International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 832-839, Oct-Dec 2009

Balanced dialysis and uv-vis spectroscopic investigation of zinc interferences in absorption and transformation of iron in apo-transferrin

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Abstract: In this research , the binding characteristics of zinc and iron to serum transferring were investigated and compared by using dialysis technique and spectrophotometer titration technique. Both metal ions bound to serum transferrin and compete for the same binding sits on the transferrin. Zinc transferrin (Zn-Tf) and iron transferrin (Fe-Tf) complexes had maximum UV-Vis absorption of zinc transferrin (Zn-Tf) and iron transferrin (Fe-Tf) complexes were detected using 222nm and 465nm, respectively. Bicarbonate addition was necessary for binding activities and citric acid facilitate the binding of both metals to transferrin. Tyrosine residues were involved in iron binding but not in zinc binding to transferrin. The complete saturation of transferrin with iron or zinc was performed within 60 min. Iron uptake by transferrin was reduced in the presence of zinc. The binding of both metals to the apo-tf appears to be pH dependent. These results suggest that zinc may complete with iron in binding to apo-transferrin and influence iron metabolism and its related biochemical parameters.
Key word: zinc, iron, transferrin , balanced dialysis, UV-Vis spectroscopy.

Introduction

Recently, a number of publications indicating the presence of high concentration of zinc in serum of anemia patients that was caused lack of iron absorption and a series of disorders[1-3]. The relationship between zinc toxicity[4] and the appearance of a number of path Physiological [5-6] disorders in this patients has been well documented, might be related to zinc binding to amino acids that have been in apo-transferrin structure.[7-8] owing to the chemical similarities between these two metal ions, zinc may compete with iron for binding to apo-transferrin there by, it may interface with the major iron metabolic pathways.[9] the present study was undertaken to investigate the effect of zinc on iron metabolism by determination of some biochemical factors, including serum iron, total iron binding capacity (TIBC)[10-16], ferritin, and hemoglobin to study the consequent effect of zinc on iron status in the serum of dialysis patients, zinc was added to the human serum as a model, and the rate of iron uptake was measured. [17-22] Spectro photo metrically, an attempt has also been made to find out witch of the amino acid located in iron binding sites of transferrin be involved in this activity.[5]

Material and Methods

All chemical used in this study were obtained from sigma (Dialysis cysts) and Merk (material) chemical companies .

Human apo-transferrin (5 mg/ml) with pH=7.4 [23-24] in H_2O solvent . This buffer solution (100mM) with pH= 7.4 (was formulated the pH of buffer solution with sodium bicarbonate). Ferric chloride solution (1 mg/ml) or standard Fe solution was prepared from dissolve of 100mg of ferric chloride in 10ml strong nitric acid, then turn it to the volume. The protein precipitating solutions in clued glycolic acid w/v 3%, tri chloroacetic acid w/v 10%, and hydrochloric acid 2M, that mixture was kept in a dark bottle, ferreousin reagent 105mg/dl that was dissolved in the soak solution of sodium acetate and was kept in a dark bottle. Sodium bicarbonate solution 0.1 M and 0.02 M, sodium citrate and sodium oxalate that is used for producing Fe-oxalate complex and Fe-citrate complex with different molar ratios. Dialysis cyst and metalothaionin (5mg/ml), EDTA solution, HNO_3 5% for washing and sulfuric acid (2N) for controlling pH, and zinc nitrate solution (1mg/ml) were used in this project.

Procedure

Firstly, the binding of zinc to apo-transferrin was studied by the incubation of aliquots of apo-transferrin solution (5 mg/ml) in the presence of (1.5 μ mol/L) iron (Fe-citrate 1:20) before reading the absorbance by UV-Vis spectrophotometer at 562 nm, that addition to outside of dialysis cyst was fill of 1800ml this buffer (pH=7.4) and after 24hr absorbance of samples, outside and inside of the dialysis cyst, were measured. In the second stage, competition between iron and zinc was performed by incubating apo-transferrin protein in the same situation with different concentrations of iron (1.5 μ mol/L) the iron solution was added to outside the dialysis cyst in periods of 24hr for 4 days in the presence of 500mL zinc solution 1mg/ml (as zinc-citrate 1:20) which was added to inside of the dialysis cyst in the beginning corresponding to Brittenhan technique.[25] Spectrophotometric titration technique was used to study the binding of zinc to each individual amino acid present in the binding sites of transferrin. For this purpose 1ml aliquot of 1mg/ml zinc (in the form of complex with citric acid) was added to an equal volume of the amino acid solution (100 μ g/ml) and mixture incubated at room temperature for 1hr. Absorption spectra of the prepared solution were then taken using an Agilest UV-Visible spectrophotometer.

Results

Effect of time

Absorbances of the samples were determined after time incubation of apo-transferrin in the presence of iron a results are shown in Fig.1.

Effect of pH

The pH is a very important factor for iron-transferrin formation.[26] Therefore the effect of the pH on the retention of iron and apo-transferrin (Fe-Tf) was studied in the range of 4-9 by adding sulfuric acid or sodium hydroxide solutions. The results are shown in Fig.2. Biological pH (7.4) are generally preferred for this studied, because the binding of iron-transferrin are dissociated in acidic situation.

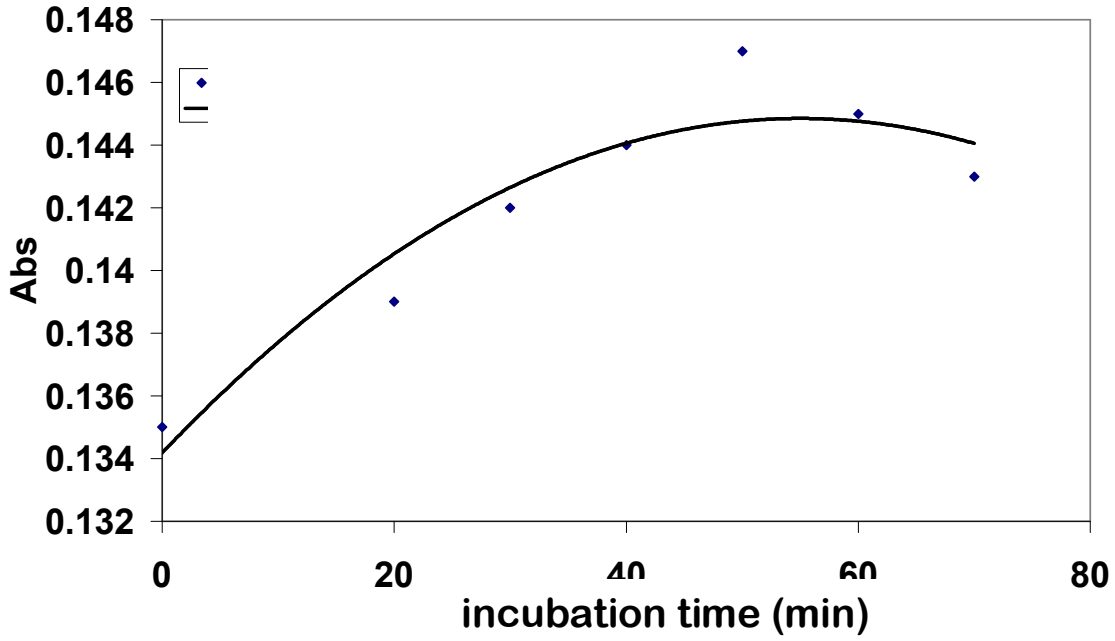


Fig.1. Time course for binding iron to human apo-transferrin in pH 7.4. Each point is the mean of three observations.

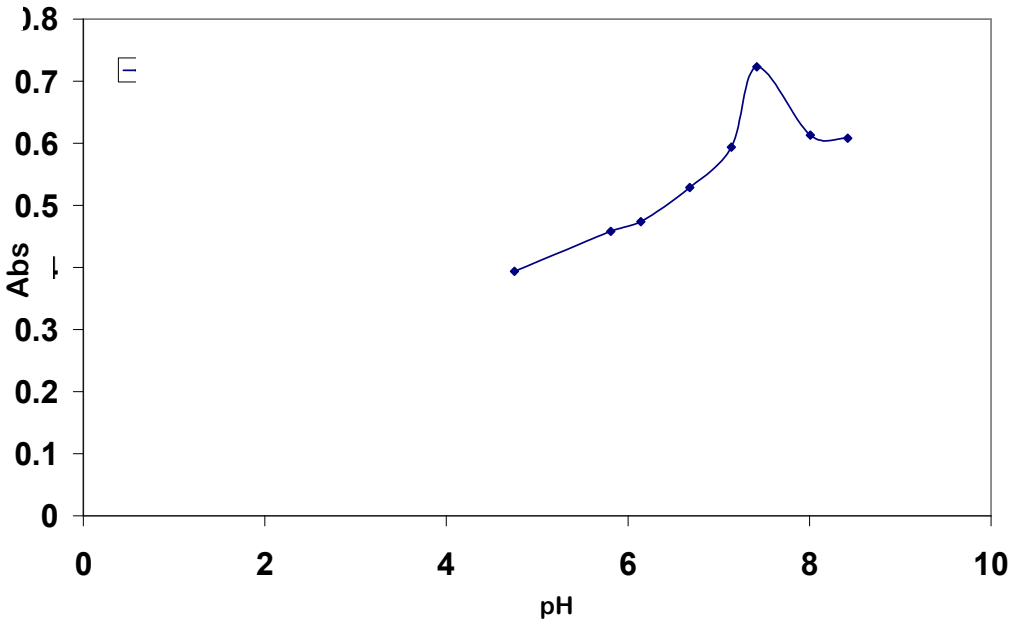


Fig.2. Effect of pH on iron binding to apo-trasferrin. Each point is the mean of three separate experiments.

Effect of zinc on iron absorption.

In order to investigate the effect of zinc on Fe(III) uptake by apo-transferrin , to series of volumetric flasks containing tris-buffer and 1mg/ml Fe (III) as a complex with citric acid, 10-20 μ g/L of zinc with citric acid 1:20 was added. The reaction mixtures were incubated for 60min. At the end of incubation time the iron concentration inside the sacs were measured and compared with control in which no zinc was added. It was found that addition of10-20 μ g/L of zinc to reaction mixture decreased iron uptake .

Effect of bicarbonate ion

The highest absorption spectra was shown, when the concentration of bicarbonate anion was 0.02M in system, we had increasing binding between iron and apo-transferrin in the best situation.

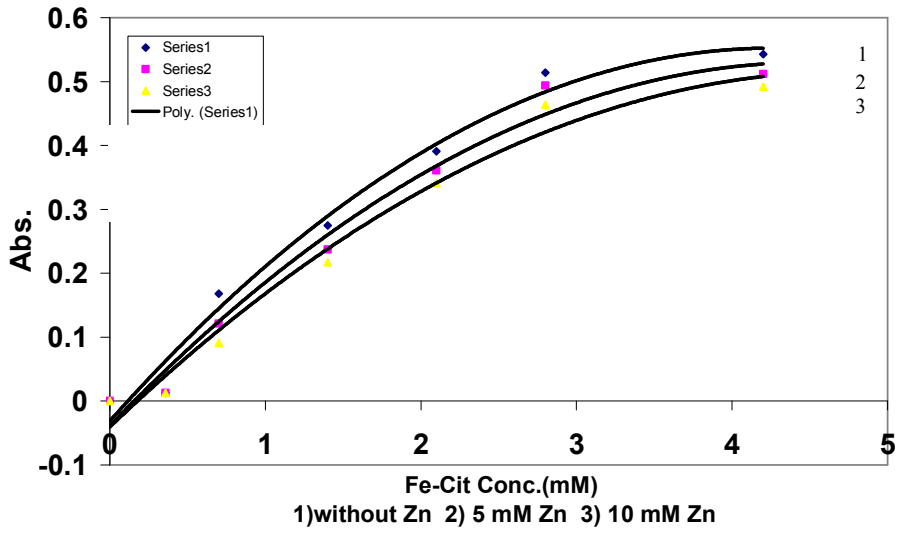


Fig.3. Effect of zinc on iron binding to ap-tf without Zn^{2+} (1), 5mM Zn^{2+} (2), 10mM Zn^{2+}(3).

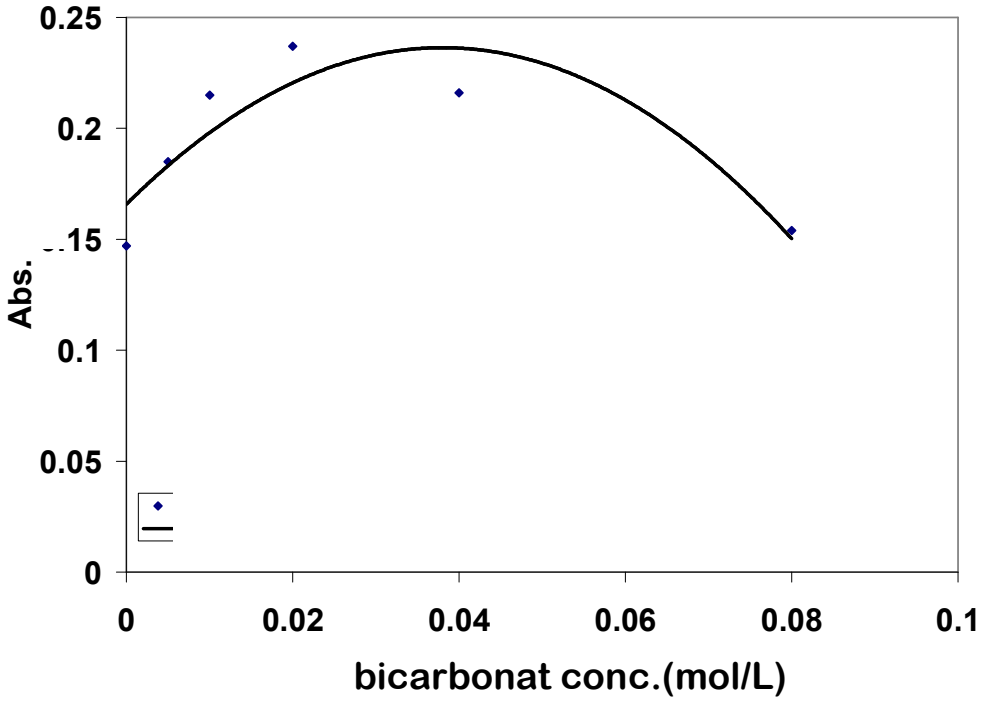


Fig.4. Effect of bicarbonate on iron binding to apo-Tf. Each point is the mean of three observations.

The concentration of zinc from in vitro study are summarized in Tables .

Table1. Total bound iron and internally bound iron as a function of iron concentration.

time (hr)	0	25	50	75	100	125
total iron $\mu g/dl$	3.025	10.715	14.984	16.812	20.604	22.120
free iron $\mu g/dl$	3.712	9.007	12.305	13.224	14.011	10.296
bound iron $\mu g/dl$	0.313	1.708	2.679	3.588	6.593	11.824
free Fe/bound Fe	8.660	5.273	4.593	3.680	2.120	0.870

(y=A+Bx,A=6.77647, B=-0.57902, r=-0.89174, r^2=0.79521)

Table 2. Total bound iron in the presence of zinc and there is internally bound iron dependency of zinc concentration.

time (hr)	0	25	50	75	100	125
total iron $\mu g/dl$	3.775	6.405	9.756	13.561	14.147	15.662
free iron $\mu g/dl$	2.613	4.907	6.802	8.501	7.197	4.489
bound iron $\mu g/dl$	0.652	1.498	2.954	5.060	6.950	11.173
free Fe/bound Fe	4.650	3.270	2.300	1.680	1.030	0.400

(y=A+Bx,A=3.93192, B=-0.36392, r=-0.92364, r^2=0.85311)

The samples were incubated for 5min with varying concentrations of Fe at room temperature. Uptake was measured either before or after the addition of 1.5 mM iron for each 24hr.

The uptake of iron was reduced in the presence of zinc, suggesting that this metal ion may compete with iron in binding to proteins, particularly transferrin.

Thus, it was interesting to study the binding of zinc to transferrin and to find out the most suitable ligand for zinc binding to transferrin. The addition of zinc to arginin in the presence of bicarbonate make changing in the absorption spectrum of this amino acid, Fig.6.

The same observations were found the amino acid lysine, tryptophum, and tyrosine.[27] In contrast, when the experiment was repeated with metalotionine. It was found that the addition of zinc led to significant decrease in the maximum absorption of these amino acids, so suggesting that all of them were probably suitable legends for zinc binding to transferrin, the result were shown in Fig.7.

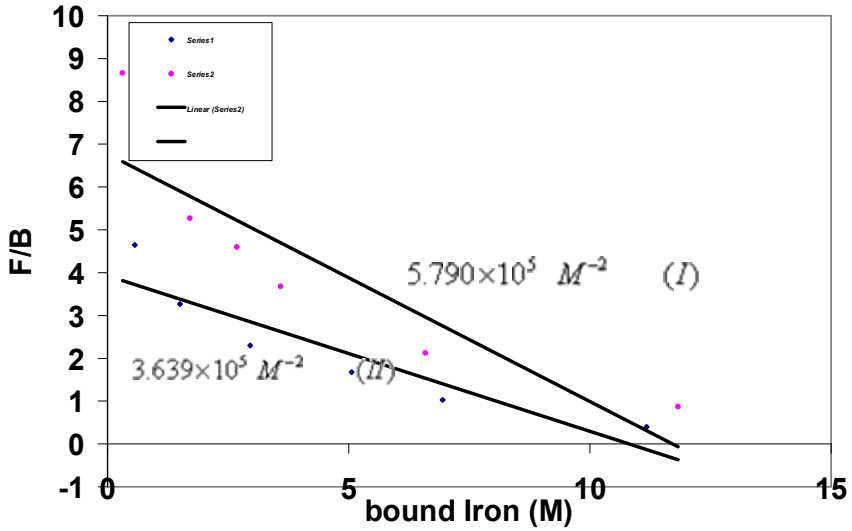
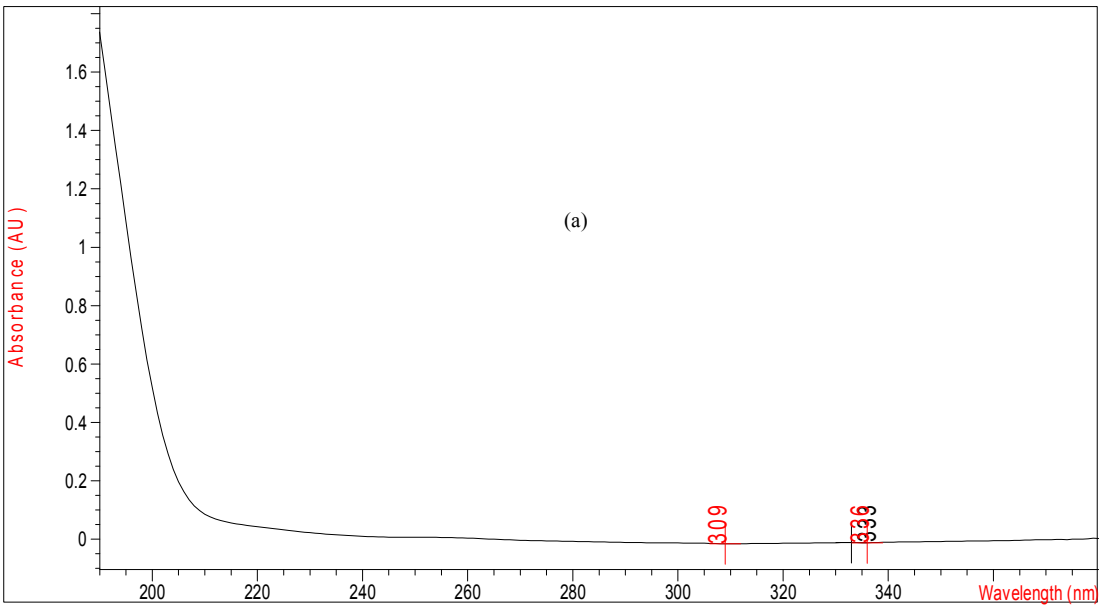


Fig.5.Comparative study of iron absorption by transferrin in balanced dialysis (I) without zinc, (II) in the presence of zinc)



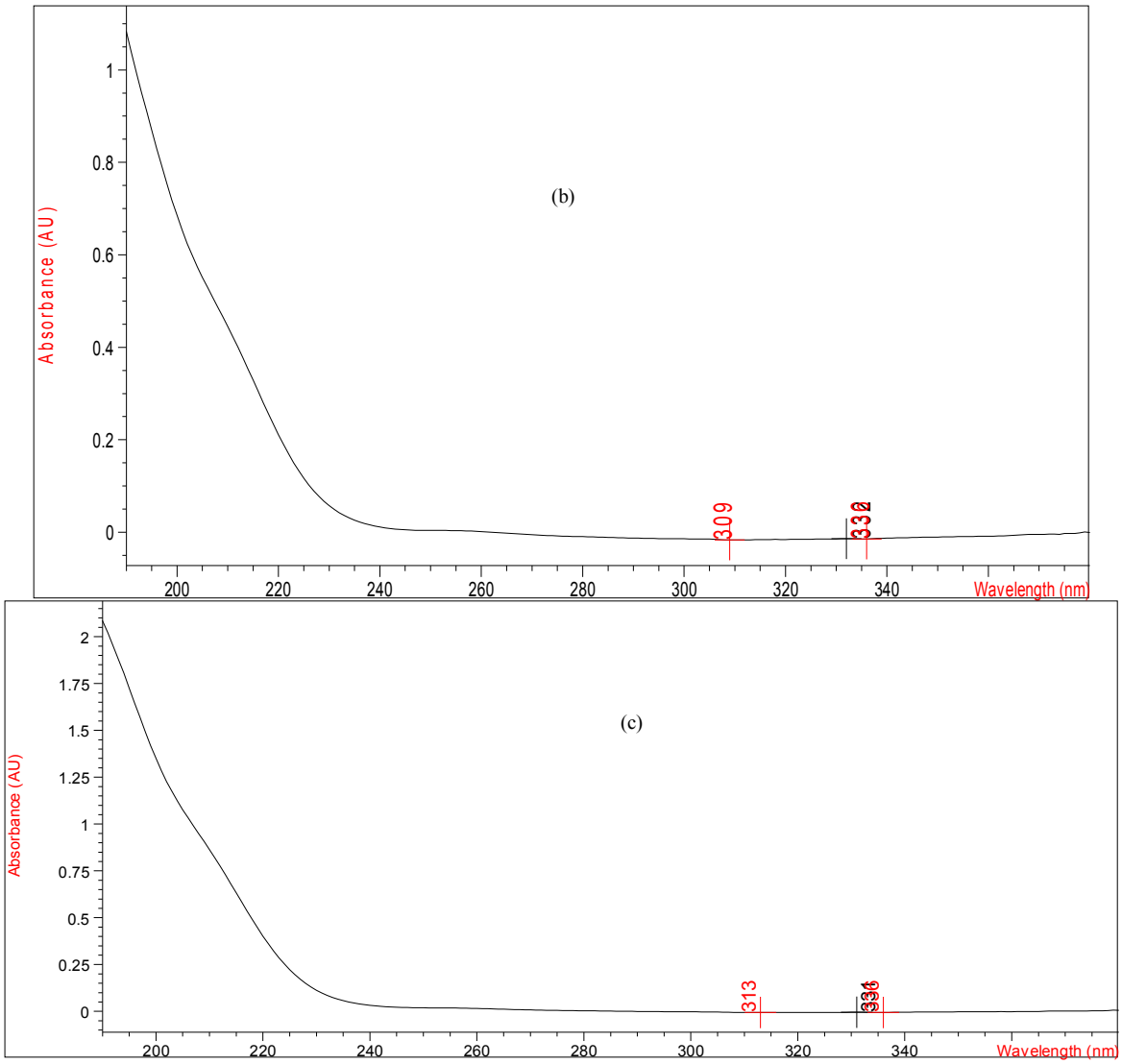
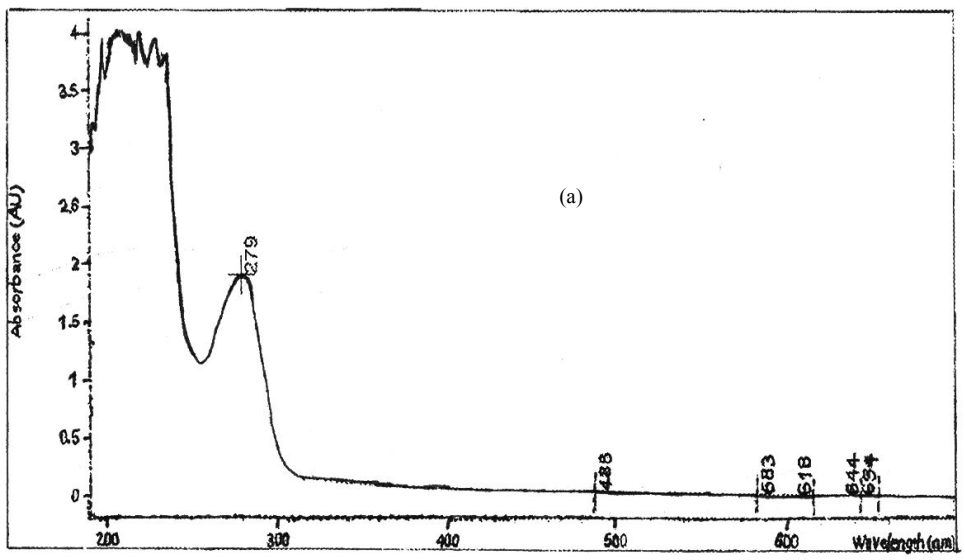


Fig.6. Absorption spectra of: (a) arginin solution, (b) arginin in the presence of zinc, and (c) arginin in the presence of iron



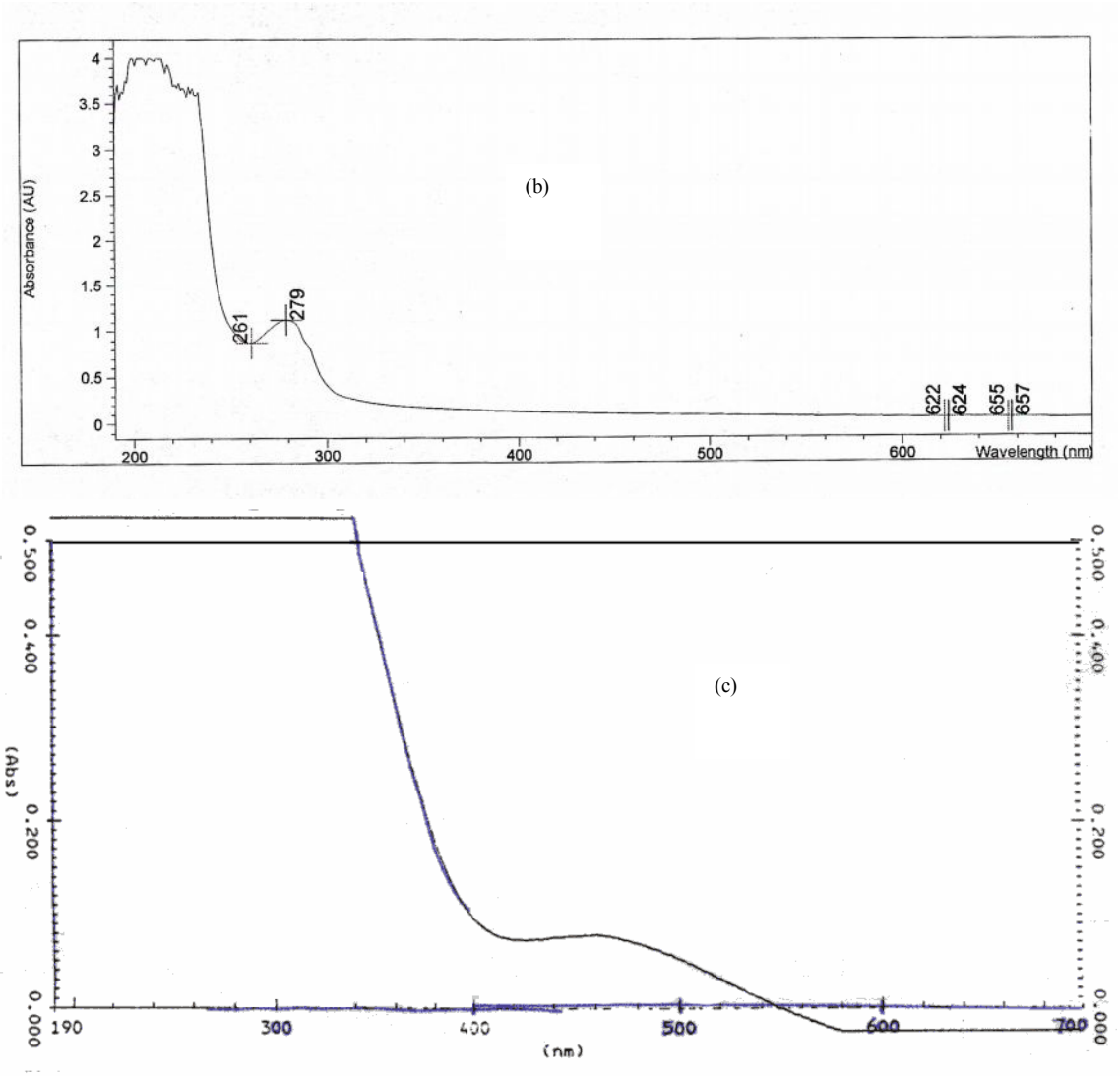


Fig.7. Absorption spectra of: (a) transferrin solution, (b) transferrin in the presence of zinc, and (c) transferrin in the presence of iron.

Discussion

The data that has been presented in this article elucidated the probable mechanism by which Zn and Fe bind to apo-Tf. Absorption spectra obtained from zinc-apo-Tf complexes indicated minimum absorbance at 279nm, when the effects of pH on the binding of zinc to apo-Tf were studied, it was found that the maximum binding activity of zinc occurred at pH:7.4. The binding activity of zinc seems to be more similar to iron.

Thus a chelating agent is necessary for the binding of iron to apo-Tf.[28] The chemical similarities between zinc and iron are thought to follow the same mechanism for binding to transferrin .[29] Results of the present study showed that, in the absence of bicarbonate or citric acid no significant binding of zinc to transferrin occurred. The decreasing in iron uptake by zinc and competition of iron with zinc suggest that these two ions compete for the same binding site on the transferrin. However, still more investigations should be done to clarity that exact mechanism by which these processes occur.

Acknowledgements

Financial support from Payame Noor University is gratefully acknowledged.

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