until the complete formation of $HP_{i-1}$. This situation permits us to divide the titration steps into distinct categories. In the first category, only $HP$ is formed and $Q_n$ should be equal to $Q_n^{(1)}$. In the second set, all of the free histone converts to $HP$ complex and further addition of porphyrin results in formation of $HP_2$ complex. So $Q_n$ for the second set should be equal to the total evolved heat for complete conversion of histone into $HP$, $Q_n(1)$ plus the evolved heat from partial conversion of $HP$ to $HP_2$, $Q_n^{(2)}$. The similar relations can be written for the third, forth and so on, sets of injections, as follows:

$Q_n = Q_n^{(1)}$ first set of injection $0 \le n \le n_1$

$Q_n = Q_n(1) + Q_n^{(2)}$ second set of injection $n_1 < n \le n_1 + n_2$

$Q_n = Q_n(1) + Q_n(2) + Q_n^{(3)}$ third set of injection $n_1 + n_2 < n \le n_1 + n_2 + n_3$

$\vdots$

$Q_n = Q_n(1) + Q_n(2) + \cdots + Q_n(i-1) + Q_n^{(i)}$ ith set of injection $n_1 + n_2 + \cdots + n_{i-1} < n \le n_1 + n_2 + \cdots + n_i$

where $Q_n(i)$ is the total heat evolution for complete formation of $HP_{i-1}$ complex to $HP_i$ and $n_1, n_2 \ldots, n_i$ are the numbers of injections in the first, second, ... and ith sets, respectively. In the first injection set that only $HP$ complex is formed, $K_1$ should be equal to:

$$K_1 = \frac{[HP]_n}{([H_0]_n - [HP]_n)([P_0]_n - [HP]_n)} \quad (4)$$

where, $[H_0]_n$, $[P_0]_n$ and $[HP]_n$ are the total concentration of histone ($[H] + [HP]$), the sum of concentration of bound and unbound ligand and the concentration of the complex following the nth addition of ligand, respectively. With respect to equation (3), $Q_n^{(1)} (n < n_1)$ should be equal to:

$$Q_n^{(1)} = \Delta H_1 \cdot V_n \cdot [HP]_n \qquad \text{where } n < n_1 \quad (5)$$

Combination of equations (4) and (5) leads to:

$$\frac{1}{K_1} = \frac{[H_0]_n \cdot [P_0]_n \cdot \Delta H_1 \cdot V_n}{Q_n} - [H_0]_n - [P_0]_n + \frac{Q_n^{(1)}}{\Delta H_1 \cdot V_n} \quad (6)$$

Equation (6) contains two unknowns, $K_1$ and $\Delta H_1$. A series of reasonable values for $\Delta H_1$ are inserted into equation (6) and the corresponding values for $1/K_1$ are calculated and the graph of $1/K_1$ versus $\Delta H_1$ is constructed. If this is done for all titration steps by considering the assumption about the formation of a 1:1 complex with respect to $K_1 >> K_2$, in ideal situation, all the curves intersect at one point. This point represents the true value for $1/K_1$ and $\Delta H_1$. Due to measurement errors, the intersection will in practice cover a limited area. The best pair of values within this area can then be obtained by minimizing the value of $\sum (Q_n^{exp.} - Q_n^{calc.})^2$. This method has been previously used for a simple system in which only a 1:1 complex is formed.[20]

Figures 2a, 2b and 2c represent the variation of $1/K_1$ versus $\Delta H_1$ for TSPP, TAPP and MnTSPP, respectively. All of the curves in these Figs. intersect in a limited area which confirms our analysis. The values of $K_1$ and $\Delta H_1$ can be estimated from these Figs.

The value of $Q(1)$ should be equal to:

$$Q(1) = \Delta H_1 \cdot [H_0] \cdot V_0 \quad (7)$$

where $[H_0]$ and $V_0$ are initial concentration and volume of histone solution in the cell, respectivily. By estimating $Q(1)$, the value of $Q_n^{(2)}$ for the second set of titration have been

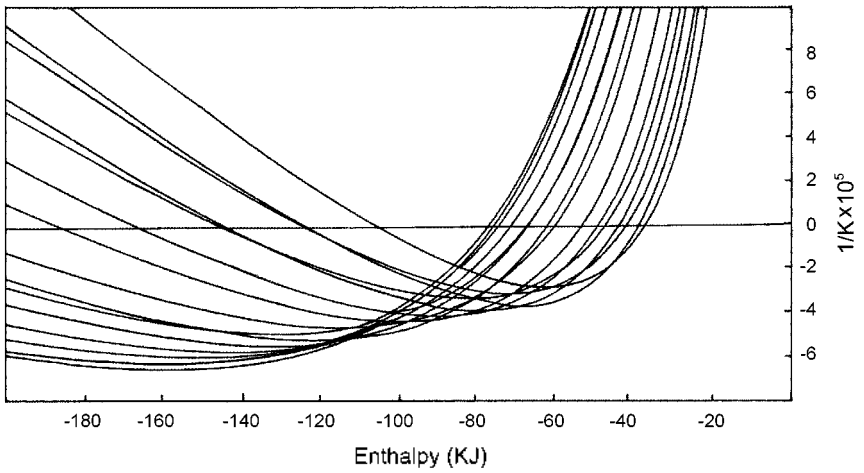



**Figure 4.** Evaluation of $K_3$ and $\Delta H_3$ for interaction of TAPP with $H_2B$. The curves in this figure do not intersect in one point and represents the fail of our assumption. The total concentration of TAPP is more than 0.132 mM. The injections of 19 to 40 are correspond to this set. Some of the curves have been deleted for better presentation of figure.

**Table 1**. Thermodynamic parameters for interaction of porphyrins to histone, $H_2B$, in 5 mM phosphate buffer, pH 6.8 and 27 ℃

| Porphyrin | Number of injection set | Numbers of injection in ith set ($n_{i-1}$–$n_{i(1)}$) | [Porphyrin]/[Histone] (molar ratio) | Concentration range of porphyrin ( mM ) | $\Delta H_i$ (kJ mol) | $K_i \times 10^{-5}$ | $\Delta G_i$ (kJ mol) | $\Delta S_i$ (J/mol·K) |
|---|---|---|---|---|---|---|---|---|
| TSPP | 1 | 1-10 | 1.10 | 0-0.074 | −8.00 ± 0.560 | 2.700 ± 0.189 | −31.193 ± 0.175 | −88.31 ± 2.44 |
| TSPP | 2 | 11-21 | 2.30 | 0.081-0.144 | −32.564 ± 1.465 | 0.611 ± 0.027 | −27.488 ± 0.110 | −16.92 ± 5.25 |
| TAPP | 1 | 1-12 | 1.32 | 0-0.088 | +1.060 ± 0.065 | 33.580 ± 1.780 | −37.480 ± 0.129 | −128.47 ± 4.40 |
| TAPP | 2 | 13-18 | 1.97 | 0.094-0.126 | −1.680 ± 0.143 | 2.800 ± 0.244 | −31.284 ± 0.192 | −98.68 ± 1.12 |
| MnTSPP | 1 | 1-15 | 3.30 | 0-0.194 | −33.240 ± 1.629 | 1.210 ± 0.060 | −29.191 ± 0.121 | −13.5 ± 5.83 |

estimated. A similar analysis can be done for second set of titration steps by considering the definition of $K_2$. Figures 3a, 3b and 3c show the variation of $1/K_2$ versus $\Delta H_2$ for TSPP, TAPP and MnTSPP, respectively. All of the curves in Figures 3a and 3b reasonably intersect in a limited region; however, Figure 3c does not show this situation. It represents that the assumption of $K_2 >> K_3$ is not valid for MnTSPP but acceptable for TSPP and TAPP. The values of $\Delta H_2$ and $K_2$ for TSPP and TAPP were estimated from these figures. The value of $Q(2)$ should be equal to:

$$Q(2) = \Delta H_2 \cdot [H_{(1)}] \cdot V_0 \qquad (9)$$

Hence, the values of $Q_n^{(3)}$ have been calculated for third set of injection. The corresponding curve for this set does not intersect in a limited region and represents the fail of our assumption. The curve for TAPP at this set was shown in Fig. 4. The calculated thermodynamic parameters for binding of TSPP, TAPP and MnTSPP were listed in Table 1.

## Conclusions

Our results represent that histone induces the self-aggregation of porphyrins. These observations have been previously confirmed by spectroscopic techniques.[19] Estimated thermodynamic parameters represent the binding processes of TSPP and MnTSPP are considerable exothermic but for TAPP, it is endothermic for first ligand and unconsiderable exothermic for second ligand. With respect to positive charge of histone and negative charge of TSPP and MnTSPP, it can be concluded that columbic attraction has considerable role in the process of binding. However, the considerable positive value of entropy changes, especially for binding of TAPP and binding of first TSPP, represent the role of hydrophobic environment in the process. Hence, histone ($H_2B$) induces self aggregation of porphyrins and both columbic and hydrophobic interactions act as self-aggregation driving forces for the formation of aggregates.

**Acknowledgements**. This work was supported by Research Council of the Isfahan University. We thank Dr. M. K. Amini for editing the manuscript.

## References

1. Fiel, R. J.; Howard, J. C.; Mark, E. H.; Dattagupta, N. *Nucl. Acids Res.* **1979**, *6*, 3093.
2. Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. *Biochemistry* **1983**, *22*, 5409.
3. Pasternack, R. F.; Garrity, P.; Ethrlich, B.; Davis, C. B.; Gibbs, E. J.; Orloff, G.; Giartosio, A.; Tturano, C. *Nucl. Acids. Res.* **1986**, *14*, 5919.
4. Nelson, J. A.; Schmiedl, U. *Magn. Reson. Med.* **1991**, *22*, 366.
5. Marchal, G.; Ni, Y.; Herijgers, P.; Flameng, W.; Petre, C.; Bosmans, H.; Yu, J.; Ebret, W.; Hilger, C. S.; Pfefferer, D.; Semmler, W.; Baert, A. L. *Eur. Radiol.* **1987**, *6*, 2.
6. Karantza, V.; Freire, E.; Moudriankis, E. N. *Biochemistry* **1996**, *35*, 2037.
7. Delange, R.; Smith, E. L. *J. Biological Chem.* **1973**, *248*, 3248.
8. Cole, D. *Ann. Rev. Biochem.* **1979**, *48*, 837.
9. Tristram, G. R.; Smith, R. H. *The Proteins*. 2nd Ed.; Neurath, Ed.; Academic Press: London, 1963; Vol. 1.
10. Weber, G. *Adv. Protein Chem.* **1975**, *29*, 1.
11. Saboury, A. A.; Moosavi-Movahedi, A. A. *J. Enz. Inhib.* **1997**, *12*, 273.
12. Ghadermarzi, M.; Saboury, A. A.; Moosavi-Movahedi, A. A. *Polish J. Chem.* **1998**, *72*, 2024.
13. Saboury, A. A. *Thermochim. Acta* **1998**, *320*, 97.
14. Saboury, A. A.; Dahut, M. U.; Ghobadi, S.; Chamani, J.; Moosavi-Movahedi, A. A. *J. Chin. Chem. Soc.* **1998**, *45*, 667.
15. Karbassi, F.; Saboury, A. A. *Thermochim. Acta* **2000**, *362*, 121.
16. Saboury, A. A. *J. Chem. Soc. Pak.* **2000**, *22*, 204.
17. Saboury, A. A. *Ind. J. Biochem. Biophy.* **2000**, *37*, 347.
18. Saboury, A. A.; Sarri-Sarraf, N.; Sacidian, S. *Thermochim. Acta* **2002**, *381*, 147.
19. Long, K.; Kubat, P.; Mosinger, J.; Wagnerova, D. M. *J. Photochemistry & Photobiology A: Chemistry* **1998**, *119*, 47.
20. Chen, A.-t.; Wadso, I. *J. Biochem. Biophys. Method* **1982**, *6*, 307.
21. Fleischer, E. B.; Palmer, J. M.; Srinivastava, T. S.; Chaterjee, A. *J. Am. Chem. Soc.* **1971**, *93*, 3162.
22. Krishnamurthy, M. *Indian J. Chem. B* **1977**, 964.
23. Harriman, A.; Porter, G. *J. Chem. Soc. Faraday Trans. II* **1979**, *75*, 1532.