Journal of Physical and Theoretical Chemistry
of Islamic Azad University of Iran, 4 (3) 187-193**:** Fall 2007
(J.Phys.Theor.Chem.IAU Iran: Fall 2007)
ISSN: 1735-2126

Interaction of Phthalocyanine with Egg albumin and Bovine serum albumin

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ABSTRACT

The interaction of bovine serum albumin (BSA) and egg albumin with water soluble phthalocyanine, cobalt (II) 4, 4′ , 4″, 4‴- tetrasulfophthalocyanine (CoTSPc) , has been studied by the UV- Vis method at pH 7.0 and five different temperatures 20, 25, 30, 35 and 40˚C. The formation constants have been elucidated by using spectrophotometric titration and computer SQUAD program data refinement. The thermodynamic parameters ΔH°, $\Delta G^{\circ}$and ΔS° at 5 different temperatures were calculated. The results showed that the best fitting corresponds to a 1: 1 complex model between BSA and egg albumin with CoTSPc. Formation constants decrease with increasing temperature.The formation constants showed that bonding effect between egg albumin and CoTSPc is stronger than BSA.

Keywords: BSA; Egg albumin; Phthalocyanine; SQUAD; Formation constants

INTRODUCTION

Albumin is a class of simple, water- soluble proteins that is found in egg white, blood serum, milk, and many other animal and plant fluids and tissues. Albumin has been the subject of many investigations because of its important roles in maintaining normal biological functions. Albumin is known to have a secondary structure that includes alpha-helices, parallel beta sheets, antiparallel beta sheets, and random coils [1,2] . Serum albumins (Fig.1) are the major soluble proteins in the circulatory system. They play an important role in the transport and deposition of many drugs molecules in the blood. Since the overall distribution , metabolism and efficacy of many drugs in the body are correlated with their affinities towards serum albumin, the investigation of pharmaceutical with respect to albumin- drug binding is imperative and of fundamental importance. Bovine Serum Albumin (BSA) has been extensively used for binding studies with small molecules because of its structural homology with human serum albumin (HAS) [3-6]. Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms, being the major macromolecule contributing to the osmotic blood pressure [7]. In addition to blood plasma, serum albumins are also found in tissues and bodily secretions throught the body ; the extravascular protein comprises 60% of the total albumin [8] .

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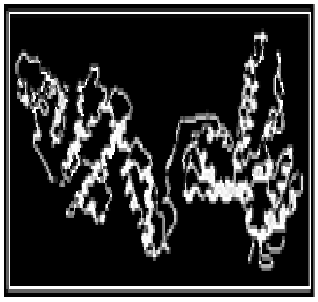
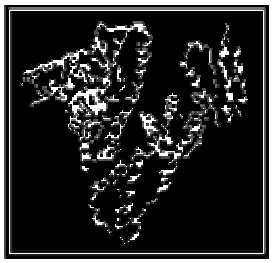
Egg albumin is complex and contains up to 40 different proteins[9]. Ovalbumin, globulins and ovommucoid are the major proteins involved in the foaming of whipped egg albumin[10] . It is used in many food formulations as a foaming ingeridient. The foaming properties of a foaming material are evaluated by its foaming properties, in return, determine the use of the foaming material in a food product[11]. Based on their interaction modes, adsorptive membranes could be divided into three or more categories: affinity, ion exchange, hydrophobic interaction and reversed phase, etc. Among these interaction modes, affinity membranes have attracted the major attention because of their biospicificity[12-16].



F form

E form



N form

Fig.1. Ribbon diagram of serum albumin in its N form, and in its proposed F and E forms. (Cartner and Ho, 1994).

Phothalocyanines posses outstanding stability to light, heat, acids and alkalis; more recently, they have been used as organic semiconductors for gas sensors, infrared dyes for laser technology, anti- cancer agents, in optical data storage and as low- dimensional conducting materials[17-30]. A number of lipophilic and negatively- charged hydrophobic photosensitizers have been investigated for the cell killing both in vitro and in vivo. These include porphyrin derivatives[31] and phthalocyanine derivatives[32]. Cobalt tetrasulfophthalocyanine (CoTSPc) (Fig.2) has however, not been explored for the catalytic oxidation of chloro phenols, even though it is produced industrially in large amounts as a catalyst for the oxidation of mercaptans in gasoline fraction[33-36].

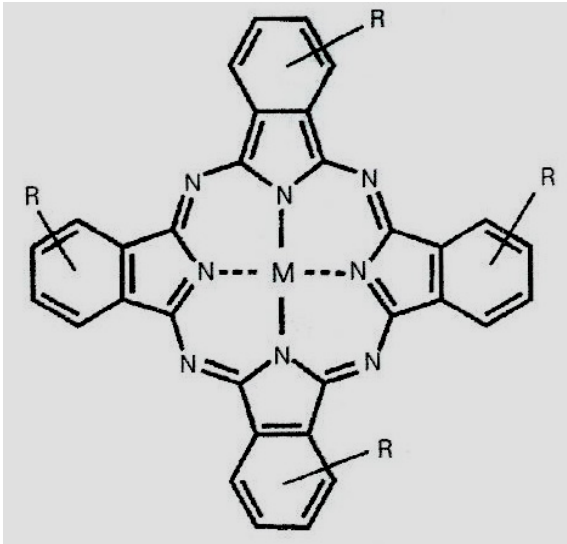


Fig.2. Typical phthalocyanine structure where M is the central metal ion (Al^{3+}, Zn^{2+}, Co^{2+}, Ga^{3+},…) and R represents a multitude of possible ring substituents including SO_3H, F, COOH, etc.

Several experimental techniques such as Calorimetry, Viscosity, UV spectroscopy, Surface tension, Potentiometry, etc. have been used to study the protein interactions [37- 45].

The objectives of the present study are the thermodynamic analysis of bonding process between BSA- CoTSPc and Egg Albumin-CoTSPc, and determining the stoichiometry of binding and formation constants.

MATERIAL AND METHODS

Egg albumin and BSA(> 98% , Roche) were purchased from Merk company and was used

without further purification. Cobalt tetrasulfophthalocyanine (CoTSPc) was synthesized, purified and characterized by chemistry department of Shahid Beheshti University of Iran. The samples were prepared using double- distilled water.Sodium phosphate buffer(1mM pH 7.0), was used as buffer. The concentration rang of phthalocyanine were between (1.5×10^{-5}- 7×10^{-5}) and were freshly prepared before experiment. The concentrations of BSA and egg albumin were between (2×10^{-4}- 3×10^{-4} M) . Spectrophotometer titrations of phthalocyanine solutions as a function of BSA and egg albumin concentrations were carried out at 1mM phosphate buffer pH 7.0 and 5 different temperatures(20, 25, 30, 35 and 40˚C). The UV spectra were recorded by UV-Vis Shimadzu 2101 PC spectrophotometer equipped with 1.0 cm quartz cells and thermostat cell compartment that control the temperature around the cell within ± 0.1˚C. The titration experiment was continued until the absorbance of phthalocyanine solution remained constant. Equilibrium formation constants and stoichiometry of complexes were determined by using SQUAD software.

RESULTS AND DISCUSSION

Phthalocyanine

The UV- Vis spectra of CoTSPc are shown in Fig.3. It is clear from the visible region that the absorption peaks of CoTSPc are in region 631.5 and 670.0 nm.

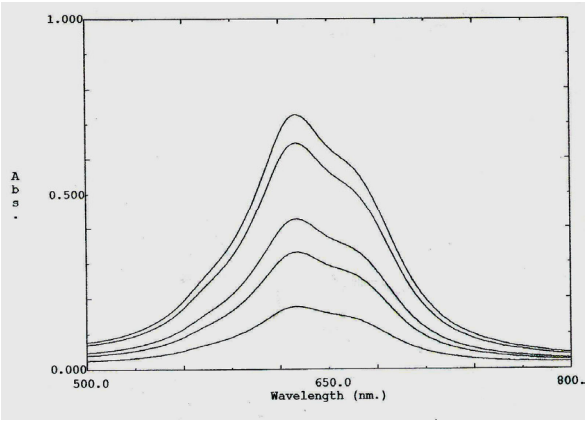


Fig.3. UV- Vis absorption spectra of the CoTSPc with different concentrations at 25˚C.

According to Fig.3 and plot of absorbance versus concentration of CoTSPc (fig. 4) one can deduce that the maximum band obeys Beer's Law over concentration range between 1.5×10^{-5}-7×10^{-5}M.

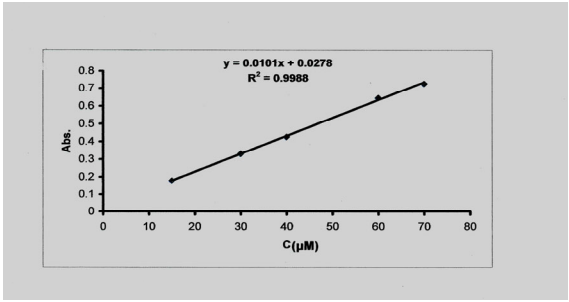
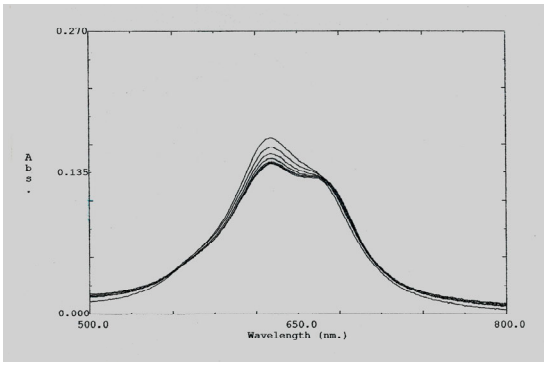


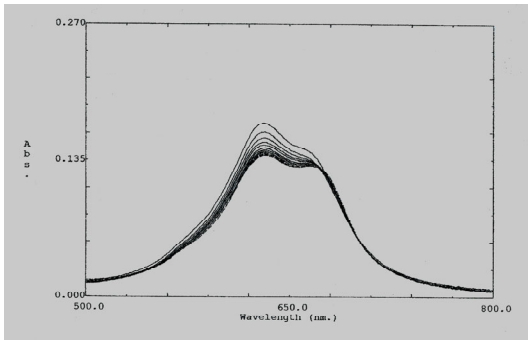
Fig.4. Plot of absorbance vs. concentration of CoTSPc.

Interaction with BSA and egg albumin

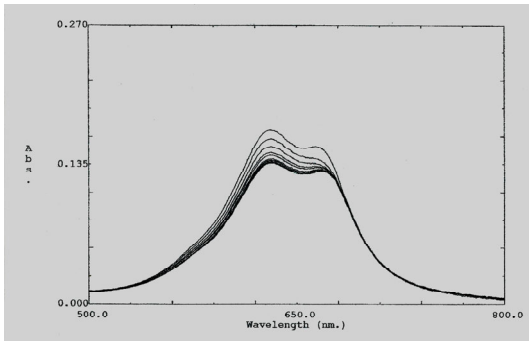
At first step, every phthalocyanine solution was titrated by adding 40 µl (and 15 times aliquots portions) of an stock solution of BSA and egg albumin into a quartz cell containing 2ml of concidered phthalocyanine solution. The titration was continued until the absorbance of the phthalocyanine solution remained constant. The UV-Vis absorbance spectra of CoTSPc at various BSA and egg albumin concentrations are shown in Fig.5 and Fig.6 respectively, at 5 different temperatures.The UV-Vis absorption spectra indicate that an interaction occurs between BSA and phthalocyanine and egg albumin and phthalocyanine to form BSA-phthalocyanine and egg albumin-phthalocyanine complexes respectively.



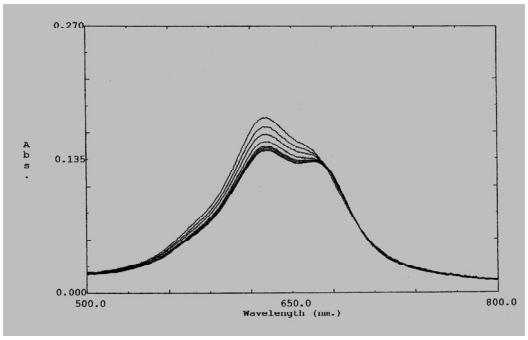
20˚C



30˚C

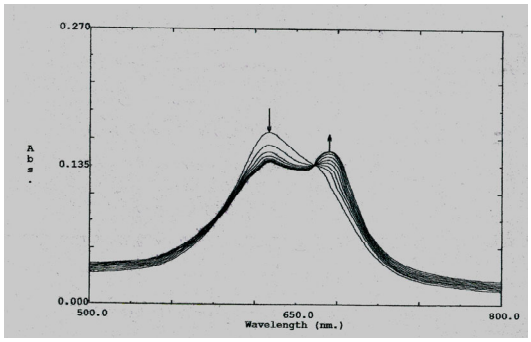


35˚C

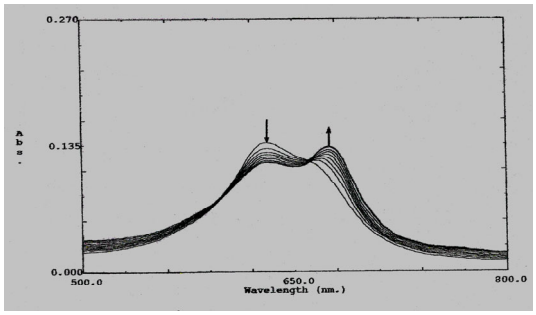


40˚C

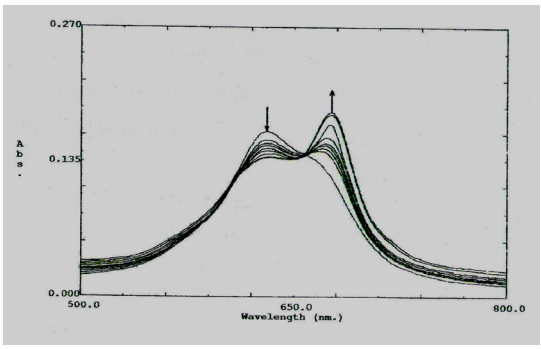
Fig.5. UV- Vis absorbance spectra of CoTSPc in the presence of various concentrations of BSA at pH 7. 0 and different temperatures.



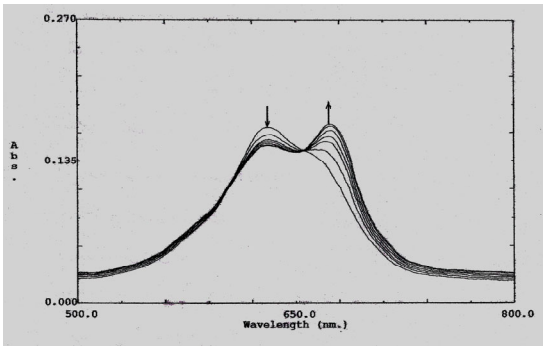
20˚C



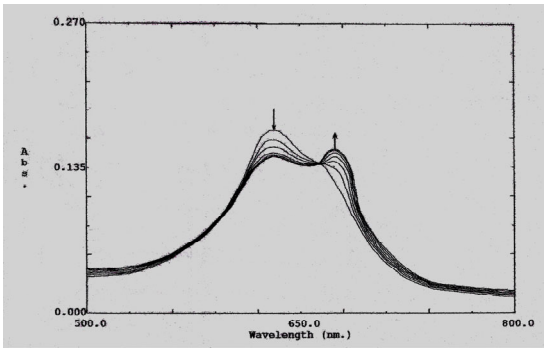
25˚C



30˚C



35˚C



40˚C

Fig.6. UV- Vis absorbance spectra of CoTSPc in the presence of various concentration of egg albumin at pH 7.0 and at different temperatures.

The thermodynamic parameters, such as enthalpy change, ΔH°, entropy change, ΔS°, and free energy change, ΔG°, play important roles in estimating the binding mode. The reaction enthalpy change is regarded as a constant if the temperature changes are small. In this condition, parameters can be determined from the Van't Hoff plot:

$$\ln K=-\Delta H^\circ/RT+\Delta S^\circ/R \quad (1)$$

where K is the binding constants, R and T reffering to the gas constant and the Kelvin temperature, respectively. The value of ΔG° can be obtained by the following equation:

$$\Delta G^\circ=\Delta H^\circ-T\Delta S^\circ \quad (2)$$

Regarding Figs 7 and 8 for BSA- CoTSPc and egg albumin- CoTSPc complexes, the concidered ΔH° can be calculated from the slope.

The binding constants and thermodynamic parameters for the binding of BSA- CoTSPc and egg albumin- CoTSPc complexes are listed in Tables 1 and 2.

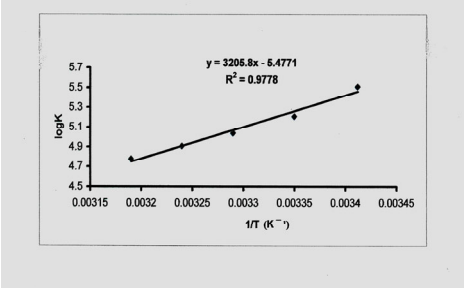


Fig.7. Van't Hoff plot for the interaction of BSA with phthalocyanine at pH 7.0

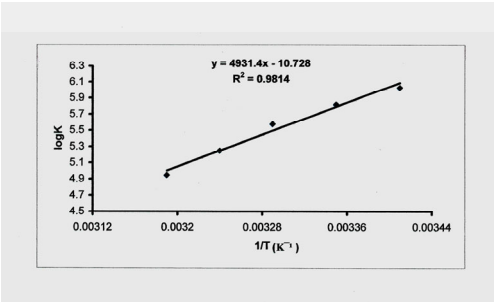


Fig.8. Van't Hoff plot for the interaction of egg albumin with phthalocyanine at pH 7.0.

Table 1. Thermodynamic functions for the binding of BSA to CoTSPc at pH 7.0

Θ/°C	$K\times10^{-4}$	ΔG°/ kJmol^{-1}	ΔH°/ kJmol^{-1}	ΔS°/ Jmol^{-1}K^{-1}
20	38.02	-31.32	-61.382	-102.56
25	13.18	-29.22	-61.382	-107.86
30	10.96	-29.25	-61.382	-105.99
35	8.13	-28.96	-61.382	-105.21
40	6.02	-28.65	-61.382	-104.52

Table 2. Thermodynamic functions for the binding of egg albumin to CoTSPc at pH 7.0

Θ/°C	$K\times10^{-4}$	ΔG°/ kJmol^{-1}	ΔH°/ kJmol^{-1}	ΔS°/ Jmol^{-}
20	107.15	-33.84	-94.422	-206.66
25	66.07	-33.22	-94.422	-205.27
30	38.02	-32.38	-94.422	-204.65
35	17.78	-30.97	-94.422	-205.91
40	8.91	-29.67	-94.422	-206.76

SQUAD PROGRAM

In order to analyze the titration experiments data of BSA- phthalocyanine and egg albumin- phthalocyanine systems, the 50 wavelengths were selected. These 50 different wavelengths were from 15 phthalocyanine solutions that showing suitable absorbance. In order to calculate association constants, K, using SQUAD software, inputs data were absorbances at 50 different wavelengths of 15 phthalocyanine solutions. The outputs are the logarithm of association constants, $\log K_{ij}$, for the following reactions:

$i\text{BSA} + j\text{phthalocyanine} \leftrightarrow (\text{BSA})_i\ (\text{phthalocyanine})_j$ (3)

$K_{ij} = [(\text{BSA})_I\ (\text{phthalocyanine})_j] / [\text{BSA}]^i\ [\text{phthalocyanine}]^j$ (4)

i egg albumin + $j$phthalocyanine $\leftrightarrow$ $(\text{egg albumin})_i\ (\text{phthalocyanine})_j$ (5)

$K_{ij} = [(\text{egg albumin})_i\ (\text{phthalocyanine})_j] / [(\text{egg albumin})^i (\text{phthalocyanine})^j]$ (6)

The results of our calculation show that the 1:1complexes of BSA- phthalocyanine and egg albumin-phthalocyanine are formed.

CONCLUSIONS

In this work, the interaction of phthalocyanine with BSA and with egg albumin have been studied by UV- Vis spectrophotometeric method and SQUAD software. The results of our calculation present the formation of 1:1 complex model between CoTSPc+ BSA and CoTSPc+ egg albumin. The data of tables 1 and 2 show that the interactions in the egg albumin-phthalocyanine system are stronger than BSA- phthalocyanine system. For example at 25˚C we have the following order of formation constants:

egg albumin- phthalocyanine> BSA-phthalocyanine

$\log K_{ij}$: 6.03 > 5.58

The negative values of ΔH° are indicatig favorable adduct formations, but a negative entropy ΔS° contribution is unfavorable for adduct formation. The experimental results indicate that egg albumin can interact with CoTSPc strongly through sulfhydryl(- SH) groups, hydrogen bond and Vander Waals force, but BSA only can interact with CoTSPc through hydrogen bond and Vander Waals force.

ACKNOWLEDGMENT

We are grateful to Science and Research Compus of Islamic Azad University for their financial support.

REFERENCES

1. K.A. Dill and D. Shortle, Annu. Rev. Biochem. 1991, 795- 825.
2. E.Bramanti and E. Benedett, Biopolymers , 1996, 38, 639- 653.
3. J.N. Tian, J. Q. Liu, X. Tian, Z. D. Hu and X. G. Chen, Mol. Struct., 2004, 97-202.
4. Y.J. Hu, Y. Liu, X. S. Shen, X. Y. Fang and S. S. Qu, J. Mol. Struct., 2005, 738, 143-147.
5. H.L. Guan, H. P. Yuan, Z. T. Pan and J. Instrum., Anal ., 2006 , 25, 25- 28.
6. A.Papadopoulou, R. J. Green, R. A. Frazier and J. Agric., Food. Chem., 2005, 53, 158-163.
7. D.C. Carter and J. X. Ho, Adv. Protein Chem., 1994, 45, 153-203.
8. X.M. He and D. C. Carter, 1992, 358, 209-215.
9. A.Kilaro, and V. R. Harwalkar, "Food proteins. Properties and Characterization." VCH publishers, New York, 1996, 71- 165.
10. G.Linden and D. Lorient, "New ingredients in food processing: Biochemistry and agriculture", Woodhead publishing, Cambridge UK, 1999, 121- 138.
11. R.Nakamara and E. Doi, " Food proteins processing applications" Wiley, New York, 2000 , 171- 207.
12. D.K. Roper and E. N. Linghtfoot, J. Chromatogr. 1995, A 702, 3.
13. C.Charcosset, J. Chem. Technol. Biotechnol., 1998, 71, 95.
14. E.Klein, Affinity membranes: a 10- year review, J. Member. Sci., 2000, 179, 1.
15. H.Zou, Q. Luo and D. Zhou, J. Biochem. Biophys. Methods, 2001, 49, 199.
16. R.Ghosh, J. Chromatogr., 2002, A 952, 13.
17. AD Zhao, QX Li, L Chen and et al science, 2005, 309, 1542.

18. DCotter, RJ Manning, KJ Blow and et al science, 1999, 286, 1533.
19. JW Perry, K Mansour, IYS Lee and et al science, 1996, 273, 1533.
20. JE Falk," porphyrins and metalloporphyrins",1964, Elsevier, Amesterdam,.
21. BN Achar, PK Jayasree and Met Synth, 2000, 114, 219.
22. J.Griffths and J. Schofield, Dyes and pigments , 1997, 33, 65.
23. JH Zagal, Coord. Chem. Rev., 1992, 119, 89.
24. Sakamoto Keiichi and Kato Taku., Dyes and Pigments, 2005, 64, 63.
25. XD Gong, HM Xiao and H.J. Tian, Mol. Struct. (THEOCHEM), 2002, 593, 93.
26. Yasin Arslanoglu, Altug Mert Sevim."Dyes and pigments", 2006, 68, 129.
27. Nagao Kobayashi., Phthalocyanines , 1999, 4, 345.
28. DH Ohnholz, S Steinbrecher and M Hanack. Journal of Molecular Structure, 2000, 521, 231.
29. M. Matsuoka, " Infrared absorbing dyes." Plenum Prees, New York, 1990.
30. H Tian and KC Chen., "Dyes and Pigments", 1995, 27, 191.
31. R.M. Ion, A. Planner, K. Wiktorowicz and D. Frackowiak, Acta Biochimica Polonica, 1998, 45, 833.
32. R.M. Ion, I. Yilmaz and O. Bekaroglu, Serb. J. Chem. Soc., 1999, 64, 453- 462.
33. T.Buck, H. Bohlen, D. Wohrle, G. Schulz-Ekloff and A. Andrew, J. Mol. Catal. 1993, 80, 253.
34. V.Iliev, A. Andreev, D. Wohrle and G. Schulz-Ekloff, J. Mol.Cata. ,1991, 66 ,5.
35. V.Iliev, J. Mol. Catal. 85, 1993, L 269.
36. Z.wart, H. C. Vander weide, N. Broker, C. Rummens, G. C. A. Schnit and A. L. German, J. Mol. Catal. 3, 1997, 151.
37. M.N. Jones and P. Manely , 1980, J. Chem. Soc.Faraday Trans. I , 76, 654.
38. M.N. Jones and P. Manely, 1980, J. Chem. Soc.Faraday Trans. I , 77, 827.
39. M.N. Jones, H. A. Skinner and E. Tipping , 1975, Biochem. J., 147, 229.
40. K.Kale, G. C. Kresheck and G. Vandekooi , 1978, Biochim. Biophys. Acta, 535, 334.
41. W.J. Knox and T. O. Parshall , 1970, J. Coll. Int. Sci., 33, 16.
42. T.Rades, W. Schuetze and C. C. Muller-Goymann, 1993, Pharmazaie, 48, 425.
43. H.Gharibi, A. A. Rafati , A. Feizollahi, B. M. Razavizadeh and M. A. Safarpour ,1998, Colloids and surfaces (A), 145, 47.
44. H.Gharibi, M. A. Safarpour and A. A. Rafati , 1999, J. Cell. Int. Sci., 219, 217.
45. H.Iloukhani, A. A. Rafati and F. Ravari , 2000, Phys. Chem. Liq., 39, 533.