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Sorption of Myoglobin by Tris - (2-aminoethyl) amine bound polymer complexed with transition metal ions

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Sorption of Myoglobin by Tris-(2-aminoethyl) amine bound polymer complexed with transition metal ions

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Dedication

To the memory of my grandfathers (Ismael and Abdul Ghani) , grandmothers (Khadra and Fatima) and My Uncle Eassa Ismael Abufara

To my beloved my father and my mother

To my sister Huda

To my dear husband Osaid

To my sons Osama and Mohammad

Rahma Samih Arar

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:…….

Rhama Samih Abdulghani Arar

Date: 28/5/2016

Acknowledgment:

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Abstract:

In this study, polystyrene modified with tris –(2-aminoethyl) amine and its complexes of (Ni (II) and Cu (II)) were used to remove Myoglobin. The optimum condition of initial concentration of Cu (II) or Ni (II), adsorbent dose, contact time, ionic strength, the initial concentration of Myoglobin and pH were investigated. The initial and final concentration of Mb was analyzed by UV spectrophotometry to confirm the removal of Mb. The characterization of tris-(2-aminoethyl) amine bound polymer and its complexes were performed by scanning electron microscope and electron dispersive spectrophotometry. The results indicated the optimum concentration of Ni (II) and Cu (II) were 200 and 400 ppb respectively. Maximum adsorption efficiency % was achieved by 0.5 mg/ml adsorbent dose, contact time 180 min, 0.1 M NaCl ionic strength for tris-(2-aminoethyl) amine bound polymer and 0.05 M NaCl for its complexes Cu (II) and Ni (II). The tris – (2-aminoethyl) amine bound polymer modified with Ni (II) fitted Langmuir adsorption model with maximum adsorption capacity 14.79 mg /g, while the tris – (2-aminoethyl) amine bound polymer modified with Cu(II) fitted Freundlich model with capacity of 21.55mg /g. The desorption percent for tris- (2-aminoethyl) amine bound polymer, its Cu (II) complex and its Ni (II) complex were 25 .42%, 35.38 % and 32.39 % respectively.

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Abbreviations:

Mb	Moglobin
kDa	Kilodaltion
His	Histidine
AMI	Acute myocardial infraction
min	Minute
IMAC	Immobilized metal ion affinity chromatography
IDA	Iminodiacetic acid
TREN	Tris – (2-aminoethyl) amine
NTA	Nitrilotriacetic acid
HEWL	Hen egg white lysozyme
Hb	Hemoglobin
BSA	Bovine Serum Albumin
BHb	Bovine Hemoglobin
PHEMA	Poly(2-hydroxyethylmethacryle)
HisU	Polyhistidine tagged Ubiquitin
SEM	Scanning electron microscope
SEI	Secondary electron image
Kv	Kilovolte
Eds	Energy- dispersive X ray spectrometry
µl	Microliter
µm	Micrometer
$HPO_4^{2-}/H_2PO_4^-$	Hydrogen phosphate / dihydrogen phosphate ion pair
SDS/PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
rpm	revolution per minute

ppm	part per million
ppb	part per billion

Chapter One

Introduction

1. Introduction:

1.1.Myoglobin and protein purification:

Nowadays, proteomic and protein purification takes the interest of scientists and researchers in many fields such as biology, medicine and food processing [Zhang et.al, 2010]. Myoglobin (Mb) is one of the proteins that got interested in detection and separation especially in medical research. Mb is a major protein of the sarcoplasm with molecular weight 17.8kDa. It is composed of 153 amino acid .Tertiary structure of Mb is composed of eight helical segments (A-H). (Fig 1). These segments are joined with non-helical segments. The single heme is inserted in a pocket lined with hydrophobic amino acid side chains. The iron atom of heme is bound covalently to nitrogen of His 93 (F8:the eighth residue of helix F). It is called proximal histidine. The sixth coordination position of the other side of heme plane is occupied by oxygen. Another histidine residue is called distal histidine (His 64,E7) stabilizes the oxygen via hydrogen bond (Sieb et.al, 2004). The importance of Mb is considered as one of cardiac biomarker in case of acute myocardial infraction (AMI). AMI occurs when the blood supply to heart muscles is blocked for extended time. As a result, the myocardium suffers cell damage and muscles death. The concentration of cardiac enzymes increases dramatically, as the dead cells release their content in blood stream. There are three cardiac enzymes which are used to assess AMI: Myoglobin, caetine kinase M13 and cardiac troponin. The Mb is the smallest one of these three proteins and rapidly diffuses through the vascular system, so it is the first biomarker appears (30 min after onset of chest pain). Mb is also rapidly cleared by kidney and return to normal level in 16 to 36 hrs after heart attack (Denniston et.al, 2007). Mb is absent or present in very concentration in the urine of healthy people (< 0.01 µg/ml). It increases rapidly in urine in case of muscles damage (750 µg/ml) (Zhang et.al.,2011).

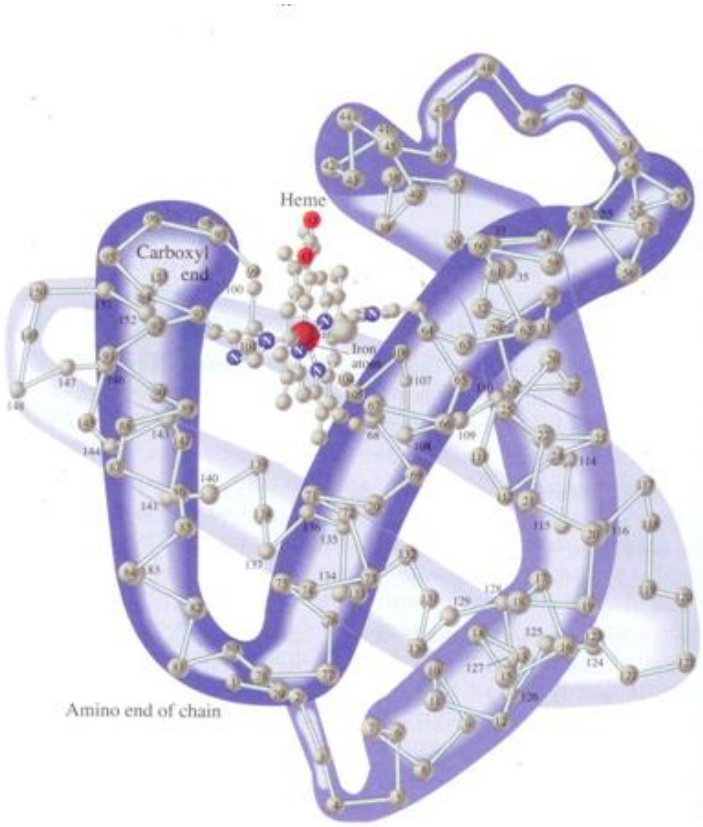


Fig 1.1 : Tertiary structure of Myoglobin (Denniston et.al.2008)

There are many traditional methods that are used in protein isolation such as centrifugation, filtration and membrane separation. However, these techniques need several stages of pretreatment (Zhang et.al, 2010). New field in protein purification have been opened by the introduction of Immobilized metal ion affinity chromatography (IMAC) (Porath, 1992). IMAC is a highly versatile separation method based on interfacial interaction between biopolymer in solution and metal ions fixed to a solid support which is usually a hydrophilic cross-linked polymer (Porath, 1988). The concept of IMAC is based on a chemical affinity of a certain side chain groups on the surface of proteins for metal ions fixed on stationary phase. The side chain groups examples are imidazole group of histidine and thiol group of cysteine (Sharma et.al. 2001). The interacting metal ions must be localized in exposed positions in the reticular

network of the polymer to allow complexation (Porath , 1992) . The formation of the matrix –adsorbate complex is represented as following:

Pmx – Sp- Ch ~M ·····P (Porath et.al.,1988)

Where:

Pmx : polymer matrix , Sp : spacer arm , Ch : chelator , M: metal ion , P: protein , ~ is stronger than ···

The formation of adsorption complex involves two steps: 1) immobilization of the metal ion by chelate formation. 2) Protein adsorption. The interaction between the immibolized metal ions and protein is governed by several forces such as electrostatic, donor acceptor (coordination) and hyrdophopic interactions. The dominant interaction force depends on a number of variables such as the nature of chelating ligand, metal ions surface, amino acid composition and the surrounding chemical environment (buffer salts, pH, ionic strength) (Sharma et.al., 2001). The advantages of IMAC are ligand stability, high protein loading, simple regeneration and low cost (Jain et.al. 2007). Many chelator ligand are used in IMAC such as : iminodiacetate (IDA), nitrilotriacetic acid and Tris-(2-aminoethyl) amine (TREN) (Porath, 1992). IDA chelator is tridentate and forms a double five membered ring chelates with tetra and hexa coordinate metal ions (Porath, 1992). Nitrilotriacetic acid is quadridentate chelating adsorbent. It occupies four positions in the metal coordination sphere and the two remaining ligand positions in the octahedral coordination are free for selective protein interaction (Hoechuli et.al., 1987). Tris-(2-aminoethyl) amine (TREN) is symmetric and will give uniform adsorption center (fig:1.2). This chelator is tetradentate but it can be tri or tetra dentate. This idea is still unclear (Porath et.al.,1992). TREN also has four nitrogen (three of them are primary and the fourth one

in tertiary). Two free site are available to bond with metal ions (Sharam et.al.,2001). All metal ions that interact specifically with proteins are potential partner in the adsoption center. The metals are classified according to Peason's polarizability into soft, hard and intermediate (Porath 1992). Hard metal ions prefer oxygen while soft metal ions prefer sulfur. The intermediate types such as Cu (II) and Ni(II) coordinate nitrogen (Porath, 1988). The intermediate metal ions have been intensely studied in IMAC and they formed very useful IMA adsorbents (Porath, 1992).

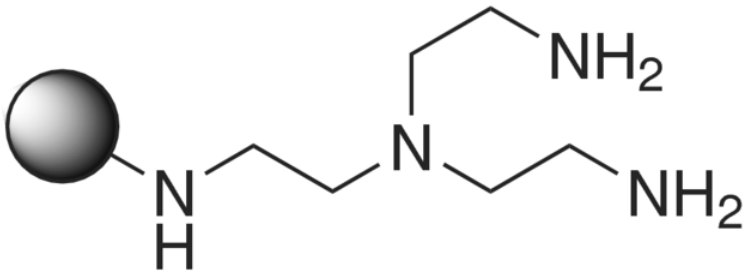


Fig 1.2: Tris – (2 aminoethyl) amine polymer bound (TREN)

Several studies were preformed on the adsorption of proteins and particularly on Mb. Hochili et.al. used chelating adsorbent contains the functional group nitrilotriacetic acid (NTA).The adsorbent was complexed with Ni (II). The results indicated that the complex that was formed was in the ratio 1:1 metal ligand. The protein affinity for NTA –Ni (II) adsorbent was studied on Cytochrome c from horse heart, trypsin from bovine pancreas and interferon α 2a synthesized in bacteria. None of these proteins were adsorbed by the adsorbent because of the absence of surface exposed histidine. Furthermore, NTA-Ni(II) selectivity adsorded proteins and peptides that had structural element neighboring histidines on the surface (Hochuli et. al.1987).

Sharama et. al. studied the general applicability of isotherm adsorption models (Langmuir, Freundlich, Temkin and Langmuir –Freundlich) in explaining the adsorption

behavior of several proteins on different IMA-M(II). The interaction between several model of proteins (lyosozyme, ovalbumin, bovine serum albumin, conalbumin and wheat germ agglutinin) with metal Cu (II) , Ni(II) chelated iminodiacetic acid and tris-(2-aminoethyl) amine (TREN) were investigated. Most of the systems fit Langmuir – Freundlich adsorption isotherm model in describing the interactions of IMA- M (II) with proteins (Sharma et.al.,2001).

Xu et.al. investigated the ability of nitrilotriacetic acid modified nanoparticles immobilized with Ni (II) to bind histidine tagged proteins. The aim of the study was to determine the selectivity of these surface modified nanoparticles in binding histidine tagged proteins in cell lysate. The results of electrophoresis analysis indicated the specificity of NTA-Ni(II) to bind histidine tagged proteins. The SDS/PAGE showed that NTA-Ni (II) represented high specifity to His tagged proteins since no other proteins being washed off by low concentration of imidazole. Also, the NTA-Ni(II) is not affected by reusing or recovering. The binding capacity of protein was 2-3 mg protein/g NTA nanoparticles (Xu et.al., 2004 a).

Xu et.al. used dopamine to anchor nitrilodiacetic acid (NTA) on iron oxide shell of magnetic nanoparticles. The system was saturated with Ni (II). The results of SDS/PAGE analysis shows the specificity of NTA- Ni (II) to bind with histidine – tagged proteins (Xu et.al. 2004b).

Trojer et.al. used silica particles with different porosity. These particles were functionalized with iminodiacetic acid and loaded with Cu (II). The system was used to remove hen egg white lysozyme (HEWL). The highest binding capacity was for particles with mean pore diameter of 12 nm and reaching about 350 mg/g (Trojer et.al., 2005).

Guo et.al. designed two molecularly imprinted polymers using hemoglobin as the imprinting molecule, acrylamide as the functional monomer, chitosan and maleic anhydride modified chitosan as matrixes. These two systems were used to remove hemoglobin (Hb). The maleic anhydride chitosan system has maximum capacity of 35.84 mg/g while chitosan beads has 33.2 mg /g capacity of (Hb). Both system separate with high selectivity Hb from a mixture of Hb and BSA (Guo et.al., 2005).

Ma et.al. synthesized magnetic silica nanoparticles functionalized with iminodiacetic acid (IDA) chelating agent. This adsorbent was charged with Zn (II) cations to be used to separate mixtures of bovine hemoglobin (BHb), and bovine serum albumin (BSA). The results indicated high selectivity for BSA with maximum adsorption capacity 207.2 mg/g while BHb was not adsorbed (Ma et.al., 2006).

Jain et.al. used a modified porous membrane with poly (2-hydroxyethylmethacrylate) (PHEMA) which is derivatized with nitriloacetetate – Ni (II) to purify polyhistidine tagged ubiquitin (HisU). The results indicated that the PHEMA-NTA –Ni (II) brushes in membrane pores show binding capacity of HisU 120 mg /cm^3. Furthermore, PHEMA –NTA-Ni (II) is selective for HisU (the binding capacity for myoglobin and bovine serum albumin (BSA) were 0.05 , <0.01 mg/cm^3 respectively. The separation of HisU from protein mixtures was also evidence that PHEMA –NTA-Ni (II) is selective for HisU. HisU was separated from mixture contained 20 fold excess of BSA. The SDS-PAGE analysis showed separation of HisU from 10% bovine serum spiked with 0.3 mg/ml. On electropherogram, strong band appeared for HisU and very faint band for BSA (Jain et.al., 2007).

Lin et.al. developed an infrared chemical sensor for selective determination of tyrosine in urine. It was based on the complexation of acidified tris-(2-aminoethyl) amine with

Ni (II) to detect tyrosine in urine. This sensing phase had linear range of signal intensities up to 600 µM with detection limit of 30 µM (Lin et.al.,2008).

Cete et.al studied the adsorption of myoglobin (Mb) by poly(glycidyl methacrylte) (PGMA) microbreads with the surface functionalized with iminodiaceic acid in packed bed column system. Several parameters were studied such as flow rate, pH, ionic strength and initial concentration of Mb. The results showed that the optimum flow rate was 0.5 ml/min, pH was 7. The maximum adsorbed Mb (810 µg/g polymer) was observed at ionic strength of 2.0×10^{-2} M NaCl. The adsorption of Mb by IDA modified PGMA exhibited good fit with langmuir isotherm model with maximum capacity 1845 µg/g. The frendlich isotherm model constants K_f and n showed that the Mb can be easily adsorbed by IDA modified PGMA with high capacity. n>1 indicated favorable adsorption condition for microbreads and heterogeneous nature of adsorption (Cete et.al., 2009).

Zhang et.al. prepared magnetic silica coated sub-microspheres immobilized with iminodiacetic (IDA) saturated with Cu (II). This was used to remove bovine Hemoglobin (BHb) from bovine blood. The adsorption capacity was 416.6 mg /g with low nonspecific adsorption (Zhang et.al. 2010).

1.2.Problem:

Myoglobin (Mb) is a biomarker for Acute Myocardial Infarction (AMI). As a result, there is a need for an efficient, fast method for Mb detection. This thesis is aimed to investigate the ability of tris – (2-aminoethyl) amine bound polymer and its metal complexes to bind Mb at low concentration from aqueous solutions. This will help in identifying muscle injury particularly that is caused by myocardial infarction and thus save patients from severe injury.

1.3.Objectives:

In this study, the polystyrene tris- (2 – aminoethyl) amine is used to remove myoglobin from aqueous solutions. The latter is also modified with Ni(II) and the resulting metal immobilized complex compared with polystyrene tris-(2-aminoehtyl)amine in removing Myoglobin. (Mb). The myoglobin has 11 histidine (His) residue at least four of them are exposedon the surface of the protein (Zhang et.al.,2011) . These histidine residues can coordinate with transition metals such as Ni (II) and Cu (II).

The specific objectives that should be achieved:

- Investigate the ability of tris – (2-aminoethyl) amine bound polymer, Cu (II) complex of tris – (2-aminoethyl) amine and Ni(II) complex of tris – (2-aminoethyl) amine to remove Mb.
- Determine the optimum condition (metal ion concentration, contact time, adsorbent dose, ionic strength, Mb concentration and pH) to each adsorbent.
- Investigate the ability of isotherm models (Langmuir and Freundlich) to explain the adsorption behavior of each adsorbent.
- Determine the adsorption capacity of each adsorbent.

Chapter Two

Experimental

2. Experimental:

2.1.Reagents:

Polystyrene tris-(2-aminoethyl) amine (TREN), Nickel(II) nitrate hexahydrate, copper (II) nitrate trihydrate, Myoglobin (Mb) from equine skeletal muscle, Sodium hydroxide, Dipotassium hydrogen phosphate, potassium dihydrogen phosphate, imidazole (Assay :100 %) were of analytical grade and were purchased from Sigma Aldrich. All solutions were prepared using distilled deionized water.

2.2. Instruments :

Initial and final concentrations of Mb were determined using UV-Visible spectrophotometer (mrc, spectro UV-16). The pH measurements were recorded on a HANNA pH instrument (HI 9812-5). Characterization of TREN modified with heavy metals was done by Scanning Electron Microscope (SEM) (JEOL model, JSM-5410 LV, accelerating voltage of 25 kV, at high vacuum (HV mode) and secondary electron image (SEI)) and energy dispersive X-ray spectrometry (Eds) analysis (Oxford system, liquid Nitrogen cooled solid state Energy Dispersive Spectrometer detector, linked ISIS software). The samples were prepared on metal stubs and coat with gold. Separation of polymer particles from solutions was performed by Centrifugation (Precision, centrifuge MOD durafuge 100). The masses were determined by analytical balance (Mettler Toledo, AB104 with error: ± 0.001g). The volumes of the solutions were measured by mircopipet (100-1000 µl).

2.3. Procedures :

2.3.1. Preparation of heavy metal solutions:

Stock solutions of 1000 ppm of each Cu (II) and Ni (II) were prepared individually by dissolving the appropriate amount of copper (II) nitrate trihydrate Nickel(II) nitrate hexahydrate respectively in deionized distilled water. Solution with 100 ppm concentration from each metal salt was prepared by transferring an appropriate volume from the stock solution to a 100 ml volumetric flask and made up with distilled water to the mark.

2.3.2. Characterization of modified TREN bound polymer:

TREN polymer (60 mg) was placed in two separate Erlenmeyer flasks. 50.00 ml of 100 ppm of Cu(II) and Ni(II) was added individually to Erlenmeyer flasks. The samples were stirred for three hours at room temperature with moderate speed. Then, the samples were centrifuged at 4000 rpm for 30 mins. The filtrate was removed and the polymer samples were dried in a desiccator and then analyzed by SEM and Eds.

2.3.3. Preparation of phosphate Buffer solution:

The buffer was prepared according to Henderson- Hasslbach equation:

$$pH = pk_a + \log\left(\frac{[A^-]}{[HA]}\right)$$ ……..(Chang , 2007)

pH: negative logarithim of hydronium concentration

pK_a: negative logarithim of acid association constant (pKa=7.21 (Change , 2010))

$[A^-]$: equilibrium concentration of conjugated base

[HA]: equilibrium concentration of weak acid

The weak acid and its conjugated base that was chosen was dipotasium hydrogen phosphate/potassium dihydrogen phosphate (K_2HPO4/KH_2PO_4). The concentration of buffer that used in the calculation was 0.2 M with pH 7.4 or 8.1. with pKa = 7.21. By applying the values of pH and pKa in Henderson- Hasslbach equation, the ratio of ($HPO_4^{2-}/H_2PO_4^-$) will be used to determine the concentration of each species. The pH of the buffer was adjusted by 2 M NaOH.

2.3.4. Ionic strength:

The ionic strength was controlled by NaCl concentration in the buffer solution.

2.3.5. Determination of Myoglobin (Mb) :

Six standard solutions were prepared. Firstly, an appropriate amount of Mb was transferred to a 100 ml volumetric flask to prepare stock solution of 200 ppm Mb in phosphate buffer (pH 7.4, 0.01 M NaCl). The Mb solution was filtered with Millipore 0.45 µm filter. Then, aliquots of (25, 12.5, 6.5, 2.5, 1.25 ml) were transferred to 50 ml volumetric flasks individually to prepare 100, 50, 25, 10, 5 ppm Mb solutions respectively. The volume was made up with phosphate buffer. The absorbance of the solutions (200, 100, 50, 25, 10, 5 ppm) was taken by UV spectrometer at 410 nm and the calibration curve was constructed.

2.3.6. Testing the adsorbent response of tris-(2aminoethyl)amine (TREN) bound polymer to Mb:

An appropriate amount of Mb was used to prepare 100 ml of 100 ppm Mb solution in phosphate buffer. (pH 7.4, 0.01 M NaCl). The Mb solution was filtered with Millipore filter. 40 ml of 100 ppm Mb was added to 40 mg of adsorbent and stirred for three hours. The Mb solution was centrifuged at 4000 rpm for 30 mins. The filtrate was analyzed directly by UV spectrophotometer. The initial concentration of

Mb was determined colorimetrically by using 1ml of 100 ppm Mb. The experiment was performed in triplicate.

2.4.Adsorption isotherm:

2.4.1. Effect of initial concentration of heavy metals:

A 100 ml of 50,100, 200, 400 and 600 ppb Cu (II) and Ni (II) were prepared individually from 100 ppm stock solution. 50 ml of each solution was placed separately in 250 ml Erlenmeyer flask with 40 mg of adsorbent. The solutions were left to stir for three hours at room temperature. The solutions were centrifuged at 4000 rpm for 30 mins. The experiment was performed in triplicate. The modified adsorbent was transferred to 250 ml Erlenmeyer flask containing 40 ml of 100 ppm Mb solution which was prepared in a phosphate buffer (pH 7.4, 0.01 M NaCl) and filtered using a Millipore 0.45µm filter. The samples were stirred for three hours at room temperature with moderate speed. The solutions of Mb were centrifuged at 4000 rpm for 30 min. The filtrate was transferred to plastic tubes. The initial and final concentrations of Mb were determined by UV spectrophotometer at 410 nm. The Mb samples were triplicate for each metal.

2.4.2. Effect of adsorbent dosage:

2.4.2.1. Using TREN bound polymer modified with Cu (II):

A concentration of 400 ppb Cu (II) was prepared. 50 ml the metal solution was placed in each of five Erlenmeyer flasks. 20,40,60,80,100 mg of TREN polymer was added to the flasks and stirred for three hours with moderate speed. The samples were centrifuged at 4000 rpm for 30 mins. Then, the filtrate was decanted. The samples were run in triplicate. The modified TREN with Cu(II) samples were placed in Erlenmeyer flasks with 40 ml of 100 ppm Mb which was prepared in phosphate buffer

(pH 7.4, 0.01M NaCl) and filtered using a Millipore filter 0.45µm. The samples were stirred for three hours at room temperature with moderate speed. The treated Mb solutions were filtered using Millipore filter with 0.45 µm. The adsorbent remained in the solution was separated by centrifugation and the solution was kept in refrigerator. The initial and final concentrations of Mb were measured by UV spectrophotometer at 410 nm.

2.4.2.2. Using TREN bound polymer modified with Ni (II):

The experiment was repeated by using Ni (II) in place of Cu (II) with concentration of 200 ppb.

2.4.2.3. Using TREN bound polymer :

20,40,60,80,100 mg of TREN polymer was added to the flasks with 40 ml of 100 ppm Mb which was prepared in phosphate buffer (pH 7.4, 0.01M NaCl) and filtered using a Millipore filter 0.45µm. The samples were stirred for three hours at room temperature with moderate speed. The treated Mb solutions were filtered using Millipore filter with 0.45 µm. The adsorbent remaining in the solution was separated by centrifuge and the solution was kept in the refrigerator. The initial and final concentrations of Mb were determined by UV spectrometer at 410 nm. Mb samples were triplicate for each adsorbent dosage.

2.4.3. Effect of contact time :

2.4.3.1. Using TREN bound polymer modified with Cu (II):

A 20 mg of TREN bound polymer adsorbent was transferred to five Erlenmeyer flasks. 50 ml of 400 ppb Cu (II) was added separately in five Erlenmeyer flasks. The samples were stirred for different time intervals (30, 90,150,180 and 240 minutes). The metal solutions were removed and the TREN bound polymer adsorbents were kept in

desiccator. The samples were run in triplicate. The modified TREN bound polymer was treated with 40 ml of 100 ppm Mb prepared in phosphate buffer (pH 7.4, 0.01M NaCl) and filtered using a Millipore 0.45µm filter. The time intervals were kept the same. The samples were filtered using Millipore 0.45µm filter. Then, the initial and final concentrations of Mb were determined by UV spectrophotometer at 410 nm. The adsorbent remained in the solution was separated by centrifuge and the solution was kept in the refrigerator.

2.4.3.2. Using TREN bound polymer modified with Ni (II):

The experiment was the same, but the metal was changed to Ni (II) with concentration of 200ppb.

2.4.3.3. Using TREN bound polymer alone :

20 mg of TREN bound polymer adsorbent was transferred to five Erlenmeyer flasks. 40 ml of 100 ppm Mb prepared in phosphate buffer (pH 7.4, 0.01M NaCl) and filtered using a Millipore 0.45µm filter was added to each flask. The samples were stirred for different time intervals (30, 90,150,180 and 240 minutes) at room temperature with moderate speed. The samples were filtered using 0.45µm Millipore filter. Then, the initial and final concentrations of Mb were determined by UV spectrophotometer at 410 nm. The adsorbent remained in the solution was separated by centrifuge and the solution was kept in the refrigerator

2.4.4. Effect of ionic strength:

2.4.4.1. Using TREN bound polymer modified with Cu (II):

20 mg of TREN bound polymer adsorbent was transferred to four Erlenmeyer flasks. 50 ml of 400 ppb Cu (II) was added to each of the five Erlenmeyer flasks. The samples were stirred for three hours at room temperature with moderate speed. The metal solutions were removed and the TREN bound polymer adsorbents were kept in a desiccator. The modified TREN bound polymer samples were treated with 40 ml of 100 ppm Mb prepared in phosphate buffer (pH 7.4 with different NaCl concentrations 0.01, 0.025, 0.05, 0.1 M) and filtered using 0.45µm Millipore filter. The samples were filtered using Millipore 0.45µm filter. Then, the initial and final concentrations of Mb were determined by UV spectrophotometer at 410 nm. The adsorbent remaining in the solution was separated by centrifuge and the solution was kept in the refrigerator.

2.4.4.2. Using TREN bound polymer modified with Ni (II):

The experiment was the same, but the metal was changed to Ni (II) with concentration of 200 ppb.

2.4.4.3. Using TREN bound polymer alone:

20 mg of TREN bound polymer adsorbent was transferred to four Erlenmeyer flasks. 40 ml of 100 ppm Mb prepared in phosphate buffer (pH 7.4 with different NaCl concentration 0.01, 0.025, 0.05, 0.1 M) and filtered using a Millipore 0.45µm filter. The samples were allowed to stir for three hours at room temperature. The samples were filtered using 0.45µm Millipore filter. Then, the initial and final concentrations of Mb were determined by UV spectrophotometer at 410 nm. The adsorbent remaining in

the solution was separated by centrifuge and the solution was kept in the refrigerator. The experiment was repeated in triplicate.

2.4.5. Effect of initial Mb concentration at pH 7.4:

2.4.5.1. Using TREN bound polymer modified with Cu (II):

20 mg of TREN bound polymer adsorbent was transferred to each of five Erlenmeyer flasks. 50 ml of 400 ppb Cu (II) was added to each of the Erlenmeyer flasks. The samples were stirred for three hours at room temperature with moderate speed. The metal solutions were removed and the TREN bound polymer adsorbents were kept in a desiccator. The modified TREN bound polymer samples were treated with 40 ml of 50, 100, 200, 400,600 ppm Mb prepared in phosphate buffer (pH 7.4 0.05 M NaCl) which was filtered using a Millipore 0.45µm filter. The samples were stirred for three hours at room temperature. The samples were filtered using 0.45µm Millipore filter to remove the adsorbent. Then, the initial and final concentrations of Mb were determined by UV-Vis spectrophotometer at 410 nm. The adsorbent remaining in the solution was separated by centrifuge and the solution was kept in refrigerator.

2.4.5.2. Using TREN bound polymer modified with Ni (II):

The experiment was performed in the same manner, but the metal was changed to Ni (II) with concentration of 200 ppb.

2.4.5.3. Using TREN bound polymer alone:

20 mg of TREN bound polymer adsorbent was transferred to each of five Erlenmeyer flasks containing 40 ml of 50, 100, 200, 400, 600 ppm Mb prepared in phosphate buffer (pH 7.4, 0.1 M NaCl) which was filtered using 0.45µm Millipore filter. The samples were allowed to stir for three hours at room temperature. The samples were

filtered using 0.45µm Millipore filter to remove adsorbent . Then, the initial and final concentrations of Mb were determined by UV spectrophptometer at 410 nm. The adsorbent remaining in the solution was separated by centrifuge and the solution was kept in the refrigerator. Each experiment was run in triplicate.

2.4.6. Effect of initial concentration of Mb at pH 8.1:

2.4.6.1. Using TREN bound polymer modified with Cu (II):

20 mg of TREN bound polymer adsorbent was transferred to each of five Erlenmeyer flasks. 50 ml of 400 ppb Cu (II) was added to each of the Erlenmeyer flasks. The samples were stirred for three hours at room temperature with moderate speed. The metal solutions were removed and the TREN bound polymer adsorbents were kept in a desiccator. The experiment was run in triplicate. The modified TREN bound polymer samples were treated with 40 ml of 50, 100, 200,400,600 ppm Mb prepared in phosphate buffer (pH 8.1, 0.05 M NaCl) and filtered using a Millipore 0.45µm filter. The samples were allowed to stir for three hours at room temperature. The samples were filtered using 0.45µm Millipore filter. Then, the initial and final concentrations of Mb were determined by UV spectrophotometer at 410 nm. The adsorbent remaining in the solution was separated by centrifuge and the solution was kept in a refrigerator.

2.4.6.2. Using TREN bound polymer modified with Ni (II):

A similar procedure was followed for the effect of pH using TREN bound polymer modified with Ni (II). The concentration of Ni (II) was 200 ppb.

2.4.6.3. Using TREN bound polymer alone:

20 mg of TREN bound polymer adsorbent was transferred to each of five Erlenmeyer flasks. 40 ml of 50, 100, 200, 400, 600 ppm Mb prepared in phosphate buffer [(pH 8.1, 0.1 M NaCl) and filtered using a 0.45µm Millipore filter]. The samples were stirred for three hours at room temperature. The samples were filtered using 0.45µm Millipore filter. Then, the initial and final concentrations of Mb were determined by UV spectrophotometer at 410 nm. The adsorbent remaining in the solution was separated by centrifuge and the solution was kept in refrigerator. Each sample was run in triplicate.

2.5.Regeneration of adsorbent:

0.5 mg/ml of TREN bound polymer, TREN bound polymer modified with Cu (II) and TREn bound polymer modified with Ni (II) with 50 ppm Mb prepared in phosphate buffer (pH 7.4, 0.01M NaCl for TREN bound polymer modified with Cu (II) and TREN bound polymer modified with Ni (II) and 0.05 M NaCl for TREN bound polymer). The samples were stirred for three hours at room temperature. The adsorbent were separated by centrifuge. The initial and final concentrations were determined by UV-Vis spectrophotometer at 410 nm. The adsorbents were left in desiccators to dry. The dried adsorbents were regenerated by 0.2 M amidazole prepared in phosphate buffer (pH 8.1 , 0.05 M NaCl). The samples were stirred for three hours. The concentrations of Mb in imidazole were determined by UV-Vis spectrophotometer at 410 nm.

Chapter Three

Results and Discussion

3. Results and discussion :

3.1. Determination of Myoglobin (Mb) :

Absorbance of Mb solutions was taken for different concentrations at λ =410 nm. The values of absorbance were plotted against the concentration of Mb. The curve was linear through the selected concentrations of Mb with correlation coefficient R^2 was 1. It indicated a strong positive linear relationship between the parameters. The samples were diluted in order to have concentrations lying in the linear dynamic range. The concentrations of Mb were calculated by the linear equation of the calibration curve and the absorbance taken by UV-Vis spectrophotometer at λ =410 nm. The date of the calibration curve in mentioned in appendixes (A1). The efficiency of protein adsorbed by the adsorbent particles was calculated by the following equation:

$$\%E_{ads} = \left(\frac{(C_i - C_f}{C_i} \right) \times 100$$

Where E_{ads} is the efficiency of protein adsorbed by a unit mass of dry particles, C_i and C_f initial and final concentrations of Mb solutions (ppm) respectively. (Lee et.al.,2012)

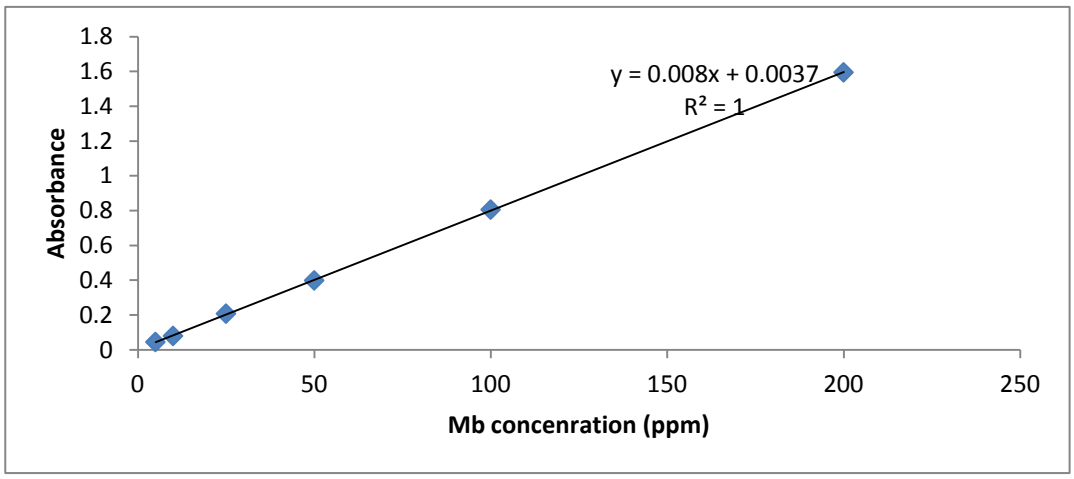


Fig 3.1: Calibration curve for determination of Myoglobin at λ=410 nm

3.2. Characterization of TREN bound polymer and its complexes:

The TREN bound polymer and its Cu (II) and Ni (II) complexes were characterized by SEM and Eds.

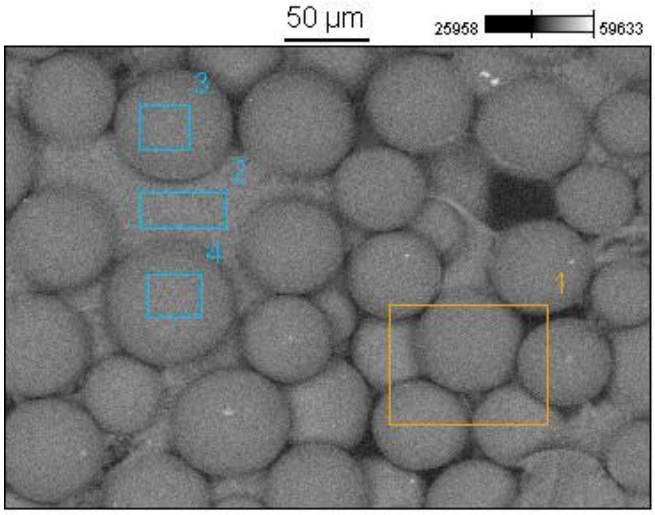
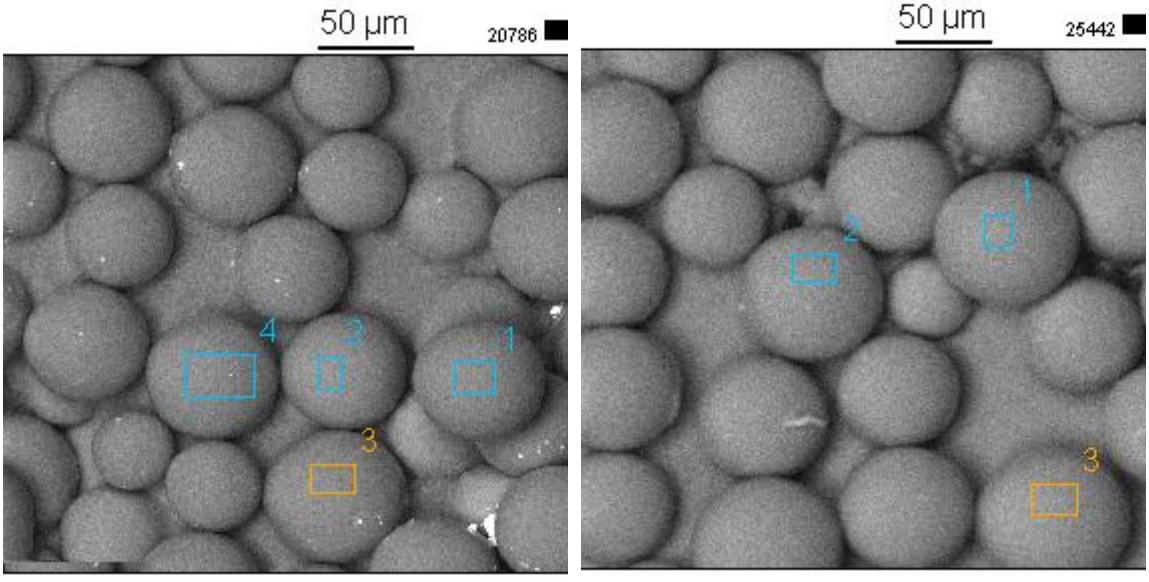


Fig 3.2: Scanning electron microscope image for Tris -(2-aminoethyl)amine bound polymer



a b

Fig 3.3: Scanning electron microscope image for TREN modified with a) Cu (II) b) Ni (II)

The scanning electron images (fig. 3.2, 3.3) showed an increase in average particle size from 58.91 µm for tris-(2aminoethyl amine bound polymer (TREN) to 59.51 µm (1.02%) and 59.86 µm (1.61%) for Cu (II) and Ni (II) complexes respectively. The increasing in average particle size indicates the complexation of Cu (II) and Ni (II) to TREN.

	C-K	N-K	O-K	Al-K	Cl-K
TREN(1)_pt1	64.90	22.88	11.77	0.32	0.13
TREN(1)_pt2	55.72	16.13	27.97	0.18	
TREN(1)_pt3	99.66			0.34	
TREN(1)_pt4	99.58			0.28	0.14

Table 3.1: Elemental analysis of Tris -(2-aminoethyl) amine bound polymer

	C-K	N-K	O-K	Al-K	Cu-K
3 CU(3)_pt1	50.13	17.72	27.11	1.18	3.85
3 CU(3)_pt2	51.99	18.06	25.46	1.13	3.36
3 CU(3)_pt3	49.67	17.75	27.84	1.22	3.53
3 CU(3)_pt4	50.31	18.63	26.77	1.24	3.05
3 CU(3)_pt5	41.47	14.44	34.88	1.43	7.78

Table:3.2:Elemental analysis of Tris- (2-aminoethyl) amine bound polymer modified with Cu^{2+}

	C-K	N-K	O-K	F-K	Al-K	Si-K	Cl-K	V-K	Ni-K
3 NI(2)_pt1	43.94	14.34	35.74	0.11	0.48		0.07		5.33
3 NI(2)_pt2	41.40	17.35	36.12		0.46			0.05	4.62
3 NI(2)_pt3	45.61	12.10	34.06	3.30	0.45	0.06			4.42
3 NI(2)_pt4	46.59	9.56	37.59	1.08	0.47				4.72

Table:3.3: Elemental analysis of Tris- (2-aminoethyl) amine bound polymer modified with Ni^{2+}

The elemental analysis (Eds) demonstrated the presence of C and N in the TREN bound polymer as major components (table 3.1).The weight % of Cu (II) in the Cu (II) bound tris amine polymer ranged between 3 % and 8 % while that bound to Ni (II) showed weight % of Ni (II) that ranged between 4 % and 5 %. This complexation is very good

taking into consideration that the loading of tris amine on the polymer backbone is relatively low (3.5-5 mmol/g) (tables 3.2,3.3).

3.3. Effect of metal ions concentration:

The effect of metal ions concentration complexed with TREN on Mb adsorption efficiency was studied. The concentration of Cu (II) or Ni (II) range was 50-600 ppb with contact time 180 min ,adsorbent dose 0.5 mg/ml, concentration of Mb was 100 ppm which was prepared in phosphate buffer with 0.02M , pH 7.4 and ionic strength (IS) = 0.01 M NaCl. As shown in fig (3.4) (A2), it is indicated that the Cu (II) concentration increased, the Mb adsorption efficiency increased. However, there was a fluctuation in Mb adsorption efficiency between 50 ppb and 200 ppb. This phenomenon was postulate to occur due to Metal Ion Trasfer (Porth, 1992). He explained that when the protein had a very strong affinity for metal ion, metal ions may leave the adsorbent according to the following equation (eq 3.1). This is most probably what is happening in the present where the Cu (II) and Ni (II) are known to be very good complexing ions for proteins residue on their surface such as Myoglobin

$$\underline{Ch}\sim M + P \longrightarrow \underline{Ch} + MP \qquad \text{eq.3.1}$$

Where: Ch : the immobilized chelator, M : metal ion, P: Protein

The concentration of Cu (II) was chosen at 400 ppb when the adsorption efficiency reached equilibrium.

The Ni (II) demonstrated the same pattern as Cu (II) with variation in values between 50-200 ppb (fig3.5) (A3). The concentration of Ni (II) that the adsorption efficiency reached equilibrium was 200 ppb. The TREN bound polymer adsorption efficiency was

tested to investigate the nonspecific interaction effect. The interaction between protein and immobilized metal affinity was divided to two types: specific and non-specific interaction. The specific interaction caused by affinity binding provided by the electron donating capacity of the exposed histidine residue on the protein surface with immobilized metal ions. The non specific interactions involved electrostatic and hydrophobic interactions between the charged proteins and the negatively charged and hydrophobic sites on the adsorbent. (Zhange et.al ,2010). The TREN bound polymer had adsorption efficiency 2.3% indicating that that non-specific interaction were also involved.

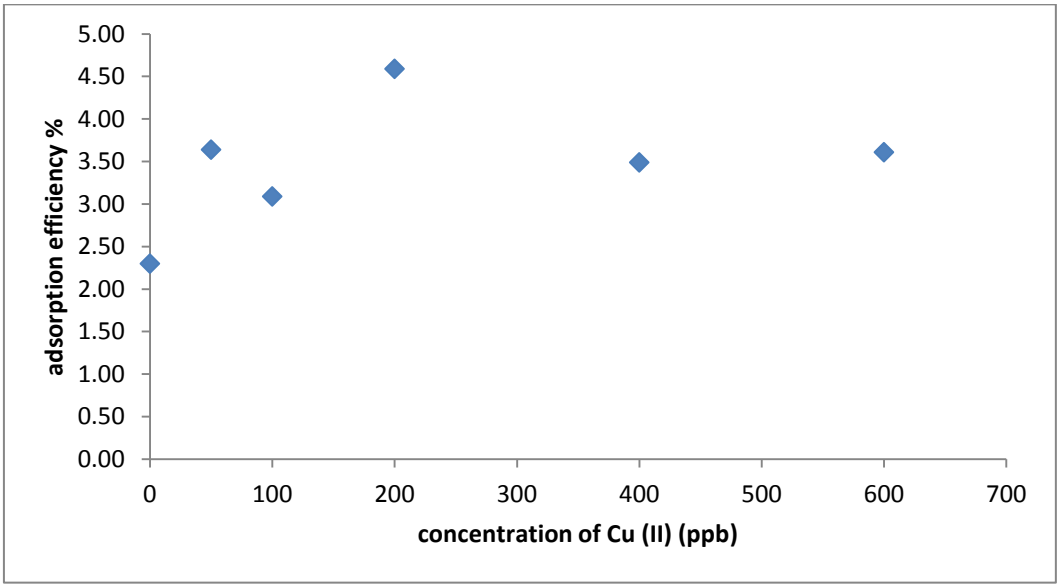


Fig 3.4: The effect of Cu (II) concentration on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Cu(II) (pH : 7.4, contact time: 3 hours , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl adsorbent dose : 0.5 mg/ml)

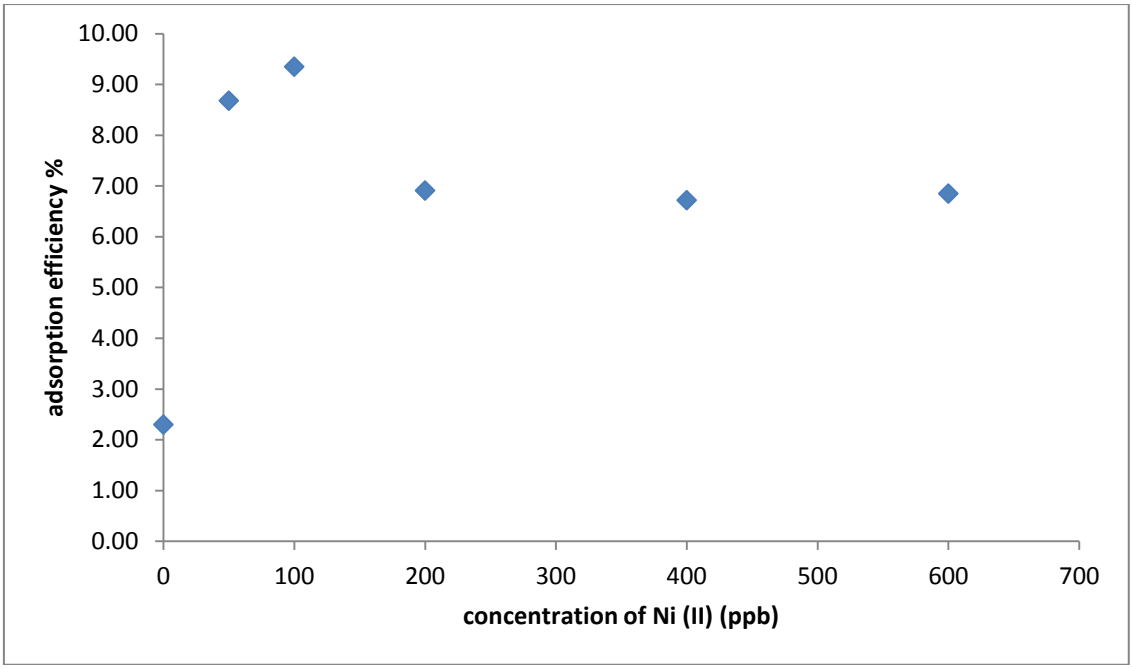


Fig 3.5: The effect of Ni (II) concentration on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Ni(II) (pH : 7.4, contact time: 3 hours , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl adsorbent dose : 0.5 mg/ml)

3.4. Effect of adsorbent dose:

The effect of adsorbent dose on the removal of Mb by the tris – (2-aminoethyl) amine modified with Cu (II) and Ni (II) was studied over the range 0.5 to 2.5 mg/ml. From (fig 3.6) (A4) , in case of The TREN modified with Cu (II), the adsorption efficiency decreased as the adsorbent dose increased. Although, the increasing in adsorbent dose gave larger surface area, the decreasing in adsorption efficiency can be due to metal ion transfer mentioned earlier .The optimum adsorbent dose was 0.5 mg/ml. Fig 3.7 , showed the effect of adsorbent dose in case of TREN modified with Ni (II). The adsorption efficiency decreases between the adsorbent doses 0.5 and 1 mg /ml. Then, the value increased til the adsorbent dose of 2 mg/ml. The optimum adsorbent dose was chosen at 0.5 mg /ml in order to minimize the amount of the adsorbent used. The data of the adsorption efficiency of TREN modified by Ni (II) and effect of adsorbent dose is the appendices (A5). The TREN bound polymer also showed same behavior like

TREN modified with Cu (II) (Fig 3.8) (A6). The optimum adsorbent dose was chosen to be at 0.5 mg /ml.

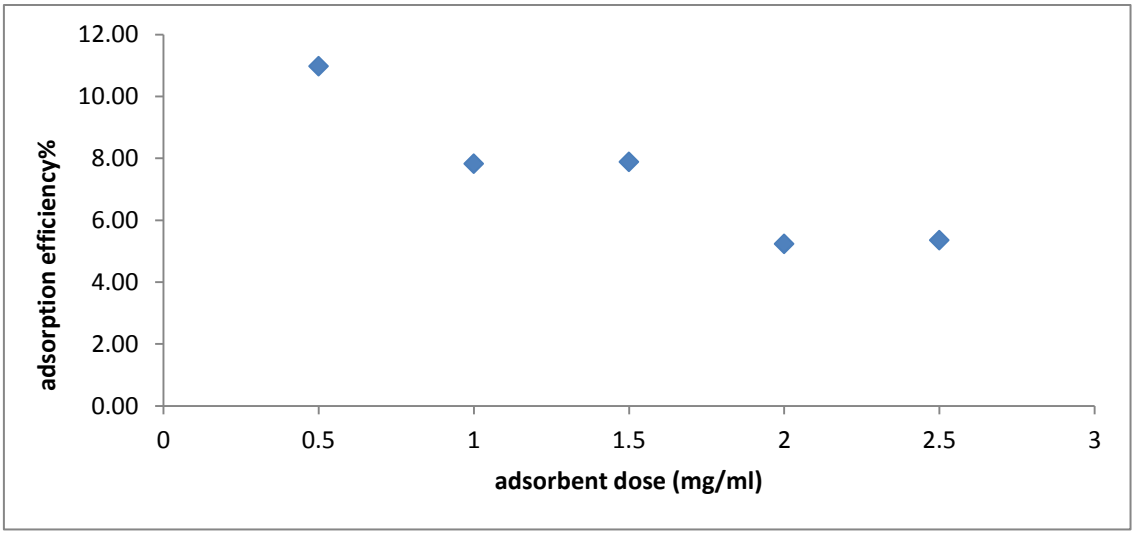


Fig 3.6: The effect of adsorbent dose on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Cu(II) (pH : 7.4, contact time: 180 min , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl, Cu (II) concentration : 400 ppb)

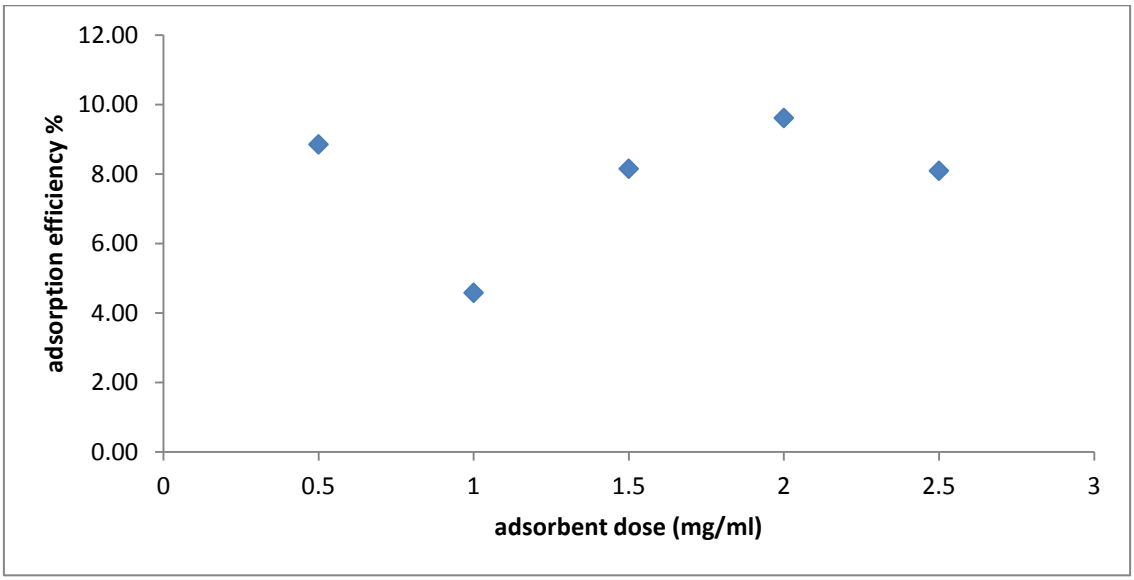


Fig 3.7: The effect of adsorbent dose on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Ni (II) (pH : 7.4, contact time: 180 min , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl, Ni (II) concentration :200 ppb)

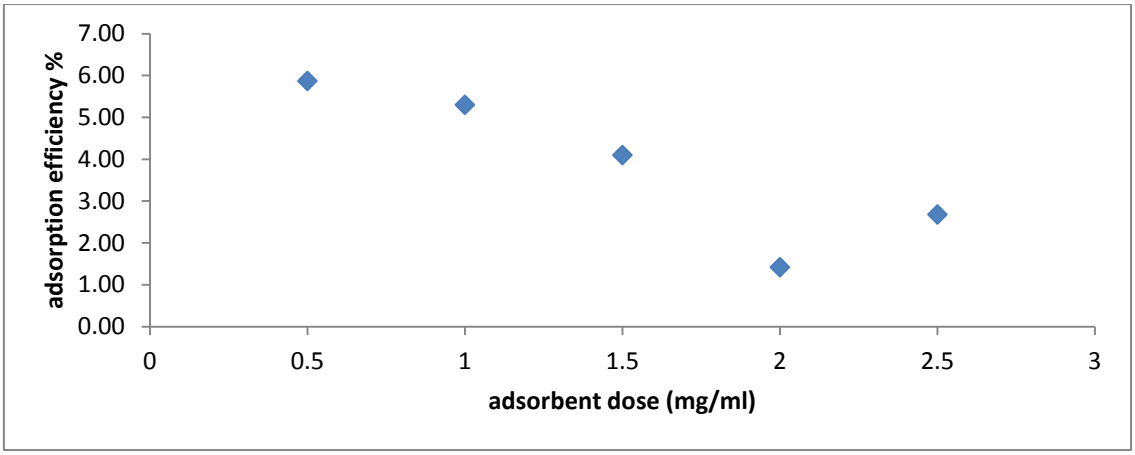


Fig 3.8: The effect of adsorbent dose on Mb removal by Tris- (2-aminoethyl) amine polystyrene (pH : 7.4, contact time: 180 min , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl)

3.5. Effect of contact time:

The effect of time contact was studied at different time interval ranged from 30 min to 250 min at pH : 7.4, adsorbent dose: 0.5 mg/ml , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl, Cu (II) concentration : 400. (Fig 3.9) showed adsorption efficiency of Mb by TREN modified with Cu (II). It was clear that the adsorbent reached the optimum adsorption by 30 min, but there was a decrease in adsorption at 150 min. The equilibrium time was chosen to be 180 min. The effect of contact time was studied for TREN modified with Ni (II). The data of fig 3.9 is mentioned in A7. As in (fig 3.10) (A8) , the adsorption efficiency increased as the time contact increased and reached equilibrium at 180 min. The optimum time contact was chosen at 180 min. The effect of contact time on adsorption efficiency of Mb by TREN bound polymer was also studied. The adsorption reached highest adsorption efficiency value at 180 min (fig 3.11) (A9).

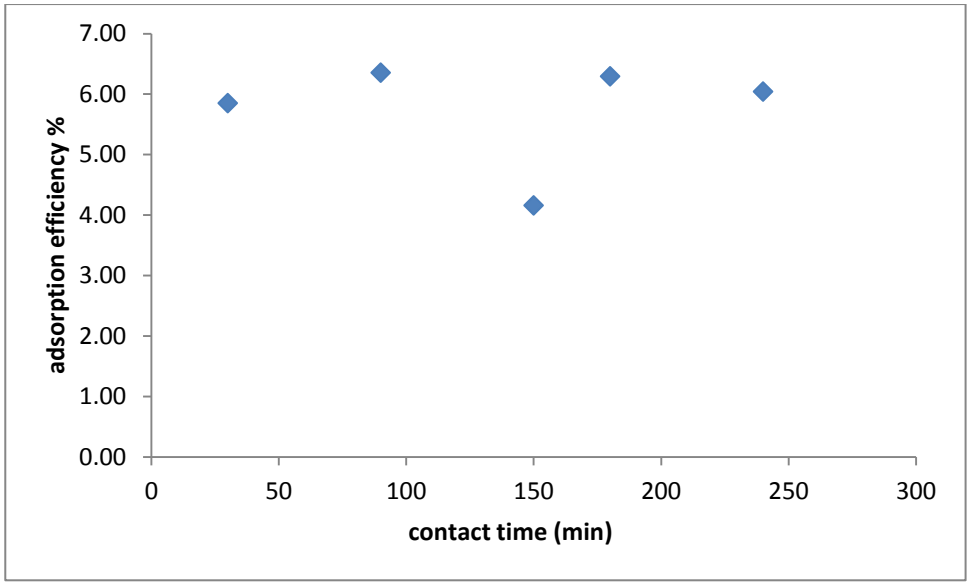


Fig 3.9 : The effect of contact time on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Cu(II) (pH : 7.4, adsorbent dose: 0.5 mg/ml , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl, Cu (II) concentration : 400 ppb)

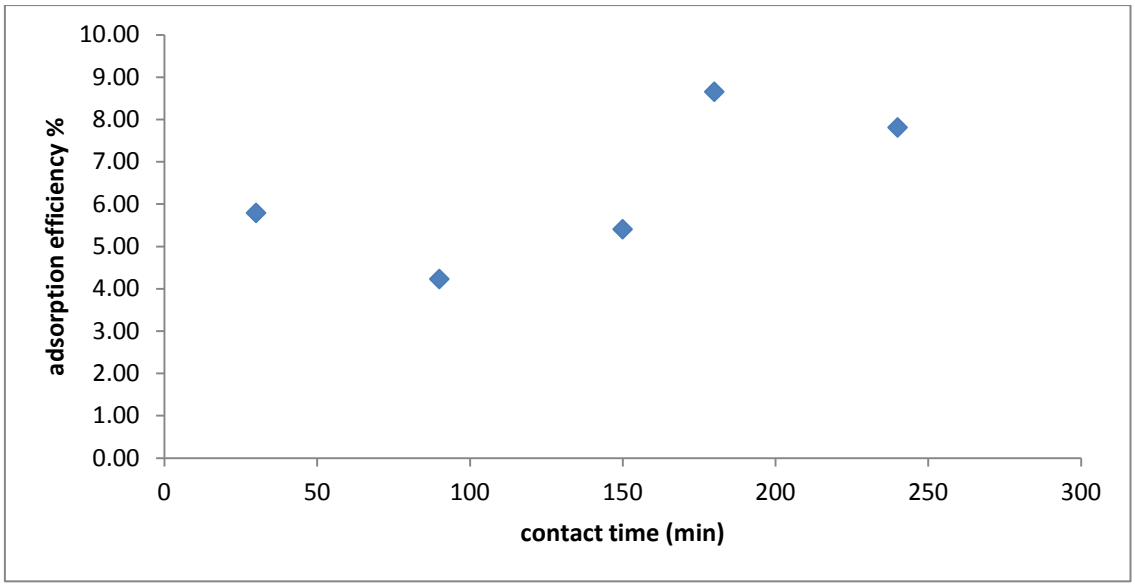


Fig 3.10: The effect of contact time on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Ni (II) (pH : 7.4, adsorbent dose : 0.5 mg/ml , Mb concentration : 100 ppm , ionic strength :0.01 M NaCl, Ni (II) concentration :200 ppb)

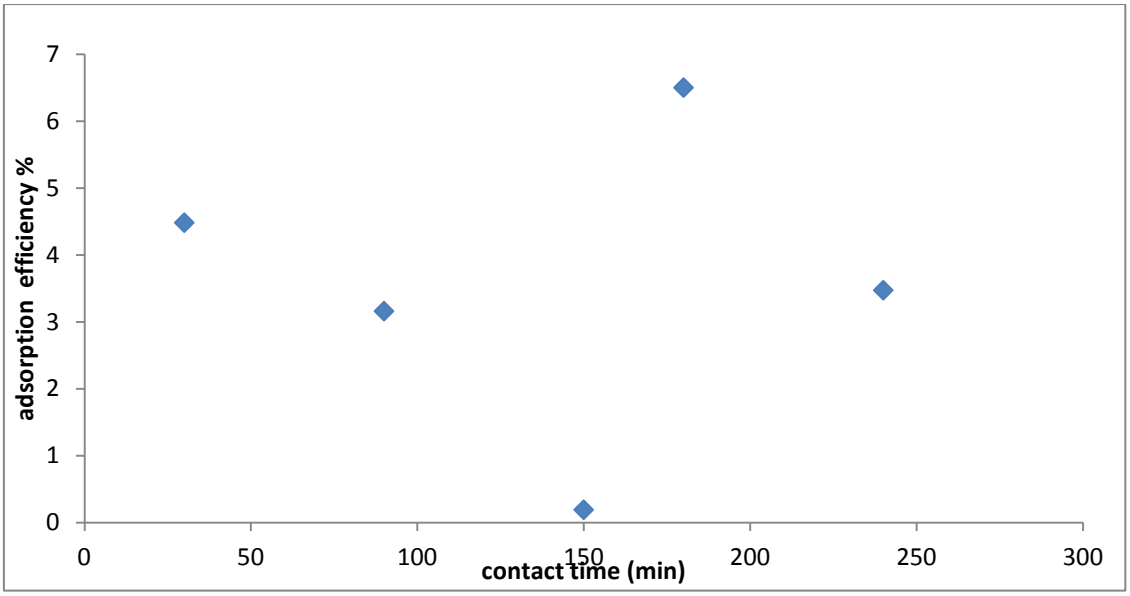


Fig 3.11: The effect of contact time on Mb removal by Tris (2-aminoethyl) amine polystyrene (pH : 7.4, adsorbent dose : 0.5mg /ml , Mb concentration : 100 ppm ,salt concentration :0.01 M NaCl)

3.6. Effect of ionic strength (salt concentration):

The effect of salt concentration on the adsorption of Mb by TREN modified with Cu (II) microparticles was also studied. As demonstrated in (fig 3.12) the salt concentration increased from 0.01 M to 0.05 M, the adsorption efficiency of the TREN polymer modified with Cu (II) to Mb increased from 6.00 to19.00. Then it decreased at higher concentration. A similar result was reported by Cete et.al. in investigating the effect of salt on removal of Mb by PGMA modified with IDA. The data is provided in (A10) The effect of salt concentration on Mb removal by TREN bound polymer modified with Ni (II) was also studied. The adsorption of Mb increased as the salt concentration increased until a concentration of 0.05 M is attained. At higher concentrations, the adsorption decreased (fig 3.13). The data is given in (A 11). However, the effect of salt when the TREN bound polymer was used without modification was different. The adsorption of Mb deceased from 0.01 M NaCl to 0.05 M but increased at 0.1 M NaCl (fig 3.14) The data of the adsorption efficiency is recorded in A12 . These results

indicated that at 0.05 M NaCl the interactions between Mb at its maximum value. The effect of salt concentration on adsorption of proteins has been widely studied over the past thirty years. Different theories have been proposed to explain the interaction of proteins under various salt concentrations (Cete et.al. 2009). It was postulated that salt molecules increase the surface tension of the solution, reduce the electrostatic attraction between protein molecules and change their surface activity, resulting in variable adsorption behavior of proteins on surface.

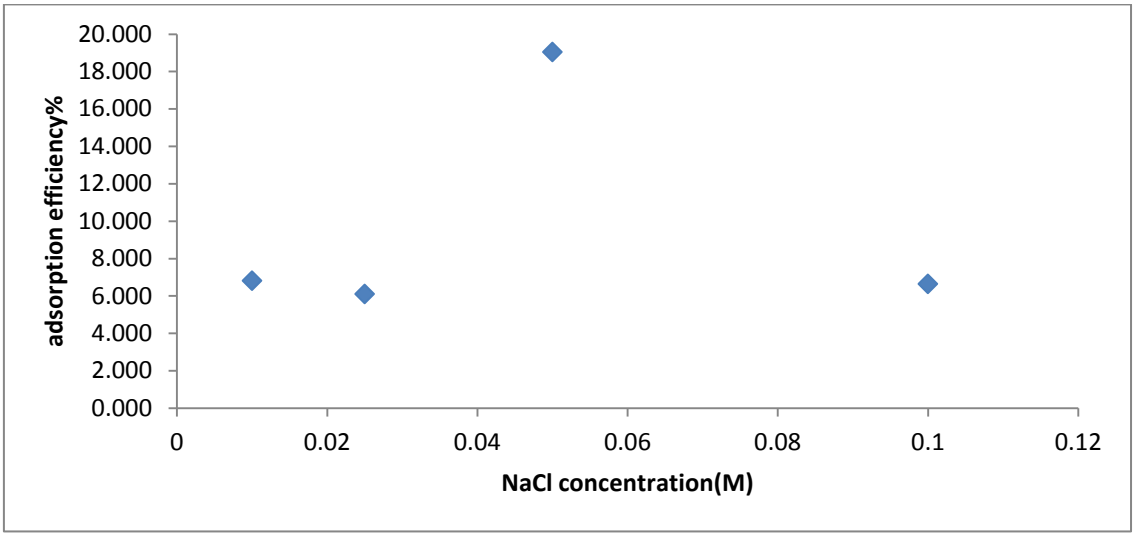


Fig 3.12 : The effect of ionic strength (salt concentration) on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Cu(II) (pH : 7.4, adsorbent dose: 0.5 mg/ml , Mb concentration : 100 ppm , contact time :180 min , Cu (II) concentration : 400 ppb)

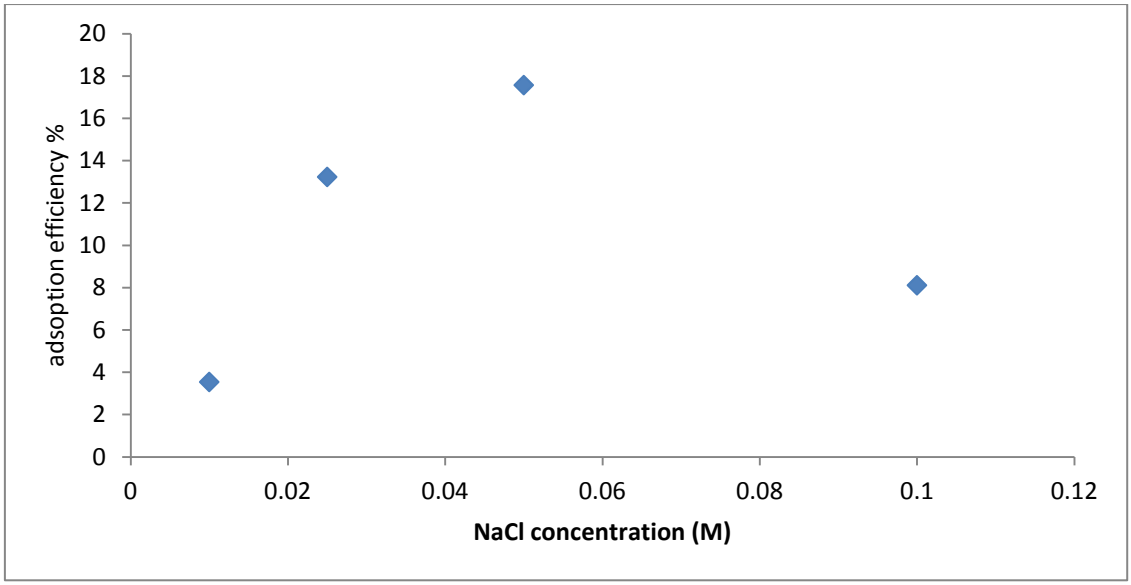


Fig 3.13: The effect of ionic strength (salt concentration) on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Ni (II) (pH : 7.4, adsorbent dose : 0.5 mg/ml , Mb concentration : 100 ppm , contact time: 180 min , Ni (II) concentration :200 ppb)

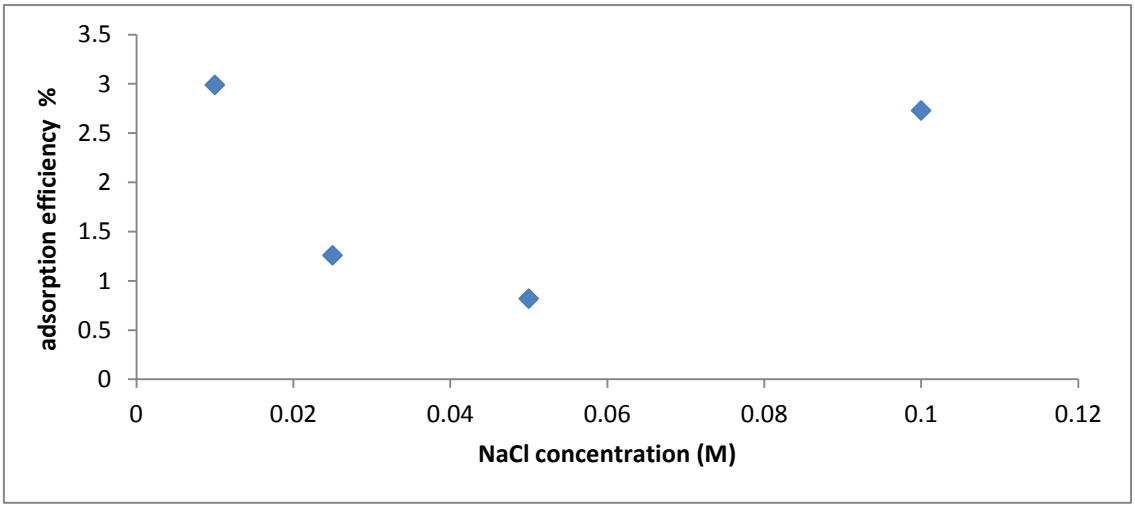


Fig 3.14: The effect of ionic strength (salt concentration) on Mb removal by Tris (2-aminoethyl) amine polystyrene (pH : 7.4, adsorbent dose : 0.5mg /ml , Mb concentration : 100 ppm , contact time:180 min)

3.7. Effect of Mb concentration at pH 7.4:

The effect of initial Mb concentration was studied at different pHs (7.4 and 8.1). The values of pH were chosen according to the type of histidine that needed to be dominant. From fig 3.15, At pH < 1.82, A is the dominant form. In the range 1.82 < pH < 6.02 B is the dominant form. In the range 6.02 < pH < 9.17 C is the dominant form, and when pH > 9.17, D is the major form in solution. As the availability of the two electrons of nitrogen of the imidazole ring is essential for coordination with transition be free to coordinate with transition metals, the pH chosen was in accordance with the dominant form of histidine. In this case form C.

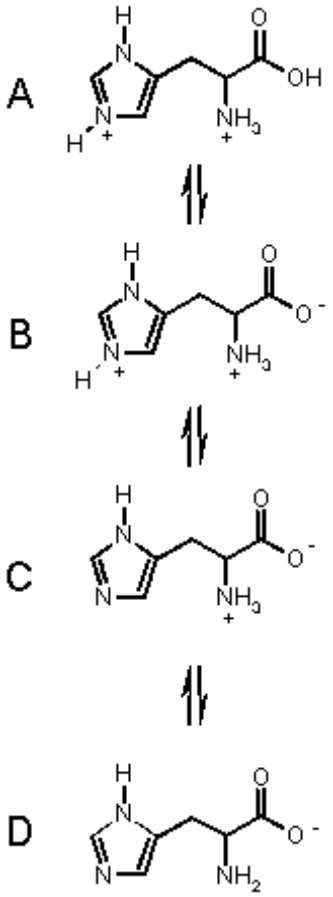


Fig 3.15 : forms of histidine (Department of Chemistry/ University of Calgary)

At pH 7.4, the adsorption efficiency decreased as the initial concentration of Mb increased for TREN modified with Cu (II) (fig 3.16) (A13) and Ni (II) (fig 3.17) (A14). For the TREN bound polymer, the performance for initial concentration of Mb with adsorption efficiency was random and appeared to be related to non specific interaction (fig 3.18). The table of the data is mentioned in (A15).

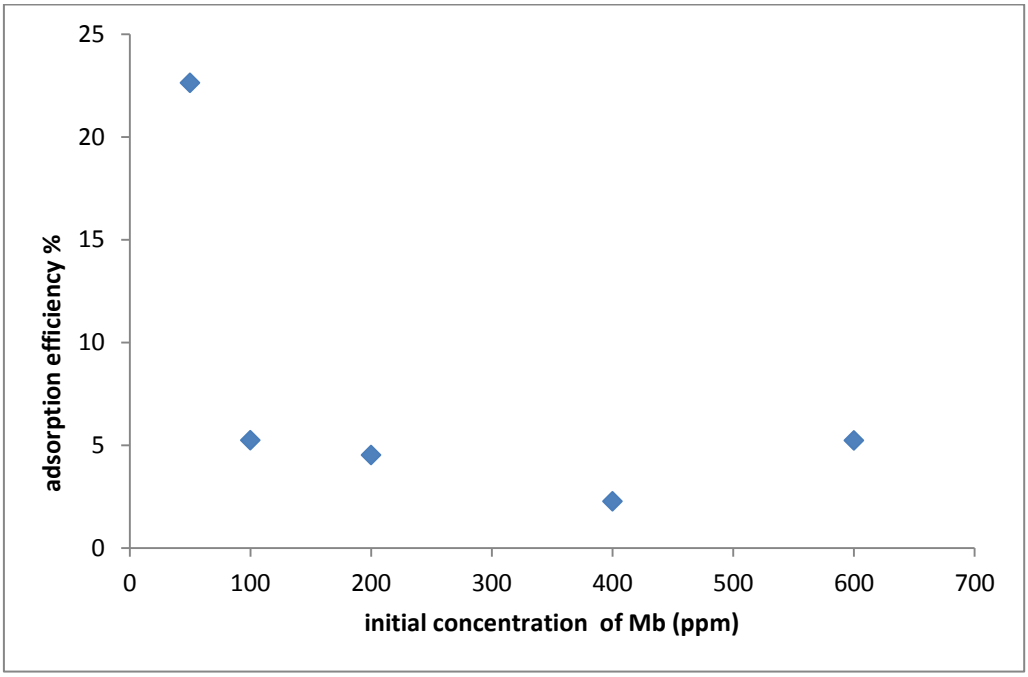


Fig 3.16 : The effect of initial concentration of Mb on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Cu(II) (pH : 7.4, adsorbent dose: 0.5 mg/ml , ionic strength 0.05 M NaCl contact time:180 min , Cu (II) concentration : 400 ppb)

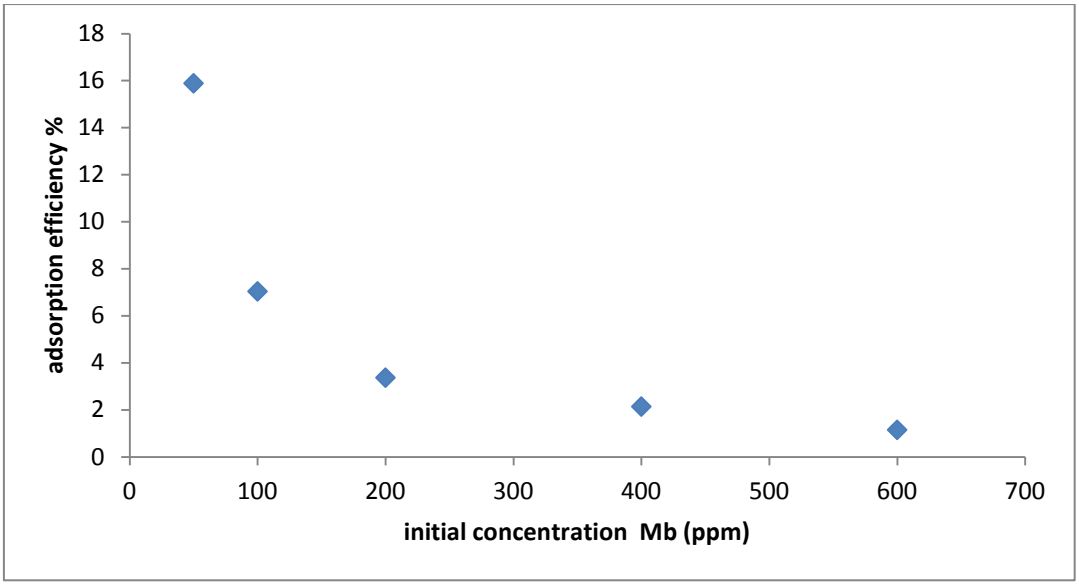


Fig 3.17: The effect of initial concentration of Mb on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Ni (II) (pH : 7.4, adsorbent dose : 0.5 mg/ml ,ionic strength : 0.05 M NaCl , contact time: 180 min , Ni (II) concentration :200 ppb)

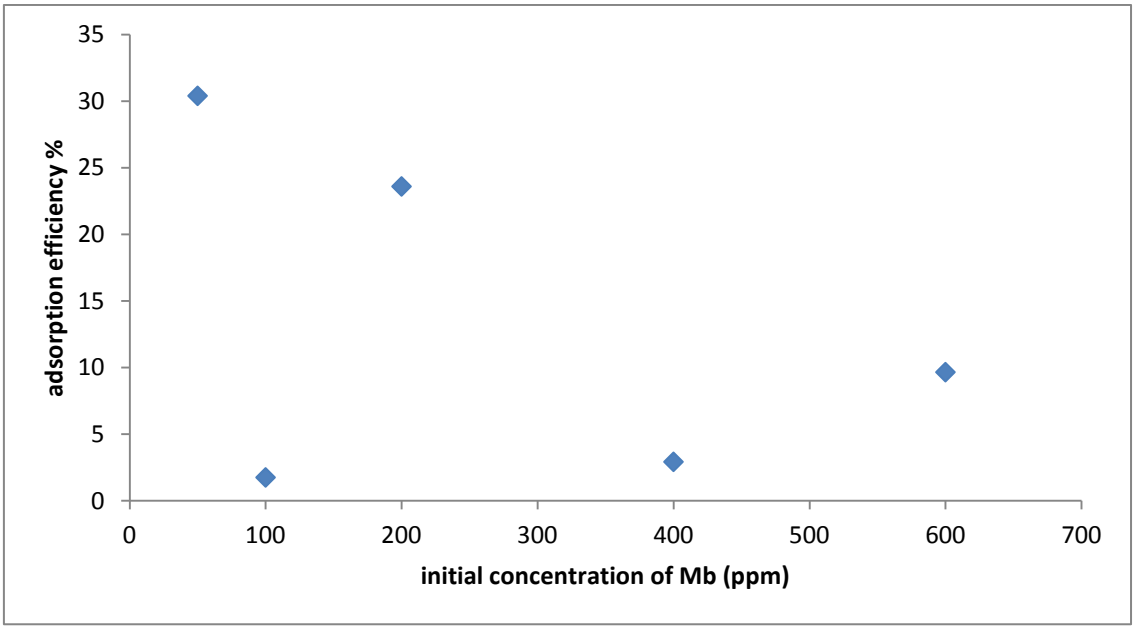


Fig 3.18: The effect of initial concentration of Mb on Mb removal by Tris (2-aminoethyl) amine polystyrene (pH : 7.4, adsorbent dose : 0.5mg /ml , Mb concentration : 100 ppm , contact time :180 min, ionic strength : 0.1 M NaCl)

3.8. Effect of Mb concentration at pH 8.1:

Again, the effect of initial concentration of Mb on the adsorption efficiency of Mb by TREN modified with Cu (II) at pH 8.1 was studied. The results indicated random behavior with an overall increased in adsorption efficiency (fig 3.19). The data are in the appendices (A15). The effect of initial concentration of Mb on adsorption efficiency in case of TREN modified with Ni (II) was also studied. The adsorption efficiency increased as the initial concentration of Mb increased (fig 3.20) (A16) . For TREN bound polymer without metal, the adsorption efficiency was random (fig 3.21) (A17). It was apparent that the adsorption of Mb at pH =8.1 was much better than that at pH =7.4 both for Cu and Ni immobilized trisamine polymer. This behavior might be attributed to the reduction of the coordination sites occupied by the immobilized tris amine at pH =8.1 and thus a better accessibility of Mb to the transition metals, Cu and Ni. It was of utmost importance here to note that the behavior of the immobilized Cu (II) and Ni (II) ions on to the tris amine polymer was different form their solution chemistry in effect, due to the steric hindrance by the polymer chain, the complexes formed will be distorted and this will certainly affect the adsorption efficiency of the metal modified polymer.

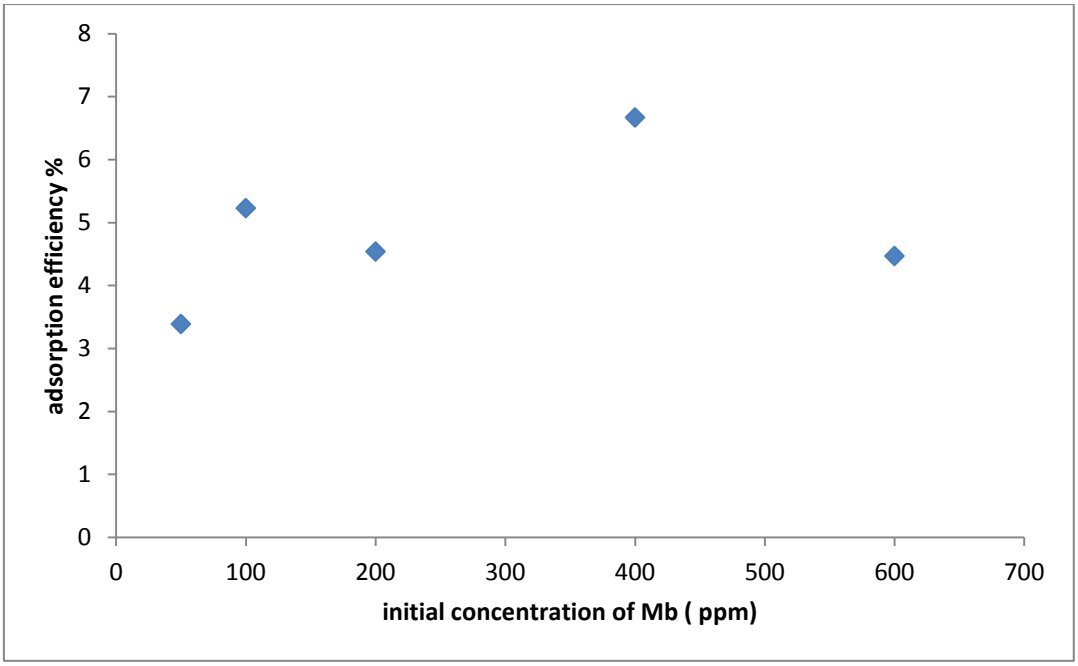


Fig 3.19 : The effect of initial concentration of Mb on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Cu(II) (pH : 8.1, adsorbent dose: 0.5 mg/ml , ionic strength 0.05 M NaCl , contact time:180 min , Cu (II) concentration : 400 ppb)

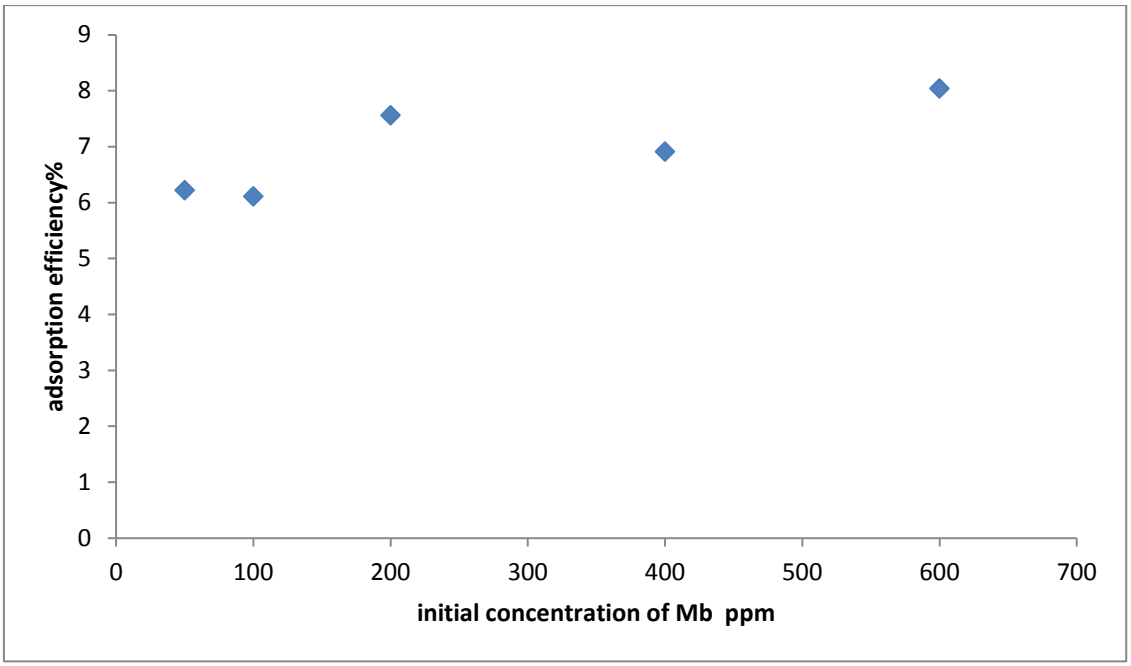


Fig 3.20: The effect of initial concentration of Mb on Mb removal by Tris (2-aminoethyl) amine polystyrene modified with Ni (II) (pH :8.1, adsorbent dose : 0.5 mg/ml ,ionic strength : 0.05 M NaCl , contact time: 180 min , Ni (II) concentration :200 ppb)

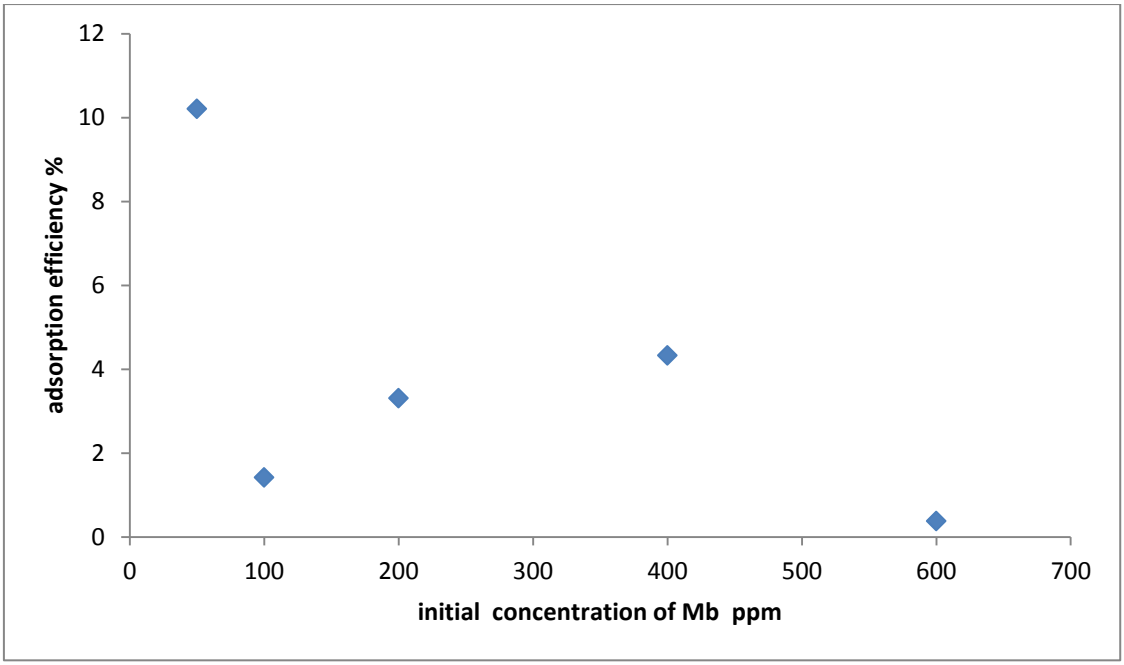


Fig 3.21: The effect of initial concentration of Mb on Mb removal by Tris (2-aminoethyl) amine polystyrene (pH : 8.1, adsorbent dose : 0.5mg /ml , Mb concentration : 100 ppm , contact time :180 min, ionic strength : 0.1 M NaCl)

3.9. Adsorption isotherm:

Adsorption isotherms were used to model the adsorption behavior. Two adsorption isotherm were used : Langmuir isotherm and Freundlich isotherm.

3.9.1. Langmuir isotherm model:

Langmiur equation had several forms and can be written as :

$$\frac{C_e}{q_e} = \frac{1}{Q_{max}K} + \frac{C_e}{Q_{max}}$$

Where :

q_e: is the amount of solute adsorbed per unit weight of adsorbent (mg/g)

C_e : the equilibrium concentration of solute in the bulk solution (mg /L)

Q_{max} : is the maximum adsorption capacity (mg/g)

K is constant related to the free adsorption energy (L/g). (Odeh, 2012)

3.9.1.1. TREN modified with Cu (II):

The plot of C_e /q_e versus C_e gave very low correlation coefficient (R^2 = 0.2724) at pH 7.4 fig (3.22) . At pH 8.1 the correlation coefficient R^2 =0.1622 with negative slope. This indicated that the adsorption of Mb by TREN modified with Cu (II) does not fit langimur model, as the latter was not able to account for the values at low and high concentration ranges. This behavior was previously observed and related to the high affinity of proteins for the immobilized Cu (II) ions resulting in multiple non identical interaction (Sharma et.al., 2001).

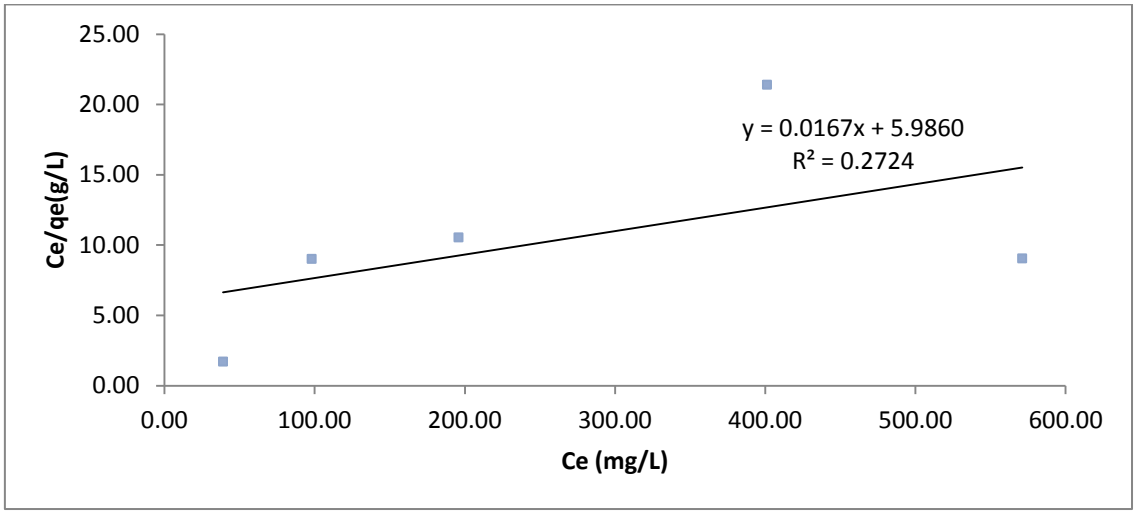


Fig 3.22: Langmuir isotherm for the adsorption of Mb by TREN bound polymer modified with Cu (II) (pH: 7.4 , ionic strength (salt concentration) : 0.05 M NaCl, adsorbent dose:0.5 mg/ml, contact time : 180 min)

3.9.1.2. TREN modified with Ni (II):

The plot of Ce/q_e versus C_e as shown in fig (3.23) showed high correlation coefficient R^2 =0.978. It means that the Mb adsorption by TREN modified with Ni (II) fitted the Langmuir model very well. The values of Q_{max} and K were determined from the slope and y intercept respectively. Q_{max} was 14.79 mg/g and K = 0.202 at pH 7.4 with adsorbent dose 0.5 mg /ml ionic strength 0.05 M NaCl and contact time 180 min Ni (II) concentration 200 ppb.

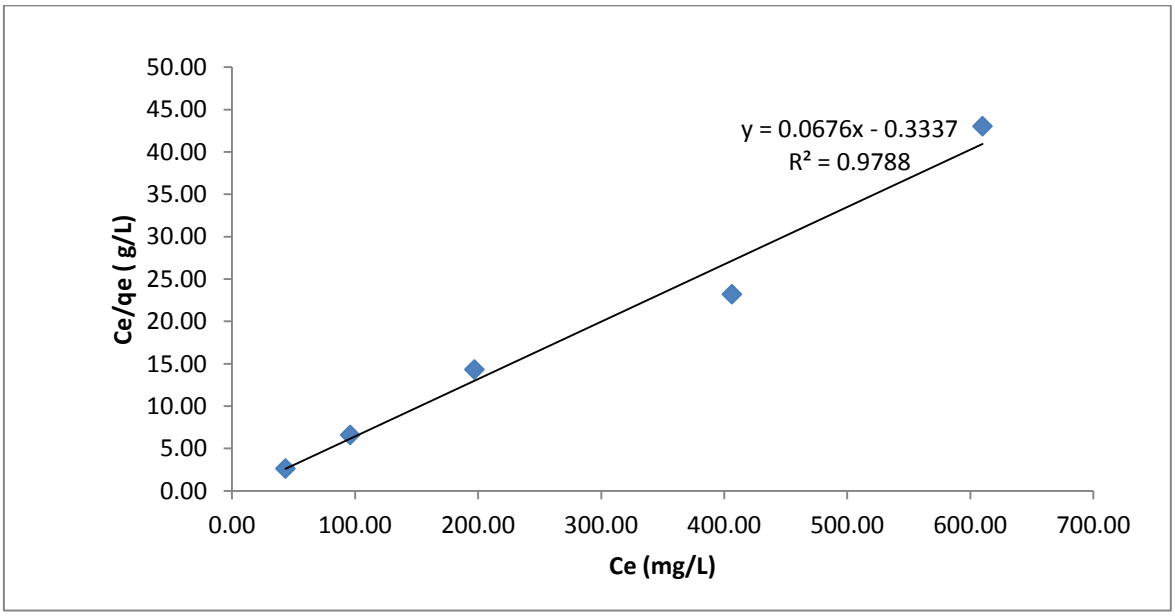


Fig 3.23: Langmuir isotherm for the adsorption of Mb by TREN bound polymer modified with Ni (II) (pH: 7.4 , ionic strength (salt concentration) : 0.05 M NaCl, adsorbent dose:0.5 mg/ml, contact time : 180 min)

3.9.1.3. TREN bound polymer alone:

The TREN bound polymer showed poor fit with Langmuir model at pH 7.4 with R^2 =0.0041. At pH 8.1, the fitting of TREN bound polymer with Langmuir model also was not good with R^2 = 0.61 (fig 3.24). The previous results demonstrated that the adsorption of Mb on TREN bound polymer modified with Cu (II) ions deviated from Langmuir mode. Apparently, the nature of the metal ion, the chelating ligand density and the solution environment were playing important roles in determining the adsorption efficiency.

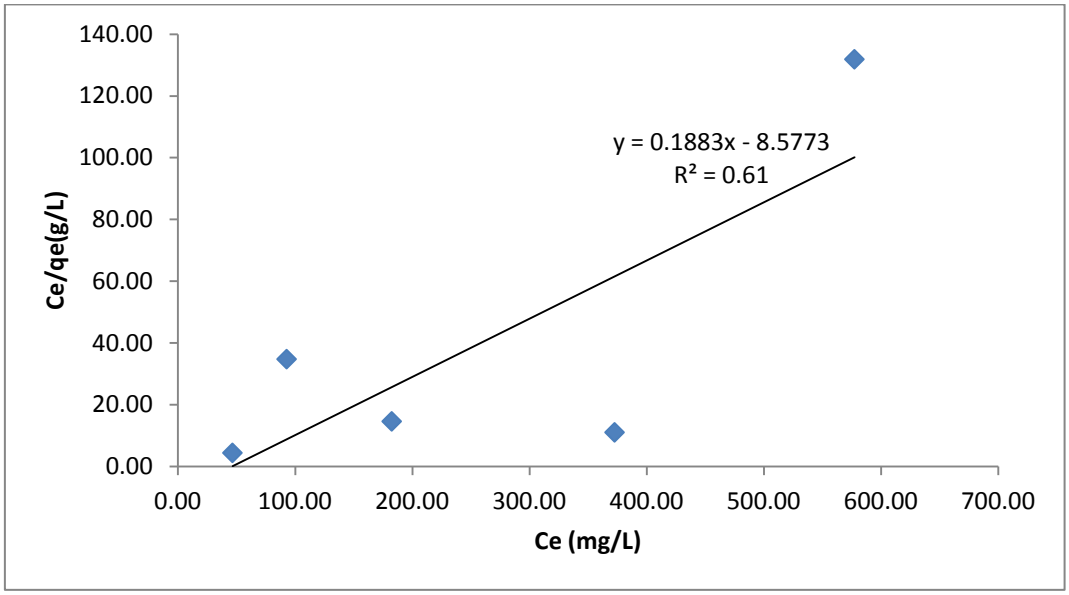


Fig 3.24: Langmuir isotherm for the adsorption of Mb by TREN bound polymer (pH: 8.1 , ionic strength (salt concentration) : 0.1 M NaCl, adsorbent dose:0.5 mg/ml, contact time : 180 min)

3.9.2. Freundlich isotherm:

The Freundlich isotherm is expressed by the following equation:

$$\log q_e = \log K_f + \frac{1}{n}\log C_e \qquad \text{(Odeh, 2012)}$$

Where :

q_e: is the amount of solute adsorbed per unit weight of adsorbent (mg/g)

C_e : the equilibrium concentration of solute in the bulk solution (mg /l)

K_f: ia aconstant indicated the relative adsorption capacity of the adsorbent(mg/L)

n: the intensity of the adsorption

3.9.2.1. TREN modified with Cu (II):

The TREN modified with Cu (II) fitted the Freundlich model at pH 8.1 with correlation coefficient R^2 0.9657 .The n = 0.87and K_f= 21.5 mg/g were calculated from slope and intercept of linear equation respectively fig (3.25).

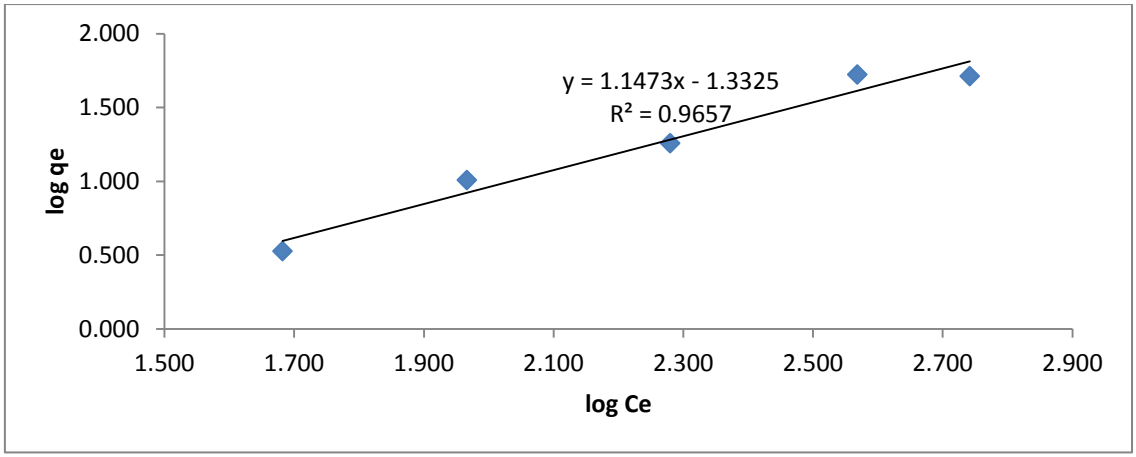


Fig 3.25: Freundlich isotherm for the adsorption of Mb by TREN bound polymer modified with Cu (II) (pH 8.1 , ionic strength (salt concentration) : 0.05 M NaCl, adsorbent dose:0.5 mg/ml, contact time : 180 min)

3.9.2.2. TREN modified with Ni (II):

The TREN modified with Ni (II) fitted the Freundlich isotherm with correlation coefficient $R^2 = 0.794$. The values of n=0.908 and K_f=14.618 mg/g.(fig 3.26).

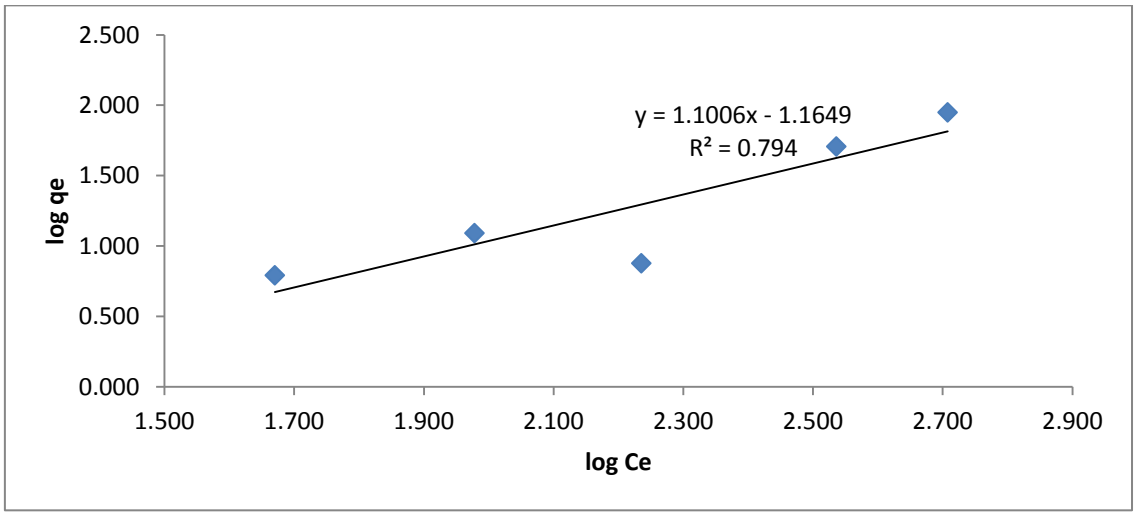


Fig 3.26: Freundlich isotherm for the adsorption of Mb by TREN bound polymer modified with Ni (II) (pH 8.1 , ionic strength (salt concentration): 0.05 M NaCl, adsorbent dose:0.5 mg/ml, contact time : 180 min.)

3.9.2.3. TREN bound polymer alone:

The TREN bound polymer didn't fit the Freundlich at both pH 7.4 R^2 = 0.1543 and pH 8.1 R^2 = 0.0314. In this case, at higher pH (pH =8.1), we can speculate that in addition to the larger contribution of form C of the histidine surface residue on the surface of the protein (fig 3.15). The latter is being more exposed to the TREN bound polymer modified with Ni (II) ion. This behavior resulted in larger immobilized metal ion protein interaction.

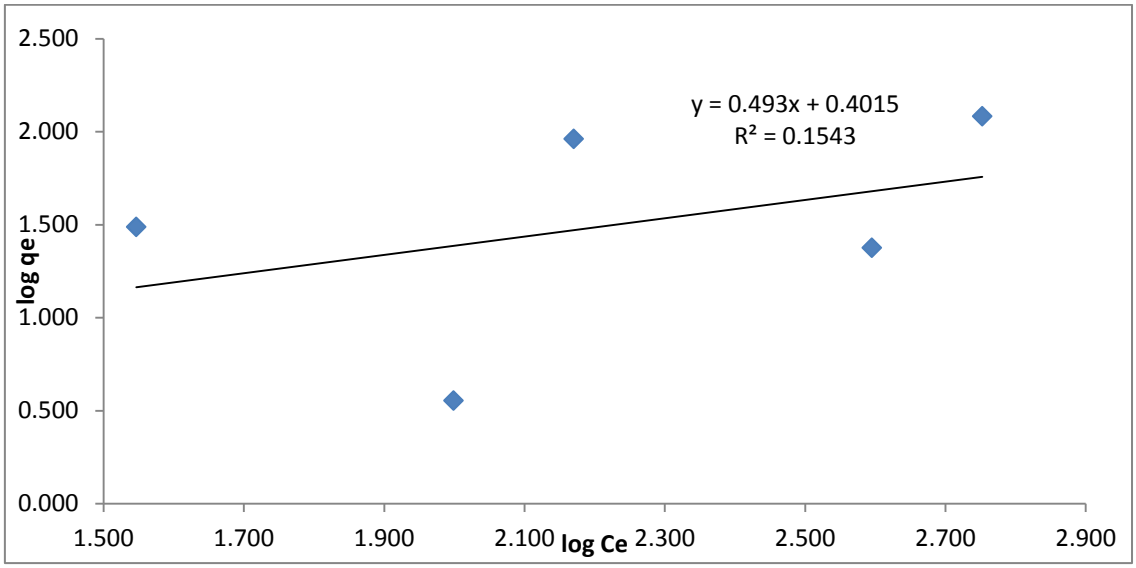


Fig 3.27: Freundlich isotherm for the adsorption of Mb by TREN bound polymer (pH: 7.4 , ionic strength (salt concentration) : 0.1 M NaCl, adsorbent dose:0.5 mg/ml, contact time : 180 min)

TREN bound polymer modified with Ni (II) fitted Langmuir model with maximum adsorption capacity of 14.79 mg Myoglobin/ g polymer while the maximum adsorption capacity of poly(glycidyl methacylate) microbeads funtionalized with iminodicetic acid which fitted Langmuir model with maximum adsorption of 1845 µg/g (Cete et.al., 2009). Furthermore, TREN bound polymer modified with Cu (II) fitted Freundlich model with capacity of 21.55mg /g while poly(glycidyl methacylate) microbeads

funtionalized with iminodicetic acid fitted Freundlich model with capacity of 4536 µg/g. It is worth to note here that the concentration of Mb was (50-600 ppm) but the cited study use concentrations (10-50 ppm).

3.10. Regeneration of adsorbents:

The desorption % of adsorbents was performed using 0.2M imidazole (Zhang et.al. 2010) due to the affinity of transition metals such as Cu (II) and Ni (II) to complex with imidazole. The desorption % was calculated using the following equation:

$$desorption\ \% = \frac{C_D}{C_i - C_f} \times 100$$

Where

C_D : the concentration of Mb in desorped sample (mg/l)

C_i : the initial concentration of Mb(mg/l)

C_f : the final concentration of Mb (mg/l)

The desorption % for TREN modified with Cu (II), TREN modified with Ni (II) and TREN bound polymer were 35.38 %, 32.39 % and 25.42% respectively. The assumed mechanism for the competing ligate (imidazole) to remove the protein molecules which is the protein replaced by the ligate

$$\underline{Ch}\text{-M}\cdots\text{P+L} \rightarrow \underline{Ch}\text{ML+P} \qquad \text{(Porath , 1992)}$$

Where: Ch: the immobilized chelator, M: metal ion, P: protein , L: competing ligate

Chapter Four

Conclusion

4. Conclusion:

This study constitutes an initial attempt to understand the nature of the interactions of immobilized heavy metal ions of TREN bound polymer with proteins namely Mb. It is well known that the interactions between immobilized metal ions and proteins are complex in nature and result from a combination of electrostatic, hydrophobic, and donor/acceptor effects. In this work, Myoglobin was chosen for two main reasons: Its role as a biomarker for Myocardial infarction and the availability of histidine residues on the surface of the protein. The latter fact led us to believe that the main type of interaction in our study will be due to the electron pair donor behavior of the imidazole ring of the histidine residue. Our results showed a reasonable affinity of Mb to the Ni (II) and Cu (II) immobilized on TREN bound polymer. The maximum adsorption efficiency of Mb was at Cu (II) concentration of 400 ppb for TREN modified with Cu (II) and 200 ppb for Ni (II) for TREN modified with Ni (II). Furthermore, the maximum adsorption efficiency at adsorbent dose of 0.5 mg /ml, contact time 180 min and ionic strength of 0.05 M NaCl for TREN bound polymer modified with Cu (II) and Ni (II) and 0.1M NaCl TREN bound polymer. The polymer modified with Cu (II) fitted Freundlich model and showed acceptable capacity (21.55 mg/g) while TREN bound polymer modified with Ni (II) fitted Langmuir model with maximum capacity 14.79 mg /g. I think that further work is required in order to study the interaction of Cu (II) and Ni (II) immobilized on TREN polymer with other proteins having surface bound histidine such as BSA. Furthermore, It is important to explore higher concentration of protein (Mb) in order to reach the level of Mb in urine of patients with damaged muscles due to myocardial infarction (750 µg/ml).

Chapter Five

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5. References:

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Chapter Six

Appendices

6. Appendices:

Concentration of Mb (ppm)	Absorbance
5	0.044
10	0.079
25	0.207
50	0.398
100	0.805
200	1.594

A1: calibration curve of Mb using UV –Vis spectrophotometer at λ=410 nm

- **Effect of Metal concentration :**

Cu (II) concentration (ppb)	Adsorption efficiency % (average ± SD)
0	2.30 ± 0.12
50	3.64 ± 0.9
100	3.09 ± 0.55
200	4.59 ± 2.02
400	3.49 ± 0.55
600	2.30 ± 0.92

A2: effect of Cu (II) concentration on Mb removal by TREN polymer bound (pH 7.4,adsorbent dose : 0.5 mg /ml, Ionic strength : 0.01 M NaCl, contact time : 180 min,Mb concentration : 100 ppm)

Ni (II) concentration (ppb)	Adsorption efficiency % (average ± SD)
0	2.30 ± 0.12
50	8.68 ± 0.96
100	9.35 ± 3.8
200	6.91 ± 2.3
400	6.72 ± 1
600	6.85 ± 0.75

A3: effect of Ni (II) concentration on Mb removal by TREN polymer bound (pH 7.4,adsorbent dose : 0.5 mg /ml, Ionic strength : 0.01 M NaCl, contact time : 180 min,Mb concentration : 100 ppm)

- **Effect of adsorbent dose:**

Adsorbent dose (mg/ml)	Adsorption efficiency % (average ± SD)
0.5	10.98 ± 1.7
1	7.83 ± 2.1
1.5	7.89 ± 1.8
2	5.24 ± 0.06
2.5	5.36 ± 1.45

A4: effect of adsorbent dose on Mb removal by TREN polymer bound odified with Cu (II) (pH 7.4, contact time :180 mins , Ionic strength : 0.01 M NaCl ,Mb concentration : 100 ppm, Cu (II) concentration : 400 ppb)

Adsorbent dose (mg/ml)	Adsorption efficiency % (average ± SD)
0.5	8.85 ± 2.3
1	4.58 ± 0.38
1.5	8.15 ± 0.5
2	9.61 ±0.06
2.5	8.09 ± 0.57

A5: effect of adsorbent dose on Mb removal by TREN polymer bound modified with Ni(II) (pH 7.4, contact time :180mins, Ionic strength : 0.01 M NaCl ,Mb concentration : 100 ppm, Ni (II) concentration : 200 ppb)

Adsorbent dose (mg/ml)	Adsorption efficiency % (average ± SD)
0.5	5.87 ± 0.85
1	5.30 ± 0.28
1.5	4.1 ± 0.34
2	1.42 ± 0.06
2.5	2.68 ± 0.40

A6: effect of adsorbent dose on Mb removal by TREN polymer bound (pH 7.4, contact time :180mins, Ionic strength : 0.01 M NaCl ,Mb concentration : 100 ppm)

- **Effect of contact time :**

contact time (min)	Adsorption efficiency % (average ± SD)
30	5.85 ± 0.06
90	6.36 ± 1.8
150	4.15 ± 2.14
180	6.29 ± 0.5
240	6.04 ± 0.001

A7: effect of contact time on Mb removal by TREN polymer modified with Cu (II) (pH 7.4,adsorbent dose : 0.5 mg /ml, Ionic strength : 0.01 M NaCl ,Mb concentration : 100 ppm, Cu (II) concentration : 400 ppb)

contact time (min)	Adsorption efficiency % (average ± SD)
30	5.79 ± 0.7
90	4.23 ± 1.6
150	5.4 ± 1.4
180	8.66 ± 0.6
240	7.81± 0.9

A8: effect of contact time on Mb removal by TREN polymer modified with Ni(II) (pH 7.4,adsorbent dose : 0.5 mg /ml, Ionic strength : 0.01 M NaCl ,Mb concentration : 100 ppm, Ni (II) concentration : 200 ppb)

contact time (min)	Adsorption efficiency % (average ± SD)
30	4.48 ± 0.4
90	3.16 ± 0.5
150	0.19 ± 0.06
180	6.5± 0.9
240	3.47 ± 1.1

A9: effect of contact time on Mb removal by TREN polymer (pH 7.4,adsorbent dose : 0.5 mg /ml, Ionic strength : 0.01 M NaCl ,Mb concentration : 100 ppm)

- **Effect of ionic strength:**

Concentration of NaCl (M)	Adsorption efficiency % (average ± SD)
0.01	6.81 ± 1.27
0.025	6.10 ± 1.42
0.05	19.04 ± 0.1
0.1	6.64 ± 1.7

A10 : effect of Ionic strength on Mb removal by TREN polymer bound modified with Cu (II) (pH 7.4, contact time: 180 mins, adsorbent dose : 0.5 mg /ml ,Mb concentration : 100 ppm Cu (II)concentration : 400 ppb)

Concentration of NaCl (M)	Adsorption efficiency % (average ± SD)
0.01	3.54 ±1.00
0.025	13.23 ± 4.85
0.05	17.57 ± 0.27
0.1	8.11 ± 1.3

A11: effect of ionic strength on Mb removal by TREN polymer bound modified with Ni (II) (pH 7.4, contact time :180 mins , adsorbent dose : 0.5 mg /ml ,Mb concentration : 100 ppm Ni (II) concentration : 200 ppb)

Concentration of NaCl (M)	Adsorption efficiency % (average ± SD)
0.01	2.99 ± 1.82
0.025	1.26 ± 0.05
0.05	0.82 ± 0.00
0.1	2.73 ± 0.29

A12: effect of ionic strength on Mb removal by TREN polymer bound (pH 7.4, contact time :180 mins, adsorbent dose : 0.5 mg/ml, Mb concentration : 100 ppm)

- **Effect of Mb concentration (pH 7.4):**

Mb concentration (ppm)	Adsorption efficiency % (average ± SD)
50	22.63 ± 15.3
100	5.25 ± 1.83
200	4.53 ± 1
400	2.28 ± 1.17
600	5.24 ± 1.23

A13: effect of Mb concentration on Mb removal by TREN polymer bound modified with Cu (II) (pH 7.4, contact time :180 mins, adsorbent dose : 0.5 mg/ml, ionic strength : 0.05 M NaCl , Cu (II) concentration :400 ppb)

Mb concentration (ppm)	Adsorption efficiency % (average ± SD)
50	15.89 ± 1.3
100	7.04± 0.02
200	3.37 ± 3.68
400	2.14 ± 0.71
600	1.15 ± 1.23

A14: effect of Mb concentration on Mb removal by TREN polymer bound modified with Ni (II) (pH 7.4, contact time :180 mins, adsorbent dose : 0.5 mg/ml, ionic strength : 0.05 M NaCl , Ni (II) concentration :200 ppb)

Mb concentration (ppm)	Adsorption efficiency % (average ± SD)
50	30.4 ± 16.47
100	1.76 ± 0.94
200	23.6 ± 5.65
400	2.93 ± 1.08
600	9.66 ± 0.65

A15: effect of Mb concentration on Mb removal by TREN polymer bound (pH 7.4, contact time : 180 mins, adsorbent dose : 0.5 mg/ml, ionic strength : 0.01 M NaCl)

- **Effect of Mb concentration (pH 8.1):**

Mb concentration (ppm)	Adsorption efficiency % (average ± SD)
50	3.39± 2.13
100	5.23 ± 2.58
200	4.54 ± 1.1
400	6.67 ± 0.95
600	4.47 ± 1.14

A16: effect of Mb concentration on Mb removal by TREN polymer bound modified with Cu (II) (pH 8.1, contact time :180 mins, adsorbent dose : 0.5 mg/ml, ionic strength : 0.01 M NaCl, Cu concentration : 400 ppb)

Mb concentration (ppm)	Adsorption efficiency % (average ± SD)
50	6.22 ± 0.2
100	6.11 ± 1.67
200	7.56 ± 2.86
400	6.91 ± 0.25
600	8.04 ± 0.19

A17: effect of Mb concentration on Mb removal by TREN polymer bound modified with Ni (II) (pH:8.1, contact time :180 min, adsorbent dose : 0.5 mg/ml, ionic strength : 0.01 M NaCl, Ni (II) concentration :200 ppb)

Mb concentration (ppm)	Adsorption efficiency % (average ± SD)
50	10.21 ± 0.48
100	1.42 ± 0.00
200	3.31 ± 0.33
400	4.33 ± 0.00
600	0.38 ± 0.10

A18: effect of Mb concentration on Mb removal by TREN polymer bound (pH 8.1, contact time :180 mins , adsorbent dose : 0.5 mg/ml, ionic strength : 0.01 M NaCl

ازاله بروتين الميوجلوبين من المحاليل المائيه عن طريق بولي ستيرين ترس – 2-امين ايثل امين

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الملخص :

في هذا العمل ، تم استخدام بولي ستيرين ترس –٢-امين ايثل امين ومركباته المعقده مع النحاس الثنائي التكافؤ والنيكل ثنائي التكافؤ لإزاله بروتين الميوجلوبين من المحاليل المائيه . تمت دراسه تركيز النحاس والنيكل ،وكمية الماده الرابطه ووقت الاتصال ،والتأثير الأيوني، وتركيز الميوجلوبين، ودرجه الحموضه. تم قياس التركيز الأولي والنهائي للميوجلوبين بجهاز الأشعة فوق البنفسجيه – المرئيه . تم اجراء تحليل بجهازي مجهر المسح الالكتروني على المادة الرابطه قبل وبعد ربط النحاس الثنائي والنيكل الثنائي وكذلك التحليل الطيفي لتشتت الطاقة و قد ظهر التصاق ايونات المعدنين بالبوليمر و زيادة في حجم حبيباته تصل الى حوالي % ١ . عمليه الامتزاز السطحي الأقصى حصلت على تركيز النيكل والنحاس عند ٢٠٠ ملغم /لتر و٤٠٠ ملغم / لتر على التوالي .الامتزاز السطحي الأقصى حصلت على كميه الماده الرابطه ٥،٠ملغم / ميلتر، ووقت الاتصال ١٨٠دقيقه، والتأثير الايوني عند تركيز كلوريد الصوديوم ٠٥،٠ مولر لبولي ستيرين ترس – ٢-امين ايثل امين و ١،٠ مولر لمركباته المعقده مع النحاس الثنائي التكافؤ والنيكل ثنائي التكافؤ. وأوضح اختبار اعادة ازاله الهيموجلوبين عن الماده الرابطه بولي ستيرين ترس – ٢-امين ايثل امين ومركباته المعقده مع النحاس الثنائي والنيكل الثنائي إلى نسبه ازاله بلغت ٤٢،٢٥% ،٣٥, ٣٨ % ، ٣٩،٣٢ % على التوالي .