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SORPTION PROPERTIES OF FUNCTIONALIZED METAL–CHELATE RESIN TOWARD DIVALENT METAL IONS AND HUMAN IMMUNOGLOBULIN G.

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Dedicated

To the memory of my father,

To my mother,

To my wife, Djamila

To my sons:Abderrakib, Aymen, Nafaa

and my daughters: Djihad and Douaa

To the family

To all my colleagues and friends

This work is dedicated to our brothers, friends and

colleagues Pr Laid Selloum

and Khaled Rouba

(Rahimahouma Allah)

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I would like to express my appreciation to my supervisor Professor Lekhmici ARRAR for his guidance throughout the course of this work and to my co-supervisor Professor Mohammad MUBARAK for his help advice and discussions. A great part of this thesis has been carried out in his laboratory at the University of Jordan, Amman, Jordan.

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Abbreviations

AAS Atomic absorption spectrometry
AGU Anhydroglucose unit
CBB Coomassie Brillant Blue)
DP Degree of polymerization
DVB Polystyrene-divinylbenzene
EDTA Ethylene diamine tetra acetate
Fab Fragment antigen binding
Fb Binding fragments
Fc Fragment crystallisable
HPLC High performance liquid chromatography
IDA Iminodiacetic acid
IE Ion exchanger
Ig Immunoglobulin
IgA Immunoglobulin A
IgG Immunoglobulin G
IMAC Immobilized Metal Ion Affinity Chromatography
***im*-IDA** Immobilized iminodiacetic acid
RIP Resin In-Pulp
SDS Sodium dodecyl sulphate
TED Tricarboxyethylene-diamine
Tris Trishydroxymethylaminomethane
$\mathbf{V_H}$ Heavy chain
$\mathbf{V_L}$ Light chain

ABSTRACT

The sorption properties of the commercially available cationic exchange resin, Amberlite IRC-718, that has the iminodiacetic acid functionality, towards the divalent metal-ions, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} were investigated by a static batch equilibration technique at 25 ^{o}C as a function of contact time, metal ion concentration, mass of resin used, and pH. Results of the study revealed that the resin exhibited higher capacities and a more pronounced selectivity towards Fe^{2+} and that the metal-ion uptake follows the order: $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$. In addition, results indicated that the metal ion uptake increases with pH. The selectivity and binding capacity of the resin toward the various metal ions investigated are discussed

Fractionation of human serum on ion exchanger-resin showed that when Cu^{+2} and Fe^{+2} were adsorbed on the resin one or two fractions, respectively, contain purified IgG, while Zn^{+2} and Ni^{+2} retain either IgG and serum albumin or serum albumin alone. Furthermore, the Ni^{+2} –resin retention of serum proteins is too strong that the use of 700 mM Tris-HCl cannot liberate any other proteins than non adsorbed serum albumin.

In conclusion, the Amberlite IRC 718 is a good metal chelate resin, which can be used in the deletion of heavy metal from aqueous solutions like wastewaters and in the purification/deletion of immunoglobulin G from human serum for diagnostic and/or therapeutic purposes.

RESUME

PROPRIETES ADSORBANTES D'UNE RESINE METAL-CHELATE FONCTIONALISEE VIS-A-VIS DES METAUX BIVALENTS ET DE L'IMMUNIGLOBULINE G HUMAINE

Dans ce travail, les propriétés adsorbantes de la résine échangeuse cationique ; Amberlite IRC 718, qui possède la fonctionnalité iminodiacétate, vis-à-vis des ions métalliques bivalents Fe^{2+}, Cu^{2+}, Zn^{2+} et Ni^{2+} sont étudiées par la techniques en batch à 25°C en fonction du temps de contact, de la concentration en ion métallique, de la masse de la résine et du pH. Les résultats montrent que la résine possède de grandes capacités et une sélectivité plus prononcée envers Fe^{2+} et que l'adsorption des ions bivalents suit l'ordre : $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$. De plus les résultats indiquent que la rétention des métaux augmente avec le pH. La sélectivité et la capacité de liaison de la résine envers les différents ions bivalents étudiés sont discutées.

D'autre part, le fractionnement du sérum humain sur la résine échangeuse de cations montre que lorsque Cu^{2+} et Fe^{2+} sont adsorbés sur la résine, une ou deux fractions contiennent de l'IgG purifiées, alors que Zn^{+2} ou Ni^{+2} retiennent l'IgG et la sérum albumine ou la sérum albumine seule. De plus, la rétention des protéines sériques par la résine-Ni^{2+} est très forte jusqu'à un point où même l'utilisation du Tris–HCl 700 mM n'a pas pu libérer les protéines adsorbées à l'exception de la sérum albumine qui n'est pas retenue.

En conclusion, l'Amberlite IRC 718 est une bonne matrice chelatrice de métaux bivalents et peut être utilisée pour la délétion des métaux lourds des solutions aqueuses comme les eaux usées et également, pour la purification/délétion des IgG des sérums humains pour des fins diagnostiques et/ou thérapeutiques.

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INTRODUCTION

Heavy metals, including zinc, copper, nickel, iron and other harmful elements are commonly found in large quantities in industrial waste effluents. They are some of the most persistent pollutants in the environment. Unlike organic pollutants, they are non-biodegradable in nature and, therefore, accumulate throughout the food chain, producing potential human health risks and ecological disturbances. For this reason, the recovery of metal ions present in wastewaters is necessary for environmental protection and economical reasons. Solid organic and inorganic ion exchangers constitute the basis of widely employed chemical separation procedures, with applications ranging from analytical and environmental chemistry research to water purification, waste management and material technologies. Strict environmental regulations require treatment of wastewater to remove heavy metals; this requirement is very costly for the industry. The use of synthetic resins for chelating toxic metal ions in wastewater is considered as a possible solution for preventing environmental pollution.

Synthetic chelating ion exchange resins are receiving considerable attention due to their application in different areas such as the removal of heavy metals from the aquatic environment, heterogeneous catalysis, solid electrolytes, ion exchange membranes, ion selective electrodes, and purification of industrial wastes. The use of chelating resins for selective removal of heavy metals from waste streams has been extensively studied. Resins with iminodiacetic acid (IDA) functional group such as Chelex 100, Amberlite IRC 718, Purolite S930, and Lewatit TP 207, were mainly applied due to their high selectivity and relatively low manufacturing cost. The IDA group could provide electron pairs for chelation; it forms stable coordinate covalent bonds with divalent metal-ions.

On the other hand, IMAC is largely employed to purify or deploy one or several proteins from plasma and sera. It is well known that serum plays a central role in clinical diagnosis. Serum is thought to contain tens of thousands of proteins along with their cleaved or modified forms.

Serum proteins may often serve as indicators of diseases and is a rich source for biomarker discovery. However, the large dynamic range of proteins in serum makes the analysis very challenging because of the high abundance of proteins including albumin, immunoglobulins (IgG and IgA), which constitute a major problem in proteome studies. Depletion of abundant serum proteins will help in the discovery and detection of less abundant proteins that may prove to be informative disease markers.

In addition, the selective retention of IgG in human serum is successfully employed for purifying this molecule which has applications in diagnostic and treatment of immune disorders. Immobilized metal ions affinity chromatography and metal ions charged alginate beads have been directly used as an IMAC medium for purification of IgG from human serum.

The work presented in this thesis is divided into two parts:

- In the fist part, the batch equilibration technique and atomic absorption spectrometry have been employed to evaluate sorption properties of the commercially available chelating resin, Amberlite IRC-718, toward the divalent metal ions Fe^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} which are present at trace levels in natural aquatic systems. The effect of the different experimental factors such as pH, contact time, metal ion amounts, and mass of resin on the sorption capacity of the resin were studied and discussed.
- In the second part, the efficiency of metal-chelated beads for improving the depletion/purification of human immunoglobulin G from serum has been investigated. The effect of the metal ion immobilized on IRC-718 on the adsorption of IgG molecule has been studied in an attempt to choose the more efficient and selective metal ion(s).

CHAPTER I: CHELATING POLYMERS

1. INTRODUCTION

Chelate-forming polymers can incorporate multidentate chelate-forming ligands by covalent bonding (Hodgkin, 1985), and are capable of reacting strongly with metal ions in solution by forming complexes and thus, providing greater selectivity as ion exchangers. The Chelate-forming polymers constitute an important class of versatile polymeric materials which have found widespread applications in environmental remediation; separation and monitoring of trace heavy metal ions from aqueous solution (Colella *et al.*, 1980; Ebraheem, *et al.*1985; Kabay and Egawa, 1994). Such chelating exchangers show high selectivity toward toxic divalent metal cations (Zhu *et al.*, 1990).

Chelating polymers are normally produced by incorporating active chelating groups into a polymeric matrix (Warshawsky, 1987). Such chelating groups may be covalently bound to a polymer matrix as pendent groups or incorporated into the repeating units of the polymer backbone by polymerization of a suitable monomer containing the required chelating group. It has been demonstrated that the nature of intervening groups connecting the active chelating ligands in chelating polymers plays an important role in the chelation process (Warshawsky, 1987; Kantipuly *et al.,* 1990). The range of chelating ligands capable of interacting with ions in solution is very wide and many have been successfully converted to polymers. The electrostatic exchange is accomplished by a rapid chemical reaction, leading in many cases to metal-ligand bonds. The intensity of the chelating interaction is governed by such properties of metal ions such as their oxidation state, electronic configuration, in addition to stereochemistry, basicity and polarization of ligand on the resin (Schmuckler, 1976).

Characterization of chelating polymers involves the use of chemical and physical techniques to determine the density, particle size, water content, ash content, thermal stability, porosity and

surface area. Additionally, the distribution and selectivity coefficients of chelating polymers depend on the degree of crosslinking, specific capacity, and nature of chelating groups (Liu and Chen, 1993). Although, the number of known chelating and complexing agents is very large, the functional group and/or donor atom(s) capable of forming complexes with metal ions is of fundamental importance in metal extraction by polymers. The functional group of the chelating agent usually includes non-metallic elements of groups V and VI: oxygen, nitrogen and sulfur. Nitrogen can be present in primary, secondary or tertiary amines, nitro, nitroso, azo, diazo, nitrile, amide and other groups. Oxygen is usually present in the form of phenolic, hydroxyl, ether and some other groups, while sulfur is in the form of thiol, thioester, thiocarbamate and disulphide groups (Kantipuly *et al.,* 1990).

2. CLASSIFICATION OF CHELATING POLYMERS

Chelating polymers can be classified according to their origin into two main categories; natural chelating polymers, which are, in general, present naturally in plants and living organisms and synthetic chelating polymers that are prepared from simpler chemical units to produce more complex materials (**Figure 1**).

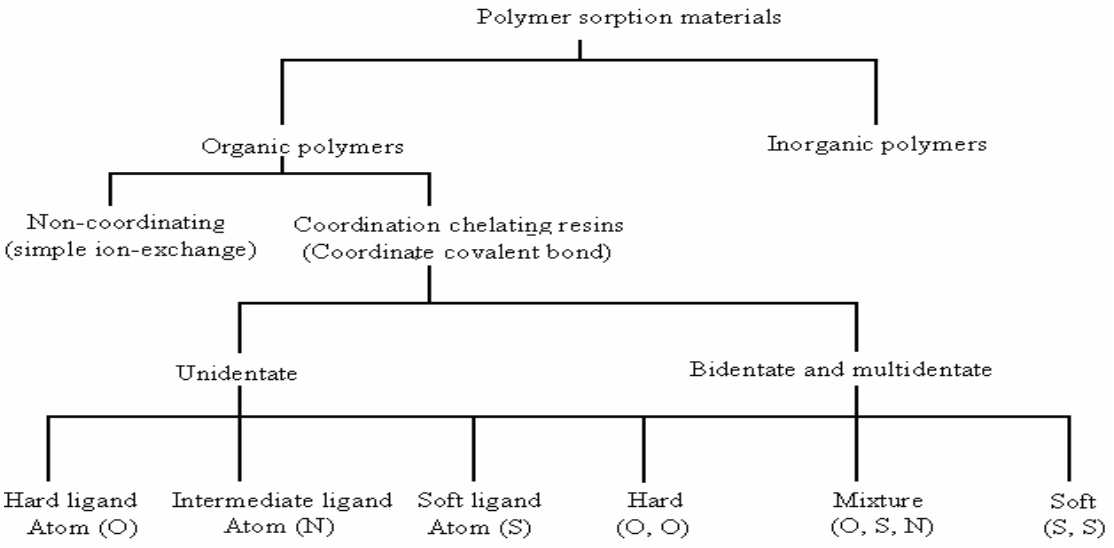


Figure 1. Classification of chelating polymers

2.1. Natural chelating polymers

Naturally occurring polymers such as lignin, cellulose, starch and pictic, alginic acid (Katsutoshi, 2007) and humic acid…, are largely available in natural resources of plants and living organisms. They have received significant attention over other types of polymers due to their unique combination of low cost, non-toxic starting materials, biodegradability, biocompatibility and bioactivity, which made them attractive in the field of trace metal removal from aqueous solutions (Muzzarelli, *et al.* 1970; Ashbrook, 1975). Some of these natural chelating polymers are mentioned below.

2.1.1. Pectic and alginic acid

The technology for removing toxic metals such as lead, cadmium, arsenic and radioactive elements from environment as well as recovering valuable metals such as precious metals from various wastes was developed by effectively using various natural products and biomass wastes (Katsutoshi, 2007). Katsutoshi employed pectic and alginic acids contained in fruits like orange and apple, and in brown seaweeds to remove cationic metal ions such as lead (II), copper (II) and iron (III). On the other hand, those loaded with high-valence metal ions such as iron (III) selectively adsorb anionic species of arsenic (III and V) according to ligand exchange reactions. On the basis of these adsorption behaviors of pectic acid, adsorption gel was directly prepared from orange waste at cheap cost to prove the effective removal of arsenic from actual acid drainage. Another adsorption gel was prepared from persimmon waste, which exhibited selective adsorption to thorium and uranium over rare earth elements. It is expected to be used for the removal of these radio active elements from environment including tailings of rare earth ores (Katsutoshi, 2007). Alginic acid (**Figure 2**), $(C_6H_8O_6)_n$, is a naturally occurring hydrophilic colloidal polysaccharide obtained from the various species of brown seaweed (*Phaeophyceae*). It is a linear copolymer consisting mainly of residues of 1,4-linked D-mannuronic acid and 1,4-linked L-glucuronic acid. These monomers are often arranged in homopolymeric blocks separated

by regions of an alternating sequence of the two acid monomers (Filipiuk, 2005). It is a water-insoluble polyuronide carbohydrate found in brown seaweeds. It precipitates and becomes a gel in the presence of polyvalent cations such as calcium (Pandolfelli, *et al.* 2002); this precipitation can be explained by the cross-linking of Ca^{2+} ions between alginate chains forming a macromolecule (Muzzarelli, 1973). This natural polymer is important in the removal of heavy metal ions (lead, cadmium, arsenic and radioactive elements) from wastes (Furushima, 1999; Katsutoshi, 2007).

2.1.2 Cellulose

Cellulose constitutes the most abundant and renewable polymer resource available worldwide. The molecular structure of cellulose as a carbohydrate polymer consists of repeating β-D-glucopyranose units which are covalently linked through acetal linkages between the OH group of the C4 and C1 carbon atoms (β-1,4-glucan) (**Figure 3**). Cellulose is a large, linear-chain polymer with a great number of hydroxyl groups (three per anhydroglucose (AGU) unit) and is present in the preferred chair conformation. To accommodate the preferred bond angles, every second AGU unit is rotated 180° in the plane. The length of the polymeric cellulose chain depends on the number of constituent AGU units (degree of polymerization, DP) and varies with the origin and treatment of the cellulose raw material (Klemm *et al.*, 2002).

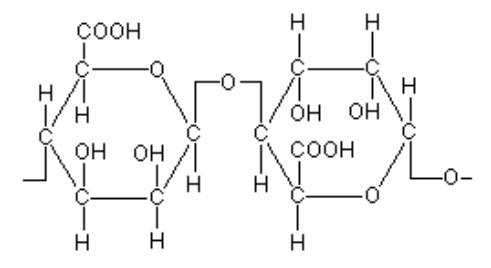


Figure 2. Alginic acid

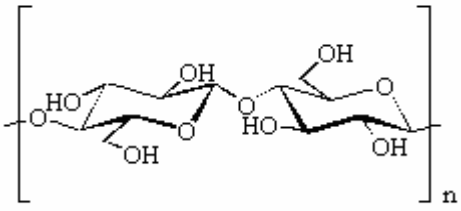


Figure 3. Chemical structure of cellulose

Cellulose has a ribbon shape which allows it to twist and bend in the direction out of the plane, so that the molecule is moderately flexible. There is a relatively strong interaction between neighboring cellulose molecules in dry fibers due to the presence of the hydroxyl groups, which stick out from the chain and form intermolecular hydrogen bonds. Regenerated fibers from cellulose contain 250–500 repeating units per chain (Klemm *et al.*, 2002). This molecular structure gives cellulose its characteristic properties of hydrophilicity, chirality and degradability. Chemical reactivity is largely a function of the high donor reactivity of the OH groups (Klemm *et al.*, 2005). Two main approaches have been tried in the conversion of cellulose into compounds capable of adsorbing heavy metal ions from aqueous solutions. The first of these methods involves a direct modification of the cellulose backbone with the introduction of chelating or metal binding functionalities producing a range of heavy metal adsorbents. Alternative approaches have focused on grafting of selected monomers to the cellulose backbone by either directly introducing metal binding capability or with subsequent functionalization of these grafted polymer chains with known chelating moieties.

Unmodified cellulose has a low heavy metal adsorption capacity as well as variable physical stability. Therefore, chemical modification of cellulose can be carried out to achieve adequate structural durability and efficient adsorption capacity for heavy metal ions (Kamel *et al.*, 2006). Chemical modification can be used to vary certain properties of cellulose such as its hydrophilic or hydrophobic character, elasticity, water sorbency, adsorptive or ion exchange capability, resistance to microbiological attack and thermal resistance (McDowall *et al.*, 1984). The β-D glucopyranose units which make up the cellulose chain contain one primary hydroxyl group and two secondary hydroxyl groups. Functional groups may be attached to these hydroxyl groups through a variety of ways.

The principle and main routes of direct cellulose modification in the preparation of adsorbent materials are etherification, esterification, halogenation, and oxidation.

2.1.3. Chitin and chitosan

Chitin and chitosan are linear polysaccharides containing 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-D-glucopyranose (GlcN) units joined by β (1→4) glycosidic bonds (**Figure 4**) (Muzzarelli, *et al.* 1970, 1980; Signini and Campana- Filho, 1999; Lamarque *et al.*, 2004).

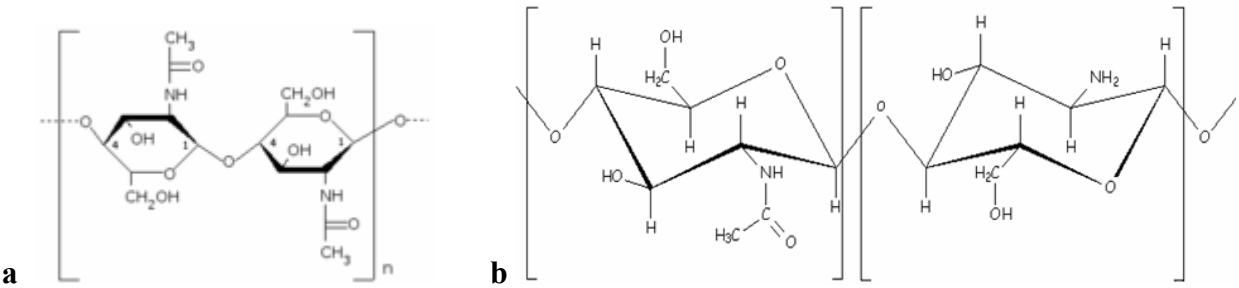


Figure 4. Structure of chitin (a) and chitosan (b).

The proportion of GlcNAc in relation to the GlcN units is defined as the degree of N-acetylation (DA) (Chatelet *et al.*, 2001; Tsaih and Chen, 2003) and differentiates chitin (DA > 0.5) from chitosan (DA ≤0.5). However, this definition is just an approximation and the difference is also defined practically by solubility in aqueous acidic medium, in which chitosan is soluble while chitin is not (Kurita *et al.*, 1991; Dung *et al.*, 1994; Signini and Campana Filho, 1999; Lamarque *et al.*, 2004). Consequently, this limits the possibility of using this biopolymer for extraction of metal ions from industrial solutions (Hsien and Rorrer 1995; Guibal *et al.*1998). Chitin can be easily obtained from crab or shrimp shells and fungal mycelia (Majeti and Ravi, 2000). Both chitin and chitosan, insoluble in water and organic solvents, are proposed as chromatographic supports for the collection of metal ions from organic and aqueous solutions.

The presence of high percentage of reactive primary aliphatic amino groups distributed on the polymer matrix offers innumerous chemical modification of which N-acetylation and Schiff reaction are the most important (Lee *et al.*, 2001). The cross-linking of chitosan with

glutaraldehyde increases the stability of chitosan in acid solutions. The reaction of chitosan with this bi-functional reagent leads to the formation of imine functions >C=N- between the cross-linking agent and amine groups of the biopolymer. This results in a decrease in the number of available amine groups. The imine group may be reduced by hydrogenation using sodium cyanoborohydride or sodium borohydride; the double linkage >C=N- is replaced by a single linkage ->C-N< to increase the reactivity of the nitrogen site (Muzzareli 1985; Ohga *et al.* 1987; Wan Ngah and Liang, 1999). Inoue and coworkers (Inoue *et al.*, 1993) protected amine groups by metal ion adsorption before crosslinking treatment and then desorbed the metal ions to prepare sorbents with high affinity for metal ions and greater stability in acid solutions. This technique has also been used for the preparation of a chitosan derivative that recognizes planar metal ions in to improve adsorption selectivity (Baba *et al.*, 1998). Another technique to improve the sorption efficiency of chitosan after a crosslinked treatment, involves the incorporation of complementary amine functions. This procedure has been used by Kawamura *et al.* for grafting polyethylene imine on chitosan beads (Kawamura *et al.*, 1993, 1997). Sulfur derivatives have been extensively studied due to the high reactivity of sulfur functions with metal ions (Saha and Cumming, 2000).

The various applications of chitin and modified chitin have been reported (Rathke and Hudson, 1994). One of its main applications is in the removal of heavy metal ions from aqueous solutions through chelation (Muzzarelli, 1997; Kumar, *et al.* 1998).

Chitosan has excellent properties for the adsorption of transition metals, mainly due to the presence of amino groups in the polymer matrix (Hsien, and Rorrer, 1995,1997; Ngsh, *et al.* 2002). However, its adsorption capacity is dependent on the degree of deacetylation, the nature of metal ion and the pH of solution (Kumar, *et al.* 1998). Muzzarelli and co- workers (1971) have used chitosan to collect trace quantities of divalent metal cations such as zinc, cadmium, lead and copper from seawater.

2.2. Synthetic chelating polymers

Synthesis of chelating ion-exchange resins began in 1952 when Gregor synthesized the first member of a new class of ion-exchange resins in which chelating groups where introduced in cross-linked form (Vernon, 1977). Many synthetic chelating polymers have been prepared by the incorporation of chelating agents into a variety of polymeric matrices. This class is based on the nature of the chemical reaction employed in the polymerization process (Nicholson, 1997). Synthetic chelating polymers are classified according to their polymerization mechanism into two major types; addition and condensation polymers. There are several methods for preparing chelating polymers. Some of these methods are discussed below:

2.2.1. Chemical modification of crosslinked vinyl polymers

The chemical modification of crosslinked vinyl polymers is the most common technique for obtaining chelating polymers. Chloromethylated polystyrene, crosslinked with DVB has been

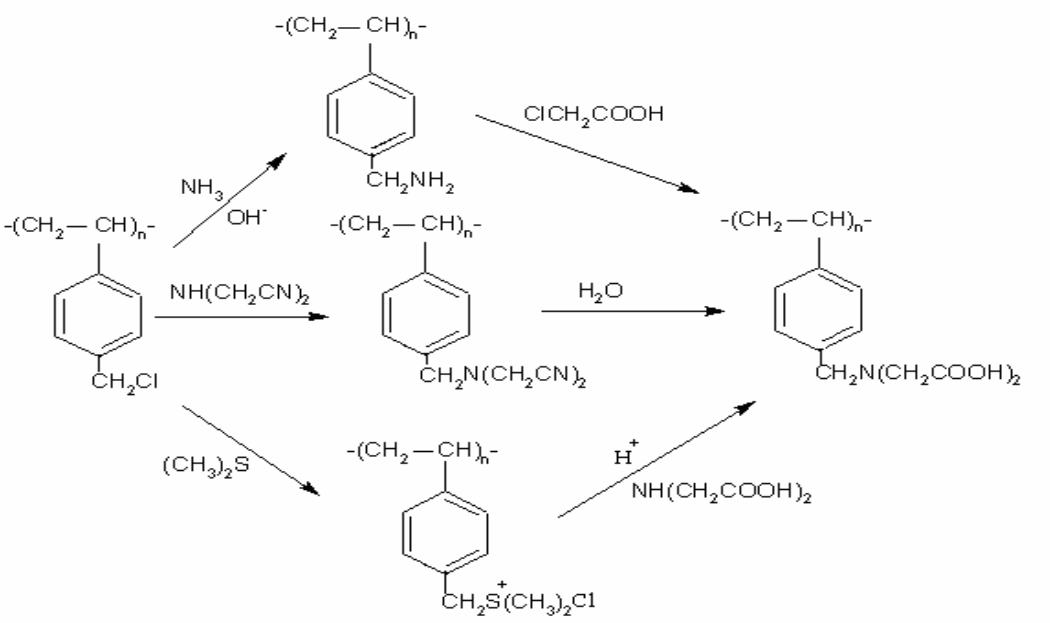


Figure 5. Chemical modification of crosslinked vinyl polymers

modified in many ways to produce chelating resins. Many other common vinyl polymers have been chemically modified to form chelating polymers such as polyacrylonitriles and polyacrylates

crosslinked with divinylbenzene. The synthesis of iminodiacetic acid resins from chloromethylated polystyrene is an example (Schmuckler, 1965) as shown in **Figure 5**.

The main advantages of this technique are the availability of the starting materials, in the form of beads or fibers, and the chemical stability of the hydrocarbon backbone chains of the resulting polymers. The main disadvantage of this method is the lack of reproducibility (Doskocilova and Shneider, 1982).

2.2.2. Chemical modification and crosslinking of linear vinyl polymers.

In this method, chemical modification of polymer occurs before cross-linking. Several chelating resins have been prepared using the same type of reactions used to crosslink resins. Other examples of this method involve crosslinking of linear polymers with monofunctional groups around a particular multivalent template (Kabanova and Efendieve, 1982). Polyvinylpyridine crosslinked with difunctional alkyl halides is an example of this system as shown in **Figure 6**.

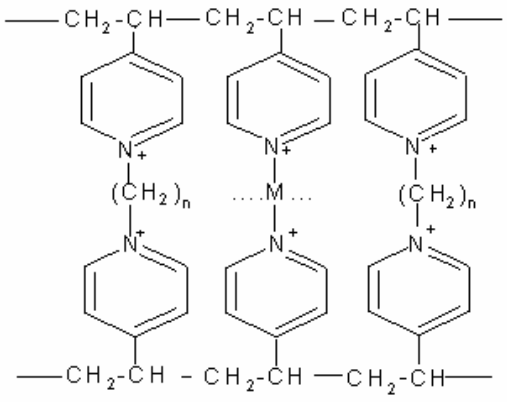


Figure 6. Chemical modification and crosslinking of linear vinyl polymers

2.2.3. Polymerization of vinyl monomers containing functional groups

This method of polymerization involves vinyl monomers containing chelating groups, which are readily synthesized and purified. A series of polymers made of vinyl crown ether compounds has been obtained using this technique (Hodgkin, 1985). Monomers containing

reactive β-diketone groups, for examples (**Figure 7**), have been polymerized and the products are well characterized (Kabanove and Efendiev; 1982).

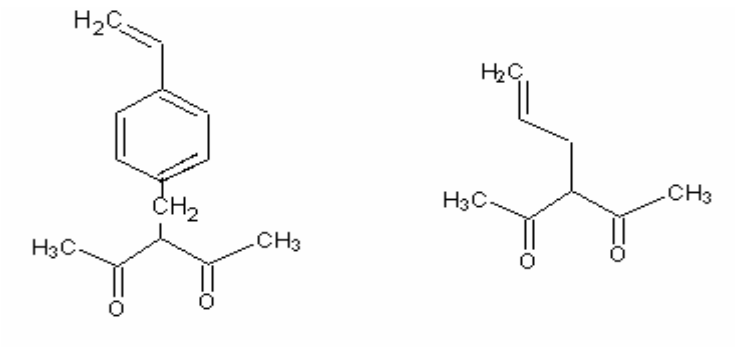


Figure 7. Monomers containing reactive beta-diketone groups

2.2.4. Condensation of monomers incorporating chelating ligands

Condensation polymerization of monomers with chelating ligands is the earliest method of preparing chelating polymers. Examples including condensation of formaldehyde with reactive phenols and /or amines such as 8-hydroxyquinoline, anthranilic acid and *o*-aminophenol have been reported (Gregor et al, 1952). Several anion-exchangers obtained by condensation of phenol, formaldehyde and polyamines are now commercially available. Other types, resulting from condensation of EDTA (ethylene diamine tetra acetate) anhydride with various diamines to produce a series of chelating polyamide polymers, have also been obtained (Marhor and Cheng, 1974).

2.2.5. Modification of natural polymers

Chemical modification of natural polymers, previously mentioned, yields chelating polymers which are almost selective for all types of metals. A number of commercially available cellulose resins with selective chelating ability have been produced (Muzzarelli and Tubertini, 1969).

3. APPLICATIONS OF CHELATING POLYMERS

Although chelating polymers have many applications their main uses are based on the selectivity toward particular metal ions, where polymers that contain appropriate donor groups can be used to remove these ions from water solutions (Florence and Battley, 1975; Buckley, 1985). Applications of chelating polymers can be classified into two main domains; environment protection and purification of proteins.

3.1. Application of chelating polymers in environment protection

Heavy metals are commonly found in large quantities in industrial wastewaters. For this reason, the recovery of the metal ions present in these wastewaters is necessary for environmental protection but stringent environmental regulations require the treatment of wastewaters; this requirement is very costly for the industry. Solid organic and inorganic ion exchangers constitute the basis of widely employed chemical separation procedures, with applications ranging from analytical and environmental chemistry research to water purification, waste management and material technologies (such as in nuclear and electroplating industries) (Helfferich, 1965; Karger *et al.*, 1973; Florence and Batley. 1975; Brower, *et al.* 1997). The most common metals found in wastewater are copper, cadmium, nickel, lead and zinc which are toxic at high concentrations (Egawas *et al.*, 1992; Vaughan *et al.* 2001). The use of synthetic resins for chelating toxic metal ions in wastewater is considered as a possible solution for preventing environmental pollution. These resins are mostly based on petroleum synthetic polymers (Liu *et al.,* 1992; Agrawal *et al.*, 2003).

Resins with iminodiacetic acid (IDA) functional group such as Chelex 100, Amberlite IRC 718 (formerly, IRC 718), Purolite S930, and Lewatit TP 207 were mainly applied due to their high selectivity and low manufacturing cost (Dabrowski *et al.,* 2004). The IDA group could provide electron pairs for chelation. It forms stable coordinate covalent bonds with divalent metal-ions. For example, Chelex 100 and Amberlite IRC-718 have been used to treat the waste effluent

discharged from printed circuit board manufacturing, which contains Cu^{2+}, Ni^{2+}, Co^{2+}, and Cd^{2+} (Diniz *et al.,* 2002). One of the few commercial chelating ion exchange resins available is Amberlite IRC-718; its chelating ability is attributed to iminodiacetic groups (Park and Cha, 1998). This acidic chelating resin has a high affinity and selectivity for heavy metal cations; this is achieved by an iminoacetic acid functionality chemically bonded to resin matrix (Kocaoba and Akicin, 2002; Agrawal *et al.*, 2003). The macroreticular structure of Amberlite IRC-718 provides a number of advantages over traditional gel resins; it is highly resistant to osmotic shock and has improved kinetics of ion exchange (Kocaoba and Akicin, 2002). IRC-718 was used for several purposes such as extraction of heavy metals (Cu^{2+}, Ni^{2+}, Fe^{2+}…) from solutions like wine (Kern and Wucherpfennig, 1993), liquor (Wucherpfennig *et al.*, 1992), stored phenol (Gonzalez *et al*, 1995) and sludge (Lee, 2006), and for ion exchange chromatography (Cha *et al.* 1998). In some studies, it was considered as a reference to study other resins (Gonzalez *et al.*, 1995).

3.2. Application of chelating polymers in wastewater treatment

Ion-exchange resins containing the IDA functional group have been investigated for a range of applications including removal of metals from industrial wastewaters (Leinonen *et al.,* 1994; Leinonen and Lehto, 2000; Valverde *et al.*, 2001), ion-chromatographic separation of metals (Biesuz et al, 1998; Bashir and Paull, 2001, 2002) and preconcentration of trace metals in seawater to aid in analysis.(Nicolau *et al.*, 2001; Jimenez *et al.*, 2002 ; Grotti *et al.*, 2002). However, investigations into the ability of IDA resins to retain metals at low temperatures, i.e. below 10°C, have not been found in the literature. Studies of the effect of salinity on the retention of metals by IDA resins have generally focused on the control of ionic strength or pH (and temperature) to enhance the separation of different metals in ion chromatographic techniques. Preconcentration techniques generally involve the control of pH to overcome matrix interference effects for quantitative trace metal analysis in seawater. However, the application being considered here is limited by the extent to which buffering chemicals can be used to control pH. It

is therefore, important to determine whether high concentrations of seawater matrix ions will swamp ion-exchange sites on the resin, thereby adversely affecting the retention of the contaminant metal ions. To evaluate this and the effect of low temperature, the removal of contaminant metals from water by Amberlite IRC748 was investigated under batch equilibrium conditions.

3.3. Application of chelating polymers in protein purification and isolation

Immobilization of chelating compounds onto solid matrices was introduced as a separation procedure in 1948 by Meinhardt, although aspects of the interaction of metal ions with poorly characterized chelating substances impregnated into paper can be traced back at least to 1855 to the pioneering studies of Runge. The high selectivity offered by multidentate compounds, particularly toward metal ions, resulted in immobilized chelate-based adsorbents which had rapidly gained popularity in industry (Sahni, 1985). In 1974, a cation exchanger equilibrated with Al^{3+} ions was used to fractionate RNA (Shankar, 1974) while 8-hydroxyquinoline, covalently attached to an agarose support and complexed with Zn^{2+} ions, was used to isolate metalloproteins (Evenson, 1974). Subsequently, the use of immobilized metal ion chelate complexes was significantly expanded by the investigations of Porath and co-workers with immobilized iminodiacetic acid (*im*-IDA) (Karger, 1973) and close analogues such as tricarboxyethylene-diamine (TED) as the chelating compounds for the isolation of proteins; this has led to the introduction by this research group of the descriptive term coined for this separation technique; "immobilized metal ion affinity chromatography" (IMAC) (Porath, 1975, 1983,1990). Various applications of IMAC with borderline Lewis metal ions have been reviewed in details in recent years (Sulkowski, 1988; Arnold, 1991; Wong, 1991). Currently, investigators frequently favor the use of IDA as the chelating agent of choice with covalent attachment of this compound either to a soft gel matrix such as cross-linked agarose (Sulkowski, 1988; Porath, 1990; Wong, 1991) or alternatively to a chemically modified inorganic matrix such as silica or zirconia (Wirth, 1993),

with subsequent complexation of borderline Lewis metal ions (as defined by Pearson in 1990) such as Cu^{2+}, Ni^{2+}, or Zn^{2+}. Several proteins with surface accessible histidine, tryptophan, or cysteine residues have been purified with these so-called borderline *im*-M^{n+}-IDA adsorbents (Sulkowski, 1988; Kagedal, 1989; Arnold, 1991; Wong, 1991). Zachariou (1992) described the binding behavior of three proteins in the presence of the hard Lewis metal ions Al^{3+}, Ca^{2+}, Fe^{3+}, and Yb^{3+}, and the borderline Lewis metal ion Cu^{2+} as a control cation, using *im*-IDA, *im*-8-HQ, and introduced ligand *im*-OPS (Zachariou, 1993) as the chelating species at pH 5.5 and 8.0. The adsorption and elution behavior of tuna heart cytochrome *c*, horse skeletal muscle myoglobin, and hen egg white lysozyme, at different ionic strengths and pH values, with these *im*-M^{n+}-chelates have been examined to establish trends in relative affinities for the protein-*im*-M^{n+}-chelate complexes. This behavior was then compared to the binding and elution behavior of the same proteins with the conventional cation exchanger CM-Sepharose. Zachariou demonstrated that the hard Lewis M^{n+}-chelate complexes show higher affinity and different selectivities for the tested proteins when low-ionic strength buffers were used than when high-ionic strength buffers were employed. Moreover, when different types of *im*-chelate complexes were used with the same hard metal ion, the IMAC selectivities for these proteins were also significantly different over the same operational pH range.

3.3.1. Metal Chelate Affinity Chromatography

More than 30 years ago it was discovered that many natural proteins have metal binding sites which can be used for purification (Porath et al, 1975; Arnold, 1991). The concept of this type of purification tool is rather simple. A gel bead is covalently modified so that it displays a chelate group for binding a heavy metal ion such as Ni^{2+} or Zn^{2+}. The design of the chelating group on the gel bead is such that it provides only half of the ligands needed to hold the metal ion. So when the protein with a metal binding site finds the heavy metal, the protein will bind by providing ligands from its metal binding site to attach to the metal ion displayed on the chelating

arm of the gel bead. This is very similar to affinity chromatography and can be viewed as a group selective tool for purifying the metal-binding class of proteins (Arnold, 1991)

3.3.2. Immobilized Metal Ion Affinity Chromatography (IMAC)

Immobilized metal-ion/metal-chelate affinity chromatography is a separation technique based on coordinate covalent binding between proteins and metal ions. Proteins have a wide variety of amino acids composition which, in fact, generates a range of different affinities toward metal ions. However, not many naturally occurring proteins have affinity for metal ions, therefore, the technique is mainly used to purify recombinant proteins. For example, proteins can be engineered to contain a poly-histidine tail (histidine can generally act as a ligand towards divalent metal cations). If the stationary phase is immobilized with divalent metal cations, a mixture of proteins can be separated based on their ability to interact with the metal ions. The proteins (glutathione transferase) containing a higher number of histidine residues would be able to bind to the column more tightly than those with fewer histidine residues (Schmidbauer and Strobel, 1997). Several different types of immobilized metal ion (e.g. Fe, Co, Cd, Ni, or Zn) columns have been developed to separate various proteins. Protein separation in IMAC generally depends on the strength of the metal ion-protein bond. Thus, choosing the type of immobilized ion is crucial to the success of protein separation. By far, the most widely-used technique is the use of an immobilized nickel column, and to engineer poly-histidine tags of six or more residues onto the recombinant proteins of interest. One thing to keep in mind is that the binding between metal ions and protein must be reversible, allowing elution of bounded protein at later steps. Three different elution strategies can be applied to IMAC; competitive elution, stripping elution, and pH adjustment (Paunovic, 2005).

4. EXCHANGE RESINS

Ion exchange resins are polymers that are capable of exchanging particular ions within the polymer with ions in a solution that is passed through them. This ability is also seen in various

natural systems such as soils and living cells. Although the synthetic resins are used primarily for purifying water, they are also used for various other applications including the separation of some elements such as Hg^{2+}, Cd^{2+}, , Mn^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, , Ca^{2+}, Pb^{2+}, Al^{3+}, Fe^{3+}…

Heavy metals belong to the major environmental concerns in waste waters and effluents, because of their toxic or even carcinogenic characteristics. Among other methods (e.g. membrane separation, solvent or reactive extraction), ion exchange processes have been established for the treatment of industrial waste waters with low to moderate heavy metal concentrations (less than 1000 mg/L); for instance flushing or etching effluents. For elimination and reduction of heavy metal ions from waste waters, a number of special artificial polymeric ion exchangers (IE) are offered. However, natural polymeric products (wood, other cellulose fibers) or their modifications have also the ability to exchange heavy metal ions – usually with a reduced ion exchange capacity.

The advantages of ion exchange processes are the very low running costs. Very little energy is required, the regenerated chemicals are cheap and if well maintained resin beds can last for many years before replacement is needed. There are, however, a number of limitations which must be taken into account very carefully during the design stages. When itemized, these limitations appear to represent a formidable list and the impression can be given that ion exchange methods might have too many short comings to be useful in practice. However, this is not the case as the advantages mentioned above are very great and compensation can readily be made for most restrictions.

4.1. Development of exchange resin

Ion exchange is a natural phenomenon which occurs in soil, mineral, plant and animal tissues. This process was identified and described in 1850 (Kichener, 1957) when ammonia was found to be exchanged with calcium in soil. Later, natural ion exchangers such as zeolites and

clays were used in water treatment. However, the instability in both acidic and alkaline solutions has limited the application of these aluminosilicate minerals to other industries (Simon, 1991).

In 1944, organic ion exchange resins based on the copolymerization of styrene and divinylbenzene copolymer were successfully developed (Helfferich, 1962). Since then, many adaptations of the process and variations of properties of the original products have been made to suit the applications of these materials (Korkisch, 1989; Walton and Rocklin, 1990). Today, numerous ion exchange resins are available commercially and are widely used in water treatment, separation and purification in hydrometallurgy, food industries, pharmaceutical products and even in drug delivery.

4.2. Chelating resins

Chelating resins which are also known as complexing or specific ion resins are designed to have high specificity for an ion or a group of ions. These types of ion exchange resins adsorb metal ions through a combination of ionic and coordinating interactions instead of the simple electrostatic interactions in conventional cation or anion ion exchangers (Harland, 1994). As a consequence, chelating resins present greater selectivity than conventional resins.

It is assumed that chelate rings are formed during metal adsorption. The possibility of the formation of chelate rings depends not only on the nature of active chemical group but also on the physical properties of the polymer matrix as well as on sorption conditions (Myasoedova and Savvin, 1986). The groups taking part in the formation of chelate rings include nitrogen (as in the form of amine, diazo and nitro groups), oxygen (in form of phenol, carboxyl) and sulfur (in the forms of thiol, thiocarbamide and disulfide groups). It is also important to realize that because of their high selectivity, these resins may create difficulties in the stripping process depending on the strength of the chelate rings formed with the particular metal ions. The development of chelating resins was attributed to Skogseid (Harland, 1994) who described the chemistry of such an ion exchanger in 1947. This was followed by several attempts of incorporating a variety of chelating

groups onto different types of polymeric matrices (Rahm, and Stamberg, 1961; Manecke and Danhaeuser, 1962). Since then, the field of chelating resins has been developing steadily. Today, numerous types of chelating resins are available commercially as shown in **Table 1**.

Table1. Commercial chelating ion exchange resins (Harland, 1994; Harju and Krook, 1995).

Functional groups	Functional groups	Commercial designation	Capacity (eq/l)	Manufacturer
Iminodiacetic acid $-CH_2-N(CH_2COOH)_2$	Fe, Ni, Co, Cu	IRC 748 (formerly known as IRC 718 or XE 718)	1.25	Rohm and Hass
		TP 207	2.4	Bayer
		TP 208	2.7	Bayer
		S 930	1.1	Purolite
		SR-5	1.7	Ionac
		Dowex IDA-1	1.1	Dow chemical
		IN SIR	0.9	Amantech
Aminophosphonic $-CH_2-NH-CH_2-PO(OH)_2$	Pb, Cu, Zn	C 467	1.0	Doulite
		S 950	1.2	Purolite
		S 940	1.24	Purolite
		Dowex APA-1	1.1	Dow chemical
		IN BSR	1.2	Amantech
Bispicolylamine $-NH(CH_2C_5NH_4)_2$	Fe, Cu, Ni, Co	XFS 4195	n.a	Dow Chemical
N-picolylamine N-hydroxyethyl $-N(CH_2C_5NH_4)C_2H_4OH$		XFS 4196	n.a	Dow Chemical
Thiocarbamide $-CH_2-SC(NH)NH_2$	Pt, Pd, Au	SR2	n.a	Ionac
		S 920	n.a	Purolite
N-methylglucamine - $CH_2N(CH_3)[(CHOH)_4CH_2OH]$	B	IRA 743	0.6	Rohm and Haas
		MK 51	0.6	Bayer
Thiol $-CH_2SH$	Hg	GT 73	1.2	Rohm and Haas
		IN MSR	1.4	Amantech
		SR4	2.0	Bayer

n.a: not available

Their applications can be found in water and waste water treatment (Hirai and Ishibashi, 1977; Moriya *et al.* 1987; Jenneret-Gris, 1989 ; Vater *et al.*, 1991; Mijangos and Diaz, 1992; Beatty *et al.*, 1999; Moriya, 2000; Stetter *et al.* 2002), analytical chemistry (Yuchi *et al.* 2002; Zagarodni *et al*., 1997), metal recovery (Johns and Mehmet, 1985; Green *et al.* 1998; Outola *et al.* 2000; Duyvesteyn *et al.* 2000; 2001; Wang *et al.* 2001) and separation and purification (Leinomen *et al.,* 1994; Hubicki, 1998; Park *et al.,* 2000; Sigh, 2001).

4.2.1. Synthesis of chelating resins

The synthesis of ion exchange resins started with the production of polymeric matrices. These matrices consist of three dimensional networks of hydrocarbon chains to which functional groups (in this case chelating ligands) are attached. The most common polymers are produced from polystyrene-divinylbenzene in which DVB is the crosslinking agent, typically present in proportions of 2 to 16 %. Other polymers include acrylates, methacrylates, phenol-formaldehyde and epichlorohydrin amine condensates. The functional groups are then chemically introduced through various routes, depending on the type of resin to be prepared as shown in **Figure 8**. A very comprehensive and detailed review that covers the theoretical aspects and methods involved in the synthesis of chelating resins has been published by Sahni and Reedijk (1984). The two most common types of resin matrices are styrene and acrylic. The styrene structure is composed of aromatic rings while the acrylic structure involves straight chain hydrocarbons. For chelating resins, a styrene copolymer with a macroporous structure is used as a starting material. This structure provides better permeability of the matrix which enhances kinetic properties than conventional gel-type polymers. The properties of the polymer matrix used and its structure determine the type and physical properties of chelating resins while the method of synthesis and the type of functional groups control its capacity and selectivity (Mysasoedova and Savvin, 1986).

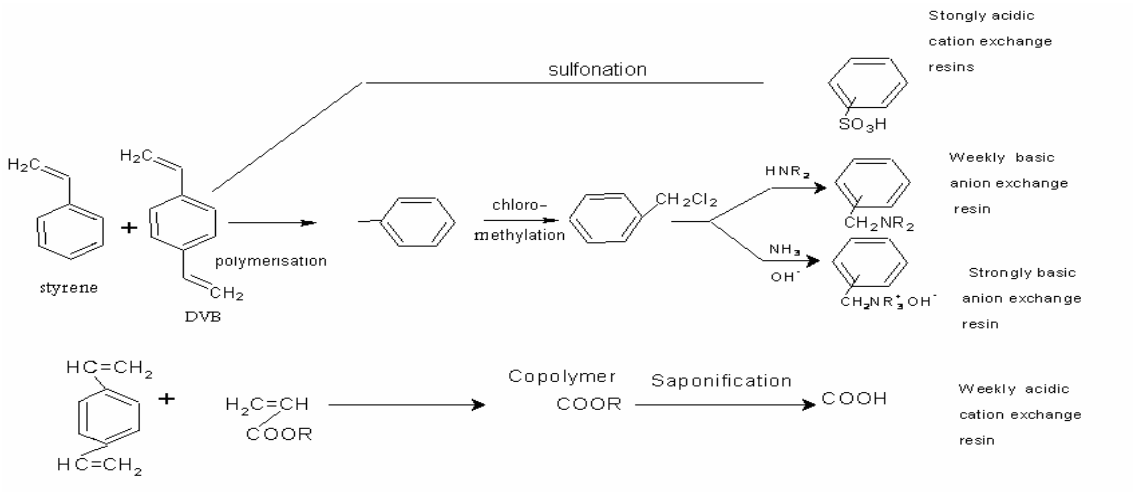


Figure 8. Synthesis routes for ion exchange resins (Korkisch, 1989)

The majority of chelating resins have been prepared by attaching a chelating ligand onto a polymer (Eccles and Greemwood, 1992). In this approach, the starting aromatic polymer is first converted into its chloromethylated derivative. This is carried out by treating the cross-linked polystyrene with chloromethyl ether (or other non-carcinogenic reagents) as shown in the route for preparing anion exchange resin (**Figure 10**). The produced chloromethyated polymer is then reacted with ammonia to convert it to the amine followed by chloroacetic acid to form chelating groups (**Figure 9)**.

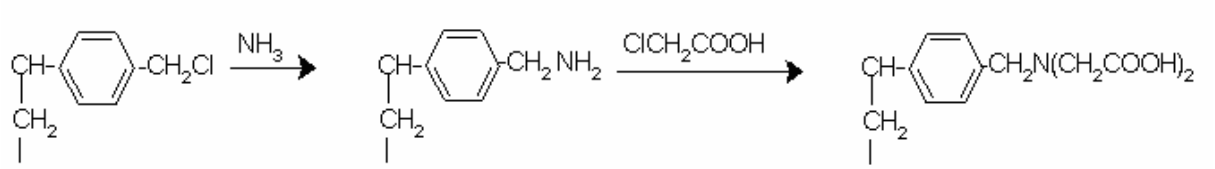


Figure 9. Synthesis of iminodiacetic resins by attaching a chelating ligand.

Another method in synthesizing chelating resins involves nitration of the polystyrene (Eccles and Greewood, 1992). The nitropolystyrene produced is reduced to amino polystyrene followed by treatment with sodium nitrite and hydrochloric acid to form the diazo resin which can then couple with various chelating ligands in alkaline solution. **Figure 9** presents the sequence of reactions in synthesizing chelating resins using this option. Using the same approach, iminodiacetic resins can be prepared by reacting the amino polymer with chloroacetic acid as given in **Figure 10**.

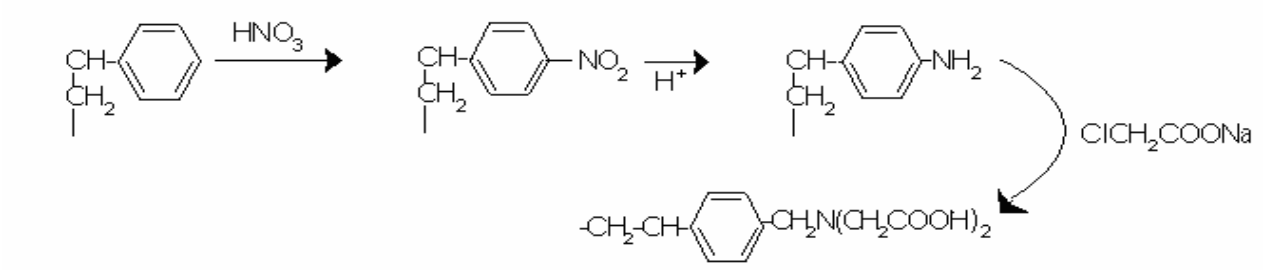


Figure 10. Synthesis of iminodiacetic resin by chloroacetic acid

4.2.2. General structure and properties of chelating resins

One of the main considerations in ion exchange applications in hydrometallurgy is the physical stability of the resin. In ion exchange, the nature of the solution changes during the adsorption and elution processes which causes periodic swelling and shrinking of the resin beads. This can induce high osmotic shock in the beads which can lead to cracking and breaking. This problem is exacerbated in the tougher environment of a resin-in-pulp application. Shattering of the resin beads due to mechanical operations such as pumping, screening and mixing and also abrasion of the beads by pulp can all lead to excessive resin breakage.

Chelating ion exchange resins generally benefit from the macroporous structure of the matrix. Macroporous polymers are prepared in a way that allows the beads to have a high degree of crosslinking without significantly affecting the exchange kinetics (Walton and Rocklin, 1990). Greater crosslinking promotes greater physical strength and toughness of the resins. The macroporous structure avoids excessive swelling and shrinking of resin beads, thus minimizing breakage caused by osmotic shock. The macroporous structure also enhances the kinetic performance of the resins as it allows more rapid diffusion of ions and molecules within the resin phase. The higher surface area of this structure also means that chelating resins have higher resistance to organic fouling. Unfortunately, the pores in this structure reduce the number of possible exchange sites and thus result in a lower exchange capacity compared to the gel type resins. The pores reportedly can take up to 10 to 30% of the volume polymer (Walton and Rocklin, 1990), which reduces the ion exchange capacity proportionately. Acrylic-based resins are particularly softer and more elastic compared to the styrene-based polymer. Their main advantage is claimed to be the ability to withstand fouling by organic ions.

4.3. Exchange Capacity

The exchange capacity is one of the most important chemical characteristics of an ion exchanger since it is a measure of the resin's capability to carry out useful ion exchange work. It is defined as the number of equivalents of exchangeable ions or molecules per unit mass or volume of the exchanger (Grimshaw and Harland, 1975).

The exchange capacity or total capacity is usually measured by an ion exchange reaction involving the functional group on the resin (Korkisch, 1989). The most widely used method in the case of ion exchange reactions which involves protons is a titrimetric method which makes use of direct or back acid-base titrations. The exchange capacity can be calculated using the following formula:

$$\textbf{Exchange capacity (Qr)} = (V_t \times C_t / M_r \text{ or } V_r)$$

Q_r = Total exchange capacity of resin (eq/l or eq/kg), V_r = Volume of titrant, Ct = Concentration of titrant (mol/l), Mr = Mass of resin (kg), and Vr = volume of resin (l)

4.3.1. Equilibrium loading

The total exchange capacity is hardly achieved even in a typical competitive exchange environment encountered in hydrometallurgical operations such as an in-pulp application. In this environment, the equilibrium loading of a particular metal ion is the relevant working capacity achievable by a resin.

This equilibrium loading is the maximum number of equivalents of a given ion that can be removed from solution per unit volume of resin under specified condition. Equilibrium is achieved when the thermodynamic potential for each ion is the same inside and outside the resin phase (Slater, 1991). The equilibrium relationship between the concentration of the metal ion in the ion exchange phase and that in the solution phase is generally described by an equilibrium

adsorption isotherm from which the actual maximum loading in a comparative competitive environment can be determined.

The adsorption (or elution) isotherm can be obtained by carrying out a series of batch tests in which a fixed volume of solution or pulp is contacted with different volumes of resin for an extended period of time. Analysis of both the eluate and the solution or pulp can be used to calculate the equilibrium metal loading capacity of the resin at various equilibrium concentrations in solution. Typical isotherms are depicted in **Figures 11** and **12** for the loading onto an iminodiacetic chelating resin.

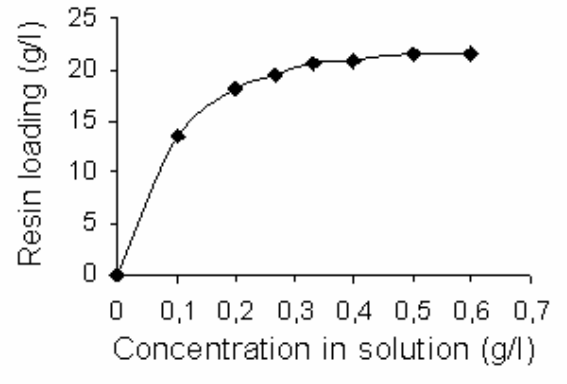


Figure 11. Equilibrium adsorption isotherm of an iminodiacetic chelating resin (Slater, 1991).

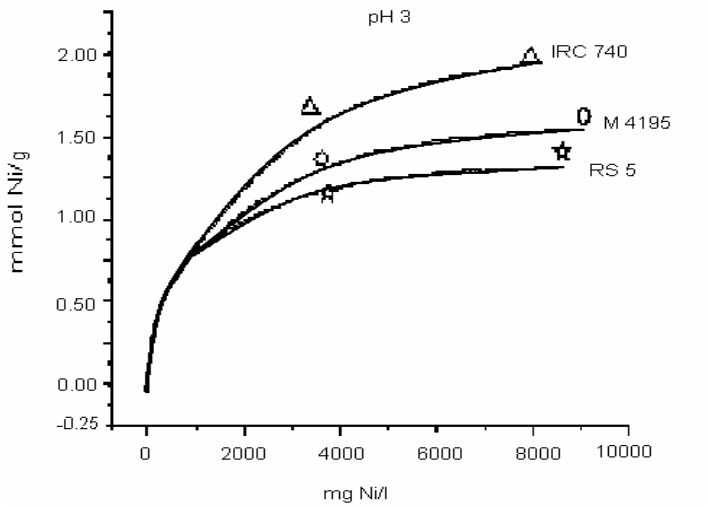


Figure 12. Nickel sorption isotherms for three resins (4 h) (Mendes and Martins, 2004)**.**

Apart from the obvious requirement to operate at maximum loading capacity as mentioned in the above section, equilibrium adsorption isotherm is an important characteristic of a particular metal ion and allows the prediction of the equilibrium state in a system which in turn provides data for design calculations.

There are a number of models which are generally used to describe the adsorption isotherms and to fit experimental equilibrium data. The two models which are most frequently used for chelating resin adsorption are the Langmuir and Freundlich isotherms (Wang *et al.*, 2001; 2002).

4.3.2. The kinetics of ion Exchange Processes

Kinetic studies of ion exchange adsorption allow a better understanding of the mechanisms which control or contribute to the rate of the overall exchange process. The development of equilibrium and rate expression models based on experimental kinetic data can be used together with fitted numerical parameters such as equilibrium and rate constants, and mass transfer coefficients in process design.

Kinetic tests are performed in a similar manner to the equilibrium loadings in batch tests. A volume of wet resin is added to a stirred contactor containing a fixed pulp. The concentration of metal in solution is monitored as a function of time. The results are analyzed by fitting the data to a kinetic model. Generally, the rate of exchange or adsorption in an ion exchange can be controlled by:

1. Diffusion through the aqueous film surrounding the particle (film diffusion).
2. Diffusion through the resin bead (particle diffusion)
3. Chemical reaction at the sites of the functional groups within the resin bead (Harland, 1994).

Film diffusion dominates when ion exchange resin beads of small particle size are in contact with dilute solutions and particle diffusion is expected when large beads are contacted with more concentrated solutions. It is possible, however, for both diffusion reactions to affect the rate of the

extent of exchange changes. In such a case, it is expected that the initial stage of the ion exchange process will be controlled by film diffusion and the later stages by particle diffusion (Slater, 1991).

Chemical reactions have been suggested to be the rate controlling step in chelating ion exchange reactions. Harland (Harland, 1994), however, reported that the evidence for this is inconclusive. Several kinetic studies involving adsorption of metal ions on iminodiacetic type chelating resins have established that the rate in these cases is controlled by particle diffusion (Feng and Hoh, 1986; Yoshida *et al.*, 1986; Fernandez *et al.*, 1995; Arevalo *et al.*, 1998).

The tendency for a chemical reaction to be the rate controlling step will depend on the ability of the metal ion to be adsorbed. It was found that the complexes formed by zinc, cobalt and copper with the iminodiacetic group are labile and therefore, the rate is controlled by diffusion (Schwarz *et al.*, 1964; Heitner-Wirguin and Urbach, 1965). On the other hand, metal ions such as iron(III) chromium(III) which are kinetically inert react more slowly with the chelating group and therefore, chemical reaction is often the slow step in these cases. The inertness of these complexes also creates problems in that they are difficult to elute once adsorbed on the resin.

4.3.3. Ion exchange resins for the adsorption of metal ions

Nickel and cobalt are normally present as divalent cations in aqueous solutions. Therefore, cation exchange resins and weakly acidic resins have been suggested and used for the adsorption of nickel and cobalt from hydrometallurgical process solutions. Weakly acidic resins with carboxylic groups offer high loading capacities, but their use is limited due to poor selectivity over alkaline earth metals and they are only suitable for use in weakly acidic or alkaline solutions. Cation exchange resins with phosphonic acid functional groups can be used in the slightly acidic range. However, their selectivity which makes them readily adsorb copper, zinc, cadmium, makes them less effective for nickel and cobalt (Qiu and Zheng 2008)**.**

Chelating resins carrying iminodiacetic acid groups have been long known to selectively recover nickel and cobalt from sulfate solutions. For common divalent non-ferrous metal cations, the selectivity of this type of resin ranks nickel second after copper. Comparative selectivity orders of carboxylic, phosphonic and iminodiacetic acid chelating resins are given in **Table 2.**

Other chelating resins have been found to selectively adsorb nickel and cobalt such as bispicolylamine, aminophosphonic acid and amidoxime.

Table 2. Selectivity order of the iminodiacetic acid chelating resins compared to carboxylic and phosphonic acid resins

Resin type	**Selectivity order**
Carboxylic resins	Cu^{2+}>Pb^{2+} >Zn^{2+} >Cd^{2+} >Ni^{2+} >Ca^{+2}
Phosphonic resins	Cu^{2+}> Zn^{2+} > Cd^{2+}> Mn^{2+}> Co^{2+} > Ni^{2+} >Ca^{+2}
Iminodiacetic acid chelating resins	Cr^{3+} > In^{3+} > Fe^{3+}> Al^{3+}> Hg^{2+} > UO^{2+}> Cu^{2+}> Pb^{2+}> Ni^{2+}> Cd^{2+} >Zn^{2+}>Co^{2+}>Fe^{2+}>Mn^{2+} >Ca^{+2} >Mg^{2+}

4.4. Iminodiacetic acid (IDA) chelating resins

Chelating resins with iminodiacetic acid (IDA) groups are commercially available since the late 1950's. These resins have an affinity for many polyvalent and transition metal cations. The functional group contains one amino and two carboxylic groups as shown in **Figure 13**.

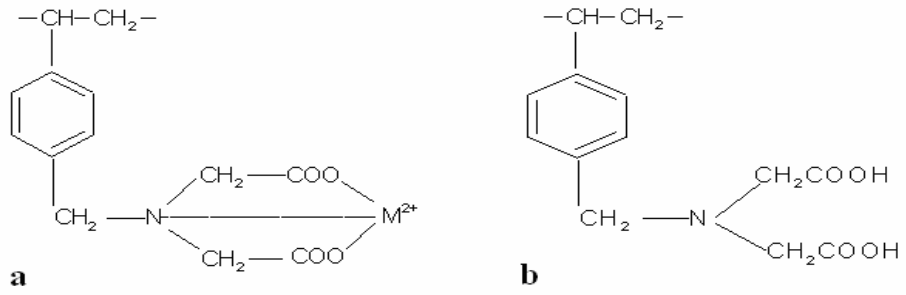


Figure 13. Chemical structure of iminodiacetic acid resin (a) with and (b) without divalent cation

The order of selectivity of these resins may vary depending on the method, materials and matrix used in the synthesis. Thus, it is expected that the chelating ability of the resins may differ

slightly from one manufacture to another. The weakly acidic nature of carboxylic acid groups in the iminodiacetic resins makes them inefficient for operation at low pH pH 1–2). (The true ion exchange capacity can only be achieved in the range of pH 2 to 6 (**Figure 14**) depending on the metal ion. Theoretically, this limits the applicability of the resin in hydrometallurgical operations since many metal cations are insoluble at pH values above 5. However, because of the high selectivity shown for many metal ions combined with its macroporous structure that provides high resistance to osmotic shock, fast kinetics and high mechanical strength, this type of resin has been found to be superior to conventional cation exchange resins in slightly acidic (pH 4 to 5) solutions. Elution of adsorbed metals from this resin is relatively straightforward in that it can be achieved by lowering the pH using dilute acid (Heitner-Wirguin and Ben-Zwi, 1970).

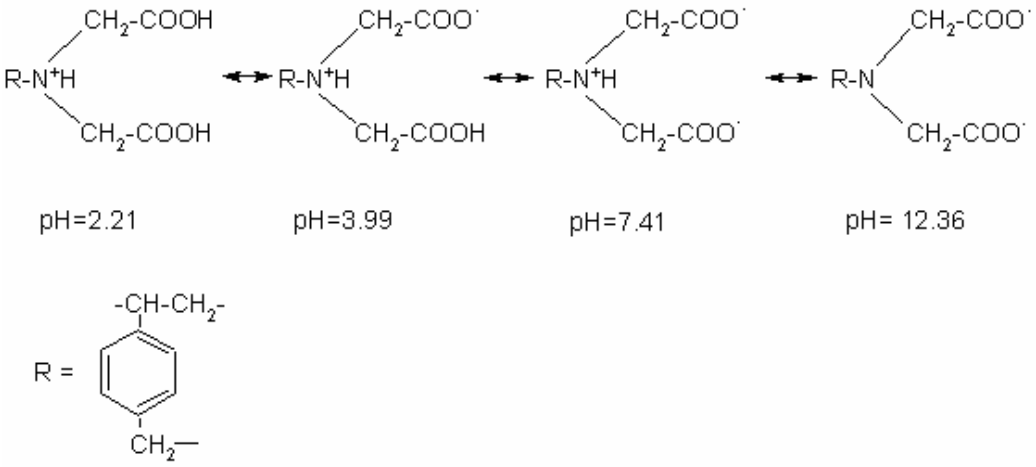


Figure 14. Protonations of the iminodiacetic species (Heitner-Wirguin and Ben-Zwi, 1970)**.**

4.4.1. Adsorption Behavior of metal ions onto iminodiacetic acid (IDA) chelating resins

Metal ions are adsorbed onto iminodiacetic chelating resins through the following simplified ion exchange reaction.

$$\overline{RH_2} + M^{2+} \rightleftharpoons \overline{RM} + 2H^+$$

where overbars represent the species in the resin phase and M^{2+} represents metal cation.

From the stoichiometry of this reaction, it is expected that for every metal cation loaded, two protons will be released into solution. The release of these protons will result in lowering of the pH of the bulk solution and thus affect the resin loading capacity and kinetics. The resin is therefore, able to extract nickel without pH control from solutions containing less than about 500 ppm of nickel at pH values between 4 and 5. Thus, for solutions typical of laterite tails which normally contain less than 500 ppm nickel, pH control is not necessary. However, for higher nickel concentrations such as those in the pregnant leach slurries, pH control will be necessary.

Theoretically, the loading capacity of the iminodiacetic resin will depend on its initial form (Moore, 2000). Conditioning or pre-treatment with strong bases like sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) and ammonium (NH_4OH) will deprotonate the carboxylic acid groups and will result in a higher operating capacity than can be achieved if the resin is in the acid form. For the recovery of nickel from laterite slurries, it is important to keep the pH as high as possible to maximize the adsorption of nickel and cobalt while limiting the precipitation of nickel and cobalt as hydroxides. Copper, if present in the pulp solution should also be removed prior to adsorption as it can lead to temporary resin fouling. **Figure 15** showed the adsorption capacity of these metals as a function of pH onto this type of resin.

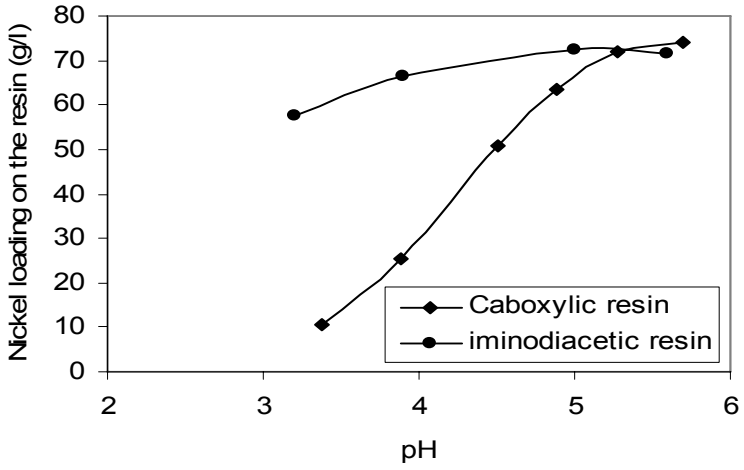


Figure 15. Effect of pH on nickel loading (Green *et al.*, 1998).

4.4.2. Recovery of metal cation by ion exchange

There is limited literature dealing with the recovery of metals cations from laterite pulps and solutions. Most of the research work was carried out with either synthetic sulfate or waste waters (Melling and West, 1984; Diaz and Mijangos, 1987; Melling and West, 1984; Green *et al.*, 1998; X Outola *et al.*, 2001). The most common iminodiacetic chelating resins reported for the recovery of metals ions are TP 207, TP 208, IRC 748, S 930 and ES 466. Chelating resins with picolylamine and aminophosphonic active groups have also been proposed and investigated for this application (Grinstead, 1984; Rawat and Bhardwaj, 1999; Duyvesteyn *et al.*, 2000).

Detailed testwork on the recovery of nickel and cobalt from low grade leach pulps was carried out by Green and co-workers (Green *et al.* 1998). Both iminodiacetic chelating and carboxylic acid resins were examined. Iron and copper present in the solution were removed by oxidation and neutralization prior to loading. Those researchers found that both resins have achieved maximum nickel loading at a pH value of 5. At lower pH values, the capacity of carboxylic acid resin decreased significantly compared to the IDA chelating resin as shown in **Figure 15**. The latter resin was also more selective for nickel over calcium and magnesium. Calcium loading should be limited to avoid precipitation of gypsum during elution with sulfuric acid.

Counter current Resin In-Pulp tests were carried out on the IDA resin in three stages. The pH of the loading was maintained throughout the test at pH 4 by caustic addition. Using this method, Duyresteyn and co-workers (2001) have achieved the nickel and cobalt recovery of 98% and 90%, respectively. Elution of the resin was easily established using less than 3 bed volumes of 1 M sulfuric acid. Metal concentrations in solution and on the resin at each stage are presented in **Table 3**. A patent on the use of chelating resin for recovering nickel and specifically from pregnant leach pulps was descried by Duyvesteyn and co-workers (2001). Their work was carried out using a bipiscolylamine type chelating resin. High concentration of impurities like iron and

copper were precipitated by neutralization prior to loading as shown in **Table 4.** The precipitated metals do not appear to interfere with the loading of nickel and cobalt onto the resin.

Table 3. Counter-current RIP of metal cations from nickel liquor* (Green *et al.* 1998)

Metal	Feed	Stage 1	Stage 2	Stage 3
		Metal in solution leaving stage, g/m^3		
Ni	3700	3400	1700	126
Co	62	58	31	5
Mg	640	650	790	770
Ca	Saturated	Saturated	Saturated	Saturated
Fe	11	<1	<1	<1
		Metal on resin, g/l		
Ni		64	43	13
Co		1.1	1.2	0.3
Mg		0.7	1.0	3.8
Ca		1.9	2.2	2.2
Fe		0.4	0.2	0.1

• *Volume of resin per stage: 150 mL; Volume of stage (contactor): 100 mL; resin transfer rate: 1 stages every 4 hr; pulp flow-rate: 700 ml/hr; pH maintained at 4 in each stage with 2 M NaOH.*

Table 4. Partial precipitation of iron copper during neutralization (Duyvesteyn *et al.* 2001)

Sample	**pH**	**Metal ions (g/l)**			
		Ni	**Co**	**Fe**	**Cu**
Leachate	1.8	8.6	0.33	2.3	0.048
Neutr.A	3.0	8.7	0.31	1.9	0.013
Neutr.B	3.5	8.7	0.33	1.9	0.007

The loading was carried out at pH around 4 and elution was carried out using 1 M sulfuric acid. This patent, however, does not report on the actual feed data and barren concentration. The low nickel concentration obtained from elution of the loading resin also does not reveal a significant enrichment of the metals on the resin. This type of resin was not considered for this study because of its high cost which would not allow for its use in a rapid, low-grade RIP application.

CHAPTER 2. TOXIC EFFECTS AND REMOVAL METHODS OF HEAVY METALS

1. INTRODUCTION

The term “heavy metals” has been queried over many years by many scientists such as Heuman (Heuman , 1955), Phipps (Phipps, 1981) and Van Loon and Duffy (VanLoon, 2000), but efforts to replace it by chemically sound terminology have so far failed (Nieboer, 1980). As will be shown below, the term “heavy metals”, however defined, always covers an extremely disparate group of elements, and an even more disparate group of compounds of the elements. Thus, any assumption of any functional similarity in biological or toxicological properties is bound to be wrong (Duffus, 2002). Bjerrum classified “heavy metals” as those metals with elemental densities above 7 g/cm^3 (Duffus, 2002). Over the years, this definition has been modified by various authors and, accordingly, any idea of defining “heavy metals” on the basis of density must be abandoned as yielding nothing but confusion (Duffus, 2002).

2. SOURCES OF HEAVY METALS

Unlike most organic pollutants, heavy metals occur naturally in rock-forming and ore minerals; a range of normal background concentrations is found for each of these elements in soils, sediments, waters and living organisms. Significant quantities of various heavy metals are produced annually from mining of their respective ores. Worldwide demand for copper reached 16,600 kt in 2004 (US Geological Survey, 2004), up 6.8% from the previous year, according to copper producer Norddeutsche Affinerie AG (NA Group). Industrial growth in China-particularly in the copper-intensive transport and infrastructure sectors-accounted for much of the global growth in consumption. Chinese demand increased 13.5% in 2004 (US Geological Survey, 2004). The country's annual copper demand of 3,500 kt accounts for roughly 21% of global demand.

Copper consumption also grew in North America in 2004, rising 7% to 3,010 kt, while European demand increased 1.6% to 4,100 kt (US Geological Survey, 2004).

Industrial uses of metals and other domestic processes (e.g. burning of fossil fuels, incineration of wastes, automobile exhausts, smelting processes and the use of sewage sludge as landfill material and fertilizer) have introduced substantial amounts of potentially toxic heavy metals into the atmosphere and into the aquatic and terrestrial environments (O'Connell, 2008). Discharged toxic metals typically include Cd, Cu, Ni, Cr, Co, Zn and Pb (Babich *et al.*, 1985). **Table 5** outlines the industrial sources and the potential for pollution for a range of metals discharged.

3. TOXIC EFFECTS OF HEAVY METALS

Heavy metals are one of the most persistent pollutants in the environment. Unlike organic pollutants, they cannot be degraded, but accumulate throughout the food chain, producing potential human health risks and ecological disturbances (Chang, 1996 ; Lovley, 2000). Only bioavailable forms of heavy metals are toxic for biological systems and toxicity correlates positively with concentration. Therefore, biomonitoring seems to be an appropriate tool to detect discharges or the presence of these pollutants in the environment. Ecotoxicological bioassays to detect or evaluate heavy metal toxicity have been carried out using freshwater ciliates, especially members in the genus Tetrahymena or ciliates from wastewater treatment plants (Nilsson, 1989. Madoni *et al.*, 1994; Sauvant *et al.*, 1999; Nicolau *et al.*, 2001), which usually do not appear in soils. Listed in **Table 5** are some significant sources of metals in the environment (O'Connell, 2008). Data about heavy metal toxicity using soil ciliates are scarce (Forge *et al.*, 1993; Janssen *et al.*, 1995; Xu *et al.*, 1997). There are, however, several studies that used soil ciliates to test the toxicity in soil elutriates with different levels of heavy metal contamination (Forge *et al.*, 1993 ; Bowers *et al.*, 1997; Campbell *et al.*, 1997).

Table 5. Significant anthropogenic sources of metals in the environment

Industry	Metals	Pollution arising	References
Metalliferous mining	Cd, Cu, Ni, Cr, Co, Zn	Acid mine drainage, tailings, slag heaps	Babich *et al.* (1985) Aswathanarayana (2003)
Agricultural materials Fertilisers	Cd, Cr, Mo, Pb, U, V, Zn	Run-off, surface and groundwater contamination, plant bioaccumulation	Nicholson *et al.* (2003) Otero *et al.* (2005)
Manures sewage Sludge	Zn, Cu, Ni, Pb, Cd, Cr, As, Hg	Land spreading threat to ground and surface water	Cheung and Wong (1983) Nicholson *et al.* (2003) Walter *et al.* (2006)
Metallurgical industries Specialist alloys and steels	Pb, Mo, Ni, Cu, Cd, As, Te, U, Zn	Manufacture, disposal and recycling of metals. Tailings and slag heaps	Alloway and Ayres (1993) Cheng (2003) Rule *et al.* (2006)
Waste disposal Landfill leachate	Zn, Cu, Cd, Pb, Ni, Cr, Hg	Landfill leachate, contamination of ground and surface Water	Kjeldson *et al.* (2002) Fernandez *et al.* (2005)
Electronics	Pb, Cd, Hg, Pt, Au, Cr, As, Ni, Mn	Aqueous and solid metallic waste from manufacturing and recycling process	Veglio *et al.* (2003)
Metal finishing industry Electroplating	Cr, Ni, Zn, Cu	Liquid effluents from plating processes.	Zhao *et al.* (1999) ; Alvarez-Ayuso *et al.* (2003) Castelblanque and Salimbeni (2004)
Miscellaneous sources Batteries	Pb, Sb, Zn, Cd, Ni, Hg	Waste battery fluid, contamination of soil and groundwater.	EU Directorate general of the Environment (2004)
Paints and pigments	Pb, Cr, As, Ti, Ba, Zn	Aqueous waste from manufacture, old paint deterioration and soil pollution.	Barnes and Davis (1996) Davis and Burns (1999) Monken (2000)

The presence of excessive amounts of toxic metals in the body contributes to several health problems. These problems can be directly linked to the metal itself as well as the toxic load stressing the body. Never before has a society been so constantly exposed to such a wide array of potentially hazardous substances permeating every aspect of the environment; this exposure begins *in utero* and continues throughout life (Preventive Medicine Group. 2008). Such effects can be cumulative and multigenerational. Many people who are seemingly healthy also have build-ups that can, with time, contribute to the development of health problems.

Toxic metals are taken very seriously in alternative medicine; testing and treating them is as important as optimum nutritional intake and a healthy lifestyle. Tests for metal levels typically include hair and urine. Hair analysis gives an indication of levels of beneficial minerals as well. Urine testing, on the other hand, may offer a more complete picture, particularly with mercury, but hair analysis should not be relied upon as the sole indicator of whether or not a person has high levels of toxic metals (Preventive Medicine Group. 2008).

3.1. Common symptoms of heavy metal toxicity

While many of the heavy metals are needed by plants and humans at the micronutrient level, higher concentrations are known to produce a range of toxic effects. At high exposure levels, lead causes encephalopathy, cognitive impairment, behavioral disturbances, kidney damage, anaemia and toxicity to the reproductive system (Pagliuca and Mufti, 1990). Chromium is widely recognized to exert toxic effects in its hexavalent form (Rowbotham *et al.*, 2000). Human exposure to Cr(VI) compounds is associated with a higher incidence of respiratory cancers (IARC, 1990). Cadmium is associated with nephrotoxic effects particularly at high exposure levels; long-term exposure may cause bone damage as well (Friberg, 1985). High concentrations of mercury can lead to neurobehavioral disorders and developmental disabilities including dyslexia, attention deficit hyperactivity disorder, intellectual retardation (Weiss and Landrigan,

2000). Excessive copper concentrations can lead to weakness, lethargy and anorexia, as well as damage to the gastrointestinal tract (Theophanides and Anastassopoulou, 2002). The toxic effects of nickel and other heavy metals are discussed in some details by Nordberg *et al.* (2007).

3.2. Toxicity of Nickel

Major sources of nickel contamination are peanut butter, margarine, nickel plating, cigarette smoking, tea, batteries, wire and electrical parts, fertilizers, food processing, fuel oil combustion, hydrogenated fats and oils, imitation whipped cream, industrial waste, nuclear device testing, oysters, stainless steel cookware, unrefined grains and cereals, and vegetable shortening (Frank *et al.*, 1982; Salt *et al.*, 1998).

The human uptake of Ni from the ambient air is neglectably low, except in industrial exposures. The main fraction of human Ni uptake is from food, nearly 50% stems from vegetables. Only about 2% of the oral uptake of Ni are resorbed and distributed over all organs investigated (Beyersmann, 2006). Nickel is implicated in anorexia, apathy, fever, hemorrhages, headache, intestinal cancer, low blood pressure, muscle tremors, nausea, oral cancer, malaise, heart attack. Ni tends to accumulate in the kidneys causing kidney damage. It can also play some physiological role related to the functions of hormone, lipid and membrane metabolism.

3.3. Toxic effects of Copper

Sources of copper include copper cookware, copper pipes, dental alloys, fungicides, ice makers, industrial emissions, insecticides, swimming pools, water (city / well), welding, avocado, beer, bluefish, bone meal, chocolate, corn oil, crabs, gelatin, grains, lamb, liver, lobster, margarine, milk, mushrooms, nuts, organ meats, oysters, perch, seeds, shellfish, soybeans, wheat germ, yeast (Xin *et al.*, 1991)

General symptoms of copper poisoning are adrenal insufficiency, allergies, alopecia, anemia, anorexia, anxiety, arthritis (osteo and rheumatoid), autism, cancer, chills, cystic fibrosis,

depression, diabetes, digestive disorders, dry mouth, dysinsulinism, estrogen dominance, fatigue, fears, fractures, fungus, heart attack, high blood pressure, high cholesterol, Hodgkin's disease, hyperactivity, hyperthyroid, low hydrochloric acid, hypoglycemia, infections, and inflammation among others (Michael *et al.*, 2007). Copper buildup can result in a tendency for hyperactivity in autistic children. It can cause stuttering, insomnia, and hypertension. An excess of copper can cause oily skin, loss of skin tone (due to its ability to block vitamin c), and can cause a dark pigmentation of the skin, usually around the face. It can also cause nails to be brittle and thin. It can contribute to the control of hair growth and loss (Pyo *et al.*, 2007).

3.4. Lead toxicity

Lead is the most common environmental pollutant. Its symptoms include hypertension, fatigue, hemolytic anemia, abdominal pain, nausea, constipation, weight loss, peripheral neuropathy, cognitive dysfunction, arthralgias, headache, weakness, irritability, impotence, loss of libido, depression, depression of thyroid and adrenal function, chronic renal failure, gout, behavioral disorders. At high exposure levels, lead causes encephalopathy, cognitive impairment, behavioural disturbances, kidney damage and toxicity to the reproductive system (Pagliuca and Mufti, 1990). A patient with lead poisoning may have a combination of symptoms - or no symptoms at all until the condition has progressed.

3.5. Iron toxicity

Sources of iron are drinking water, iron cookware, iron pipes, welding, Foods such as blackstrap molasses, bone meal, brain, chives, clams, heart, kidney, leafy vegetables, legumes, liver, meat, molasses, nuts, organ meats, oysters, and parsley (Hallberg, 1986; Roberts, 1999). Iron deficiency is a problem but too much iron in the tissues and organs leads to the production of free radicals. High levels of iron have been found in association with heart disease and cancer. Ingestion accounts for most of the toxic effects of iron because iron is absorbed rapidly in the

gastrointestinal tract. The corrosive nature of iron seems to further increase the absorption. Most overdoses appear to be the result of children mistaking red-coated ferrous sulfate tablets or adult multivitamin preparations for candy. Fatalities from overdoses have decreased significantly with the introduction of child-proof packaging (Roberts, 1999).

Symptoms and diseases caused by overdose of iron are amenorrhea, anger, rheumatoid arthritis, birth defects, bleeding gums, constipation, diabetes, dizziness, emotional problems, fatigue, headache, heart damage, heart failure, hepatitis, high blood pressure, hostility, hyperactivity, infections, insomnia, irritability, joint pain, liver disease, loss of weight, mental problems (Schauben *et al.*, 1990).

3.6. Toxic effects of Zinc

Zinc is an environmental pollutant and omnipresent in the environment (Ragaini *et al.*, 1977; Weltje, 1998). Millions of tons of zinc metal are used commercially, principally to galvanize iron and to manufacture brass (Barceloux, 1999). It is also used widely in preservative treatment, fungicidal action and medicine, etc. (Barceloux, 1999). Natural water supplies usually contain only trace amounts of zinc, but the concentration may be increased if the water flows through galvanized, copper or plastic pipes (Llobet *et al.*, 1988).

It is well known that zinc is an essential trace element and has important biological functions that control many cell processes including DNA synthesis, normal growth, brain development, behavioral response, reproduction, fetal development, bone formation, and wound healing (Calesnick and Cla, 1988; Barceloux, 1999). Zinc deficiency results in growth retardation, testicular atrophy, skin changes, and suppressed appetite, etc. (Calesnick and Cla, 1988; Hoffman *et al.*, 1988; Prasad, 1991).

Zinc or zinc salts may enter the body by inhalation, through the skin or by ingestion and induce irritation of the respiratory or digestive system, and dental deterioration and ulceration of

the skin, and zinc fumes cause fever, chills nausea and vomiting, and muscular aches and weakness (Barceloux, 1999).

4. REMOVAL OF HEAVY METALS

Various methods exist for the removal of heavy metal ions from wastewater which include chemical precipitation / coagulation, membrane technology, electrolytic reduction, ion exchange and adsorption (Wang *et al.*, 2003; Kurniawan *et al.*, 2006). The advantages and disadvantages associated with each method are listed in **Table 6**.

The most widely used method of removing heavy metals from solution is to increase the pH of the effluent, thus converting the soluble metal into an insoluble form (i.e. its hydroxide). Ion exchange is the second most widely used method for heavy metal removal from aqueous streams (Shukla *et al.*, 2002). During removal, recovery, or processing of metals, ion exchange acts as a concentrator of metals. The chemistry of the effluent stream becomes very important to the success of the ion exchange application.

Coagulation–flocculation can also be employed to treat wastewater laden with heavy metals wherein the coagulation process destabilizes colloidal particles by adding a chemical agent (coagulant) and results in sedimentation (Wang *et al.*, 2004). Coagulation is followed by flocculation of the unstable particles in order to increase their size and form into bulky floccules which can be settled out. Flotation is employed to separate solids or dispersed liquids from a liquid phase using bubble attachment (Wang *et al.*, 2004). Adsorptive bubble separation employs foaming to separate the metal impurities. Ion flotation, precipitate flotation and sorptive flotation are the main fotation process mechanisms for removal of metal ions from solution. Membrane Filtration has received considerable attention for the treatment of inorganic effluent, since it is capable of removing not only suspended solids and organic compounds, but also inorganic contaminants such as heavy metals.

Table 6. Technologies for the removal of heavy metals from wastewaters and associated advantages and disadvantages.

Technology	Advantages	Disadvantages	Reference
Chemical precipitation	Process simplicity Not metal selective Inexpensive capital cost	Large amount of sludge containing metals Sludge disposal cost High maintenance costs	Aderhold *et al.*(1996)
Ion exchange	Metal selective Limited pH tolerance High regeneration	High initial capital cost High maintenance cost	Aderhold *et al.*(1996)
Coagulation–flocculation	Bacterial inactivation capability Good sludge settling and dewatering characteristics	Chemical consumption Increased sludge volume generation	Aderhold *et al.*(1996)
Flotation	Metal selective Low retention times Removal of small particles	High initial capital cost High maintenance and operation costs	Rubio *et al.* (2002)
Membrane filtration	Low solid waste generation Low chemical consumption Small space requirement Possible to be metal selective	High initial capital cost High maintenance and operation costs Membrane fouling Limited flow-rates	Qin *et al.* (2002) Madaeni and Mansourpanah (2003)
Electrochemical treatment	No chemical required can be engineered to tolerate suspended solids Moderately metal selective Treat effluent > 2000 mg dm^{-3}	High initial capital cost Production of H_2 (with some processes) Filtration process for flocs	Kongsricharoern and Polprasert (1995) Kongsricharoern and Polprasert (1996)
Adsorption	Wide variety of target pollutants High capacity Fast kinetics Possibly selective depending on adsorbent	Performance depends on type of Adsorbent Chemical derivatisation to improve its sorption capacity	Crini (2005)

Amongst all the treatment processes mentioned, adsorption using sorbents is one of the most popular and effective processes for the removal of heavy metals from wastewater. The adsorption process offers flexibility in design and operation and in many cases produces treated effluent suitable for re-use, free of color and odor. In addition, because adsorption is sometimes reversible, regeneration of the adsorbent with resultant economy of operation may be possible (Kelleher,

2001). Activated carbon adsorbents are used widely in the removal of organic contaminants and to lesser extent, heavy metal contaminants in product purification and pollution control. Carbon is converted to activated carbon by heating in the absence of air. The activation process results in the creation of a network of fine pores in the carbon particles. In spite of its prolific use, activated carbon remains an expensive material and the higher the quality of activated carbon, the higher its cost. In practice activated carbon is employed more frequently for adsorption of organic compounds rather than heavy metal ions. **Table 7** lists some commercially available adsorbents and ion exchange materials.

Table 7. Cu(II) adsorption levels on commercial adsorbent/ion exchange materials

Adsorbent / ion exchanger Commercial adsorbents	**Chelating group**	**Cu(II) Uptake (mg g^{-1})**	**Reference**
Dowex 50WX4	Sulphonic acid	71. 4	Cochrane *et al.* (2006)
Amberlite IRC-86	Carboxylic	130	Marshall and Wartelle. (2006)
Duolite GT-73	Thiol	61.6	Vaughan *et al.* (2001)
Amberlite IRC-718	Iminodiacetic acid	127	Vaughan *et al.* (2001)
Amberlite 200	Sulphonic acid	89	Vaughan *et al.* (2001)
Lewatit TP207	Iminodiacetic acid	85	Brown *et al.* (2000)

CHAPTER 3. HUMAN IMMUNOGLOBULINS G

1. DEFINITION

Immunoglobulins (Ig) are glycoprotein molecules produced by plasma cells in response to an antigen and which function as antibodies. The immunoglobulins are also called gamma globulins because they migrate, in basic conditions, with globular proteins when normal serum is placed in an electrical field. The basic structure of IgG is illustrated in **Figure 16**. Although Immunoglobulins can differ structurally, they all are built from the same basic units (Meyer, 2008).

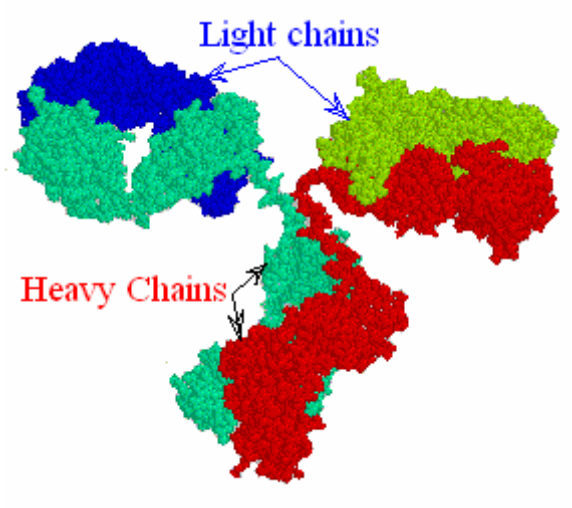


Figure 16: Basic structural unit of an immunoglobulin.

2. HEAVY AND LIGHT CHAINS

All Immunoglobulins have a four-chains structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD). The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions (Voet, *et al.*, 1999). The number of inter-chain disulfide bonds varies among

different immunoglobulin molecules. Within each of the polypeptide chains there are also intra-chain disulfide bonds. Each heavy and light chain could be divided into two regions based on variability in the amino acid sequences. These are the light chain V_L (110 amino acids) and C_L (110 amino acids) and the heavy chain V_H (110 amino acids) and C_H (330-440 amino acids). The region at which the arms of the antibody molecule forms a Y is called hinge region because there is some flexibility in the molecule at this point (Stewart, 1984).

Three dimensional images of the immunoglobulin molecule show that it is not straight as depicted in Figure 2A, rather, it is folded into globular regions each of which contains an intra-chain disulfide bond (**Figure 17**); these regions are called domains. Light chain contains V_L and C_L domains while a heavy chain contains V_H, C_H1-C_H3 (or C_H4). Carbohydrates are attached to the C_H2 domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

3. FRAGMENTS: STRUCTURE/FUNCTION RELATIONSHIPS

Immunoglobulin fragments produced by proteolytic digestion have proven very useful in elucidating structure/function relationships in immunoglobulins.

Digestion with papain breaks the immunoglobulin molecule in the hinge region before the S-S inter-chain disulfide bond **(Figure 17**). This results in the formation of two identical fragments that contain the light chain and the V_H and C_{H1} domains of the heavy chain. These fragments are called antigen binding fragments (Fab) and each Fab fragment is monovalent whereas the original molecule was divalent.

Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a C_H2 and C_H3 domain (Adamczyk *et al.*, 2000). This fragment is called Fc because it was easily crystallized. The effector functions of immunoglobulins are mediated by this part of the molecule (Voet *et al.*, 1999). Different functions are mediated by the different

domains in this fragment. Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen.

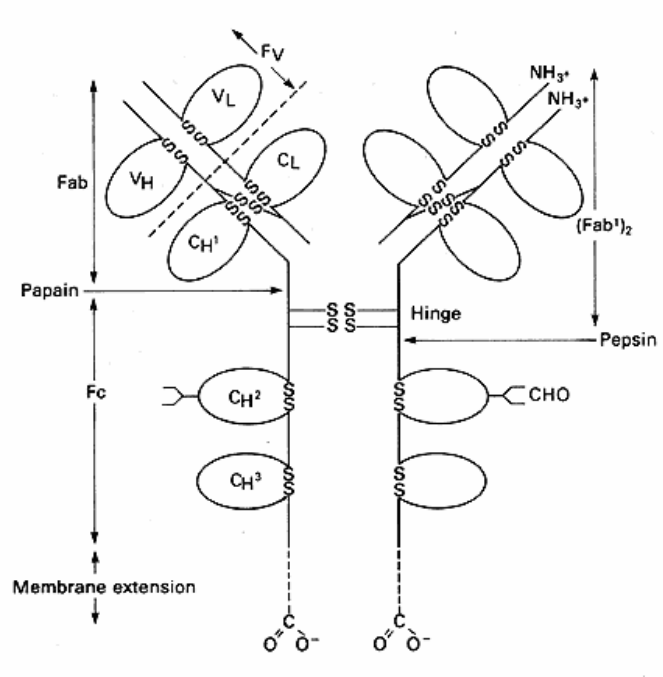


Figure 17: Structure of an IgG molecule.

Treatment of IgG with pepsin results in cleavage of the heavy chain after the S-S inter-chain disulfide bonds to give a fragment that contains both antigen binding sites. This fragment is called $F(ab')_2$ because it is divalent. The Fc region of the molecule is digested into small peptides by pepsin (Kurkela *et al.* 1988; Hagmann *et al.*, 1998).

4. STRUCTURE AND SOME PROPERTIES OF IgG SUBCLASSES

IgG subclasses are IgG1, IgG2, IgG3 and IgG4 with Gamma 1, 2, 3 and heavy chains, respectively (Plebani *et al.*, 1989). IgG is the most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules. It represents more than 75% of serum Ig and is the major Ig in extra vascular spaces and IgG is the only class of Ig that crosses the placenta. Except for IgG4 all IgG subclasses fix the complement; IgG binds to cells like macrophages, monocytes, polymorphonuclears (PMN's) and some lymphocytes have Fc receptors for the Fc region of IgG (Shakib and Stanworth, 1980). Subclasses bind differently to Fc receptors

and IgG2 and IgG4 do not bind at all. A consequence of binding to the Fc receptors on PMN's, monocytes, macrophages and dendritic cells (Amigorena, 2002) is that the cell can now internalize the antigen better. The antibody has prepared the antigen for eating by the phagocytic cells. The term opsonin is used to describe substances that enhance phagocytosis; IgG is a good opsonin. Binding of IgG to Fc receptors on other types of cells results in the activation of other functions (Amigorena and Bonnerot, 2006).

Quantitatively, the relative serum concentrations of the human IgG subclasses are as follows (Plebani *et al.*, 1989): IgG1 > IgG2 > IgG3 = IgG4. The four subclasses show more than 95% homology in the amino acid sequences of the constant domains of the γ-heavy chains. The four IgG subclasses, however, show their most differences in the amino acid composition and structure of the 'hinge region', which is the part of the molecule containing disulfide bonds between the γ-heavy chains (**Figure 18**). This region, between the Fab arms (Fragment antigen binding) and the two carboxy-terminal domains CH_2 and CH_3) of both heavy chains, determines the flexibility of the molecule (Murphy *et al.*, 2007). In the schematic structure of IgG, shown in **Figure 18**, it is clear that the molecule contains domain-like structures, in which the two identical antigen-binding

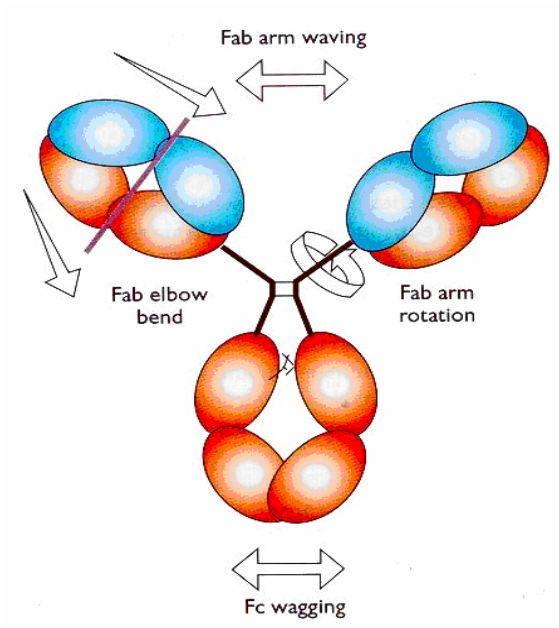


Figure 18. Flexibility of IgG molecule (Meulenbroek and Zeijlemaker, 1996).

Fab fragments and the single Fc fragment (fragment crystallisable) are quite mobile.

The upper hinge (toward the amino-terminal) segment allows variability of the angle between the Fab arms (Fab-Fab flexibility) as well as rotational flexibility of each individual Fab. The flexibility of the lower hinge region (towards the carboxy-terminal) directly determines the position of the Fab-arms relative to the Fc region (Fab-Fc flexibility). Hinge-dependent Fab-Fab and Fab-Fc flexibility may be important in triggering further effector functions such as complement activation and Fc receptor binding, and probably, the bond to polymers used in the purification of IgG (Meulenbroek and . Zeijlemaker, 1996).

The length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 encompasses amino acids 216-231 and since it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges (Pilz *et al.*, 1980). IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue and is relatively short and contains a rigid poly-proline double helix stabilised by extra inter-heavy chain disulfide bridges; these properties restrict the flexibility of the IgG2 molecule. IgG3, on the other hand, differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge); it contains 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix (Ledbetter *et al.*, 2007). In IgG3 the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2.

Another structural difference between the human IgG subclasses is the linkage of the heavy and light chain by a disulfide bond. This bond links the carboxy-terminal of the light chain with the cysteine residue at position 220 (in IgG1 or at position 131 (in IgG2, IgG3 and IgG4) of the

CH_1 sequence of the heavy chain (Williams, 2008). Because in the folded structure these positions are close in space, they preserve the essential structure of the molecule.

In addition to differences among genes encoding the IgG subclass proteins, each with different amino acid composition and derived properties, mutations within these genes have led to variations of the composition of IgG subclass proteins within the population. The latter mutations provide the basis for genetic markers called Gm allotypes (Nakao *et al.*, 1984) and correspond with minor differences in primary amino acid sequence between molecules of one IgG subclass that occur throughout a species. These allotypic determinants are polymorphic epitopes, which are inherited in a Mendelian pattern. Among individuals, different allelic forms are expressed. At present, immunoglobulin G can be typed for 18 different allotypes, located on the heavy chain. Subclass IgG3 is the most polymorphic, with thirteen Gm3 allotypes (Meulenbroek and Zeijlemaker, 1996). There are four IgG1 allotypes and one IgG2 allotype, whereas no allotypes have been detected on the heavy chains of IgG4. Allotyping of immunoglobulins can be of diagnostic use in family and parenthood investigations and can have a role in asthma and allergy affections (Oxelius, 2008).

As a consequence of the structural differences, the four IgG subclasses show differences in some of their physicochemical characteristics and biological properties.

5. PURIFICATION OF IgG

Because of the importance of IgG as the major antibodies in the biotechnology industry, they are purified from various sources with different chromatographic techniques, including high-performance liquid chromatography, size exclusion chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, histidine affinity chromatography, and thiophilic chromatography (Pery *et al.*, 1984; Burshiel *et al.*, 1984; Pavlu *et al.*, 1986; Yang *et al.*, 1996; Özkara *et al.*, 2002; Garipcan *et al.*, 2002; Özkara *et al.*, 2003). However, antibodies are purified by affinity chromatography because of its high selectivity. Among the affinity

techniques, protein A affinity chromatography is a well-known and popular method for purifying antibodies (Langone, 1982). Protein A binds with different affinities to the Fc region of immunoglobulins from a variety of sources; for example, it binds to human, rabbit, and pig immunoglobulin G (IgG) with high affinity, to horse and cow IgG with lower affinity, and to rat IgG only very weakly. It exhibits a very high specificity and can, therefore, be employed as a one-step procedure for the purification of antibodies. Because of this specificity, protein A chromatography is now commonly used on a pilot scale for the purification of immunoglobulins to be used in clinical tests and therapy (Füglistaller, 1989). However, despite its high selectivity, protein A chromatography also has some drawbacks that are worth considering: (1) a considerable amount of protein A may be released from the matrix and such contamination cannot, of course, be tolerated in clinical applications, and (2) the cost of these biomolecules tends to be very high (Denizli and Piskin, 1995). In addition, ligands such as protein A and protein G are difficult to immobilize in the proper orientation. Bioaffinity chromatography on immobilized lectins has been extensively used to purify glycoproteins (Saleemuddin and Husain, 1991; Mislovicova *et al.*, 1995; Turkova, 1999).

Considerable attention has been paid to alternative methods, for instance, when a special procedure is required in the field of veterinary or human medicine (Guse, 1994). General adsorption technique, using high performance liquid chromatography (HPLC), is attractive in this respect. Recent works have shown that high performance ion exchange and high performance size exclusion have the potential for rapid isolation and/or purification of monoclonal and polyclonal antibodies from different range of biological material (Gallo, 1986; Carty, 1988). Hydroxyapatite adsorption chromatography is very useful for simple and rapid fractionation of proteins, but has intrinsic limitations for routine use, and is difficult to scale-up for IgG purification from serum of animal species (Jungbauer, 1989; Josic, 1991). Some authors have demonstrated that IgG from human serum could be partially purified on an anion-exchange Mono Q column followed by gel

filtration on superose column (Gallo, 1986; Luo, 2002). Recently, a mixed mode ion-exchange chromatography matrix, utilizing silica gel as the support, was used for the rapid purification of immunoglobulins. This chromatographic matrix has demonstrated little or no affinity for albumin, transferrins, proteases or pH indicator dyes from tissue and culture media (Hwang, 1988).

6. PURIFICATION OF IMMUNOGLOBULIN G USING METAL-CHELATE AFFINITY CHROMATOGRAPHY

Immobilized metal-chelate affinity chromatography (IMAC) is widely used for protein purification. Transition-metal ions can form stable complexes with electron-rich compounds and may coordinate molecules containing oxygen, nitrogen, and sulfur by ion–dipole interactions. Metal-complexing ligands are first-row transition-metal ions incorporated by iminodiacetic acid, nitrilotriacetic acid, amino salicylic acid, and carboxymethylated amino acids (Johnson *et al.* 1996). IMAC introduces a new approach for selectively interacting materials on the basis of their affinities for metal ions. The separation is based on the differential binding abilities of the proteins to interact with chelated metal ions to a support (Denizli *et al.* 1999; Zhang *et al.*2000). The dominating electron-donating group in a protein is the imidazole side chain of histidine, whereas the N-terminal of the protein contributes to a lesser extent. In addition, the thiol group of cysteine would be a good electron donor, but it is rarely present in the appropriate reduced state (Arnold, 1991). The number of histidine residues in the protein is of primary importance in the overall affinity for immobilized metal ions. In addition, factors such as the accessibility, microenvironment of the binding residue, cooperation between neighboring amino acid side chains, and local conformations play important roles in biomolecular adsorption (Patwardhan *et al.*, 1997). Aromatic amino acids and the amino terminus of the peptides also have some contributions (Yip *et al.*). The low cost of metals and the ease of regeneration of the supports are the attractive features of metal affinity separation.

CHAPTER 4: MATERIALS AND METHODS

1. REAGENTS

Unless otherwise indicated, all chemicals were obtained from commercial sources and were used as received without further purification. The ion exchange resin containing iminodiacetic acid groups, Amberlite IRC-718 was purchased as a sodium salt from Rohm and Haas Company (USA). This resin has a bulk density of 750 g/L, swelling (%) 30, total exchange capacity 1.35 meq/ml and particle size of 16–50 mesh. The metal ion salts were also used without further purification: copper(II) acetate dehydrate (98%), $(CH_3COO)_2Cu$, $2H_2O$ and iron(II) chloride, $FeCl_2$ from Fluka, nickel(II) acetate tetrahydrate, $(CH_3COO)_2Ni$, $4H_2O$ from BDH (Poole, England) and zinc(II) acetate dehydrate, $(CH_3COO)_2Zn$, $2H_2O$ from Riedel de Haen (Seelze, Germany). Pure human IgG, Tris (Hydroxy methyl-aminomethane) and Morpholinopropane sulfonic acid were purchased from Sigma (Germany). Coomassie Brillant Blue G250 (CBB), HCl, NaOH and chemicals used in polyacrylamide gel electrophoresis were from Merck (Germany).

2. METHODS

2.1. Infrared Spectrophotometer

Infrared spectra of IRC-718 and IRC-718-metal ion complexes were recorded with Nicolet Impact 400 Fourier transform infrared spectrophotometer (FTIR) from 400 to 4000 cm^{-1}. KBr discs were used for all of solid samples by the mixture of 1.0 mg of the sample with about 100 mg of KBr.

2.2. Preparation of buffer solutions

A series of buffer solutions was prepared from sodium acetate, acetic acid to obtain pH values ranging from 2.0 to 7.0. The desired pH value was achieved by changing sodium acetate to acetic acid ratio according to the following equation:

pH = pKa + log [acetate]/[acetic acid]

2.3. Sorption of the metal ions on the polymer

The metal chelation characteristics of the resin for each metal ion were studied by the batch equilibrium technique. Duplicate experiments involving 0.100 g of dry resin samples were suspended in 25 ml of sodium acetate-acetic acid buffer adjusted to the desired pH with continuous shaking and left for 2 h to equilibrate. To this mixture, 25 ml of metal ion solution containing a total of 15 mg metal-ion were added. After being shaken for a definite period of time at 25 °C, the mixture was filtered, and the amount of metal ion remaining in the filtrate was determined by atomic absorption spectrometry (AAS), with the aid of a Varian Atomic Absorption Spectrophotometer Model AA-250 plus (Mulgrave, Victoria Australia). Samples were shaken using a GFL-1083 shaker thermostated water bath (Burgwedel, Germany), using standard solutions for calibration.

2.4. Effect of time and pH on ion uptake

The extent of metal-ion uptake was studied under similar experimental conditions, where the contact time was varied from 0.5 to 24 h at 25 °C after the solution was equilibrated with distilled water. Similar experiments were also carried out in buffered solutions, in which the pH was varied between 2.0 and 7.0 for a fixed contact time of 6 h.

2.5. Effect of resin mass on ion uptake

The effect of resin mass on the metal-ion uptake was also studied using the same general procedure by shaking a suspension of 0.1, 0.2, 0.3, 0.6, 0.8, or 1.0 g of the dry resin in 25 ml of the acetate buffer solution at pH 6.0 for 2 h. To this mixture, 25 ml of buffer solution containing 15 mg of metal-ion were added. The mixtures were then shaken at 25 °C for 6 h, filtered, and the amount of metal ion remaining in solution was determined by atomic absorption spectrophotometry maintained at 25 °C.

2.6. Effect of metal-ion concentration on ion uptake

The effect of metal-ion concentration was investigated in a similar manner in buffer solutions containing 0.10 g of dry resin and variable amounts of metal ions at 25°C and a fixed contact time of 6 h.

2.7. Desorption and repeated use

Desorption of metal ions was carried out in 3 M HCl solution. The chelated resin was immersed in 3 M HCl solution and stirred for 60, 180 and 360 min. Then it was immersed in 0.5 M NaOH solution and stirred for 20 min to convert carboxylic acids to sodium carboxylate. The resin was filtered and washed thoroughly with distilled water for further use. The amount of metal ion desorbed was determined by atomic absorption spectrometry.

2.8. Preparation of human plasma

Blood samples from healthy donors were collected on anticoagulant (129 mM sodium citrate). The samples were centrifuged for 5 min at 3000 rpm with a Sigma 3K30 C centrifuge at 4 °C and the supernatant was used or stored at -20 °C until use without further treatment.

2.9. Preparation of immunoglobulin G (IgG) human

Purification of IgG human was achieved by means of ammonium sulphate precipitation; precipitation was performed at 4°C. Equal volumes of diluted serum and saturated ammonium sulphate were mixed by slow addition of the ammonium sulphate solution with gentle stirring.

2.10. Chromatographic experiments

The column (1x10cm) packed with the resin saturated with metal ions was equilibrated with adsorption buffers (Tris/HCl 25mM) pH 7.0 (equilibration buffer). One milliliter of human plasma (about 60 mg of protein) was diluted (1:5) with the equilibration buffer and loaded into the column (bed volume of 5 ml). For the experiments with pre-purified human IgG, protein samples containing 1.17 to 1.83 mg of IgG diluted in 2 ml of equilibration buffer was loaded into the column. For both experiments; after protein injection, the column was washed with equilibration buffers until protein was not detected in the column out-stream by adsorption at 280 nm. Adsorbed proteins were subsequently eluted with discontinuous step gradient of Tris/HCl 25 mM at pH 7 to pH 4 in the second experiment. Regeneration of the column (total removal of remaining proteins) was achieved by washing the column with 0.5 M NaOH for both experiments. All chromatographic procedures were carried out at ambient temperature at flow rate equal 0.5 ml/min.

2.11. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Purity and protein contents of different peaks obtained by chromatography were checked using polyacrylamide gel electrophoresis 7.5 % in presence of sodium dodecyl sulphate (SDS) as described by Laemmli (1970). Samples were dissociated, by heating the solution containing SDS at 100 °C for 5 min. The migration was started at 10 mA until total entry of proteins in the concentration gel (5% acrylamide gel) then the current was increased to 40 mA. When the migration finished, the gel was coloured in Coomassie Brillant Blue R 250 solution (0.1 % in

acetic acid / methanol / water; 10/20/70) for one hour then the gel was decoloured in acetic acid / water (10/90) solution.

2.12. Protein estimation

Protein estimation was carried out according to the method firstly described by Bradford (1976) and modified by Macart and Gerbaut (1982), which is based on the binding of Coomassie Brillant Blue (CBB) G250 dye to proteins in presence of ethanol at low pH. A linear relationship between A_{595} and protein concentration is obtained using sodium dodecyl sulphate. Bovine serum albumin (BSA) was used as standard to build out the regression curve. The standard is diluted (0.1 – 1.5 mg/ml) in the same buffer used for diluting samples to be tested. Samples at appropriate dilution (100µl) were added to the reactive protein assay reagent (1.9 ml), mixed gently several times by inversion and left for 10 min. to allow colour development. The absorbance at 595 nm was determined and protein concentration was calculated from a linear calibration curve **(Figure 19**).

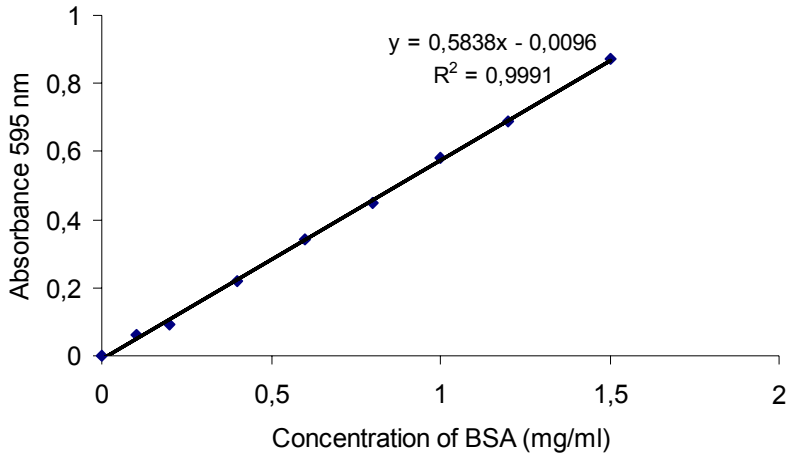


Figure 19. Calibration curve for protein estimation using bovine serum albumin as standard.

CHAPTER 5: RESULTS AND DISCUSSION

1. SORPTION PROPERTIES OF IMINODIACETATE ION EXCHANGE RESIN TOWARD DIVALENT METAL IONS

The resin used in this work is Amberlite IRC-718 which is an iminidiacetic acid polymer. This resin is available as Na form where Na can be exchanged with metal ion as shown in the following equation:

$$RCH_2N(CH_2COONa)_2 + M^{n+} \rightarrow RCH_2N(CH_2COO)_2M + nNa^+$$

The metal ions (M^{n+}) adsorbed on the resin can then be eluted with different eluents. After the metal elution was complete the resin was regenerated to its hydrogen form using mineral acids such as HCl:

$$RCH_2N(CH_2COO)_2M + nHCl \rightarrow RCH_2N(CH_2COOH)_2 + M^{n+}$$

The resin in its hydrogen form is then converted to the Na^+ form by passing a dilute sodium hydroxide solution on it. Thus, the hydrogen ion in the resin gets exchanged with the sodium ions. The resin in sodium form is then washed with distilled water and is recycled back to the system.

$$RCH_2N(CH_2COOH)_2 + 2NaOH \rightarrow RCH_2N(CH_2COO\,Na)_2 + 2H_2O$$

1.1. Infrared (IR) spectroscopy

Infrared (IR) spectroscopy provides a powerful tool to locate the coordination or the binding sites of metal ions with the resin as well as to examine the presence or absence of some characteristic groups in polymeric ligands.

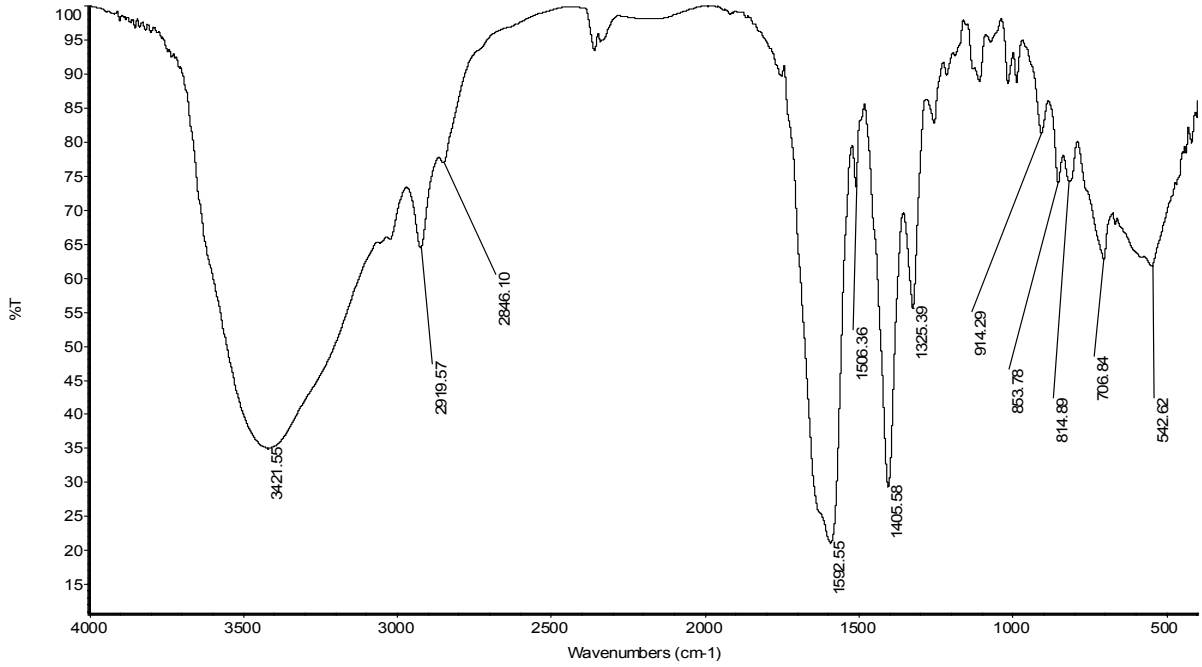
Table 8 shows different absorption bands expressing diverse functional groups of cheating covalently grafted on the backbone of polymer. The examination of the absorption C-N band of the chelating polymer and its of Fe^{2+} , Ni^{2+}, Cu^{2+} and Zn^{2+} complexes, which are respectively 1186, 1167, 1182 and 1204 cm^{-1}. Concerning the absorption bands of carbonyl groups, we note a heat increasing of its frequency when the chelating polymer coordinates to the transition metals ions mentioned above. Then, frequencies shift from 1593 cm^{-1} for the chelating polymer to 1636, 1601, 1620, 1604 $cm^{-1}$of Fe^{2+} , Ni^{2+}, Cu^{2+} and Zn^{2+} complexes, respectively.

Table 8. IR adsorption bands for the polymer loaded with, metal- ions

Probable Assignment	Typical range(cm^{-1})	Resin	Resin-Cu^{+2}	Resin-Ni^{+2}	Resin-Zn^{2+}	Resin-Fe^{2+}
C-O stretching	1080-1300	1242.42	1084.84	1095.80	1106.60	1083.33
C-N stretching	1180-1360	1234.84	1181.81	1166.66	1204.45	1186.39
C=O stretching	1540-1870	1592.55	1619.88	1601.21	1603.65	1636.40

Moreover, the frequencies of C-O bands showed a decrease to lower frequencies than chelating polymer. The absorption bands obtained for the four complexes are 1083, 1096, 1085 and 1107 cm^{-1} for Fe^{2+} , Ni^{2+}, Cu^{2+} and Zn^{2+} complexes, respectively. Finally, it is concluded that

modification occurred on the chelating polymer when it coordinates to the metal ions. **Figures 20-24** are FTIR spectra of the polymer before and after the coordination with metal ions.



Fig

Figure 20. IR spectrum resin

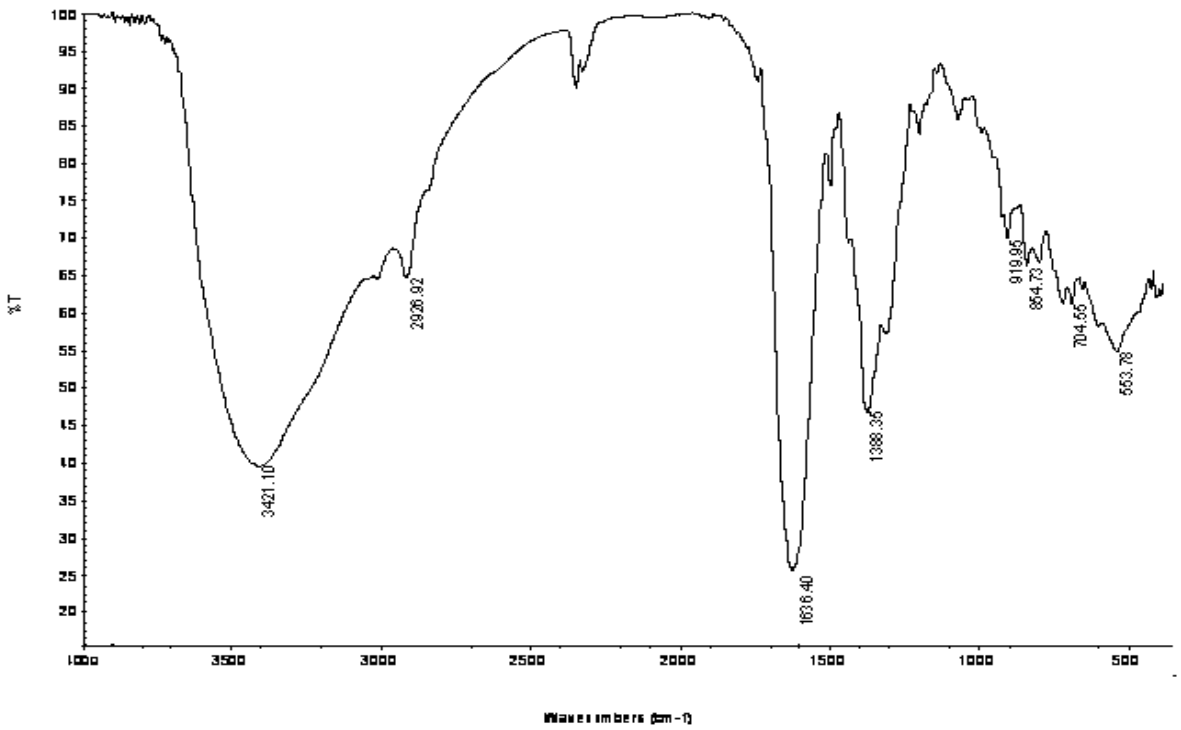


Figure 21 R spectrum resin-Fe(II)

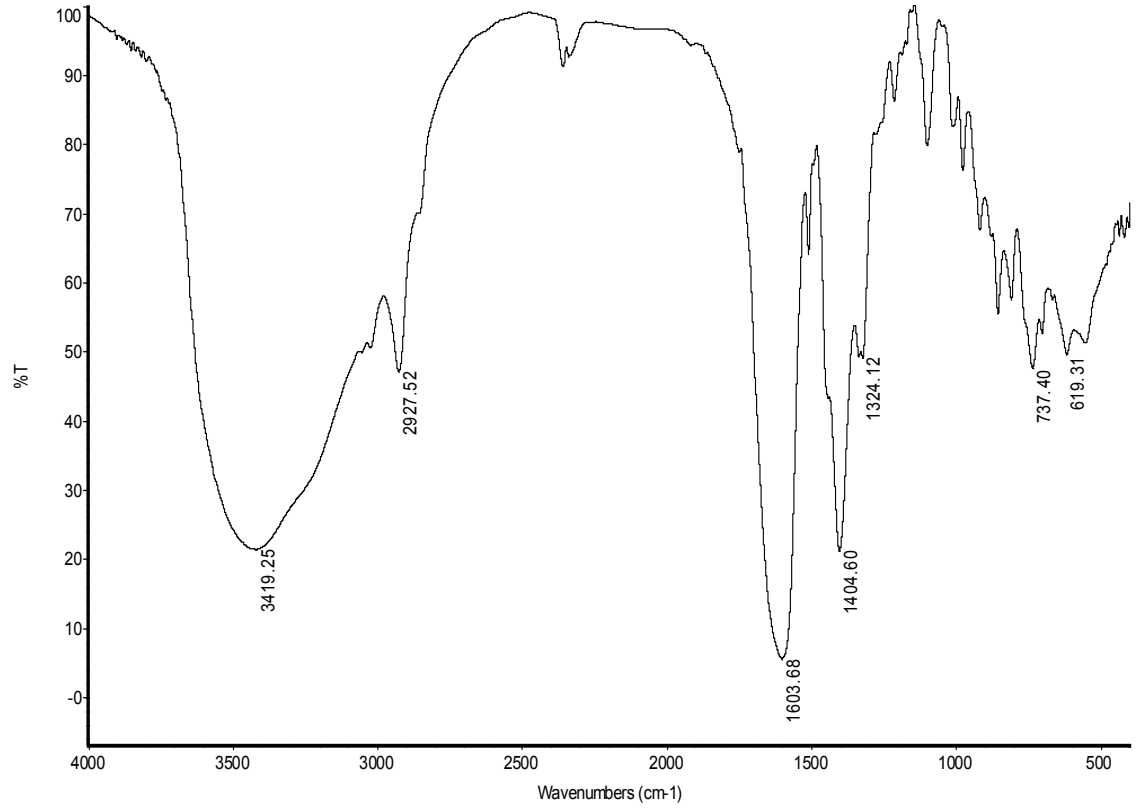


Figure 22. IR spectrum resin-Zn(II)

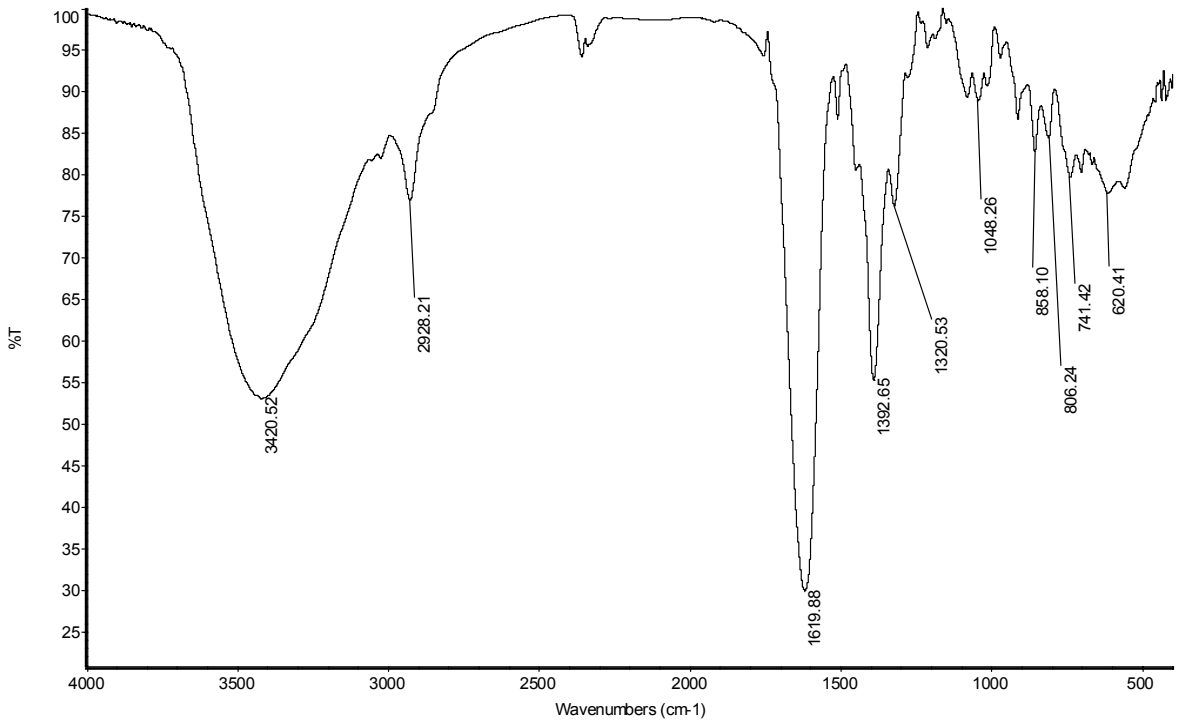


Figure 23. IR spectrum of resin-Cu(II)

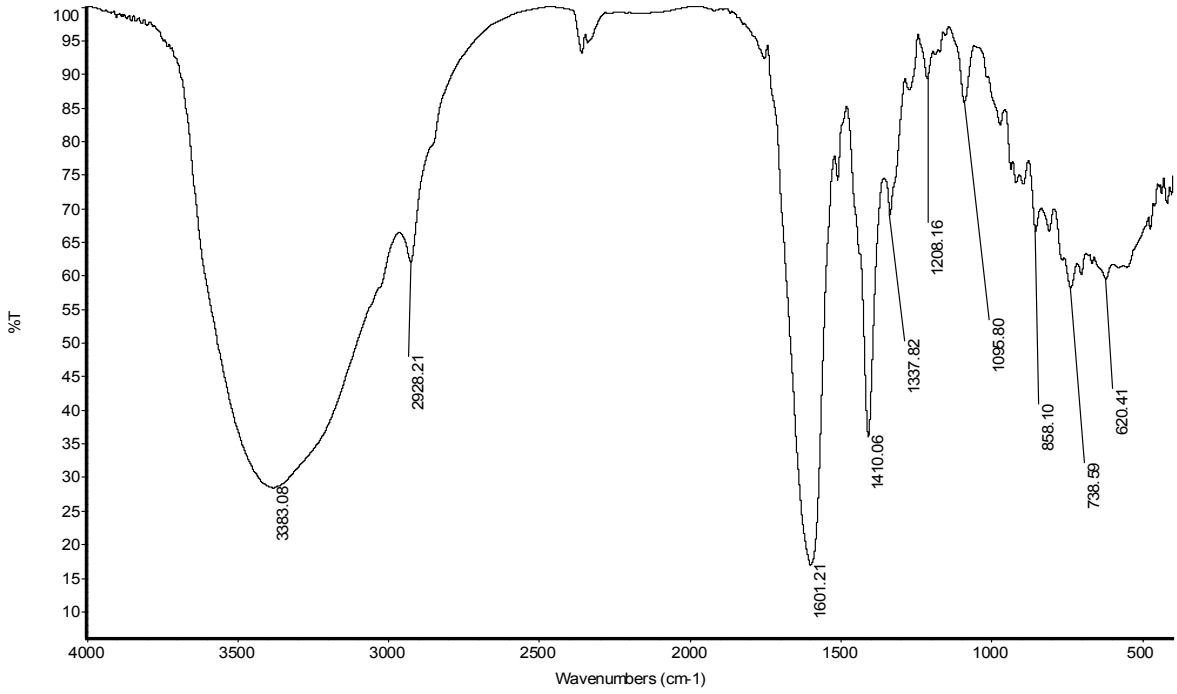


Figure 24. IR spectrum of resin-Ni(II)

1.2. RATE OF METAL-ION UPTAKE AS A FUNCTION OF CONTACT TIME

The sorption of various divalent metal ions (Ni^{+2}, Cu^{+2}, Fe^{+2}, and Zn^{+2}) on Amberlite IRC-718 iminodiacetate chelating ion exchanger as a function of contact time was investigated by a batch equilibrium technique. The results for the dependence of the metal ion uptake on contact time for resin are presented in **Figure 25**. The results indicate fast rates of equilibration; the rates of metal-ion uptake increase in the first 3 h and a steady state is reached within 5–10 h. For Fe(II), about 99% of metal-ion was achieved in the first hour.

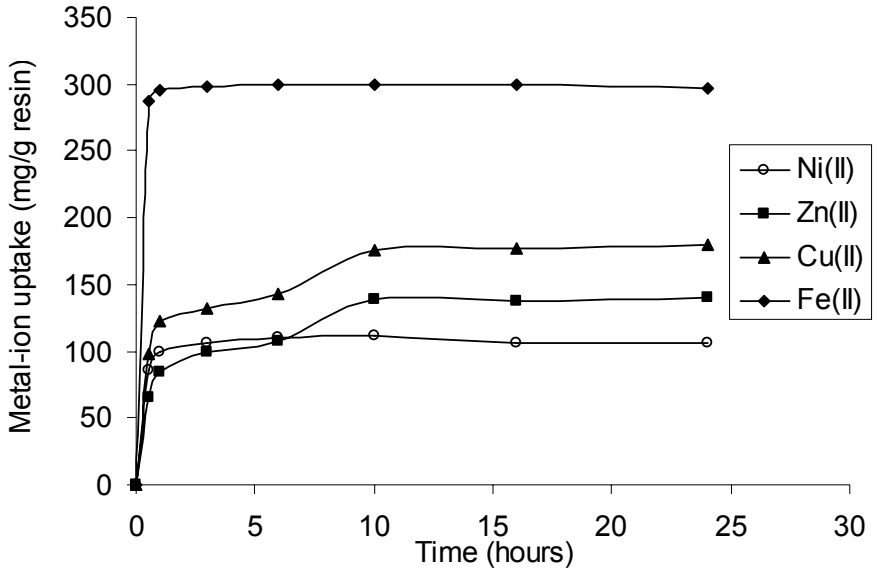


Figure 25. Metal ion uptake by resins as a function of contact time

The rates of metal-ion uptake by various classes of chelating polymers reported in the literature exhibited a wide range of adsorption rates. In general, the adsorption rates are governed by several factors such as the nature of active chelating groups and repeating units, structural properties of the polymer (porosity, surface area, size, and molar mass), the concentration of

metal-ion, the amount of polymer used, and the concentration of other ions that may compete with the metal ion of interest.

This makes rate comparisons a subject of great uncertainty (Salem *et al.*, 2004). Results also revealed that the metal-ion uptake follows the order: $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$; resin shows highest uptake capacity toward Fe(II) and lowest for Ni(II). This difference in capacities observed among the metals by the resin can be explained by the negative steric effect on coordination with the iminodiacetate group (Malla *et al.*, 2002). The ionic radius for Fe(II) is 75 pm , for Zn is 88, pm Cu(II) is 73 pm and for Ni(II) is 83 pm. The stability of the chelate is expected to be less favourable for ions of larger size; this is consistent with earlier investigations. (Ebraheem *et al.*1998; Al-Riwani *et al.*, 2003).

1.3. RATE OF METAL-ION UPTAKE AS A FUNCTION OF pH

pH dependence of the metal-ion uptake The binding capacity of the resin toward the investigated metal ions was studied in the pH range 2–7 under continuous shaking for a fixed contact time of 6 h at 25°C; typical pH-binding capacity profiles are displayed in **Figure 26**.

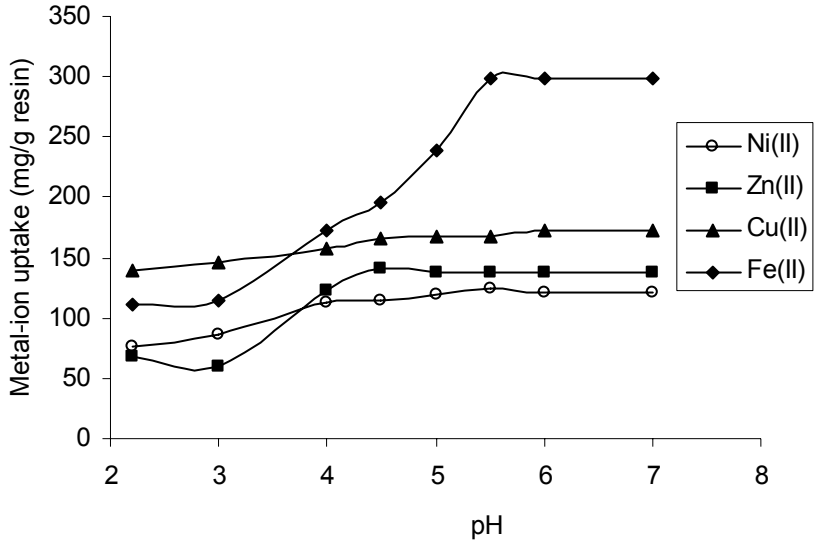


Figure 26. Effect of the pH on metals ions uptake by resin.

Results reveal that metal-ion uptake increased with pH of the medium and approached a steady state at about pH 6.0. This observation was more pronounced with Fe(II) where the binding capacity of the resin strongly increases with increasing pH, whereas the sorption of other metals ions Cu(II), Zn(II), and Ni(II) slightly increases with pH. This behavior could be explained by the nature of the chelating group; the iminodiacetate groups of the Amberlite IRC-718 are weak acids and the degree of protonation will critically affect the ability of resin to bind metal cations. For this resin, protonation of carboxylate groups and nitrogen atoms is reported to be complete at pH 2 (Schmuckler, 1965; Gode and Pehlivan, 2003). A completely deprotonated form of the resin is reached at pH 12 (Wucherpfennig, 1992; Malla *et al.*, 2002).

1.4. Effect of resin mass on metal-ion uptake

The effect of resin's mass on the rate of metal-ion uptake was investigated using a batch equilibration technique by suspending different masses (0.1, 0.2,0.3, 0.6, 0.8, or 1.0 g) of the dry resin in 25 ml of the acetate buffer solution at pH 5-7.0 for 2 h. Then, 25 ml of buffer solution containing 15 mg of metal ion was added at 25°C under continuous shaking for 6 h. Results are displayed in **Figure 27**. Results show that the amount of metal ions adsorbed on the resin increases with the increase of the mass of resin used. This may be explained by the increase of the polymer sites available for chelation when fixed amounts of metal ions are available in solution. In all cases, metal-ions are completely taken out of solution with the presence of 0.8 g of resin.

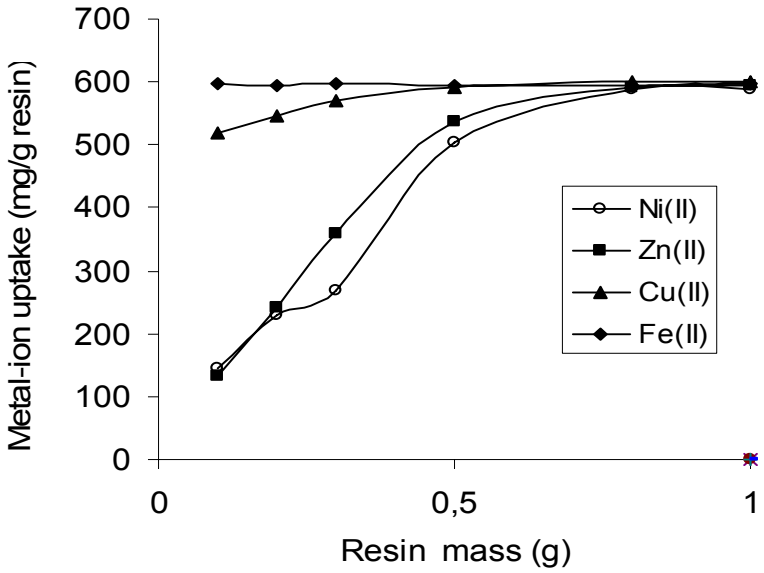


Figure 27. Effect of polymer mass on metals ions uptake by resin

1.5. The effect of metal-ion concentration on metal-ion

The effect of metal ion concentration on metal ion uptake was studied by suspending 0.10 g of the dry resin in 25 ml of the acetate buffer solution at pH 7.0 for 2 h followed by the addition of 25 ml of buffer solution containing different amounts of metal-ion. Results shown in **Figure 28** reveal that the metal-ion uptake capacity of the resin toward the metal ions does not change considerably with metal ion concentration.

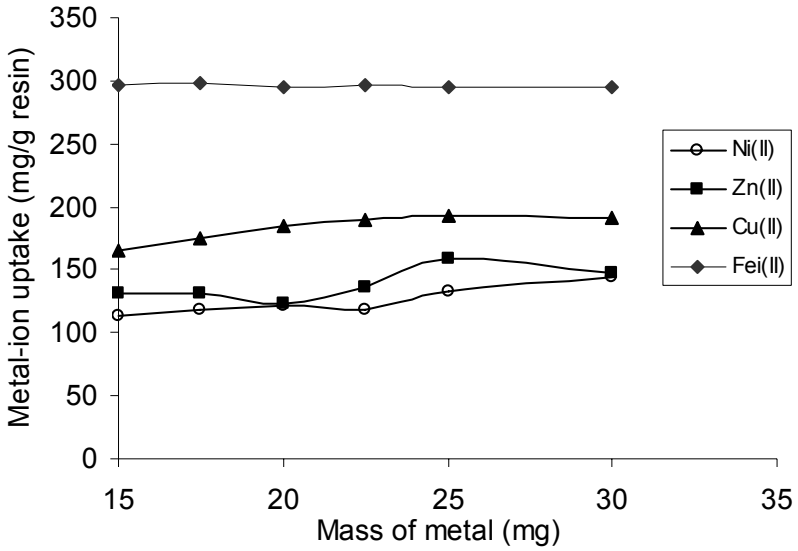


Figure 28. Effect of initial amount of metal ions on metal ion uptake by resin

1.6. Metal ion desorption from the resin

Removal of metal ions from chelated resins was achieved by 3M HCl as eluent. Desorption behaviour of the investigated M(II) ion from the polymers was carried out by batch processes at room temperature and with continuous shaking for 1, 3, and 6 hours. Example of desorption is shown in **Table 9** for copper. Data reveals that the percentage recovery of Cu(II) ion is almost complete in one hour

Table 9. Metal ion desorption for resin –Cu(II)

Time (min)	60	180	360
% recovery	94.9	98.5	99.1

1.7. Conclusion:

In this investigation, we focused on the sorption properties of the commercially available resin, Amberlite IRC-718, a chelating resin containing iminodiacetic acid as ligand attached to the copolymer of styrene and divinyl benzene of macroporous matrix structure, toward some divalent metal-ions in aqueous solutions. The effect of exposure time on the metal-ion uptake was studied by a batch equilibrium technique and showed that a time of 6–10 h was enough to achieve maximum metal-ion sorption and that the extent of metal-ion uptake followed the order Fe^{2+} > Cu^{2+}> Zn^{2+} > Ni^{2+}. The pH binding capacity profiles showed that the metal-ion uptake of the resin increased with increasing pH and reached a maximum at pH 6.0.

2. FRACTIONATION OF HUMAN IgG ON AMBERLITE IRC-718

Chromatographic column was packed with the resin saturated with bivalent metal ions as described in materiel and methods section. Initial fractionation of human serum at the flow rate of 0.5 mL/min resulted in the chromatographs corresponding to each metal ion. The adsorption and washing were carried out in 25 mM Tris-HCl buffer of pH 7.0, then elution was achieved with buffers at the same pH but with different concentrations of Tris (100-700 mM). The eluates obtained were controlled for their purity using SDS-PAGE. The regeneration of the resin for further use was performed with 0.5 M NaOH solution.

2.1. Protein recovery

Protein quantities were determined, in the initial solution and pooled peaks obtained from chromatographic fractions, using Bradford method (Bradford, 1976). Except for Zn^{2+}, which gave the least recovery of liberated proteins of 42.41 %, all other metal ions gave about 80 % of injected

proteins (**Table 10)**. Similar results have been obtained by Vançan and coworkers (Vancan, et. al., 2002) using Cu^{2+}, Fe^{2+}, Ni^{2+} and Co^{2+} immobilized on IDA-Sepharose.

Table 10. Mass balance for chromatographs of human serum eluated with Tris-HCl, pH 7

Metal		**Cu^{2+}**		**Ni^{2+}**		**Fe^{2+}**		**Zn^{2+}**	
Protein recovery		mg[a]	%[b]	mg[a]	%[b]	mg[a]	%[b]	mg[a]	%[b]
Injection		21.15	100	21.15	100	21.15	100	21.15	100
Washing	25 Mm	16.13	90.45	14.51	82.79	15.05	85.34	7.25	43.73
Elution	100 mM	0.46	2.17	0.91	4.30	0.38	1.79	0.92	4.37
	300 mM	1.02	4.82	1.17	5.53	1.31	6.19	0.67	3.16
	500 mM	0.27	1.28	0.31	1.46	0.11	0.52	0.12	0.56
	700 mM	0.23	1.08	0.03	0.14	0.19	0.89	0.01	0.04
Total		17.11	80.89	16.93	80.04	17.04	80.56	8.97	42.41

[a] Mass calculated from protein concentration determined by Bradford (1976) method
[b] Percentage relative to injected protein mass

2.2. Fractionation on Cu(II)-resin

Results of human serum fractionation on Cu(II)-resin are shown in **Figure 29**. The first peak obtained in the adsorption/washing buffer contains all proteins of the serum, as depicted in **Figure 30**. The elution with 100 mM liberates two major proteins corresponding to IgG and serum albumin (lane 2) as compared to native human serum (lane S) and purified IgG (Sigma) (lane Ig). The peak obtained with 300 mM Tris-HCl contains purified IgG (lanes 3) while at 500 and 700 mM Tris-HCl, afforded IgG contaminated with higher molecular weights proteins (lane 4, 5).

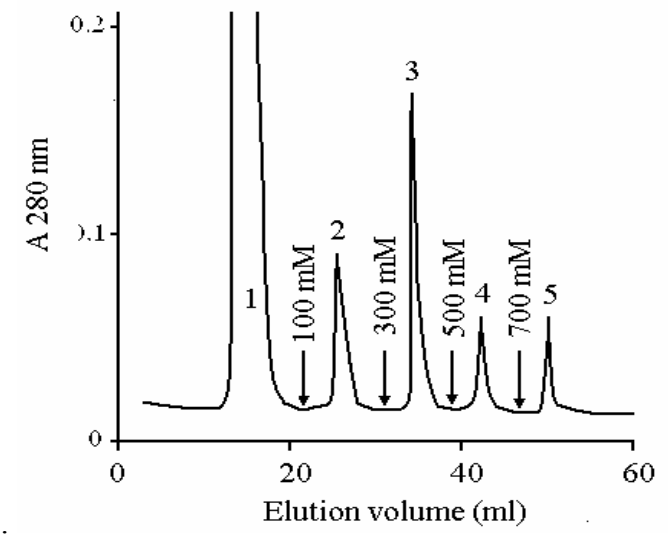


Figure 29. Low flow rate chromatography of human serum on IRC-718-Cu^{2+} resin. A sample of 1.7 ml of human serum was diluted to 1/5 in 25 mM Tris/HCl pH 7 buffer and then applied onto the resin in 5 ml column prepared as described in the materials and methods section. The column was washed with the buffer (peak 1) followed by increasing concentrations of Tris/HCl; 100 mM (peak 2) 300 mM (peak 3) 500 mM (peak 4), and 700 mM (peak 5).

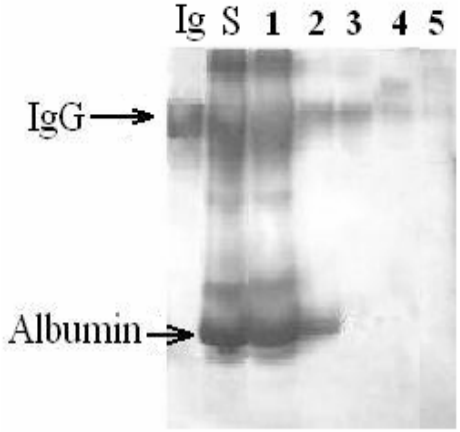


Figure 30. SDS PAGE of human serum fractions separated on IRC-718-Cu^{2+} gel at low flow rate, pH 7. Pooled fractions from the peaks presented in **Figure 29** were run on 7.5% polyacrylamide gel in nonreducing conditions and stained with Coomassie Brillant Blue (CBB) according to Laemmli (1970). Arabic numerals correspond to peak numbers from the chromatograph. Lane Ig: Human IgG (Sigma), S: human serum.

2.3. Fractionation on Ni(II)-resin

Results of human serum fractionation on Ni(II)-resin are shown in **Figure 31**. The first peak obtained in the adsorption/washing buffer contains most proteins of the serum as given in **Figure 32**. The elution with 100 and 300 mM Tris liberates purified IgG (lanes 2, 3) as compared to purified IgG (Sigma) (lane Ig). Peaks obtained with 500 and 700 mM contain proteins with higher molecular weights (lanes 4, 5).

2.4. Fractionation on Fe(II)-resin

Results of human serum fractionation on Fe (II)-resin are shown in **Figure 33**. The first peak obtained in the adsorption/washing buffer contains all proteins of the serum (**Figure 34)**. The elution with 100 mM liberates two major proteins corresponding to IgG and essentially serum albumin (lane 2) as compared to native human serum (lane S) purified IgG (Sigma) (lane Ig). The peak obtained with 300 mM Tris-HCl contains mainly albumin (lanes 3) while at 700 mM only one protein with lower molecular weight is observed (lane 4).

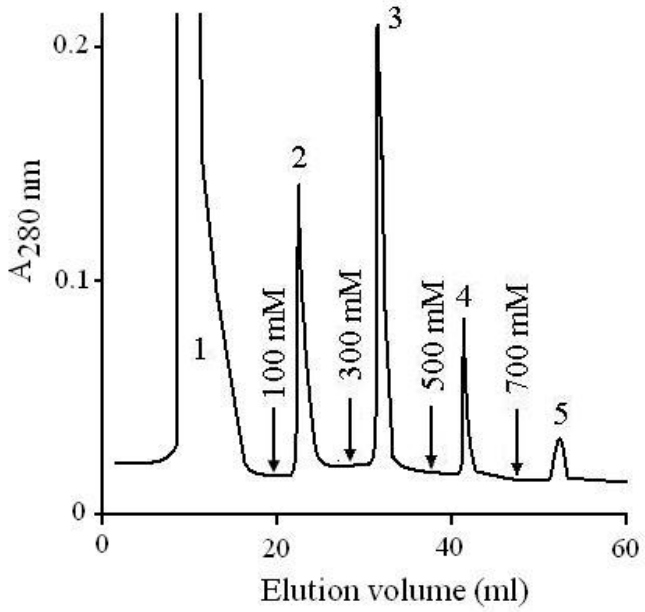


Figure 31. Effect of buffer system on adsorption and elution of human IgG chromatography on R-Ni^{2+}. Buffer composition is Tris/HCl, pH 7; 100, 300, 500 and 700 mM for 2, 3, 4, 5 peaks, respectively.

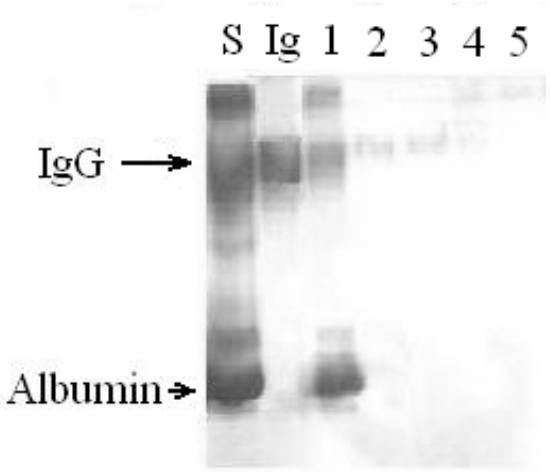


Figure 32. SDS PAGE of human serum fractions separated on R-Ni^{2+} gel at low flow rate, pH 7 **(Figure 31)**; pooled fractions from the peaks were run on a 7.5% polyacrylamide gel SDS-PAGE and stained with CBB. Arabic numerals correspond to peak numbers from the chromatograph. Lane Ig: Human IgG (Sigma), S: human serum.

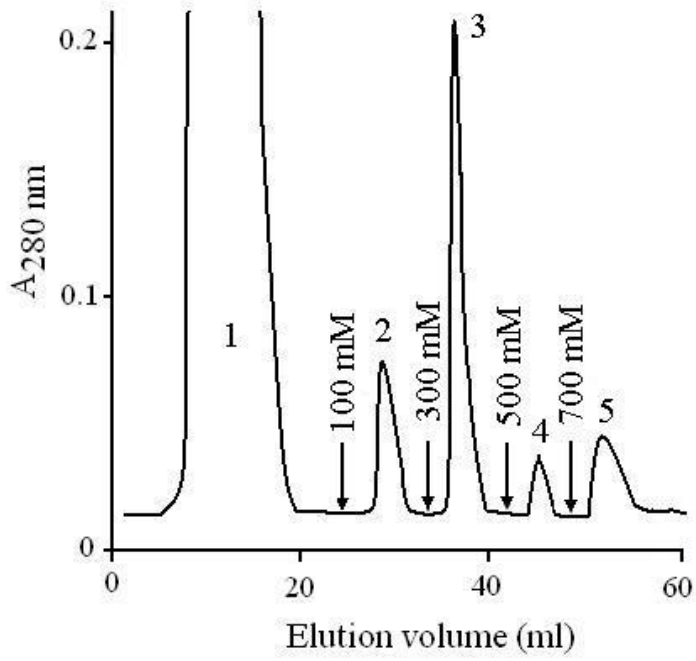


Figure 33. Effect of buffer system on adsorption and elution of human IgG chromatography on R-Fe^{2+}. Buffer composition is Tris/HCl pH 7; 100, 300, 500 and 700 mM to obtain peaks 2, 3, 4, and 5, respectively.

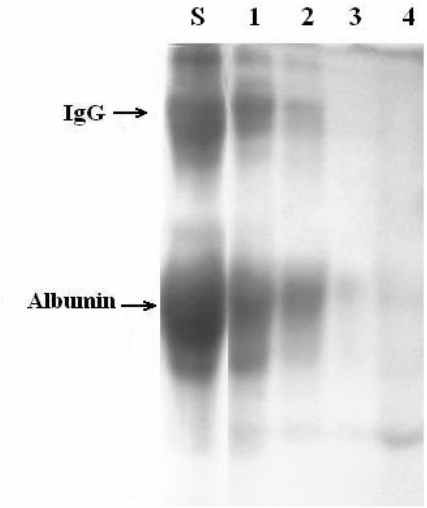


Figure 34. SDS PAGE of human serum fractions separated on R-Fe^{2+} gel at low flow rate, pH 7 (**Figure 33)**. Human serum was fractionated on R-Fe $^{2+}$ gel and pooled fractions from the peaks were run on a 7.5% polyacrylamide gel SDS-PAGE and stained with CBB. Arabic numerals correspond to peak numbers from the chromatograph. Lane Ig: Human IgG (Sigma), S: human serum.

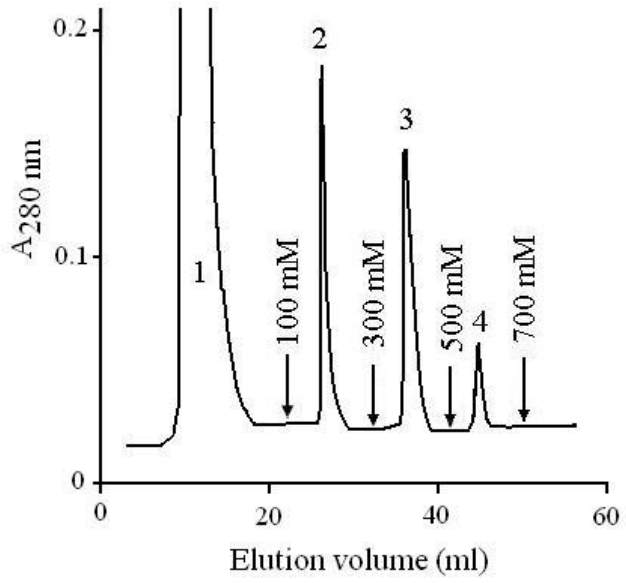


Figure 35. Effect of buffer system on adsorption and elution of human IgG chromatography on R-Zn^{2+}. Buffer composition is Tris/HCl pH 7; 100, 200, 300, 500, and 700 mM for 1, 2, 3, and 4, respectively.

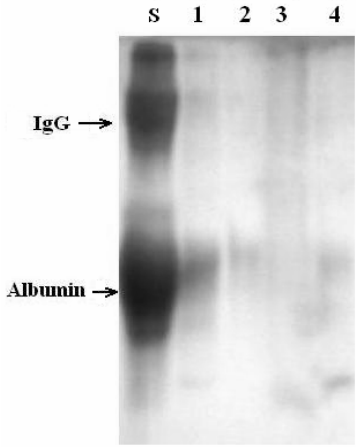


Figure 36. SDS PAGE of human serum fractions separated on R-Zn^{2+} gel at low flow rate, pH 7, human serum was fractionated on R-Zn^{2+} gel as described in Figure 32 and pooled fractions from the peaks run on an 7.5% polyacrylamide gel SDS-PAGE and stained with CBB. Arabic numerals correspond to peak numbers from the chromatograph. Lane Ig: Human IgG (Sigma), S: human serum.

2.6. Conclusion

Fractionation of human serum on ion exchanger-resin showed that when Cu^{+2} and Ni^{+2} were adsorbed on the resin one or two fractions, respectively contain purified IgG, while Zn^{+2} and Fe^{+2} retain either IgG and serum albumin or serum albumin alone. Furthermore, the Zn^{+2} –resin retention of serum proteins is too strong that the use of 700 mM Tris HCl cannot liberate any other proteins than non adsorbed serum albumin.

CHAPTER 6: GENERAL DISCUSSION

1. SORPTION PROPERTIES OF AMBERLITE IRC718 TOWARD METAL IONS

Amberlite IRC-718 is one of the few commercial chelating ion exchange resins available; its chelating ability is attributed to iminodiacetic groups (Park, 1998). This acidic chelating resin has a high affinity and selectivity for heavy metal cations; this is achieved by an iminoacetic acid functionality chemically bonded to macroreticular resin matrix (Agrawal *et al.*, 2003; Kocaoba and Akicin, 2002). The macroreticular structure of Amberlite IRC-718 provides a number of advantages over traditional gel resins; it is highly resistant to osmotic shock and has improved kinetics of ion exchange (Kocaoba and Akicin, 2002).

The investigation carried out here revealed that the resin exhibited higher capacities and a more pronounced adsorption toward Fe^{2+} and that the metal-ion uptake follows the order: $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$. This difference in capacities observed among the metals can be explained by the negative steric effect on coordination with the iminodiacetate group (Malla *et al.*, 2002). The ionic radius for Fe(II) is 75 pm and for Zn is 88 pm. The stability of the chelate is expected to be less favourable for ions of larger size; this is consistent with earlier investigations. (Ebraheem *et al.*1998; Al-Riwani *et al.*, 2003).

Results also revealed that metal-ion uptake increased with pH of the medium and approached a steady state at about pH 6.0. This observation was more pronounced with Fe(II). This behavior could be explained by the nature of the chelating group; the iminodiacetate groups of the Amberlite IRC-718 are weak acids and the degree of protonation will critically affect the ability of resin to bind metal cations. For this resin, protonation of carboxylate groups and nitrogen atoms is reported to be complete at pH 2 (Gode and Pehlivan, 2003; Schmuckler, 1965). A completely deprotonated form of the resin is reached at pH 12 (Wucherpfennig, 1992; Malla *et al.*, 2002).

The effect of resin mass on the adsorption of metal ions showed that the amount of metal ions adsorbed on the resin increases with the increase of the mass of resin used. This may be explained by the increase of the polymer sites available for chelation when fixed amounts of metal ions are available in solution. In contrast, results showed that the metal-ion uptake capacity of the resin toward the metal ions does not change considerably with metal ion concentration.

2. DEPLETION/PURIFICATION OF HUMAN IGG WITH ION EXCHANGER RESIN

IgG from human serum has been adsorbed onto different immobilized metal ions. Therefore, it was necessary to investigate the adsorption of human IgG onto different metals in a search for an ideal ligand to which IgG binding would be sufficiently strong to capture the protein selectively but not too strong that could hinder application (elution could then only be possible with EDTA as verified by Sidenius *et al.*, 1999 for selenoprotein P). Results reveal that Cu^{2+} and Ni^{2+} are more efficient in the adsorption and desorption of IgG, followed by Fe^{2+} which, retains IgG and serum albumin desorbed together with 100 mM Tris-HCl. When Zn^{2+} was employed, most proteins were retained but IgG was not liberated with Tris-HCl up to 700 mM, as presented in Figure 35. In the case of Fe^{2+}, IgG was widely contaminated with albumin. The results obtained in this study show that IMAC with Cu^{2+}, Ni^{2+} and Fe^{2+} immobilized on IDA-polystyrene has the potential to be developed as part of a process to purify IgG out of untreated human serum since acceptable adsorption and elution levels of IgG could be achieved. Experiments with human serum indicated that IgG can be efficiently separated from serum proteins using Tris as eluent at biological pH. Similar results have been obtained by Vancan and coworkers (Vançan *et al.* 2002) using Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} immobilized on IDA-Agarose as chromatographic gel and imidazole as eluent buffer.

Although, the experiments were not designed to identify the site of interaction between the IgG (and other serum proteins) molecule and the immobilized metal ion, this site could probably be

present in the Fc region of the antibody. Hale and Bleider (1994) identified the Fc region of the humanized IgG monoclonal antibody molecule as the region where the binding site to immobilized metal ions is located. Therefore, human IgG should also bind to the Fc region since the humanized antibody has a murine Fab region fused to a human Fc region. This is a desired situation since the human IgG to be produced by purification through immobilized ion affinity chromatography (IMAC) would have sites for complex formation with antigens not involved in the adsorption and elution step and therefore, would certainly be preserved. Thus, immobilized metal-ion affinity chromatography is not only one of the most popular techniques for protein purification, but also it is a very efficient method for studying protein structure in terms of histidine residue accessibility (Berna *et al.*, 1997; Chaga, 2001). As postulated by Sulkowski (1985; 1987; 1989), the affinity of proteins for chelate Cu(II) requires at least one accessible His residue. When proteins are retained on chelated Ni(II), they have more than one histidine residue and the adsorption on chelated Zn(II) and Co(II) means a cluster of histidine residues accessible for coordination (Todorova-Balvay, 2004).

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Sorption Properties of the Iminodiacetate Ion Exchange Resin, Amberlite IRC-718, Toward Divalent Metal Ions

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ABSTRACT: The sorption properties of the commercially available cationic exchange resin, Amberlite IRC-718, that has the iminodiacetic acid functionality, toward the divalent metal-ions, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} were investigated by a batch equilibration technique at 25°C as a function of contact time, metal ion concentration, mass of resin used, and pH. Results of the study revealed that the resin exhibited higher capacities and a more pronounced adsorption toward Fe^{2+} and that the metal-ion uptake follows the order: $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$. The adsorption and binding capacity of the resin toward the various metal ions investigated are discussed. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 1316–1319, 2008

Key words: Amberlite IRC-718; chelating resin; divalent metal ions exchange; sorption

INTRODUCTION

Heavy metals are commonly found in large quantities in industrial wastewaters. For this reason, the recovery of the metal ions present in these wastewaters is necessary for environmental protection and economical reasons. Solid organic and inorganic ion exchanges constitute the basis of widely employed chemical separation procedures, with applications ranging from analytical and environmental chemistry research to water purification, waste management, and material technologies (such as in nuclear and electroplating industries).[1–4] Stringent environmental regulations require the treatment of wastewater to remove heavy metals; this requirement is very costly for industries.

The most common metals found in wastewater are copper, cadmium, nickel, lead, and zinc which are toxic at high concentrations.[5,6] The use of synthetic resins for chelating toxic metal ions in wastewater is considered as a possible solution for preventing environmental pollution. These resins are mostly based on petroleum synthetic polymers.[7,8]

Synthetic chelating ion exchange resins are receiving considerable attention because of their application in different areas such as the removal of heavy metals, heterogeneous catalysis, solid electrolytes, ion exchange membrane, ion selective electrode, and purification of industrial waste. The use of chelating resins for selective removal of heavy metals from waste streams has been extensively studied.[9–25] Resins with iminodiacetic acid (IDA) functional group such as Chelex 100, Amberlite IRC 718 (formerly, IRC 718), Purolite S930, and Lewatit TP 207 were mainly applied because of their high selectivity and low manufacturing cost.[9] The IDA group could provide electron pairs for chelation; it forms stable coordinate covalent bonds with divalent metal-ions. For example, the resins Chelex 100 and Amberlite IRC-718 have been used to treat the waste effluent discharged from printed circuit board manufacturing, which contains Cu^{2+}, Ni^{2+}, Co^{2+}, and Cd^{2+}.[26]

One of the few commercial chelating ion exchange resins available is Amberlite IRC-718; its chelating ability is attributed to iminodiacetic groups.[27] This acidic chelating resin has a high affinity and selectivity for heavy metal cations; this is achieved by an iminoacetic acid functionality chemically bonded to macroreticular resin matrix.[7,28] The macroreticular structure of Amberlite IRC-718 provides a number of advantages over traditional gel resins; it is highly resistant to osmotic shock and has improved kinetics of ion exchange.[28] Amberlite IRC-718 was used for several purposes such as extraction of heavy metals (Cu^{2+}, Ni^{2+}, Fe^{2+}, and so forth) from solutions like wine,[29] liquor,[30] stored phenol,[31] sludge,[32] and for ion exchange chromatography.[33] In some studies, it was considered as a reference to study other resins.[34]

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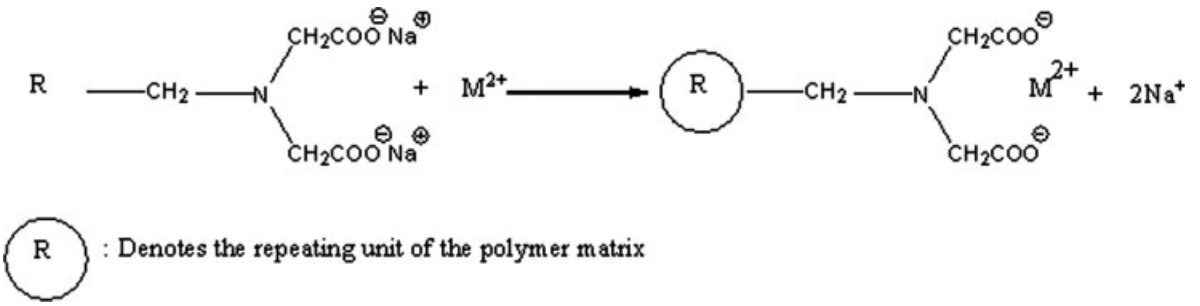

Scheme 1 Ion exchange between metal ions and sodium ions.

In the present work, we have employed the batch equilibration technique and atomic absorption spectrometry to evaluate the sorption properties of the commercially available chelating resin Amberlite IRC-718 toward the divalent metal ions, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+}, which are present at trace levels in natural aquatic systems. The effect of the different experimental factors such as pH, contact time, metal ion amounts, and mass of resin on the sorption capacity of the resin will be discussed.

EXPERIMENTAL

Reagents

Unless otherwise indicated, all chemicals were obtained from commercial sources and were used as received; the ion exchange resin containing iminodiacetate groups, Amberlite IRC-718, obtained as a sodium salt was purchased from Rohm and Haas, company (USA); bulk density 750 g/L, swelling (%) 30, total exchang capacity 1.35 mequiv/mL,[35] particle size of 16–50 mesh was used without further purification. The following metal ion salts were purchased from Fluka and were also used as received without further purification: Cu(II) acetate, Ni(II) acetate, Zn(II) acetate, and Fe(II) chloride.

Instrumentation

Atomic absorption measurements were carried out with the aid of a Varian Atomic Absorption Spectrophotometer. Samples were shaken using a GFL-1083 shaker thermostated water bath maintained at 25°C.

Sorption of the metal ions on the polymer

The metal chelation characteristics of the resin for each metal ion were studied by the batch equilibrium technique. Duplicate experiments involving 0.100 g of dry, 16–50 mesh size, resin samples were suspended in 25 mL of sodium acetate-acetic acid buffer adjusted to the desired pH with continuous shaking and left for 2 h to equilibrate. To this mixture, 25 mL of metal ion solution containing a total of 15 mg metal-ion were added. After being shaken for a definite period of time at 25°C, the mixture was filtered, and the amount of metal ion remaining in the filtrate was determined by atomic absorption spectrometry (AAS) using standard solutions for calibration.

The extent of metal-ion uptake was studied under similar experimental conditions, where the contact time was varied from 0.5 to 24 h at 25°C after the solution was equilibrated with distilled water. Similar experiments were also carried out in buffered solutions, in which the pH was varied between 2.0 and 7.0 for a fixed contact time of 6 h.

The effect of resin mass on the metal-ion uptake was also studied using the same general procedure by shaking a suspension of 0.1, 0.2, 0.3, 0.6, 0.8, or 1.0 g of the dry resin in 25 mL of the acetate buffer solution at pH 7.0 for 2 h. To this mixture, 25 mL of buffer solution containing 15 mg of metal-ion were added. The mixtures were then shaken at 25°C for 6 h, filtered, and the amount of metal ion remaining in solution was determined by AAS. The effect of metal-ion concentration was investigated in a similar fashion in buffer solutions containing 0.10 g of dry resin and variable amounts of metal ions at 25°C and a fixed contact time of 6 h.

RESULTS AND DISCUSSION

Rate of metal-ion uptake as a function of contact time

The sorption of various divalent metal ions (Ni^{+2}, Cu^{+2}, Fe^{+2}, and Zn^{+2}) on Amberlite IRC-718 iminodiacetate chelating ion exchanger as a function of contact time was investigated by a batch equilibrium technique. The metal ions displace the sodium ions inside the resin when it was equilibrated with the metal-ion solution as shown in the Scheme 1.

The results for the dependence of the metal ion uptake on contact time for resin are presented in Figure 1. The results indicate fast rates of equilibration; the rates of metal-ion uptake increase in the first 3 h and a steady state is reached within 5–10 h. For Fe(II), about 99% of metal-ion was achieved in the first hour. The rates of metal-ion uptake by various

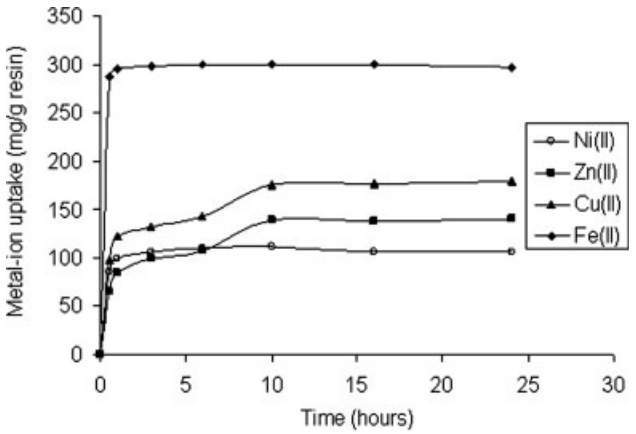

Figure 1 Metal-ion uptake by resin as a function of contact time.

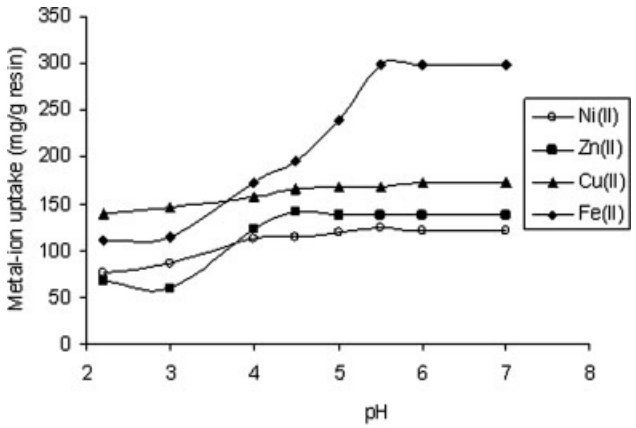

Figure 2 Effect of the pH on metal-ion uptake by the resin.

classes of chelating polymers reported in the literature exhibited a wide range of adsorption rates. In general, the adsorption rates are governed by several factors such as the nature of active chelating groups and repeating units, structural properties of the polymer (porosity, surface area, size, and molar mass), the concentration of metal-ion, the amount of polymer used, and the concentration of other ions that may compete with the metal ion of interest. This makes rate comparisons a subject of great uncertainty.[36]

Results also revealed that the metal-ion uptake follows the order: $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$; resin shows highest uptake capacity toward Fe(II) and lowest for Ni(II). This difference in capacities observed among the metals by the resin can be explained by the negative steric effect on coordination with the iminodiacetate group.[16] The ionic radius for Fe(II) is 75 pm and for Zn is 88 pm. The stability of the chelate is expected to be less favorable for ions of larger size; this is consistent with earlier investigations.[37–39]

pH dependence of the metal-ion uptake

The binding capacity of the resin toward the investigated metal ions was studied in the pH range 2–7 under continuous shaking for a fixed contact time of 6 h at 25°C; typical pH-binding capacity profiles are displayed in Figure 2. Results reveal that metal-ion uptake increased with pH of the medium and approached a steady state at about pH 6.0. This observation was more pronounced with Fe(II) where the binding capacity of the resin strongly increases with increasing the pH of the medium, whereas the sorption of other metals ions Cu(II), Zn(II), and Ni(II) slightly increases as the pH increases. This behavior could be explained by the nature of the chelating group; the iminodiacetate groups of the Amberlite IRC-718 are weak acids and the degree of protonation will critically affect the ability of resin to bind metal cations. For this resin, protonation of carboxylate groups and nitrogen atoms is reported to be complete at pH 2.21.[40] A completely deprotonated form of the resin is reached at pH 12.30.[16]

Effect of resin mass on metal-ion uptake

The effect of resin's mass on the rate of metal-ion uptake was investigated using a batch equilibration technique by suspending different masses (0.1, 0.2, 0.3, 0.6, 0.8, or 1.0 g) of the dry resin in 25 mL of the acetate buffer solution at pH = 7.0 for 2 h. Then, 25 mL of buffer solution containing 15 mg of metal-ion was added at 25°C under continuous shaking for 6 h. Results are displayed in Figure 3. Results show that the amount of metal ions adsorbed on the resin increases with the increase of the mass of resin used. This may be explained by the increase of the polymer sites available for chelation when fixed amounts of metal ions are available in solution. In all cases, metal-ions are completely taken out of solution with the presence of 0.8 g of resin.

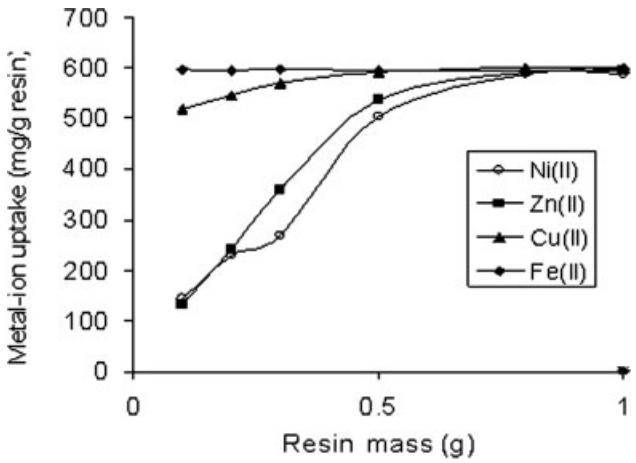

Figure 3 Effect of resin mass on metal-ion uptake by resin.

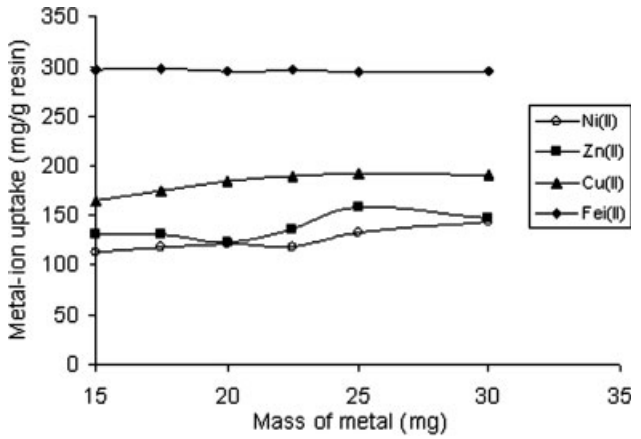

Figure 4 Effect of initial amount of metal ions on metal-ion uptake by resin.

Effect of metal-ion concentration on metal-ion uptake

The effect of metal-ion concentration on metal-ion uptake was studied by suspending 0.10 g of the dry resin in 25 mL of the acetate buffer solution at pH 7.0 for 2 h followed by the addition of 25 mL of buffer solution containing different amounts of metal-ion. Results shown in Figure 4 reveal that the metal-ion uptake capacity of the resin toward the metal ions does not change considerably with metal ion concentration.

CONCLUSION

In this investigation, we focused on the sorption properties of the commercially available resin, Amberlite IRC-718, a chelating resin containing iminodiacetic acid as ligand attached to the copolymer of styrene and divinyl benzene of macroporous matrix structure, toward some divalent metal-ions in aqueous solutions. The effect of exposure time on the metal-ion uptake was studied by a batch equilibrium technique and showed that a time of 6–10 h was enough to achieve maximum metal-ion sorption and that the extent of metal-ion uptake followed the order $Fe^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$. The pH binding capacity profiles showed that the metal-ion uptake of the resin increased with increasing pH and reached a maximum at pH 6.0. The effect of resin mass and metal-ion concentration on the extent of metal-ion uptake were also investigated.

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