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A Novel Iron-Chelating Graft Copolymer

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ABSTRACT

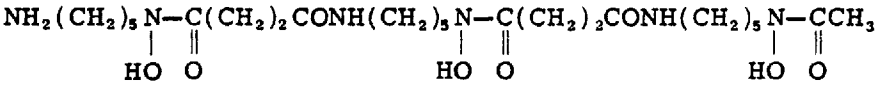
Deferoxamine B, liberated from its methane-sulfonate salt, has been linked to the chemically reactive polyacrolein to form polyacrolein-deferoximine (PADFI), whose structure may contain imino groups as well as N-acetal groups. Free aldehydes are still present in the polymer structure. The polyacrolein-deferoximine is stable in physiological saline solution for at least 24 hr. A sample of 0.3000 g of PADFI removes 40% of iron(III) from a 1.8×10^{-3} M solution. The polymer, PADFI, is insoluble in most ordinary solvents, and it failed to dissolve completely in sulfur dioxide solution. Deferoxamine B attached to a polymer backbone may be useful as an insolubilized chelating agent for the extracorporeal treatment of severe iron poisoning.

INTRODUCTION

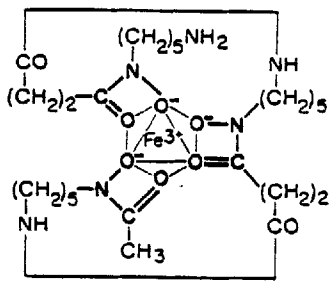
Acute iron poisoning is a common cause of poisoning among young children [1]. One of the few ways in which dangerously high levels of iron can be treated is through the use of a strong chelate for iron. One of the most specific and irreversible of the iron chelating agents is deferoxamine B, a derivative of which is a drug produced by CIBA under the brand name of Desferal (trademark, CIBA Pharmaceutical Co., Summit, New Jersey) [2].

Deferoxamine B is isolated from feroxamine B, an iron-bearing metabolite belonging to the sideramines, a group of natural occurring substances [3, 4]. Due to its great affinity for iron and to its rapid excretion by the kidneys, deferoxamine B is used clinically for the treatment of severe acute iron poisoning [1]. The relatively low molecular weight iron-deferoxamine B complex is hydrophilic and water soluble, so that it can pass through the kidneys. Roughly 10 parts by weight of the drug will bind one part of Fe(III). The stability constant of the complex is approximately 10^{31} in aqueous solutions ranging from pH 3 to 8. This value is about 15 powers of ten higher than the stability constants for other metallic complexes of deferoxamine B [5]. This difference is greater than in the case of synthetic chelating agents. On the other hand, the complexes of transferrin and ferritin (the two iron transporting proteins in the blood) with iron are more stable than feroxamine B, meaning that deferoxamine B should not remove iron from such proteins [6].

Chemically, deferoxamine B is a trihydroxamic derivative, whose structural formula is



It reacts stoichiometrically with Fe(III) to form feroxamine B, an octahedral iron complex [7]:



The pH range in which feroxamine B formation is rapid and quantitative is 2 to 4.5. Between pH 4.5 and 7.0 complex formation becomes increasingly incomplete, in direct relation to the pH and buffer capacity. Above pH 8 practically no feroxamine B is formed [7].

Lentz et al. [8] have studied the kinetics of complex formation and found that the second-order reaction had a rate constant of 2.5×10^3 M^{-1} sec^{-1}. The half life for the reaction is about 2.5 sec and the activation energy is about 12 kcal.

Studies of Whitten et al. [9] showed that equimolar quantities of Fe(II) and deferoxamine B at pH 6 yield as much complex as is formed by equimolar quantities of Fe(III). This is due either to the rapid oxidation of Fe(II) to Fe(III) in the presence of deferoxamine B, or the latter forms an unstable complex with Fe(II) which is rapidly converted to stable Fe(III) complex.

When serum iron levels exceed 500 μg %, the serum iron-binding proteins become saturated, thus free iron is present. Iron levels in the 500 to 1000 μg % range are considered high and dangerous. Levels substantially above this are potentially lethal. Serum irons as high as 30,000 μg % have been measured. In most iron poisoning situations deferoxamine B can be applied quite successfully and with minimal side effects [2, 10]; however, both the drug and its iron complex are toxic if present in large amounts. They have been shown to produce hypotension and tachycardia in dogs, cats, and rabbits [11], and in children [9, 12]. The use of deferoxamine B is thus dose-restricted [10]. In very severe poisoning cases the maximum amount of drug which can safely be used may not be sufficient for the removal of the required amounts of iron.

To avoid the problem of toxicity of large doses of deferoxamine B and its iron complex, we have bonded the drug to a polymer, thus rendering it insoluble. We propose that the chelating polymer can be fabricated in bead form; the patient's blood can then be perfused through a column packed with this material to chelate and retain the iron, thus purifying the blood from plasma iron, analagous to the method described by Andrade et al. [13, 14] for the removal of uremic toxins and certain poisons. Deferoxamine B has a free NH_2 group that is apt to react with other functional groups. This NH_2 group in the deferoxamine B molecule is not directly involved in the formation of the octahedral complex [2], since this compound acts as a hexadentate chelating agent, according to the structure described by Schwarzenbach [7].

Thus it would be possible to react polyacrolein with deferoxamine B to obtain an insoluble polymer that is able to remove iron from solution. We used polyacrolein as our polymeric substrate since it reacts with amines in the presence of organic solvents [15] without introducing acids or bases that may either hydrolyze the trihydroxamic acid or produce aldol condensation reactions [16].

Acrolein may be polymerized in two ways: by free radical polymerization to form addition polymers through the C=C double bond, and by ionic (both cationic and anionic) polymerization, where the addition is to the C=C double bond and to the C=O double bond.

In the free radical polymerization the structure of the polymer has been shown to contain free carbonyl groups (Fig. 1a) as well as hydrated carbonyl groups (Fig. 1b) and cyclic hemiacetals (Fig. 1c) [15].

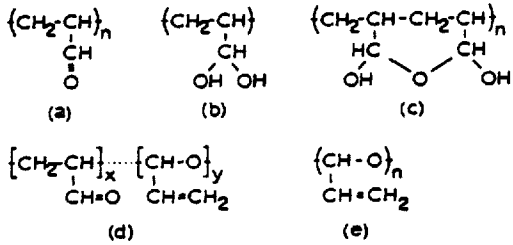


FIG. 1. Representative forms of polyacrolein polymerized under different conditions. Free radical polymerization produces a polymer containing free carbonyls (a), hydrated carbonyls (b), and cyclic hemiacetals (c). Ionic polymerization produces free vinyl and free aldehyde groups (d). The polymer can also be formed with free vinyl groups (e); see text.

Ionic polymerization of acrolein leads to the formation of polymers with free vinyl and aldehyde groups (Fig. 1d). The exception is when the polymerization is carried out with sodium or sodium cyanide in tetrahydrofuran or toluene at low temperatures [16]. In this case the polymers contain only vinyl groups (Fig. 1e).

The three different groups present in the polyacrolein obtained by free radical polymerization may react with amines as was shown by Schulz et al. [15]. By using low molecular weight model compounds they demonstrated that polyacrolein reacts with amines or hydrazines to form, in addition to azomethine groups (Fig. 2b), N-acetal groups (Fig. 2a).

EXPERIMENTAL

Polyacrolein [16]

Polyacrolein was prepared by free radical polymerization of acrolein (Polysciences, Inc., Warrington, Pennsylvania). Fifty grams

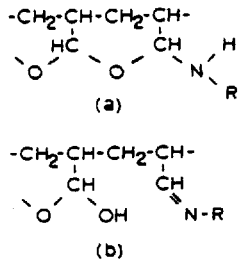


FIG. 2. Structures of the reaction products of polyacrolein and primary amines: (a) N-acetals, (b) azomethines (see Ref. 16).

of freshly distilled acrolein was dissolved in 200 ml of benzene and then 0.3 g of 2,2'-azo-bis-(2-methyl-propionitrile) (Eastman Kodak, Rochester, New York) was added to the solution. The mixture was refluxed for about 72 hr and the polymer precipitated out. A white, fine dustless powder was obtained in about 90% yield. The IR spectrum of the polyacrolein (Fig. 3) was identical to that reported in the literature [16].

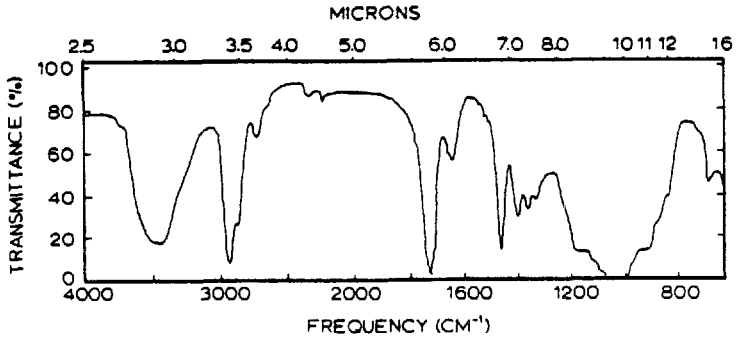


FIG. 3. IR spectrum of polyacrolein obtained by free radical polymerization in benzene (KBr pellet).

Intrinsic Viscosity

The intrinsic viscosity of polyacrolein was determined according to the procedure described by Seibert [17] by using the one point relation:

$$[\eta] = 0.96897\eta_{sp} - 0.30936\eta_{sp}^{2} + 0.09064\eta_{sp}^{3} \quad (1)$$

The polyacrolein (2.0 g) was suspended in water (50 ml) and the suspension saturated with sulfur dioxide. After 1 hr the polyacrolein was completely dissolved due to the formation of the polyacrolein-SO_2 adduct. The excess SO_2 was stripped off by bubbling nitrogen into the solution and then by vacuum. The solution was filtered through a sintered glass funnel and an aliquot of 10 ml was evaporated to dryness to determine the concentration. The concentration c measured by this procedure was 0.4288 g/dl.

The rest of the solution was made 0.25 M with sodium sulfate and diluted to 100 ml with 0.25 M Na_2SO_4 solution. The viscosity measurements were made on the polyacrolein-SO_2 solution and on the 0.25 M Na_2SO_4 solution used as solvent; the result was $\eta_{sp} = 0.16$. The intrinsic viscosity as calculated from Eq. (1) was $[\eta] = 0.38$.

Deferoxamine B

Liberation from Its Methane Sulfonate Salt (Desferal) [18, 19]

Desferal (3.9 g) was dissolved in warm water (12.5 ml). After cooling the solution in an ice bath, 2.5 ml of 2 N potassium bicarbonate was added and the container scratched while cooling until precipitation started. The solution was left at room temperature for 2 hr and then diluted with 5 ml of cold water, filtered with suction, washed with cold water, and dried, yielding 1.5 g of deferoxamine B with a mp of 138 to 140°C (mp lit. [19] 138 to 140°C). The filtrate was concentrated to dryness, then 10 ml of water was added and the product filtered again, yielding 0.6 g of deferoxamine B. The two solids were dissolved in hot methanol and recrystallized, yielding 1.9 g of deferoxamine B of mp 139 to 140°C.

Elemental Analysis

Calculated for $C_{25}H_{48}O_8N_6$: C, 53.38; H, 8.54; N, 14.95. Found: C, 52.01; H, 8.71; N, 14.86.

IR Spectrum

The IR spectrum of deferoxamine B is shown in Fig. 4. This spectrum is consistent with the structure of deferoxamine B, having $-NH_2$ group bands (3440 and 3320 cm^{-1}) overlapped with the strong OH bands of the hydroxamic acids; $-CH_2-$ groups (2940 and 2860 cm^{-1}) and $-N-C=O$ groups (1650 cm^{-1}) [20, 21].

Polyacrolein-Deferoximine (PADFI)

Polyacrolein was reacted with deferoxamine B by a procedure similar to that used by Schulz et al. [22] for the reaction of polyacrolein

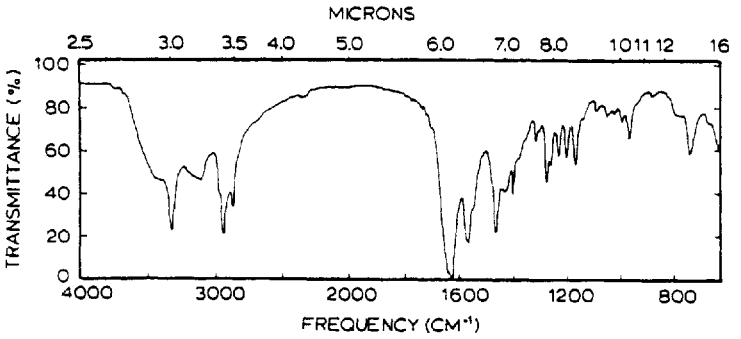


FIG. 4. IR spectrum of deferoxamine B (KBr pellet).

and amines. Thus 1 g of polyacrolein was let swell in 50 ml of dimethylformamide (DMF) and heated to 60°C; then a solution of 1 g of deferoxamine B in 50 ml of DMF at 60°C was added to the partly swollen, partly dissolved polyacrolein. The mixture was kept at 60°C for 2 hr, during which it took on a brownish color, and then stirred at room temperature overnight. Then water was added to precipitate the polymer in solution. The brown polymer was filtered, washed with water, dried, and triturated with hot methanol to eliminate the unreacted deferoxamine B, then filtered, washed with water, and dried under vacuum at 60°C.

IR Spectrum

The IR spectrum of PADFI is shown in Fig. 5. The broad, strong band centered at 3400 cm^{-1} indicates the presence of hydrogen bonded

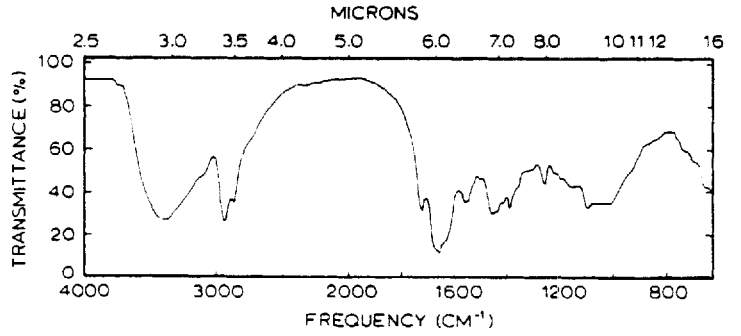


FIG. 5. IR spectrum of polyacrolein-deferoximine (PADFI), (KBr pellet).

hydroxyl groups. The band at 1650 cm^{-1} corresponds to the —N—C=O groups and that at 1720 cm^{-1} to the aldehyde groups.

Nitrogen Content of PADFI

The theoretical nitrogen content of PADFI was calculated assuming complete reaction between polyacrolein and deferoxamine B. By taking equal weights of both reactants (molar ratio of aldehyde groups/amino groups = 10/1) the calculated nitrogen content is 8.4%. Found: 7.16%.

Stability of PADFI in Physiological Saline Solution

PADFI was suspended in physiological saline solutions and stirred at 36 to 37°C for 24 hr. After this period, PADFI was filtered, washed with water, and dried. The IR spectrum of this product is identical to that of untreated material, the solution showed a decrease in Fe(III) content and when left in contact with ferric chloride solution.

Iron Chelation Test for PADFI

A sample of 0.3000 g of PADFI was left in contact with 10 ml of a solution of 0.0290 g of ferric chloride in 100 ml water for 16 hr. After this period the PADFI was filtered and the filtrate analyzed for Fe(III) at 396 mμ [23], giving an absorbance of 0.92 whereas the standard ferric chloride solution gave an absorbance of 1.5. This value corresponds to a 40% decrease in Fe(III) concentration.

RESULTS AND DISCUSSION

Polyacrolein, prepared by free radical polymerization of acrolein with 2,2'-azo-bis-(2-methyl-propionitrile) as initiator, was reacted with deferoxamine B, which was obtained by hydrolysis of its methane sulfonate salt with 2 N potassium bicarbonate solution.

The reaction between polyacrolein and deferoxamine B was carried out in dimethylformamide at 60°C. In this condition, polyacrolein swells and becomes partly dissolved, while deferoxamine B dissolves completely. The solution takes a yellow color that turns to brown as the reaction proceeds; the reaction mixtures were usually left overnight at room temperature to insure a more complete reaction. In all the reactions equal weights of the reactants were taken to leave a large number of unreacted aldehyde groups in order to dissolve the polyacrolein-deferoximine (PADFI) in sulfur dioxide solutions for molecular weight determinations, and then by comparison with the molecular weight of the starting polyacrolein have some idea of the extent of the reaction. Unfortunately, the polyacrolein-deferoximine

did not form clear solutions even after filtration through sintered glass.

When polyacrolein and deferoxamine B are in equal weight, the ratio of $-CHO$ groups to $-NH_2$ groups is 10:1, thus a maximum nitrogen content of 8.4% can be obtained. The measured nitrogen content of 7.16% indicates that under these conditions PADFI is obtained with an aldehyde/amine ratio of about 14/1, and that the reaction is about 70% complete.

The IR spectrum of PADFI (Fig. 5) showed the features of both polyacrolein (Fig. 3) and deferoxamine B (Fig. 4). The $-NH_2$ band at 3320 cm^{-1} and the three C—N vibration bands [7] at 1230, 1200, and 1165 cm^{-1} of deferoxamine B have disappeared, while the absorption corresponding to the $-N-C=O$ group at 1650 cm^{-1} is now present. The band at 1720 cm^{-1} corresponds to unreacted aldehyde groups. The bands in the range 900 to 1200 cm^{-1} have the same appearance as those of polyacrolein. After trituration with boiling methanol to dissolve any unreacted deferoxamine B, the IR spectrum of the PADFI was identical to that before, meaning that deferoxamine B had reacted with polyacrolein, probably through an imine, or N-acetal, or both.

Two possible structures for PADFI are presented in Fig. 6. Unfortunately it is not possible to distinguish them from the IR data, since the absorption bands for the $-C=N$ groups (1690 to 1640 cm^{-1} [21]) overlaps with that of the hydroxamic acid groups, and the band of the $-NHR$ overlaps with the hydroxyl group absorption. Since the IR shows

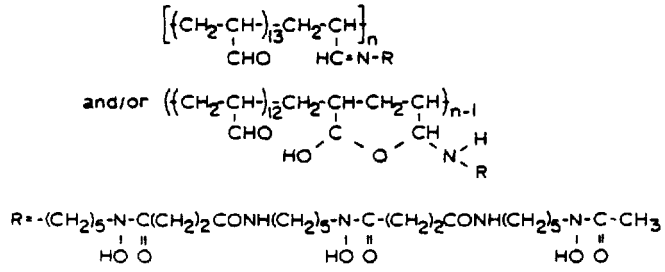


FIG. 6. Possible structures for the polyacrolein-deferoximine (PADFI) graft copolymer. The aldehyde groups may be forming cyclic hemiacetals; see text.

the presence of free aldehyde groups, they have been indicated as such in the structures of Fig. 6, but some of them may be in the form of cyclic hemiacetals, as was indicated for polyacrolein.

PADFI is insoluble in water and in common organic solvents, showing

some solubility in dimethylformamide. No change in the IR spectrum was evident after the polymer was left in contact with physiological saline solution for 24 hr at 36 to 37°C.

The iron binding capacity of PADFI was determined on samples with and without saline solution treatment by leaving them in contact with a dilute ferric chloride solution for about 20 hr. In both cases there was a decrease in the iron concentration of the solution. The 40% decrease in solution iron tends to indicate that 1) the $-NH_2$ group of deferoxamine B does not participate in the complex formation, and 2) deferoxamine B maintains its chelating affinity when attached to a polymer backbone. Polyacrolein exhibited no iron removal when tested under the same conditions.

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