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POLYMER-DRUG GRAFTS FOR IRON CHELATION

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INTRODUCTION

Accidental poisonings account for a large proportion of children's accidents. There is a need to improve the emergency treatment of poisonings, particularly to decrease the period of coma and to minimize mortality.

Iron poisoning is a common cause of poisoning among young children. Use of chelating agents, especially Deferoxamine B, to remove excess iron from the body is one of the most effective treatments. However, in very severe cases of acute iron overloads, the amount of deferoxamine required to remove the iron is so high that the drug and its iron chelate would be toxic.

Our objective is to develop a chelating polymer which could be used in extracorporeal detoxification of severe acute iron poisoning cases.

POLYMER CHELATES

Ion-Specific Resins

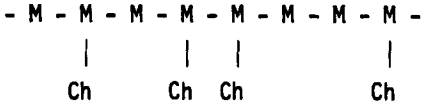
In the search for specific ion-exchange resins there has been a great deal of effort toward the synthesis of specific chelating

or complexing resins. The commercial manufacture of these resins has not yet been successful[1] with the exception of Dowex A-1[2].

Polymer chelates consist of a polymer backbone and a grafted chelating group (Fig. 1a) although resins containing the chelating group as part of the repeat unit have been reported[3-11] (Fig. 1b). Resins of the first type may be considered as random graft copolymers and those of the second type as homopolymers.

Chelating resins differ from ion exchange resins in three properties[9]: greater selectivity, higher metal-polymer interaction energy, and slower kinetics.

Applications of chelating resins have been mainly in water purification (i.e., removal of heavy metals from water), analytical determination of metal ions, chromatographic separation of ions,



(a)

(b)

Fig. 1. General structure of polymer chelates. M = repeat unit; Ch = chelating group. (a) The chelating group is grafted on the polymer chain. (b) The chelating group is part of the monomer unit.

and recovery of metals from solutions. A number of chelating groups have been grafted onto polymers (Table 1).

Synthesis of Polymer Chelates

There exist two general approaches for the synthesis of chelating polymers. One is the incorporation of the active (chelating) group in the polymer matrix by making use of reactive functional groups in the polymer. The second method is the transformation of the active group into a monomer with subsequent polymerization. In the first method the hydrophilicity of the chelating groups is sometimes incompatible with the polymer matrix (usually hydrophobic), and therefore the latter requires some modification. In the second method the presence of a larger group in a monomer may reduce and even prevent homopolymerization, making it necessary to copolymerize the transformed monomer. It is possible that some bulky chelating monomers may not polymerize or copolymerize at all.

Gregor et al.[38] suggested four conditions that the chelating group should fulfill:

1. It should be able to react with a group and become attached to the polymer matrix, or be able to polymerize for resin formation.

TABLE I

Some Chelating Groups Grafted on Polymers

CHELATING GROUP	REFERENCES
Alpha-Amino acids	13-19
Iminodiacetic and/or Iminodipropionic acids	5, 7, 14, 15, 20-34, 36
Nitrilotriacetic acid	35-37
EDTA	37-39
Hydroxamic acid	40-42
Deferoxamine B	43
8-Hydroxyquinoline	44-47
Others	3, 8, 37, 48-80

2. If the latter is the case, it should be sufficiently stable to withstand the polymerization process without alteration of its chelating function.

3. The chelating group should be compact so as not to be sterically hindered by the polymer matrix.

4. The arms of the chelate structure should be present on the same monomer unit in proper spatial configuration, for preservation of specific arrangement of the ligand.

D'Alelia et al.[28] suggest that monomeric models for the chelating polymer should be constructed, and that for an effective chelating polymer the behavior of the active group in the polymer should be similar to that of the group in the monomeric segment. They proposed the following approach for the synthesis of a chelating polymer:

1. Synthesis and determination of chelating characteristics of a model monomeric compound.

2. Synthesis and characterization of an oligomer of low molecular weight (dimer, trimer, etc.) model compound, and determination of its chelating properties.

3. When the results of the second step confirm chelation, a high molecular weight polymer should be synthesized and characterized, and its chelation capacity should be determined.

D'Alelio et al.[28,29,31-33] have followed this approach for the study of chelating polymers containing hydroxyarylaminoacetate groups as the chelating group.

A general sequence for the synthesis of grafted chelating polymers is shown in Fig. 2. In this case, polystyrene (1), usually cross-linked with divinylbenzene, is first chloromethylated, then transformed into a sulfonium salt (3), and finally reacted with sodium iminodiacetate to give the chelating polymer (4). The sulfonium salt is hydrophylic whereas the chloromethylated resin (2) is not and will not react appreciably with iminodiacetate salt.

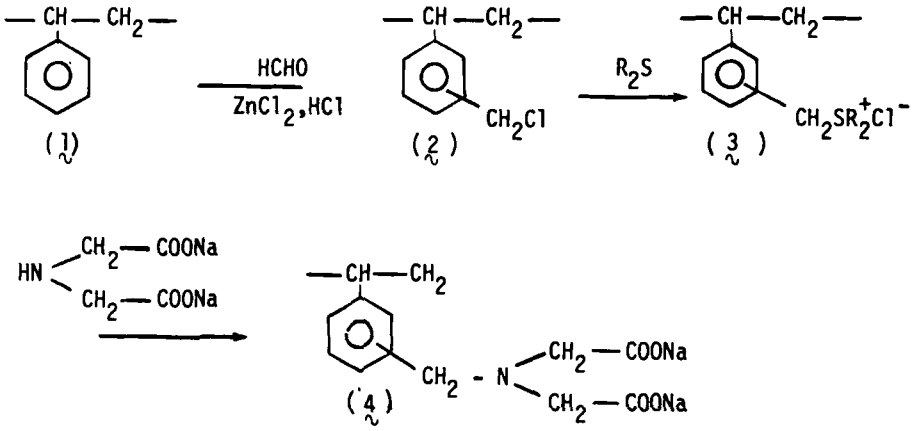


Fig. 2. Reaction sequence for the synthesis of an iminodiacetate chelating polymer [1].

Another approach to solve the problem of hydrophobicity of the chloromethylated polystyrene is to partially transform it into a quaternary salt with a tertiary amine (e.g., triethylamine), which makes the polymer more hydrophylic, and then to react it with the chelating group[81]. The reaction sequence for this process is shown in Fig. 3.

The transformation of the chelating group into a monomeric species is shown in Fig. 4[26]. In this case methacrylchloride (8) reacts with the diethyl ester of iminodiacetic acid to give amide (9), which may be homopolymerized to give (10). Monomer (9) may also be copolymerized with other vinyl monomers or graft copolymerized with radiation on preformed polymers[9] or fibers to produce chelating graft copolymers.

Distribution Coefficient and Selectivity Coefficient of a Chelating Resin

The distribution coefficient, K_D, or distribution ratio, and the selectivity coefficient, $K_{A/B}$, or concentration quotient[83],

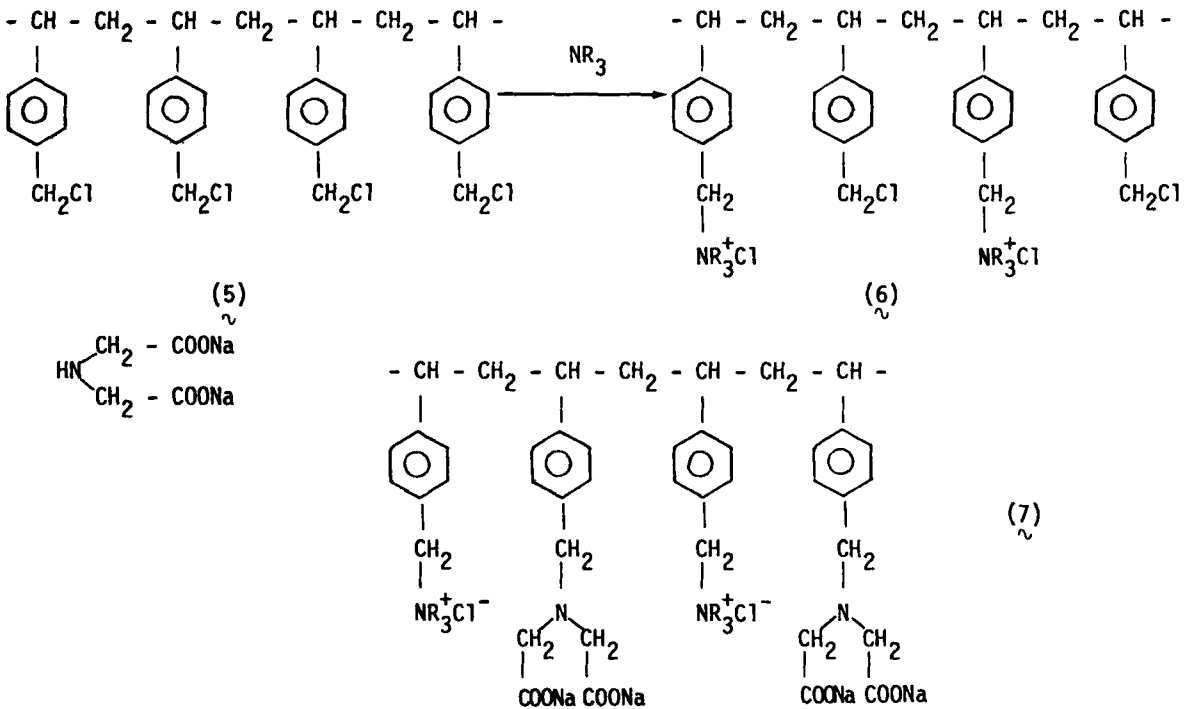


Fig. 3. Reaction sequence for the synthesis of a chelating polymer with partial substitution [1].

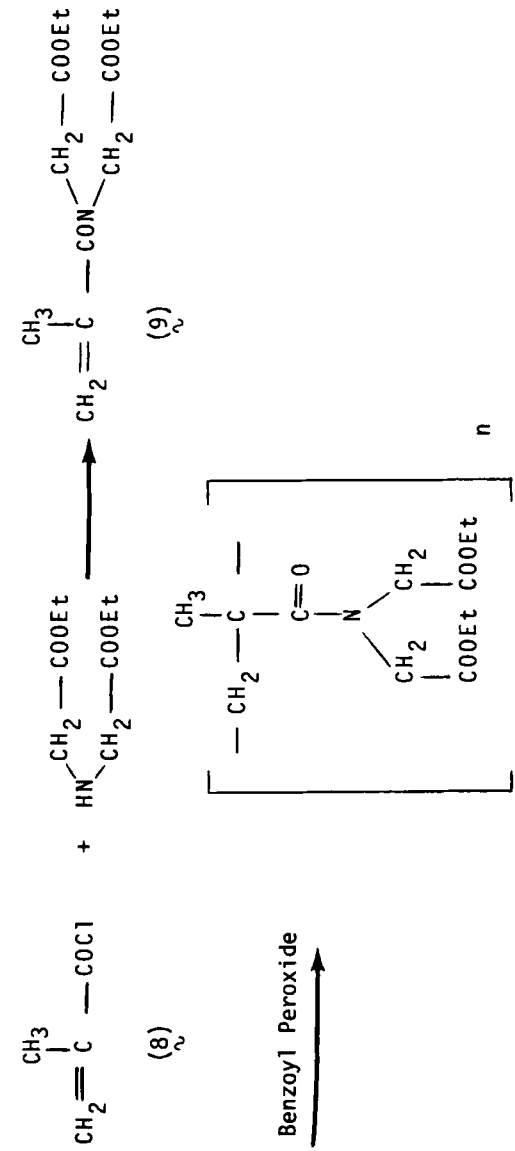


Fig. 4. Synthesis of a chelating polymer with one active group per monomer unit.

are important parameters in the characterization of ion-exchange and chelating resins.

For very dilute solutions of an ion A, the distribution coefficient for any ion exchange resin is given by

$$D = [\bar{A}]/[A] \qquad (1)$$

where $[\bar{A}]$ is the concentration of ion A in the resin phase and
$[A]$ is the concentration of ion A in the aqueous phase.

The selectivity coefficient for the exchange of two ions, A and B, of charge $|a|$ and $|b|$, respectively, is given by

$$K_{B/A} = \frac{[A]^{|b|}[\bar{B}]^{|a|}}{[\bar{A}]^{|b|}[B]^{|a|}} \qquad (2)$$

where the bars indicate the resin phase.

Equation (2) is comparable to the equilibrium constant of an ionic equilibrium reaction. Thus if one considers the exchange reaction of two ions A and B with charges a and b as being

$$|a|B^b + |b|\bar{A}^a \rightleftarrows |a|\bar{B}^b + |b|A^a \quad ; \qquad (3)$$

the equilibrium constant for this reaction is identical to $K_{B/A}$.

These two expressions, (1) and (2), have been defined for ion-exchange (nonchelating) resins; however, they can be equally applied for chelating resins[33,83].

Both the distribution and selectivity coefficients depend on the degree of cross-linking in the resin[82,84], on the specific capacity of the resin, i.e., the number of miliequivalents of fixed exchange groupings per gram of resin[84], and on the nature of the exchange (or chelating) group[84].

Detailed treatments of ion-exchange selectivity and of resin selectivity in aqueous solutions are given by Reichenberg[84] and by Diamond and Whitney[82].

CHELATING AGENTS IN MEDICINE

Chelating agents are a class of drugs for which a clear connection between action in the body and physical and chemical properties are known[85]. Several natural chelates are well known: hemoglobin (iron), chlorophyll (magnesium), vitamin B-12 (cobalt), and enzymes such as cytochrome oxidase (iron and copper). This section will be mainly concerned with synthetic chelating agents or those of natural origin that are not found in the human organism.

According to Schuber[85], chelating agents may accomplish three different functions:

1. To chelate toxic metals in blood and help the body get rid of them by excretion.

2. To deliver certain trace metals that are essential to tissues.

3. To inactivate certain bacteria and viruses by making essential metabolic metals unavailable, or by delivering metals that are harmful to them.

An effective chelating agent must form more stable complexes with the metals of interest than the biological substance holding the metals. This means that the stability constant of the metal chelate should be higher than the stability constant of the complex between the biological substance (usually proteins) and the metal. The stability constants of chelates are taken as a measure of their chelating ability. However, the stability constant determined in aqueous systems is usually different from the actual stability constants found in a living system, where interferences from proteins, enzymes, pH, and other ions may change the chelating properties of the drug[86]. Shubert[87] examined the biological implications of iron binding and described chelating agents in terms of an "effective constant", which is not actually constant but varies with the media in which the chelating agent and the metal ion are in contact.

An important property that the chelating agent should have is selectivity. It should be able to chelate the metal of interest without influencing the other essential ions present, often in larger amounts, such as Ca and Mg, as well as the trace metals, such as Zn, Mn, and Cu, whose removal could be dangerous.

Solubility of both the chelating agent and the complex in various media are important. They should be water soluble, to permeate within the intra- and extravascular spaces but this may exclude them from penetrating into the intracellular space. Water solubility also allows a more rapid excretion of the drug and its chelate.

Chelating agents used as drugs should withstand metabolic processes, and if they undergo enzymatic or other degradation, the metabolites should not have toxic side effects[86].

The administration mode of a chelating agent is also very important. For example, EDTA and DTPA (see Table 2 for formulas and chemical names) must be administered by prolonged intravenous infusions, and temporarily immobilize the patient thus restricting their use to hospitals[86]. An ideal chelating agent as a drug would be one that is taken orally, acts in the gastrointestinal tract, and is absorbed through the intestines, extending its action to the rest of the body[88].

Table 2 summarizes various chelating agents used in medicine. Some of these agents are still in the experimental stage, whereas others are presently being used for clinical treatment. Some of these chelating agents are used to remove metals from the organism (EDTA, DTPA, deferoxamine, BAL, etc.). Others are used to deliver metals into infected areas (Oxine) or to the brain to reduce fever and headaches (aspirin)[85].

One of the main uses of chelating agents in medicine has been in the treatment of acute metal poisoning, such as lead, mercury, and iron. The most success has been in the case of iron poisoning where DTPA and especially deferoxamine have proven very effective.

ACUTE IRON POISONING

Occurrence of Iron Poisoning

Young children may ingest large amounts of iron tablets (commonly ferrous sulfate) that are prescribed for their mothers. Adults less often take ferrous sulfate in suicide attempts. The estimated lethal dose of ferrous sulfate in children is 900 mg/kg of body weight[90]. Some children have survived after ingesting 15 g, and sometimes as little as 1 g has been fatal[91]. Arena[92] stated that the average human fatal dose is 200 to 250 mg of iron per kilogram of body weight. For the average 2 year-old child, that dose means the ingestion of 3 g of iron.

Crotty[93] has summarized reports from the National Clearinghouse for Poison Control Centers over a period of 32 months. Of 292,060 poisoning reports, 1194 cases were of ingestion of medicinal preparations containing more than 65 mg of iron. Of these cases, 1024 were children under 5 years of age, and ferrous sulfate ranked 6th among the medicines that caused hospitalization of children under 5. According to Crotty[93], "the reported information that 179 of 654 cases (27%) were hospitalized places iron salts in the category of the most toxic substances." This is probably because of the consideration of iron as a "vitamin" and not as a potential poison; thus parents do not take the necessary precautions.

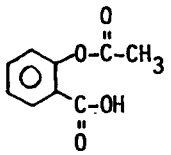
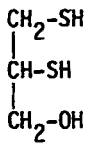
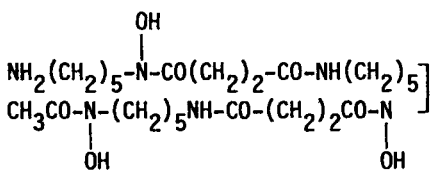
Until 1958, Aldrich[94] estimated the mortality rate of acute iron intoxication as 45%. Whitten et al.,[95] in 1965 proposed that mortality rates may be reduced with the appropriate therapy using deferoxamine as a chelating agent for iron.

Mechanism for Iron Toxicity from Iron Salt Ingestion

Iron overdoses are accompanied by both local and systematic effects. Significant amounts of either ferric or ferrous salts lead to rapid necrosis of the mucosa of the gastrointestinal tract[96]. Absorbed iron soon exceeds the bonding capacity of iron

Table 2

Some chelating agents used in medicine [85,86]

Name	Formula and Chemical Name	Uses
Aspirin	 o-Acetylsalicylic acid	To reduce fever and relief of headache, muscle and joint pain. Also for symptoms of rheumatic fever.
BAL (British Anti-Lewisite)	 2,3-Dimercaptopropanol	In cases of arsenic, antimony, mercury, and gold poisoning. Also in lead poisoning.
Deferoxamine B (DFA)	 N-{5-[3-[(5-aminopentyl)hydroxycarbamoyl]-propionamido]pentyl}-3-[[5-(N-hydroxyacetamido)-pentyl]carbamoyl]-propionohydroxamic acid	In iron poisoning cases.

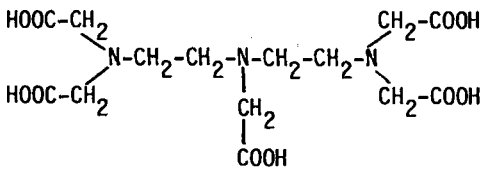
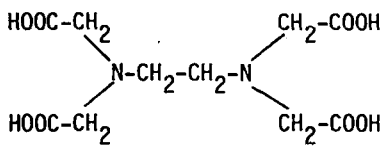
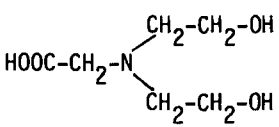
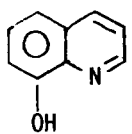
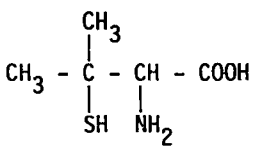
Name	Formula and Chemical Name	Uses
DTPA	 Diethylenetriaminepentaacetic acid	In poisoning by radioactive metals and in iron poisoning.
EDTA	 Ethylenediaminetetraacetic acid	In cases of lead poisoning. Treatment of skin irritation by metals. In treatment of atherosclerosis.
Fe-3 Specific (DHEG)	 N,N- Dihydroxyethylglycine	In iron poisoning.

Table 2 (concluded)

Name	Formula and Chemical Name	Uses
Oxine	 8-Hydroxyquinoline	Used for its antibacterial action.
Penicillamine	 2-Amino-3-mercapto-3-methyl butanoic acid	In cases of copper overloads (Wilson disease) and in lead poisoning. Inhibits tumor growth in mice.

proteins in blood; thus serum iron concentration increases rapidly. The liver and the spleen remove sizeable amounts of iron, and this often results in hepatic necrosis[97]. Witzleben and Buck[89] proposed a hypothesis involving indirect lipid peroxidation. In the liver and elsewhere at the cellular level, iron is a potent protoplasmic poison due to its enzymatic impact[98]. However, the biochemical mechanism of iron toxicity has not yet been well established.

Symptoms of Iron Poisoning

Symptoms of iron poisoning begin with vomiting, usually accompanied by abdominal pain and diarrhea, and followed by lethargy and a state of shock[96]. Shock is attributed to hemorrhage and fluid loss from the necrotizing action of iron on the gastrointestinal tract[99].

Vasomotor collapse is explained by Smith[100] as produced by an excessive amount of ferritin formed from apoferritin in the mucosal cells. Ferritin, considered to be a vasodepresant[101], is then released into the circulation, initiating and maintaining the state of cardiovascular collapse.

Hepatic damage, although it has been found in some fatal human cases[97,100,102-105] and in some experimental studies[97,106], cannot be attributed as a causative factor in death from acute iron poisoning since no evidence of hepatic failure has been identified[104].

Other symptons include coagulation defects reported in some cases[105,107]. However, no systematic study of the various clotting factors has been reported in humans[104]. Experimental iron poisoning has shown prolongation of clotting time or complete absence of coagulation in rabbits[108]. Whitten[109] found alteration in the different stages of blood coagulation but the clotting time was not altered.

Metabolic acidosis in experimental iron poisoning is well known, and this is probably a common occurrence in lethal iron

poisoning in children[104]. Metabolic acidosis may be explained by two factors: (1) The release of hydrogen ions from the conversion of iron(II) and iron(III) into the circulatory system[110] and (2) the accumulation of lactic and citric acid from anerobic metabolism characteristic of shock[110,111].

Shock and coma are characteristic of all the fatal cases of acute iron intoxication reported until 1965[95].

Serum iron levels greatly increase in iron poisoning, and they are usually a measure of the degree of intoxication. Normal serum iron levels in humans fluctuate between 60 to 160 µg/100 ml of blood. In acute iron poisoning, serum iron levels cover a wide range from 200 to 5000 µg/100 ml, although one case with serum iron of 30,494 µg has been reported[112]. Iron levels of 500 to 1000 µg/100 ml are considered dangerous, and above this range the iron level is potentially lethal.

Treatment of Acute Iron Poisoning

Emergency treatment of acute iron poisoning is described by many authors[92,96,113,114,116,117]. In general, the treatment consists of induction of vomitting with an emetic, stomach lavage with sodium bicarbonate, intravenous fluid therapy (blood or plasma) to correct acidosis and dehydration, use of a chelating agent (Deferoxamine B) to remove "free iron" and peritoneal or hemo dialysis to help the removal of chelated ironn.

Waxman and Brown[86] have an excellent review of the various chelating agents that have been employed in iron overloads. The emphasis has been on deferoxamine though DTPA has also been evaluated[118-122]. Since its introduction in 1960[123], at least 700 papers have described different aspects of the application of deferoxamine.

DEFEROXAMINE B IN IRON POISONING

The major objective in the treatment of poisoning with most substances is the removal of the toxic substance from the organism.

The human body is not provided with an effective mechanism for eliminating excess iron. Therefore, in acute iron intoxication only two ways of increasing iron excretion exist: phlebotomy and the use of a chelating agent[124].

One of the most specific and irreversible of the iron chelating agents is deferoxamine B, commercially available as the methane sulfonate salt. This derivative of deferoxamine is produced by CIBA under the brand name of Desferal. (Trademark, CIBA Pharmaceutical Co. Summit, New Jersey).

The high solubility of the iron complex of deferoxamine and its low molecular weight allow it to pass easily through the kidneys, making deferoxamine of great value in the treatment of acute iron poisoning.

Deferoxamine was initially proposed by Moeschlin and Schnider[125] as a two-fold approach for the treatment of acute iron intoxication: enternally and parenterally. Moeschlin et al.[26] showed in animal experiments that the survival rate can be greatly increased by the use of deferoxamine.

More recently, several authors[86,95,112,113,115,124,127,128] have reviewed the clinical use of deferoxamine in acute iron overloads, and the question arises whether deferoxamine should be used orally at all since it is only slightly absorbed from the gastrointestinal tract[129]. Oral deferoxamine would help to remove any iron that may still be in the intestines. However, more effective methods, such as use of emetics and lavage, exist for the evacuation of the gastrointestinal tract[128]. Westlin[112] states that intramuscular deferoxamine is preferable to avoid drug-induced hypotension and recommends that the intravenous route be used for severly intoxicated patents that are in coma or cardiovascular collapse.

Rapid infusion of deferoxamine has been reported to induce hypotension and tachycardia in dogs, cats, rabbits[130], and children[95,112]. Whitten et al.[95,114] studied the toxicity of the iron-deferoxamine complex in children and dogs. Rapid intravenous

administration of 30 mg/kg of the complex produced a fall in mean arterial blood pressure in children. They gave a lethal dose of 225 mg/kg of iron-deferoxamine complex to dogs, which resulted in a fall of blood pressure, a decrease in hematocrit, a decrease of blood pH, and the development of renal shutdown.

These side effects of deferoxamine and its iron complex restricted the drug dose. An intramuscular dose[131] can be given to patients not in shock (1g initial administration followed by 0.5 g every 4 hours, with the total amount not to exceed 6 g in 24 hours. The intravenous dose[131] recommended for patients in a state of cardiovascular collapse should not exceed 15 mg/kg/hour by slow infusion, and a total dose of 6 g in 24 hours[86].

In summary, deferoxamine is very useful in the treatment of acute iron poisoning as shown by the reduction of mortality reported by several authors[95,124,128]. However, delayed therapy with deferoxamine did not reduce mortality in several cases of iron overloads[117,132,133]. Therefore, McEnery[117] recommends to "treat all cases of acute iron ingestion vigorously with deferoxamine." However, there is evidence[95,114] that deferoxamine and its iron complex are toxic when the drug is used in large amounts. The use of an extracorporeal system containing immobilized deferoxamine may prove to be useful in the treatment of very severe acute iron intoxication.

EXTRACORPOREAL TREATMENT OF ACUTE IRON POISONING

Deferoxamine may be covalently bonded to polymers containing reactive functional groups[43]. These polymers act as solid supports for the drug, which maintain its chelation ability. A number of investigators[134,135] have used an extracorporeal system based on activated carbon for the removal of certain uremic toxins and other poisons such as salicylates and barbiturates. Blood is perfused through a chamber containing the solid sorbent which removes the toxin from blood. In the case of iron poisoning, the excess iron

in blood would be complexed by the immobilized deferoxamine. Then neither the drug nor its iron complex would be released into the circulatory system, avoiding the problem of toxicity of large doses of deferoxamine.

This procedure would not replace the present use of soluble deferoxamine. Instead, it would help the soluble drug in removing iron from blood in those cases of very high serum iron levels.

DEFEROXAMINE B. ORIGIN AND CHEMISTRY

Deferoxamine B is isolated from feroxamine B, an iron-bearing metabolite belonging to sideramines obtained from Streptomyces Pilosus[123,136,137]. The trivalent iron in feroxamine B is removed by chemical means[138], yielding deferoxamine.

The molecule of deferoxamine is composed of one residue of acetic acid, two residues of succinic acid, and three residues of 1-amino-5-hydroxylaminepentane, as shown in Fig. 5. The residues altogether form a trihydroxamic acid with a free amino group at one end of the molecule. This amino group gives its basic character to the molecule and allows it to react with acids (inorganic and organic) and acid derivatives (acyl chlorides and anhydrides) to form salts and amide derivatives, respectively[139].

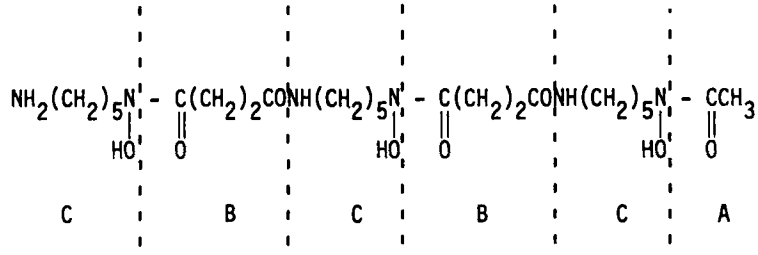


Fig. 5. Deferoxamine B, where A is acetic acid residue,
B is succinic acid residue, and
C is 1-amino-5-hydroxylaminopentane residue.

Deferoxamine B reacts stoichiometrically with Fe(III) to form feroxamine B, an octahedral iron complex, shown in Fig. 6. The ferric ion becomes attached to the three hydroxamic acid groups. The organic material surrounding the iron confers great stability to the complex[129].

The stability constant of the complex is of the order of 10^{31} in aqueous solutions ranging from pH 3 to pH 8. This value is at least 15 powers of 10 greater than for other complexes of deferoxamine and also greater than for other common chelating agents. Values of the stability constants for the complexes of various ions and chelating agents are given in Table 3.

Keberle[129] reported the effect of deferoxamine on the iron proteins ferritin, hemosiderin, transferrin, and hemoglobin. He found that deferoxamine removes iron from ferritin and hemosiderin until its maximum binding capacity is attained. He found, however, that deferoxamine removed only 10 to 15% of iron from saturated transferrin, and found no exchange from feroxamine to transferrin, showing that deferoxamine does not take up all the iron from transferrin under equilibrium conditions. These results are in disagreement with Bailar's conclusions[14]. He said that deferoxamine "is able to extract iron from nearly all of the tissues of the body, but not from transferrin or ferritin."

The kinetics of iron complexation of deferoxamine has been studied by Lentz et al.[142]. They found that the overall second-

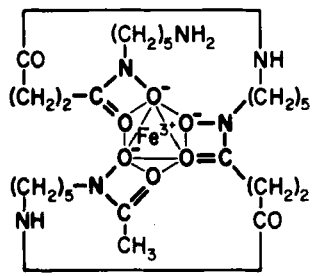


Fig. 6. Feroxamine B (also called ferrioxamine B).

TABLE III

Stability Constants for Some Metallic Complexes [86, 129, 140]

Metallic Ion	Deferoxamine B (DFA)	EDTA (a)	DTPA (b)	HEDTA (c)	EDDHA (d)	Fe-3-Specific (e)
Fe(III)	10^{31}	10^{25}	10^{29}	10^{20}	10^{32}	10^{30}
Fe(II)	10^{10}	10^{14}	10^{17}	10^{12}	-	10^{4}
Mg(II)	10^{4}	10^{9}	10^{9}	10^{5}	10^{8}	10
Ca(II)	10^{2}	10^{11}	10^{10}	10^{8}	10^{7}	-
Sr(II)	10	10^{9}	10^{11}	-	-	-
Co(II)	10^{11}	10^{16}	10^{19}	-	-	-
Ni(II)	10^{10}	10^{19}	10^{20}	-	-	-
Cu(II)	10^{14}	10^{19}	10^{21}	10^{17}	10^{16}	10^{8}
Cd(II)	10^{8}	10^{16}	-	-	-	-
Zn(II)	10^{11}	10^{16}	10^{18}	10^{14}	10^{9}	10^{5}

a Ethylenediaminetetraacetic acid

b Diethylenetriaminepentaacetic acid

c Hydroxyethylethylenediaminetriacetic acid

d Ethylenediamine-bis(o-hydroxyphenylacetic acid)

e Bis-(hydroxyethyl)-glycine

order rate constant for the complex formation of deferoxamine and iron(III) is the same in aqueous solutions of pH 3.92 as in blood plasma of pH values ranging from 4.1 to 7.1.

Whitten et al.[95] found that iron(II) also forms a complex with deferoxamine almost quantitatively at pH's of 6 or higher. For iron(III) the pH range in which complex formation is rapid and quantitative is between 4.5 to 7.0. At higher pH's it becomes increasingly incomplete, and is directly related to the pH and buffer capacity of the solution[139].

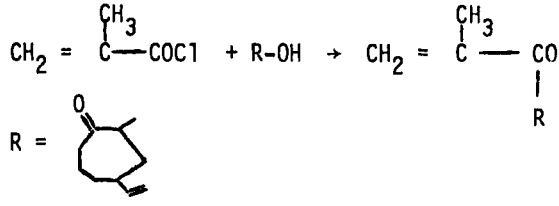
In-vivo experiments of Tripod and Keberle[143] first demonstrated that deferoxamine was also capable of removing iron from the organism. They administered ferric chloride and ferric chloride plus deferoxamine to rats and found that in the first case 0.03% of the iron was excreted in the urine and 81% of the feces with 19% absorbed. On the other hand, 0.8% of the iron administered with deferoxamine was excreted in the urine and 99.3% in feces, with no iron being absorbed.

IMMOBILIZATION OF DRUGS

Biologically Active Polymers

Immobilization of a drug by binding it to a polymer may prolong the activity of the drug. This may be achieved by grafting the drug to a reactive polymer or by converting the drug to a monomer with subsequent polymerization.

Cornell and Donamura[144] prepared monomeric drugs by reaction of methacrylyl chloride with tropolones that show biological activity. An example is



However, not all the monomers were able to polymerize, probably because of the steric effects associated with the bulky grouns[145].

Donamura and co-workers have reported, in a sequence of papers, the synthesis of biologically active formaldehyde-sulfamide copolymers[146,147] and sulfonamide- and sulfone-dimethylolurea copolymers[148,149]. They found that some of the copolymers presented antimalarial activity, and that the activity depended on the comonomer present in the polymer other than the drug[152].

Phenethylamines have been bound to polymers by converting them into vinyl monomers for homopolymerization[153] or copolymerization[154]. Monomers derived from styrene and methacrylic acid were used in this work. The biological activity of the monomers, homopolymers, and copolymers was studied by Weiner et al.[154]. They propose that the biological activity depended on the hydrolysis rate of the drug, and found that the polymer suffered slower hydrolysis than the monomer.

Polymeric salicylic acid derivatives have been studied by Weiner and Zilkha[155] for their biological action. They prepared the monomer by the reaction of methacrylyl chloride and various derivatives of salicylic acid, with subsequent homopolymerization. They tested the polymeric drugs as analgesics and found the drugs had retarded analgesic action, in some cases better than aspirin. The retarded action was suggested to be due to slow release of the salicylic acid derivatives.

Several other polymer drugs have been described in the literature[156-161].

Examples where the drug is grafted onto polymers are found in the binding of noradrenaline, isonicotinic acids, and hydrazide onto oxydextran and polyacrolein[163]. Drugs have also been bound to plasma proteins, such as albumin[163-170], including acidic[163,164] and basic drugs[166-170].

In our work we grafted a basic drug, deferoxamine, on to various polymers in order to insolubilize the drug and use it as a chelating polymer for biomedical applications.

POLYMERIC SUPPORTS FOR DEFEROXAMINE

The chemical structure of deferoxamine, which contains a primary amino group, allows it to react with other functional groups, such as chloroformyl (-COCl), benzyl chloride, and aldehyde (-CHO). Polymers and copolymers containing such functional groups were studied and the rationale for the selection of the various polymers is discussed.

Polyacrolein

In a previous paper we reported the synthesis of a polyacrolein-deferoxamine copolymer for potential use in the extracorporeal treatment of iron poisoning[43]. Polyacrolein was selected because the aldehyde groups of the polymer react with amines in neutral or slightly basic solvents to form Schiff's bases[171], while they do not react with hydroxamic acid groups, also present in the deferoxamine molecule, under these conditions.

Copolymers of Acrylyl Chloride and Methacrylyl Chloride with Styrene

Our interest in the poly(acid chlorides) is because of the high reactivity of these polymers which makes it possible to transform them into many polymeric derivatives. Copolymers are similarly useful because inclusion of only a small percent of acid chloride in a polymer chain provides sites which can be used for the attachment of grafts.

The inclusion of styrene as comonomer of the acid chloride is based on the hydrophobicity of styrene groups that renders the polymeric-deferoxamide insoluble in aqueous media. If homopoly (acid chlorides) are used and reacted with deferoxamine, a completely water-soluble system is obtained[172].

Terpolymers of Methacrylyl Chloride, Vinyl Pyrrolidone, and Styrene

To allow a more open structure for easier access of the ferric ions into the chelating sites, a swellable network is

necessary. We expected that by introducing vinyl pyrrolidone units into the polymer chains we would obtain a more hydrophylic, swellable (but not soluble) polymer. Styrene was included to provide the hydrophobicity necessary to prevent complete solubility.

Chloromethylated Polystyrene

Chloromethylated polystyrene is very commonly used as a support for the immobilization of enzymes[173] and for the preparation of ion-exchange resins[1]. The chlorobenzyl groups are very reactive with other groups containing active hydrogens, such as amine and hydroxyl groups[174]. Thus the low molecular weight analog, benzyl chloride, reacts with primary amines to form secondary amines, the polymer reacting in a similar way.

The bond that is formed in the reaction of chlorobenzyl and primary amine groups is very stable to hydrolysis. Therefore, if deferoxamine were reacted with chloromethylated polystyrene, the drug would not be released from the polymer even in conditions more severe than those found in blood.

Chloromethylated Amberlites

Amberlites XAD-2 and XAD-4, trademark of Rohm and Haas Company, Philadelphia, Pennsylvania 19105, are divinylbenzene cross-linked polystyrene resins available as porous beads[175,176]. The presence of styrene groups and the relatively high surface area of these beads allow them to be chloromethylated and aminated. Deferoxamine may be attached to the beads since the size of the pores is greater than the size of the deferoxamine molecule. This would allow us to prepare chelating resins without disturbing the scope of the beads and without having to fabricate the polymers into an appropriate physical form that would permit a good perfusion of blood.

EXPERIMENTAL RESULTS

SYNTHESIS OF POLYMER-DEFEROXAMINE GRAFTS

The reaction between the polymers or copolymers with deferoxamine utilized N,N-dimethylformamide (DMF) as solvent and triethylamine (TEA) as basic catalyst in order to prevent reaction of the hydroxamic acid groups with functional groups of the polymers. With the exception of the chloromethylated polystyrene-based polymers, they were soluble in DMF at the temperature of the reaction, 100-110°C.

Polyacrolein-Deferoxamine (VIII)[43]

Polyacrolein (I), prepared by free radical redox polymerization of acrolein[177], contains free aldehyde groups, hydrated aldehyde groups, and cyclic hemiacetal groups[178], all of which may react with an amine[171,179]. This is shown in Fig. 7, where after the reaction with a primary amine three types of groups may be found: azomethine, N-acetal, and tetrahydropyran. Thus, in the reaction of polyacrolein and deferoxamine all the possible structures may be found. Table 4 shows the yield and elemental analysis of the various polymer-deferoxamine grafts. The IR spectra of polyacrolein and polyacrolein-deferoxamine graft (VIII) are given in Fig. 8.

Poly(acryly chloride-co-styrene)-Deferoxamine (IX) and Poly(methacrylyl chloride-co-styrene-Deferoxamine (X)

The poly(acid chloride-co-styrene) copolymers were synthesized using a comonomer feed mole ratio of 1:1. The copolymer of acrylyl chloride and styrene (II) showed more styrene units then acid chloride units, as shown in Table 4. The reaction between the copolymers and deferoxamine proceeds with the formation of an amide linkage between the acid chloride group and the amine group of the drug, as shown in Fig. 9.

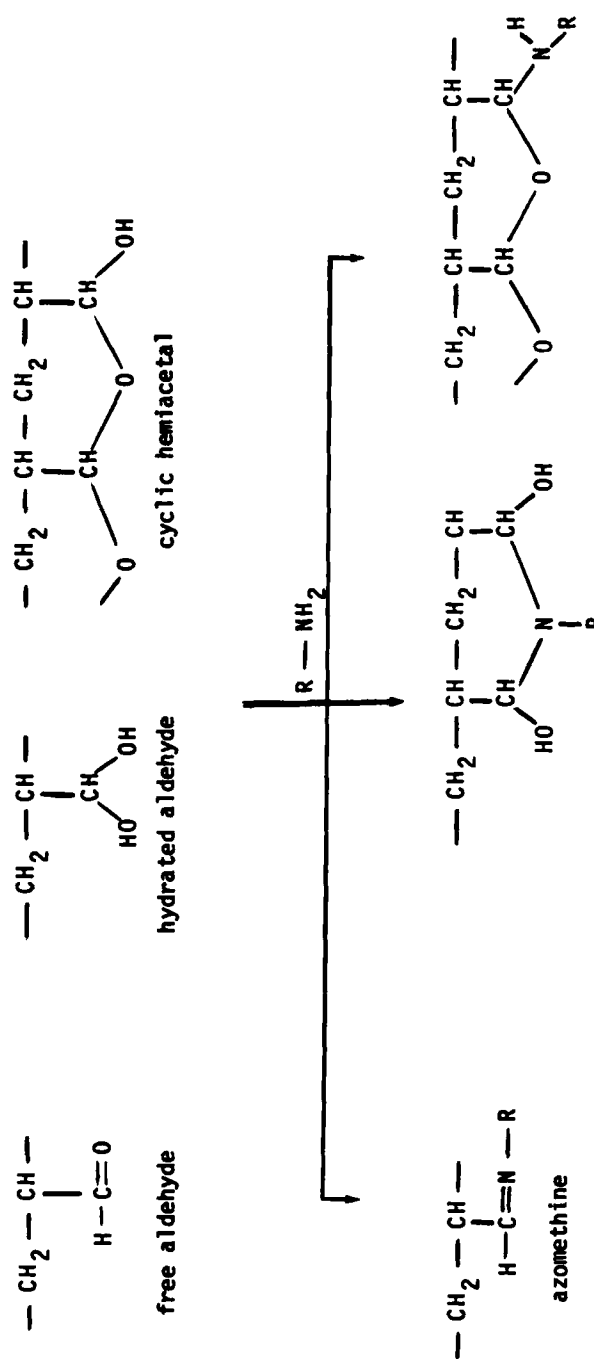
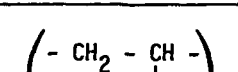
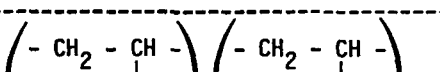
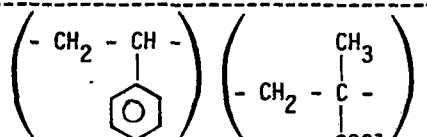
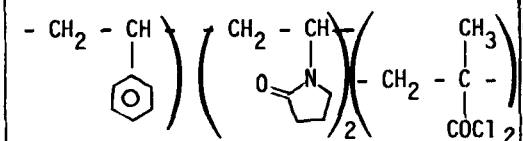
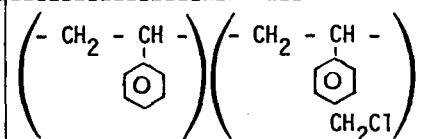


Fig. 7. Functional groups of polyacrolein and their reaction with an amine [178].

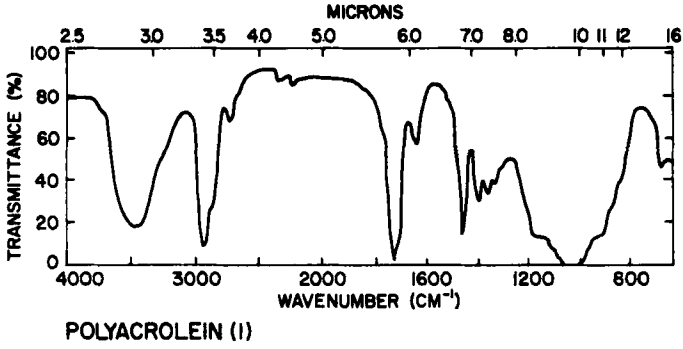
Table 4

Results of the synthesis of various polymers

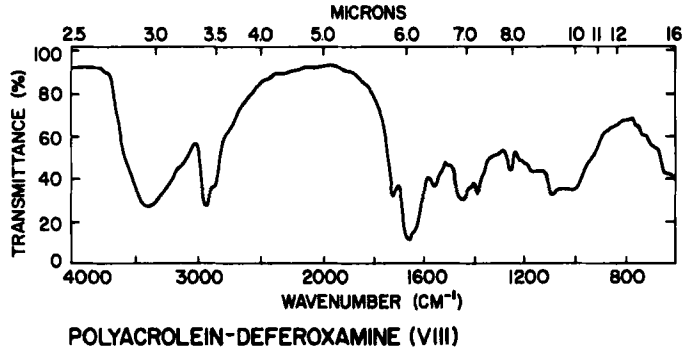
Base Polymer						Graft Polymer					
No.	Composition	Elemental Analysis				No.	Yield %	Elem. Analysis			% DFA Grafted
		C	H	N	Cl			C	H	N	
I	 Polyacrolein	Calc. 64.27 Found 61.17	7.19 6.99	- -	- -	VIII	49	Found 57.89	7.46	7.81	57 ratio* 1:5
II	 Poly(acrylyl chloride-co-styrene)	Calc. 73.02 Found 70.88	6.13 6.45	- -	14.37 14.24	IX	27	Found 75.36	7.81	3.28	30 ratio* 1:1
III	 Poly(methacrylyl chloride-co-styrene)	Calc. 69.06 Found 70.00	6.28 6.42	- -	16.99 17.52	X	51	Found 68.76	8.08	6.05	53 ratio* 1:1

Base Polymer						Graft Polymer					
No.	Composition	Elemental Analysis				No.	Yield %	Elem. Analysis			% DFA Grafted
		C	H	N	Cl			C	H	N	
IV	 Poly(methacrylyl chloride-co-styrene co-vinyl pyrrolidone)	Calc. 62.79 Found 59.14	6.78 6.62	5.23 4.75	13.24 13.31	XI	55	Found 64.87	7.79	7.98	60 ratio* 1:1
V	 Chloromethylated polystyrene	Calc. 73.95 Found 77.23	6.20 6.07	- -	18.85 19.86	XII	63	Found 72.70	8.02	6.51	68 ratio* 1:2
VI	Chloromethylated Amberlite XAD-2 (Amberlite XAD-2 is polystyrene crosslinked with divinylbenzene)	Found -	-	-	6.77	XIII	97	Found 84.91	8.21	2.26	28 ratio* 1:1
VII	Chloromethylated(Amberlite XAD-4 Amberlite XAD-4 is polystyrene crosslinked with divinylbenzene).	Found -	-	-	11.14	XIV	96	Found 83.18	8.27	3.28	33 ratio* 1:1

* Mole ratio of reactants = polymer:DFA



(a)



(b)

Fig. 8. IR spectra of (a) polyacrolein (I) and (b) polyacrolein-deferoxamine graft (VIII).

The reaction is not quantitative as shown by the percentage of deferoxamine grafted (Table 4). The unreacted acid chloride groups may be converted to carboxylic groups in the precipitation of the copolymer-drug graft with 10% hydrochloric acid. The IR spectra of poly(methacrylyl chloride-co-styrene) and of the co-polymer-drug graft are shown in Fig. 10.

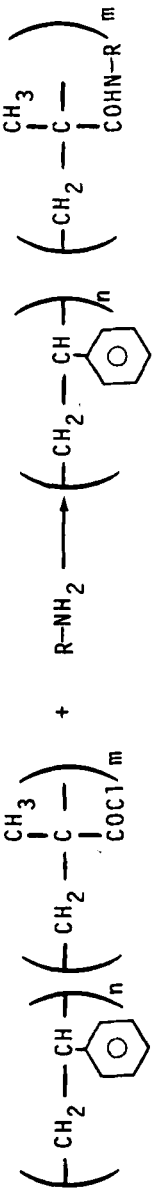
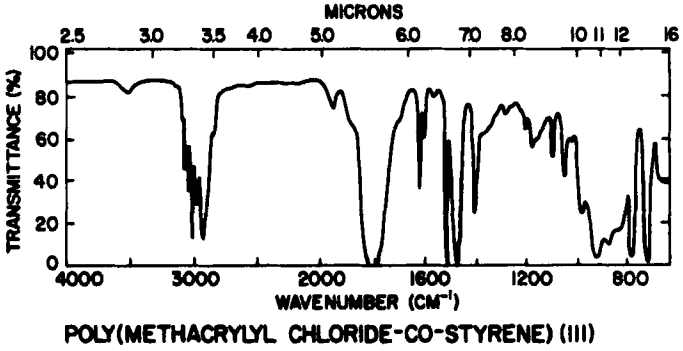
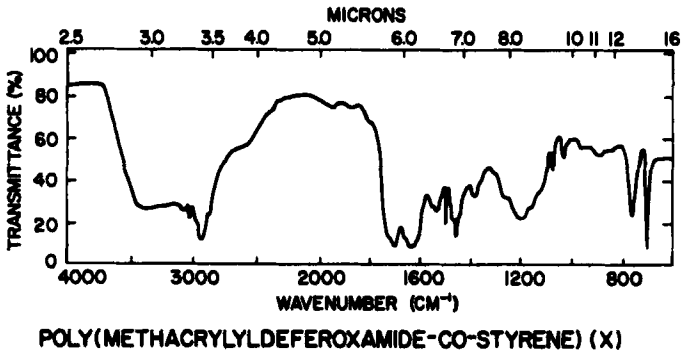


Fig. 9. Reaction between poly(methacrylyl chloride-co-styrene) (III) and deferoxamine. $R\text{-}NH_2$ is deferoxamine.



(a)



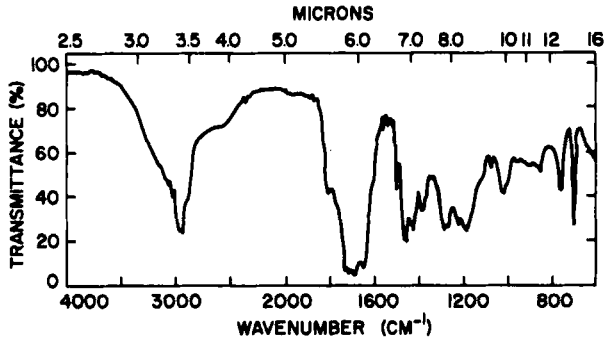
(b)

Fig.10. IR spectra of (a) poly(methacrylyl-chloride-co-styrene) (III) and (b) its deferoxamine graft (X).

Poly(methacrylyl chloride-co-vinyl pyrrolidone-co-styrene)-Deferoxamine (XI)

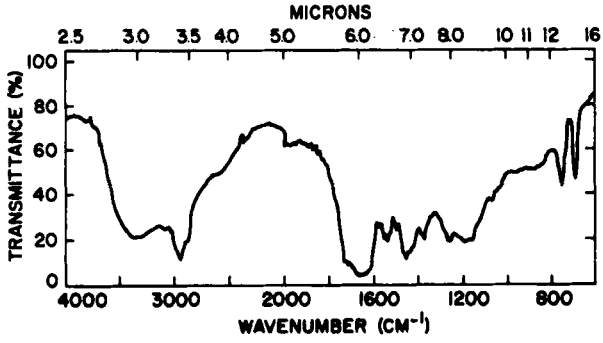
The terpolymer (IV) was obtained by free radical co polymerization of methacryl chloride, N-vinyl pyrrolidone, and styrene in a 2:2:1 feed mole ratio. A ratio of 2 acid chloride units, 2 vinyl pyrrolidone units, and 1 styrene unit is obtained from elemental analysis (Table 4). The reaction of the terpolymer with deferoxamine proceeds with the formation of an amide linkage. The

chemical equation for the reaction is similar to that of Fig. 9. Results for this reaction are summarized in Table 4, and the IR spectra of both the terpolymer (IV) and termpolymer-drug graft (XI) are shown in Fig. 11.



POLY(METHACRYLYL CHLORIDE-CO-VINYL PYRROLIDONE-CO-STYRENE) (IV)

(a)



POLY(METHACRYLYL CHLORIDE-CO-VINYL PYRROLIDONE-CO-STYRENE)-DEFEROXAMINE (XI)

(b)

Fig.11. IR spectra of (a) the terpolymer (IV) and (b) its deferoxamine graft (XI).

Chloromethylated Polystyrene-Deferoxamine (XII)

Chloromethylated polystyrene (V) was prepared by the reaction of commercial polystyrene[180] with chloromethyl methyl ether and zinc chloride. To obtain a high chlorine content, the reaction time was longer than the one normally used[181,182]. The percentage of chlorine from Table 4 indicates that about 80% of the phenyl rings have been chloromethylated.

The reaction of chloromethylated polystyrene and deferoxamine is shown in Fig. 12. In this reaction a secondary amine is formed, which provides the graft with very high hydrolytic stability. The results for this reaction are shown in Table 4. In this case, there was 3.91% of unreacted chlorine, indicating that a longer reaction time is probably necessary for higher conversion. The IR spectra of chloromethylated polystyrene (V) and poly(styrene-co-vinyl benzyl)-deferoxamine (XII) are shown in Fig. 13.

Chloromethylated Amberlite XAD-2- and XAD-4-Deferoxamine (XIII) and (XIV)

Chloromethylated Amberlites were obtained by the same procedure used for the chloromethylation of polystyrene. The reaction between deferoxamine and the chlormethylated Amberlites follows the same scheme as for the synthesis of XII. Table 4 gives the results for the reactions. IR spectra could not be obtained for the chloromethylated Amberlites or the Amberlite-deferoxamine grafts due to their particulate nature.

PROPERTIES OF THE POLYMERS

Some physical properties of the polymers are given in Table 5. Viscosity measurements could be done only on those polymers that were soluble in appropriate concentrations.

Stability of the Grafts in Saline Solutions

Stability of the grafts in the present case refers to the degree of deferoxamine liberation when the polymers are placed in

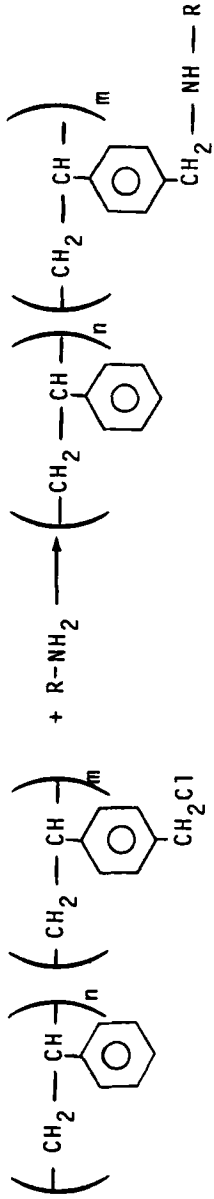
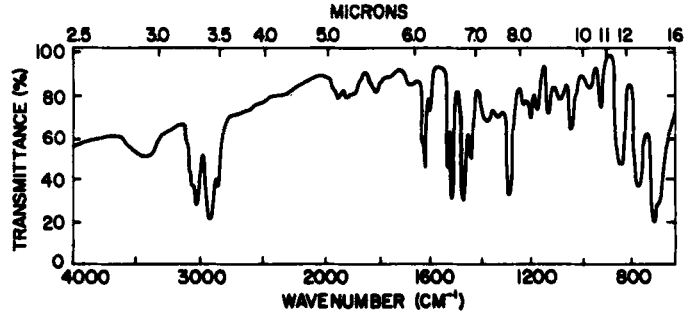
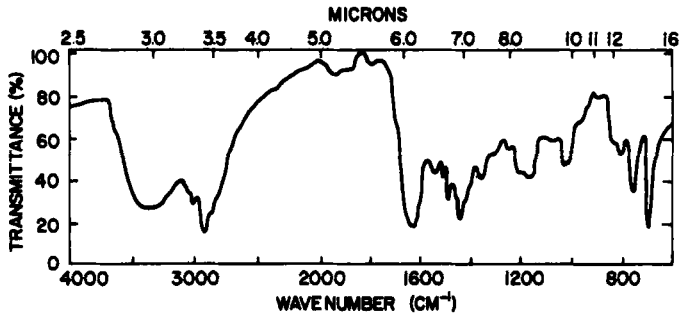


Fig.12. Reaction between chloromethylated polystyrene and deferoxamine. $R\text{-}NH_2$ is deferoxamine.



(a)



(b)

Fig.13. IR spectra of (a) chloromethylated polystyrene (V) and (b) its deferoxamine graft (XII).

contact with saline solutions for several days. The presence of deferoxamine in the solutions was determined quantitatively by UV spectrophotometry. Deferoxamine has a strong absorption with a maximum of 210 nm (epsilon max = 32,000) at pH 5.6, allowing a rapid determination of deferoxamine concentration in solution.

Fig. 14 shows the stability of the various polymer grafts in normal saline (0.9% NaCl) solution (pH 5.6). The stability of the grafts in phosphate-buffered saline solution (pH 7.4) is shown

TABLE V
Some Physical Properties of the Various Polymers

Polymer No.	[η] (Solvent)	Solubility	Transition Temperature(b) (°C)	Density(c) (g/cm³)
I	0.38(a) (water)	Xylene, DMSO	M.T. 126(d)	1.196
II	1.05 ($CHCl_3$)	Acetone, isopropanol, $CHCl_3$	M.T. 224	0.700
III	0.53 (DMF)	$CHCl_3$, xylene, DMF, DMSO, 1,2-dichloroethana, dioxane, CCl_4, acetone	M.T. 302 (some decomposition)	-
IV	0.11 (dioxane)	Swells in ethanol, xylene, DMF, DMSO	T_g 282; Dec. 358(e)	0.751
V	-	Swells in DMF(hot), xylene, DMSO, $CHCl_3$	Dec. 252	0.915
VI	-	Insoluble in all solvents tested	Dec. 335	-
VII	-	Swells in DMSO	Dec. 227	0.943
VIII	-	Xylene, DMSO, 1,2-dichlorethane	Dec. 203	1.158
IX	-	Ethanol, xylene, DMSO, Dioxane, acetone, isopropanol	M.T. 352 (with dec.)	-
X	-	Xylene, DMF	Dec. 357	1.041
XI	-	Swells in DMSO, DMF, xylene, CCl_4	T_g 261	0.964
XII	-	Swells in DMSO, DMF, xylene	Dec. 374	1.016
XIII	-	Swells in 1,2-dichloroethane	Dec. 327	0.967
XIV	-	Swells in DMSO, CCl_4 (hot)	T_g 212; Dec. 262	0.883

a Intrinsic viscosity of a polyacrolein-bisulfite adduct solution
b Determined by differential scanning calorimetry (DSC)
c Determined by pycnometry
d M.T.: Melt temperature (center of peak in DSC thermogram)
e Dec.: Temperature at which decomposition started

in Fig. 15. In both cases, the graft with the lowest stability was VIII. This is due to the easier hydrolysis of the carbon-nitrogen double bonds. In the case of graft XII, the relatively high

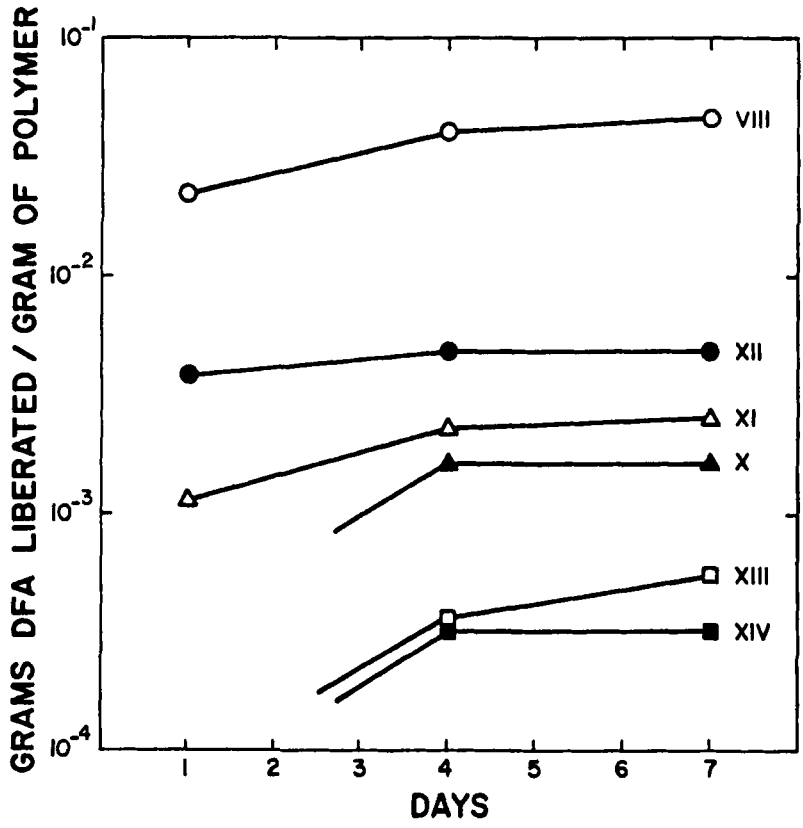


Fig.14. Stability of the various polymer-deferoxamine grafts in normal saline solution.

concentration of liberated deferoxamine is probably due to some unreacted drug entrapped in the polymer network.

The amounts of deferoxamine liberated from the polymers were low enough to allow us to expect similar behavior in biological media. Blood plasma was used to determine the stability of the grafts in the presence of other molecules, such as proteins and enzymes, which may promote or enhance degradation of the grafts.

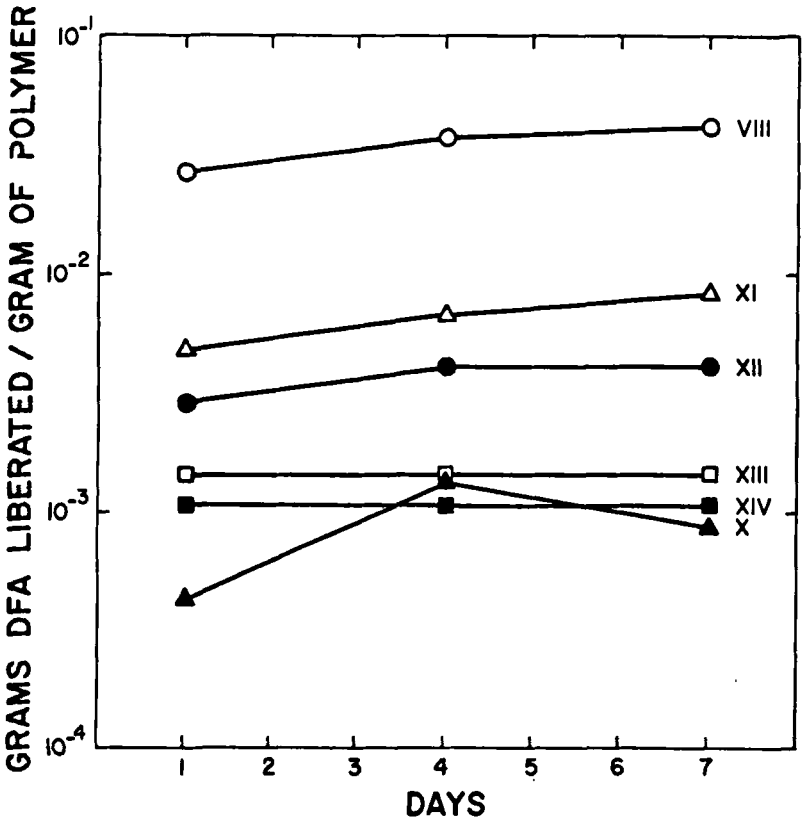


Fig.15. Stability of the various polymer-deferoxamine grafts in phosphate buffered saline solution.

Stability of the Grafts in Plasma

The same principle, deferoxamine liberation, was used to determine the stability of the polymers in plasma. In this case the liberated drug was analyzed spectrophotometrically by forming its Fe^{3+} complex and measuring absorbances at 430 nm. This method is only accurate for concentrations not lower than $9 \cdot 10^{-5}$ mole/liter as determined from a standard calibration curve. None of

the polymers showed liberation of deferoxamine in concentrations higher than the accurate limit, i.e., $9 \cdot 10^{-5}$ mole/liter, after 27 hours in contact with plasma.

Iron Chelation Ability of the Polymer Grafts

Samples of the various grafts were placed in concentrated ferric chloride solutions for 3 days. All the polymers took on a brown-red color. The iron content of the polymers was determined quantitatively by chemical analysis[183] and qualitatively by energy dispersive analysis of x-rays (EDAX) in the scanning electron microscope.

A comparison of chelating capacity found for the grafts and the one calculated from the nitrogen content is given in Table 6. These results show that the capacity of grafts XIII and XIV (derived

TABLE 6

Chelating Capacity of the Various Polymer-Deferoxamine Grafts

Polymer No.	Capacity (mg Fe/g resin)		Efficiency[a] (%)
	Calculated	Found	
VIII	49.3	18.8	38
IX	20.8	-	-
X	40.4	17.7	44
XI	30.2	10.3	34
XII	41.6	12.9	31
XIII	14.8	6.0	41
XIV	18.6	6.2	33

a Efficiency = mg Fe found/mg Fe calculated.

from Amberlite XAD-2 and XAD-4, respectively) is about half of the capacity for other polymers. However, the efficiency is comparable, indicating that the accessability of the iron into the chelating sites is approximately the same for all the grafts.

A qualitative analysis of the iron in the polymers is given in Fig. 16. The traces on the left side are controls, i.e., grafts without exposure to iron, and at the right are the results for iron-containing grafts. The peaks at 6.4 keV reveal the presence of iron[184], whereas the controls do not present any peak in that position.

Stability Constants of the Polymer Iron Chelates

When a polymer-deferoxamine iron complex (P-DFA-Fe) is placed in water, the following reaction takes place:

$$\text{P-DFA-Fe} + 3H_2O \rightleftarrows \text{P-DFA-H}_3 + Fe(OH)_3$$

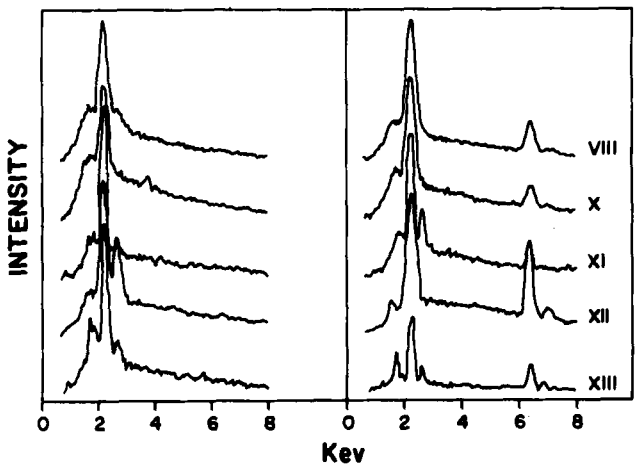


Fig.16. Energy dispersive analysis of X-rays (EDAX) of various polymer-deferoxamine-iron complexes. At the right are the traces for iron complexes, and at the left for the controls.

The equilibrium constant for this reaction is

$$K_e = \frac{[\text{P-DFA-H}_3]\,[\text{Fe(OH)}_3]}{[\text{P-DFA-Fe}]} \quad \text{, where} \qquad (4)$$

$[\text{Fe(OH)}_3] = [\text{Fe}^{3+}]\text{sol}$ = iron concentration in solution, and

$[\text{P-DFA-Fe}] = [\text{Fe}^{3+}]\text{pol}$ = iron concentration in the polymer.

Therefore

$$K_e = \frac{[\text{Fe}^{3+}]^2_{\text{sol}}}{[\text{Fe}^{3+}]_{\text{pol}}} \qquad (5)$$

and the stability constant would be

$$K_s = \frac{1}{K_e} = \frac{[\text{Fe}^{3+}]_{\text{pol}}}{[\text{Fe}^{3+}]^2_{\text{sol}}} \qquad (6)$$

The various polymer grafts were placed in concentrated ferric chloride solutions for 4 days to let them equilibrate with iron. Then a known amount of each polymer-chelate was placed in distilled water for 7 days. Iron and deferoxamine in solution were analyzed. The difference between total moles of iron and moles of deferoxamine gives the absolute amount of iron coming from the polymer-chelate according to the reaction given above. The iron content of the polymers was determined[183], and the stability constants (Table 7) were calculated using Eq. (6). The values in Table 7 demonstrates that a large decrease in stability of the deferoxamine-iron complex occurs when the drug is bound to a polymer backbone. This is probably due to steric hindrance from the polymer and a decrease in mobility of the drug molecule.

TABLE 7

Stability Constants of the Polymer-Deferoxamine-Iron Complexes

Polymer	K_S (liter/mole)
X	10^6
XI	10^5
XII	10^5
XIII	10^4
XIV	10^4

REMOVAL OF IRON FROM SOLUTION IN RECIRCULATING SYSTEMS

Iron in Aqueous Solutions

Preliminary experiments for iron removal were carried out in aqueous solutions. Ferric chloride solutions of relatively high concentration ($1.2 \cdot 10^{-3}$ M) compared to the concentration of serum iron in acute poisoning cases ($4.5 \cdot 10^{-5}$ to $1.8 \cdot 10^{-4}$ M) were used. Fig. 17 gives a schematic diagram of the system employed for these determinations.

The iron solution was recirculated through the chamber containing the chelating polymer, and samples were taken in the outflow. The results obtained for iron concentration in the solution at various times indicated that, with the iron concentrations used, the resins became saturated after 15 to 20 min. of initiating the experiment, showing no further decrease in iron concentration. The results for some of the polymers tested in these conditions are given in Table 8.

The polymer grafts that were obtained in powder form (VIII-XII) became agglomerated as a hard cylinder, and the solution could not penetrate into the bulk of the resin, flowing only through

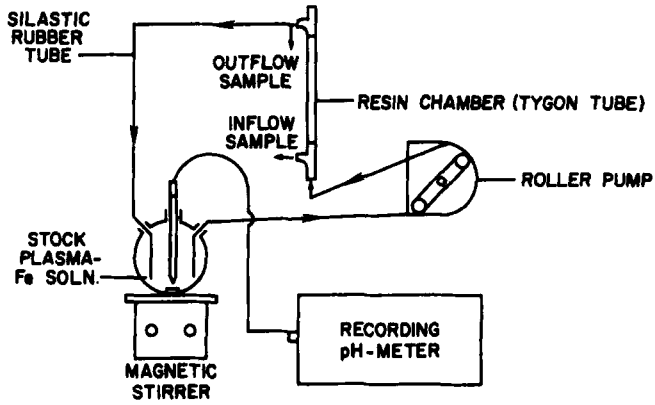


Fig.17. Diagram of the system employed in the experiments for iron removal from water and plasma.

the outer parts next to the walls of the chamber. This agglomeration effect was not present in those polymers in bead form (XIII and XIV).

Iron in Plasma Solutions

Iron was added to plasma in the form of ferric chloride in concentrations of 500 to 800 µg Fe/100 ml plasma (i.e, $8.9 \cdot 10^{-5}$

TABLE 8

Iron Removed from Aqueous Solutions by Various Polymer-Deferoxamine Grafts

Polymer	µg Fe removed/g resin
X	792
XII	2196
XIII	661
XIV	900

to $1.4 \cdot 10^{-4}$ M). Polymer X and XII gave scattered results for the rate of iron removal. Polymers VIII, IX, and XI were not tested.

Results for grafts XIII and XIV (in bead form) are shown on Figs. 18 and 19. The plot on Fig. 18 is the rate of iron removal, i.e., decrease of iron concentration in plasma with time. The two polymers show approximately the same rate. A better comparison is given on Fig. 19 where the amount of iron removed is observed during the first hours of the experiments. Then the iron removed by graft XIV starts leveling off, while the iron removed by grafts XIII continues to increase. This result agrees with the higher chelating efficiency determined for XIII (Table 6).

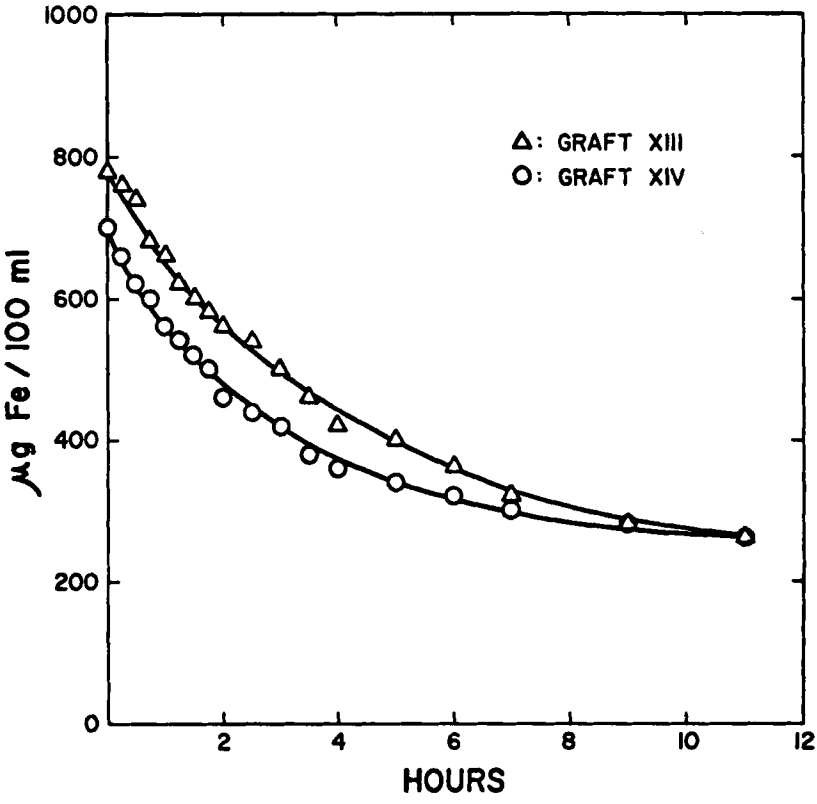


Fig.18. Rate of iron removal from plasma.

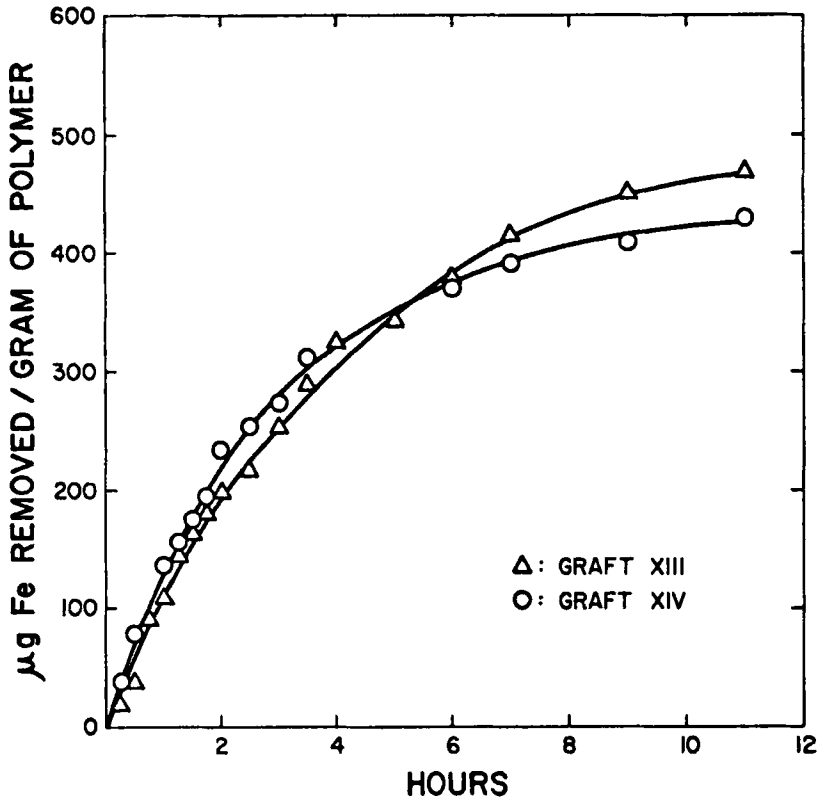


Fig.19. Iron removed from plasma.

CONCLUSIONS AND FUTURE DIRECTIONS

1. Deferoxamine B has been bound to various reactive polymers, including polyacrolein, copolymers of acrylic acid chlorides, and chloromethylated polystyrenes (uncross-linked and cross-linked).

2. The polymer-deferoxamine grafts showed good stability in saline solutions (0.9% NaCl and phosphate-buffered NaCl solutions) and in plasma.

3. The polymer-deferoxamine grafts maintained the chelation ability of the drug, although the stability of the polymer-drug-iron complex was decreased compared to the stability of feroxamine (the soluble deferoxamine-iron complex).

4. The chelation capacity of the polymer varies from 6 to 19 mg of Fe/g of resin. This value is in the range necessary for their application in acute iron poisoning where the serum iron levels vary from 0.2 to 5 mg/100 ml of blood.

5. Additional experiments should be performed in order to study the effect of the different variables that affect the chelation process in recirculating systems.

6. The ability of the polymer-deferoxamine grafts to remove iron from plasma, a protein-rich medium where iron becomes less available, opens the possibility of applying the graft copolymer to in-vivo situations. Experiments in animals will be the next logical step after complete in-vitro characterization of the grafts has been made.

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