A Novel Polynuclear Iron(III) Mixed Ligand Complex for Use in Parenteral Iron Therapy

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Received May 11, 1983

A novel, water soluble, electrically neutral polynuclear iron(III) mixed ligand complex has been prepared for use in parenteral iron therapy. The composition of the new compound has been determined by preparative and analytical methods. Osmotic pressure measurements have shown its polymeric nature. The complex molecule has a composition of $Fe_8L_2D_2AcO(OH)_{19}$ *where L denotes the lactiobionate ion, D stands for one glucopyranose unit of dextrane and Ac is the acetate ion. Iron(III) acetate is the starting material in the preparation of the complex. The acetate neutralises the protons liberated in the interaction of iron(III) and the ligands and the acetic acid formed in this reaction is removed by steam distillation. An aqueous solution of 1.8 mol* dm^{-3} *iron concentration was prepared which has an osmotic pressure below 300 mosmol.*

Introduction

In human and veterinary iron therapy injections of different iron concentrations have to be used. The highest iron content (100 mg cm^{-3} = 1.79 mol dm^{-3}) is needed in preparations used for the treatment of baby pigs. They contain iron(III) complexes which release the iron slowly over several days [1]. Since such injections must not cause local necrotic or toxic effects their solutions have to be nearly isotonic and of pH 4.5–5.0 in spite of the extremely high (~1.8 mol dm^{-3}) iron content. Only the polynuclear complexes can provide these requirements.

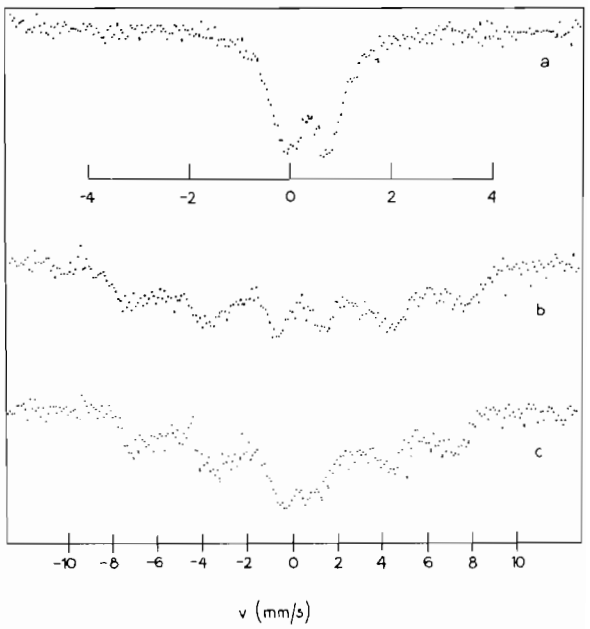

Fig. 1. Mössbauer spectra or iron solutions quenched by quick-freezing and recorded at a temperature of −180 °C. Curves: (a) iron-lactobionate containing solution; (b) β-FeO(OH) suspension (internal magnetic field 45T); (c) iron-dextrane solution containing the iron in β-FeO(OH) cores (internal magnetic field 42T).

Veterinary iron injections contain iron(III) in its high spin complexes formed with carbohydrate type ligands [2, 3]. The preparation of such complexes is accompanied by the release of protons which have to be neutralized [4, 5]. The salt formed, however, increases the osmotic pressure of the aqueous solution. Ionic exchange [4], dialysis [6] or the precipitation of the complex by organ-

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0020-1693/83/$3.00

ic solvents [5, 7] must be used therefore to separate the complex from the ions of the salt in order to get a suitable pharmaceutical preparation.

Electron microscopic, X-ray diffraction, infrared and Mössbauer spectroscopic measurements suggested that these preparations contain a β-iron oxide hydroxide core wrapped in dextrane molecules [8, 9]. The latter component stabilizes the colloidal solution in water.

Our aim was to prepare a new polynuclear iron complex in isotonic or near isotonic aqueous solutions with an iron content of 1.79 mol dm^{-3} and pH ~ 4.5–5.0 for use in parenteral iron therapy.

Preparation of the New Complex

The β-FeO(OH) core of former colloidal iron–dextrane injections has been substituted by an iron(III) mixed complex formed with lactobionate and acetate ligands. The Mössbauer spectra of its solution solidified by quick-freezing (Fig. 1) has shown a quadrupole split line in contrast with the six line pattern of β-FeO(OH) colloids and β-FeO(OH) core containing iron–dextrane injections indicating superparamagnetism in the latter two colloidal systems. Thus the Mössbauer spectra indicate a much smaller particle size in the frozen solution of the new lactobionate and acetate containing system than in those made with β-FeO(OH). This observation suggests that the mixed ligand complex forms a molecular solution.

In the preparation of the aqueous solution of the new complex iron(III) acetate served as starting material. Since commercial iron(III) acetate reacts very slowly (if at all) with lactobionic acid and dextrane and even the freshly prepared iron(III) acetate loses its reactivity in a few hours, a stable solution of iron(III) acetate in aqueous acetic acid was used in all experiments. This solution was prepared by the dissolution of freshly precipitated and washed (until completely free from anions) iron(III) hydroxide in 30 v/v % aqueous acetic acid. The required amount of ligands (lactobionic acid and dextrane) was dissolved in this solution. In the interaction between iron(III) and the ligands protons are released, due to hydrolysis and to the deprotonation of the ligand. The acetate of $Fe(OOCCH_3)_3$ buffers the protons and the acetic acid formed in this reaction together with the excess acetic acid in the solution is removed by steam distillation. In this way the pH could be raised up to 4.5–5.0 without adding any buffer compound or base leading to salt formation and increase of the osmotic pressure of the solution.

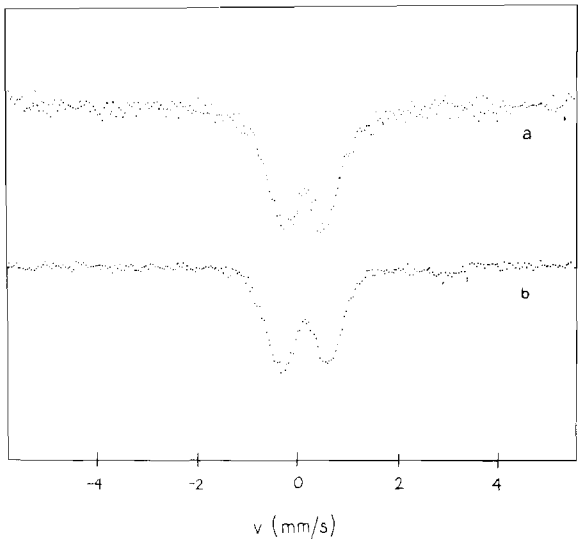

Fig. 2. Mössbauer spectra of the quick-frozen aqueous solution of $Fe_8L_2D_2AcO(OH)_{19}$ recorded at a temperature of −180 °C (L denotes lactobionate ion, D one glucopyranose unit of dextrane and Ac acetate ion). Curves: (a) after preparation, (b) after 3 months of storage at room temperature.

Characterization of the New Complex

The above procedure was used for preparing the solutions of the iron(III) complex with different ratios of iron, lactobionic acid and dextrane. The goal was to achieve 1.79 mol dm^{-3} iron(III) concentration in each system. The solutions were stored for several weeks at room temperature before analytical control measurements were made.

The optimum iron:lactobionic acid:dextrane ratio for a stable aqueous solution of 1.79 mol dm^{-3} iron level was found to be 1:0.25:0.25. (The dextrane content was calculated in glucopyranose units). A lower concentration of ligand causes the precipitation of iron hydrolysis products, whereas at a higher one dextrane precipitates. In both cases the solution separated from the precipitate contained the iron and the ligands in the above ratio.

The molecular weight (Mw) of dextrane influenced only the pH of the solution. Using dextrane of Mw ⩾ 40000 the pH of the solution increased during the steam distillation procedure up to pH 5.0–5.2 without any sign of decomposition. Using dextranes of Mw ~ 20000 insoluble hydrolysis products precipitated at pH 4.5–4.8.

The Mössbauer spectra of the solution of the new complex solidified by quick-freezing at −180 °C (Fig. 2) indicated equivalent high spin iron(III) atoms in a uniform compound. (Isomer shift is 0.45 mm/s referred to metallic iron, quadrupole splitting is 0.83 mm/s).

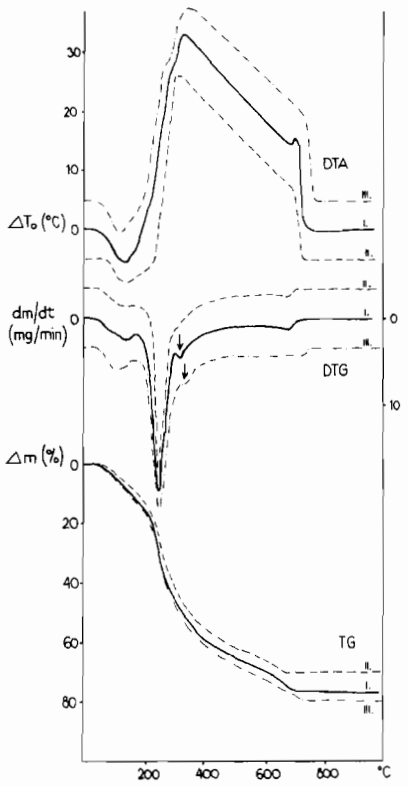

Fig. 3. Thermoanalytical (TG, DTG and DTA) curves of the $Fe_8L_2D_2AcO(OH)_{19}$ complex. Curves: I, prepared with ligand excess; II, with stoichiometric composition, and III, the stoichiometric complex mixed with ligand excess.

Thin layer chromatography (TLC) with several solvent mixtures was used for determining the eventual presence of free ligands besides the iron complex. The R_f values for the complex (R_f = 0.07), for the lactobionate ion (R_f = 0.1) and for dextrane (R_f = 0 3) were established on a Macherey Nagel Polygrom SiP G plate with a water:butanol: ammonia (8:1:0.5 ratio) solvent mixture. The chromatogram was developed by concentrated sulphuric acid. The TLC measurements did not indicate the presence of uncomplexed organic ligands.

Thermoanalysis of the solid complex prepared by the evaporation of its aqueous solution led to analogous results. On the DTG curve recorded by the Paulik–Paulik–Erdey derivatograph (MOM, Hungary) a ligand excess of only 10% resulted in the appearance of a minimum (indicated by a downward arrow on curves I and III in Fig. 3) which was absent on the corresponding curve of the stoichiometric iron complex.

Spectrophotometric equilibrium measurements performed on solutions of much lower concentration [11] also indicated that the stability of the mixed complex is high enough to ensure its quantitative formation in the absence of appreciable free ligand concentration.

All these investigations suggested that the stoichiometric ligand concentration (the 1:0.25:0.25 ratio of iron:lactobionic acid:dextrane-glucopyranose unit) needed for the complexation of iron is coordinated to this metal. The free ligand concentration is negligibly small compared with the complexed one. The Mössbauer spectrum is compatible with the presence of one uniform compound in the system.

The above ratio of the components of the complex in the solution indicated (on the basis of the former assumptions) that the complex is polynuclear. The number of iron atoms in one molecule (the grade of polymerization) was determined by osmotic pressure measurements using a Knauer halfmicro osmometer. The molar concentration of the solute was found to be 0.23 mol dm^{-3} in a solution containing 1.84 mol dm^{-3} iron(III) and in general one eighth of the iron concentration (determined in a series of experiments) indicating the presence of eight iron(III) atoms in one complex molecule. (The eventual presence of accompanying materials (*e.g.* free ligands) would increase the osmotic pressure).

Electrophoresis performed by a Labor MIM (Hungary) electrophoretic apparatus in the same solvent mixture as used in the thin layer chromatographic studies proved that the polynuclear complex is neutral (*i.e.* it has no charge) in accordance with our suggestion based on the mode of preparation.

Thus in the complex containing 8 iron(III) atoms coordinated by two lactobionate ligands and two glucopyranose units of dextrane, 24 positive charges of the iron(III) central atoms have to be neutralized by the ligand sphere. The two lactobionates neutralize only two positive charges, the other 22 may be neutralized by hydroxide ligands formed in the hydrolysis process during the preparation of the complex and/or by oxygen bridges formed by the dehydration of some of the OH groups, by acetate ligands coming from the starting material or by negative charges on the organic ligands originating from eventual deprotonation of alcoholic OH groups.

The acetate content of the complex was determined by potentiometric titration after separation (steam distillation) of the acetic acid formed during the decomposition of the complex with concentrated phosphoric acid. Acetic acid of 0.125 ± 0.01 mol for one mol of iron(III) was formed indicating that only one of the 22 positive charges mentioned above is neutralized by acetate in the ligand sphere.

The thermoanalytical study of the solid compound (Fig. 3, curve II) led to the determination

TABLE I. Water Activities in the Aqueous Solution of the New Iron(III) Complex together with some Comparative Data.

System[a]	Water activity	Solutions of equal water activity[a]
1. Solution of $Fe_8D_2L_2AcO(OH)_{19}$ [Fe] = 1.78 mol dm^{-3} [D] = [L] = 0.445 mol dm^{-3} [Ac] = 0.23 mol dm^{-3} Complex concentration in molality 0.3332 m	0.9863	0.4000 m LiCl 0.3892 m $LiClO_4$ 0.4152 m NaCl 0.4188 m $NaClO_4$ 0.7200 m saccharose
2. Solution as in 1. but without iron(III) m(D + L + Ac) = 1.4279	0.9853	0.4286 m LiCl 0.4162 m $LiClO_4$ 0.4455 m NaCl 0.4531 m $NaClO_4$ 0.7700 m saccharose

[a]Concentration data denoted by m are given in molality.

of the complete composition of the new complex. The TG curve showed that the ignition residue (Fe_2O_3 at 900 °C) of the complex is 30.0 ± 0.5%. From this the molecular weight for the complex was calculated (considering that it contains 8 Fe/molecule) as 2129 ± 36. According to the TG and DTG curves the compound loses 8.1 ± 0.1% water in the temperature interval between 100 and 160 °C which corresponds to 9–10 water molecules per one mol complex (Mw 172 ± 3). Since the thermal decomposition of two hydroxide ligands results in the release of one water molecule this proves that 18–20 of the positive charges are neutralized by OH ligands in the coordination sphere. The remaining positive charges (1–3) may be neutralized by oxygen bridges and/or by the deprotonation of alcoholic OH groups of the ligand, but considering that the pH of the solution is below 5 during the preparation procedure the latter possibility is less likely.

The difference between the composition of the new complex and those containing β-FeO(OH) cores is also well reflected by this thermoanalytical behaviour. In the latter complex the TG curve indicates the presence of only one hydroxide ligand per iron(III) in contrast to 2.4 ± 0.1 in our compound.

On the basis of all these investigations the composition of the new polynuclear iron(III) mixed complex is

$$Fe_8L_2D_2AcO(OH)_{19}$$

where L denotes the lactobionate ligand, D stands for one glucopyranose unit of dextrane and Ac is the acetate ion.

The molecular weight calculated from this formula is 2208 and the iron content 20.23%, in fair agreement with the data calculated from the thermoanalytical curves.

The dry material content of a 1.79 mol dm^{-3} iron containing aqueous solution of the complex is 49.0 w/v %. In spite of a low (51%) water content its osmotic pressure and viscosity were found to be suitable for use in parenteral iron therapy. For the characterization of the solvent the water activity of the solution was determined by isopiestic measurements [12]. For comparison the water activity of the iron free solution of otherwise analogous composition was also measured. The results together with the concentrations of some aqueous solutions with the same water activities are shown in Table I.

It can be seen from Table I that the concentrated solutions have water activites equal to that of 0.4 m salt or 0.7 m saccharose solutions. The water activity of solutions of the iron complex is higher than that containing only the ligands.

The results indicate that hydration of the vicinal OH groups of the ligand caused a structural breaking of the water cluster (the water–water associations are partially substituted by alcoholic hydroxyl–water associations) resulting in an increase in water activity. The coordination of iron led to a decrease in electron density in the alcoholic hydroxyl groups resulting in their increased polarity favouring the hydration process. The water structure breaking property of the complex explains the high water activity and low viscosity of its concentrated solution which is important for pharmaceutical applications.

Acknowledgement

The authors express their gratitude to Prof. A. Vértes, Dr. G. Liptay, Dr. G. Szepesi and Dr. J. Wajand for their help with the experimental work.

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