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A process for producing an iron-dextran compound

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(71) Applicant(s)
Pharmacosmos Holding A/S

(72) Inventor(s)
Hans Berg Andreassen; Lars Christensen

(74) Agent/Attorney
GRIFFITH HACK.GPO Box 4164, SYDNEY NSW 2001

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<p>(21) International Application Number: PCT/DK99/00425 (22) International Filing Date: 29 July 1999 (29.07.99) (30) Priority Data: PA 1998 01526 20 November 1998 (20.11.98) DK (71) Applicant (for all designated States except US): PHARMA-COSMOS HOLDING A/S [DK/DK]; Frederiksborgvej 27, DK-4000 Roskilde (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): ANDREASEN, Hans, Berg [DK/DK]; Vænget 2, DK-4130 Viby Skaelland (DK). CHRISTENSEN, Lars [DK/DK]; Frederiksborgvej 27, DK-4000 Roskilde (DK). (74) Agents: SIMONSEN, Christian, Rosendal et al.; International Patent-Bureau, Høje Taastrup Boulevard 23, DK-2630 Taastrup (DK).</p>	<p>(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. With amended claims and statement.</p>	
<p>(54) Title: A PROCESS FOR PRODUCING AN IRON-DEXTRAN COMPOUND</p> <p>(57) Abstract</p> <p>In a process for producing an iron-dextran compound for use in parenteral treatment of iron-deficiency in humans or animals a stable compound of desired relatively low molecular weight is obtained by using first hydrogenation and then oxidation to convert reducing terminal groups on the dextran molecules before reaction with the iron. By varying the ratio of hydrogenated groups to oxygenated groups the average molecular weight of the resulting iron-dextran compound can be varied.</p>		

A PROCESS FOR PRODUCING AN IRON-DEXTRAN COMPOUND

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BACKGROUND OF THE INVENTION AND PRIOR ART

10 Iron-deficiency anemia has been described as one of the most common - possibly the most common - pathological conditions among humans when viewed on a global basis. Also in modern farm-breeding of pigs and other domestic animals iron-deficiency anemia is a problem
15 unless suitable prophylactic measures are taken.

Although iron-deficiency anemia can often be prevented or cured by oral administration of iron-containing preparations, it is in many cases preferred to use parenterally administrable iron preparations to avoid
20 variations in bioavailability of oral administrations and to ensure effective administration.

Therefore, iron-containing preparations for parenteral use, i.e. subcutaneous, intramuscular or intravenous administration, have for many years been at
25 the disposal of the veterinary or human medical practitioner.

Various iron-containing substances have been used or suggested as components in parenterally injectable preparations against iron-deficiency anemia, such as
30 saccharated ferric oxide. However, the most common preparations accepted today are such which comprise a combined product of ferric oxyhydroxide (or ferric hydroxide) in association with dextran since such preparations are less toxic than for instance the
35 ferric saccharates. Dextran is a polymeric carbohydrate

produced by the microorganisms *Leuconostoc mesenteroides*.

An iron-containing preparation for parenteral injection should obviously satisfy several requirements including ready availability of the iron for haemoglobin synthesis, absence of local or general side-effects and stability on storage enabling a satisfactory shelf-life at ambient temperature.

Iron-dextran preparations for the treatment of anemia have been marketed for decades, and many variations in the manufacturing process and in the selection of starting materials have been suggested with a view to improving the stability of such preparations and to decrease the amount of side effects obtained at their administration.

As examples of patents dealing with these problems the following may be cited:

US 2,885,393 (1959) describes a basic process of producing an iron-dextran complex in which the average molecular weight of the dextran is 30,000 to 80,000 Daltons (Da) or lower. The suitability of these complexes for human therapy does not appear from this patent specification.

US Re. 24,642 (1959) comprises a detailed explanation of the requirements to an iron solution intended for intramuscular injection, incorporated herein by reference. The patent deals with a substantially nonionic complex of ferric hydroxide with a dextran having an average intrinsic viscosity at 25°C of about 0.025 to about 0.25, as well as a process for preparing such a complex by contacting a dextran as described with ferric hydroxide formed *in situ* by reaction between a ferric salt and an alkali base. No information as to the desired molecular weight of the dextran is given,

and no chemical modification of the dextran, apart from a partial depolymerisation, is suggested.

US 3,093,545 (1963). This patent discloses some details such as temperatures and pH-values in an improved method of preparing a product apparently very similar to the one prepared in the last mentioned above patent.

GB 1,200,902 (1970) teaches that in contrast to preparing the ferric hydroxide *in situ* it is advantageous to preform the ferric hydroxide under controlled conditions since such ferric hydroxide will readily form complexes with dextrans. It is stated that not only partially depolymerised dextran having a weight average molecular weight in the range of for example 500-50,000 Da, preferably in the range 1,000- 10,000 Da, but also modified forms or derivatives of dextran such as hydrogenated dextrans or oxidised dextrans or alkali treated dextrans come into consideration as theoretical possibilities. However, the only dextrans specifically mentioned are oxidized dextrans having an average molecular weight of 3,000 and 5,000 Da, resp. The ferric hydroxide is preprepared before contact with the dextran. This means that the resulting product consists of ferric oxyhydroxide on which the dextran forms a coating in contrast to the more homogeneous products formed by precipitating the ferric hydroxide *in situ*, that means in the presence of the dextran.

DK 117,730 (1970) deals with a process in which hydrogenated dextran having a molecular weight between 2,000 and 10,000 Da is reacted with ferric hydroxide in aqueous medium. The average molecular weight of the dextran used in the embodiment examples is not indicated. However, the intrinsic viscosity is stated as approximately 0,05 which could correspond to an average molecular weight of approximately 5,000 Da.

DK 122,398 (1972) also discloses the use of hydrogenated dextran for preparing complex compounds with ferric hydroxide, and it is explained that a substantially lower toxicity is obtained than when non-5 hydrogenated dextran is used. The subject of the patent is a process in which moist ferric hydroxide is mixed with dry hydrogenated dextran, and after optional addition of citric acid or citrate the mixture is heated and purified.

10 US 3,697,502 (1972) discloses a process for producing an iron-dextran preparation in which citric acid is added to the dextran and a simultaneous addition of alkali metal hydroxide solution and ferric chloride solution is made. The average molecular weight of the 15 dextran is between 3,000 and 20,000 Da. The dextran used in the embodiment examples has a molecular weight of 7,000 and 10,000 Da, resp.

DK 129,353 (1974) is directed on an analogy process for producing a ferric hydroxide-dextran deriva-20 tive at an average molecular weight of the dextran of at the most 50,000 Da, and the terminal groups of the polymer chains thereof have been modified to convert the terminal reducing anhydroglucose unit into a corresponding carboxylic acid group. Although the 25 limits indicated for molecular weight of the dextran are very broad, viz. from 500 to 50,000 Da, preferably from 1,000 to 10,000 Da, the only exemplified dextran has an average molecular weight of 5,000 Da.

DK 129,942 (1974) has similarity to the above 30 last-mentioned DK patent and deals with the manufacture of ferric hydroxide complexes with dextran hepton acid or dextrine hepton acid. The hepton acids are prepared by hydrolyzing the corresponding cyanhydrids.

US 4,827,945 (1989) and 5,102,652 (1992) both deal 35 with superparamagnetic metal oxides such as iron oxides

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coated with or associated with polymeric materials such as dextran. The polymer is contacted with a mixture of the metal oxides in two different oxidation stages to produce a superparamagnetic combined product 5 which is afterwards oxidized to transform all the metal oxide into the highest of said oxidation steps. The product is especially useful as contrast agent in magnetic resonance imaging in medical diagnosis. However, it is also mentioned that it can be used for 10 treatment of iron-deficiency anemia. The molecular weight of the polymers, including carbohydrates such as dextran, are preferably from 5,000 to 250,000 Da.

US-A-4,370,476 describes the preparation of dextran polycarboxylic acids ferric hydroxide 15 complexes. A dextran that may be hydrogenated is treated in a two step oxidation process using periodate in the first step and another oxidating agent in the second step to form pairs of intermediate carboxylic groups along the dextran molecule and 20 either a hydroxyl or an carboxylic group as the terminal group. The formed dextran polycarboxylic acid is combined with ferric hydroxide for formation of the complexes.

25 In spite of the several attempts to improve iron-dextran preparations for treatment of anemia, as reflected in the above patents, the preparations prepared according to the state of the art still have some drawbacks.

30 This is a result of the fact that in some patients the preparations may cause delayed hypersensitivity, or severe anaphylactic side effects, resulting e.g. in dyspnea, hypotension, shock and death. Also other toxic reactions might be observed.



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Besides, several of the prior art preparations are not able to meet current requirements as to 5 stability. Lacking stability may manifest itself as gelatination of the liquid or precipitation of iron hydroxide or oxyhydroxide.

Moreover, the promoting action of the commercially available iron-dextran preparations on 10 the haemoglobin synthesis in the patients receiving said preparations presents itself rather late after administration, and reestablishment of desired haemoglobin levels takes place more slowly than often desired.

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COPENING ART

Copending non-published Danish patent application 420/98 (incorporated herein by reference) discloses an invention by means of which certain of the above mentioned drawbacks are overcome. Said invention is based on the recognition that many of the specified drawbacks are associated with the presence of insufficiently hydrolyzed, relatively high-molecular weight dextran in the dextran used as starting material as well as with the presence of low-molecular weight saccharides therein.

This recognition is utilized to produce, i.a. by means of membrane technique, an iron-dextran compound which is characterized in that it comprises hydrogenated dextran having a weight average molecular weight (Mw) between 700 and 1,400 Da, preferably approximately 1,000 Da, a number average molecular weight (Mn) of 400 to 1,400 Da and wherein 90% by weight of the dextran has molecular weights less than 2,700 Da and the Mw of the 10% by weight fraction of the dextran having the highest molecular weights is below 3,200 Da, in stable association with ferric oxyhydroxide.

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SUMMARY OF THE INVENTION

Although the product of the above cited Danish Patent Application 420/98 presents a substantial improvement as to decreased toxic reactions and reduced tendency of causing hypersensitivity or anaphylactic side effects and also involves improvements as to stability, there still is a need for a means of controlling the average molecular weight of the final iron-dextran compound, and thus the availability of the

iron for haemoglobin synthesis in the human or animal organism.

If an iron-dextran compound having an iron content of e.g. 15-45% b.w. is prepared using a dextran having a weight average molecular weight of approximately 1,000 Da, in which dextran substantially all reducing aldehyde groups have been hydrogenated to alcohol groups, the apparent peak molecular weight (Mp) will typically be approximately 140,000 Da.

It is desired to be able to produce iron-dextran compounds of lower molecular weight and improved stability, especially to obtain compounds in which the iron is readily available for haemoglobin synthesis in the human or animal organisms.

In a first aspect, the present invention provides a process for producing an iron-dextran compound, in which the molecular weight of a dextran is reduced by hydrolysis, and functional aldehyde terminal groups thereof converted into alcohol groups by hydrogenation; said dextran as an aqueous solution is combined with at least one water-soluble ferric salt; base is added to the resulting solution to form ferric hydroxide, and the resulting mixture is heated to transform the ferric hydroxide into ferric oxyhydroxide as an association compound with the dextran, characterized in that the hydrogenation is only partial, leaving, however, at the most 15% by weight reducing sugar, calculated on the total amount of carbon hydrates, and said dextran before being combined with the ferric salt, and after being subjected to hydrogenation is subjected to an oxidation, said hydrogenation and oxidation being performed to obtain dextran having substantially all aldehyde groups converted into alcohol and carboxylic groups.

In a second aspect, the present invention provides a process for producing a dextran preparation, in which process the molecular weight of a dextran is reduced by hydrolysis, and functional aldehyde terminal groups



thereof converted into alcohol groups by hydrogenation; characterized in that the hydrogenation is only partial, leaving, however, at the most 15% by weight reducing sugar, calculated on the total amount of carbon hydrates, and said dextran is subsequently subjected to oxidation, said hydrogenation and oxidation being performed to obtain dextran having substantially all aldehyde groups converted into alcohol and carboxylic groups.

In a third aspect, the invention also provides an iron-dextran compound produced according to the above defined process, characterized in that its apparent peak molecular weight (Mp) is 50,000-150,000 Da, preferably 70,000-130,000 Da, more preferably 80,000-120,000 Da, and its iron content is 15-45% b.w.

In a fourth aspect, the invention provides a pharmaceutical composition for prophylaxis or treatment of iron-deficiency by parenteral administration comprising a compound as defined above.

In a fifth aspect, the invention provides a use of an iron-dextran compound as defined above for preparation of a parenterally administrable therapeutical composition for prophylaxis or treatment of iron-deficiency by parenteral administration.

In a sixth aspect, the invention provides a method for prophylaxis or treatment of iron-deficiency in a human or animal organism comprising parenterally administering an iron-dextran compound as defined above.

The present invention is based on the recognition that a stable iron-dextran of relatively low molecular weight may be obtained if the reducing aldehyde groups of the hydrolyzed dextran, before the reaction with the iron component, are only partially hydrogenated into alcohol groups whereas substantially all the remaining aldehyde groups are oxidized into carboxylic groups. The molecular weight of the iron-dextran formed when the dextran has received such a pretreatment is substantially lower than the molecular weight of an iron-dextran produced using a



similar hydrolyzed dextran having been pretreated only by
a, possibly complete, hydrogenation. By adjusting the
ratio of the amount of reducing groups hydrogenated to the
amount of reducing groups oxidized, it is possible to
5 influence the average molecular weight of the resulting
iron-dextran compound. However, if the proportion of
oxidized groups in the dextran is too high the iron-
dextran will have insufficient stability. It has turned
out that to obtain a stable product, the amount of

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reducing groups in the dextran before oxidation must not exceed a value corresponding to 15% by weight.

Thus, the present invention deals with a process for producing a stable iron-dextran compound having a relatively low molecular weight and a narrow molecular weight distribution, in which process the molecular weight of a dextran is reduced by hydrolysis, and functional aldehyde terminal groups thereof are converted into alcohol groups by hydrogenation, the hydrogenated dextran as an aqueous solution is combined with at least one water-soluble ferric salt, base is added to the resulting solution to form ferric hydroxide, and the resulting mixture is heated to transform the ferric hydroxide into ferric oxyhydroxide as an association compound with the dextran, which process is characterized in that the hydrogenation is only partial, leaving, however, at the most 15% by weight reducing sugar, calculated on the total amount of carbon hydrates, and said dextran before being combined with the ferric salt, and after being subjected to hydrogenation is subjected to an oxidation, said hydrogenation and oxidation being performed to obtain dextran having substantially all aldehyde groups converted into alcohol and carboxylic groups.

Thus, the hydrogenation is performed before the oxidation as a partial hydrogenation leaving a portion of the aldehyde groups of the dextran unreacted, and the oxidation is performed subsequently to obtain a substantially complete conversion of said portion of aldehyde groups into carboxylic acid groups.

It is believed that by this sequence of the hydrogenation and oxidation an advantageous distribution of the resulting alcohol and carboxylic acid group is obtained, since by performing the hydrogenation as an initial operation, the alcohol forming

hydrogenation primarily takes place in those aldehyde groups attached to the relatively low molecular weight dextran molecules, whereas the aldehyde groups on the higher molecular weight dextrans are primarily reacted 5 in the oxidation step which means that the carboxylic acid groups formed by the oxidation will to a large extent be introduced in the dextran of higher molecular weight.

This distribution of the alcohol groups and the 10 carboxylic acid groups on the lower molecular weight fraction and the higher molecular weight fraction, resp., is an advantage because it is to expect that the stability of the resulting product will be better than if the alcohol and carboxylic acid groups were distri- 15 buted at random, and especially better than if the carboxylic acid groups were primarily present on the lower molecular weight portion of the dextran.

However, this invention is not limited to any specific theory concerning the reason for the 20 satisfactory stability of the product produced by said preferred embodiment.

In relatively low molecular weight dextrans as those primarily coming into consideration according to the present invention the influence of the terminal 25 groups (aldehyde groups hydrogenated into alcohol groups or oxidated into carboxylic acid groups) on the polymer chains is substantially more pronounced than in dextrans of higher molecular weight, since the fraction (on weight basis) of functional terminal groups is 30 higher. Therefore, it is important that the carboxylic acid groups, which otherwise could cause instability, are present on the relative high molecular weight fraction of the dextran molecules.

It is preferred to perform the hydrogenation by 35 means of sodium borohydride in aqueous solution.

The oxidation is preferably performed by means of a hypochlorite, preferably sodium hypochlorite, in basic, aqueous solution.

It is important that an oxydant is used having an
5 oxydative capacity suitable for transforming the aldehyde groups into carboxylic acid groups without attacking other sites of the dextran molecules. By tests based on NMR-analysis of the resulting dextrans it has turned out that sodium hypochlorite is a
10 suitable oxydant in this respect, since it seems that all oxygen atoms introduced by the oxidation are present in the carboxylic acid groups.

The process of the present invention is in principle not limited to the use of dextrans having
15 specific molecular weights and molecular weight distribution, however it is preferred to use a dextran having before the formation of the iron-dextran a molecular weight lower than 7,500 Da. To obtain a product which by overall considerations is regarded as
20 most suitable for treatment of iron-deficiency anemia, an embodiment of the process is preferred which is characterized in that after the hydrolysis but before being combined with the water soluble ferric salt, the dextran is purified by one or more membrane processes
25 using a membrane having a cut-off value suitable for holding back dextran of molecular weight above 2,700 Da, possibly followed by further hydrolysis, and followed by one or more membrane processes using membranes with a cut-off between 340 and 800 Da
30 removing the smaller molecules.

A more specifically preferred embodiment comprises the following terminal steps of the process:

preparing an aqueous solution comprising the purified hydrogenated and oxydized dextran and at least
35 one water-soluble ferric salt;

adjusting the pH of said aqueous solution to a value above 10 by addition of a base;

heating the mixture to a temperature above 100°C until it turns to a black or dark brown colloidal solution which can be filtered through a 0.45 μm filter; and

further neutralization, purification and stabilization using filtration, heating and membrane processes and addition of one or more stabilizers, and optionally drying the solution to obtain the desired iron-dextran compound as a stable powder. Injection liquids may be produced by redissolving this powder, adjustment of pH, sterilizing by filtration and filling into ampoules or vials. Sterilization may also be accomplished by autoclaving the filled ampoules or vials.

Alternatively the drying operation is omitted, and an injection liquid is produced from the purified solution without intermediate drying thereof.

As explained above, a feature of the invention is the adjustment of the ratio of hydrogenated dextran aldehyde groups to the oxidized aldehyde dextran groups, as well as the total percentage of such groups.

It is essential that substantially all reducing groups in the hydrolysed dextran used as starting material are converted by the hydrogenation or the oxidation. This is because any remaining reducing groups react with the ferric compounds when contacted therewith to form ferro compounds which by parenterally administration are more toxic than ferric compounds.

Thus, a further preferred embodiment of the process of the invention is characterized in that the oxidation of the hydrolyzed and hydrogenated dextran is performed to decrease the content of reducing sugar to

not above 4% b.w. The amount of reducing sugar in the hydrolysed dextran before hydrogenation is in no way critical and will typically be in the range 20-50% b.w.

The invention also comprises an iron-dextran
5 compound produced according to the above defined process which compound is characterized in that the apparent peak molecular weight (Mp) thereof is 50,000-150,000 Da, preferably 70,000-130,000 Da, more preferably 80,000-120,000 Da, and its iron content is
10 15-45% b.w. When an aqueous preparation of such an iron-dextran compound is injected intra-muscularly to a patient suffering from iron-deficiency anemia, a positive influence on the haemoglobin production can be observed earlier than when a corresponding amount of
15 iron is injected in a preparation based on the commercial iron-dextran compounds having an apparent peak molecular weight of not below 150,000 Da.

In the present specification and in the attached claims the indications of molecular weights refer to
20 such weights determined by gel-permeation chromatography.

Stability was evaluated as the absence of visible detrimental changes, such as gel formation or precipitation, of the product after heating to 70°C or
25 more for 10 min.

The invention further comprises a pharmaceutical composition for prophylaxis or treatment of iron-deficiency by parenteral administration, which composition is characterized in that it comprises a
30 compound as defined above.

Such pharmaceutical composition preferably further comprises a salt of an organic hydroxy acid, preferably selected from citrates and gluconates as stabilizer.

Finally, the invention comprises the use of an
35 iron-dextran compound as defined above for the

preparation of a parenterally administerable therapeutical composition for prophylaxis or treatment of iron-deficiency by parenteral administration.

The invention is further illustrated by means of 5 the following non-limiting examples.

EXAMPLE 1

10 (i) **Hydrolysis, hydrogenation and oxidation of dextran**

2,522 kg hydrolyzed dextran collected as permeate from a membrane having a cut-off value < 5,000 Da, is hydrolyzed at pH 1.5 at a temperature of 95°C.

The hydrolysis is monitored chromatographically 15 using gel permeation chromatography (GPC), and is terminated by cooling when the molecular weight of the material being hydrolyzed is estimated to have achieved the desired value, i.e. a weight average molecular weight of 700-1,400 Da.

20 By the hydrolysis low molecular weight dextran is produced but also glucose is formed. After cooling and neutralization the amount of glucose and very low molecular weight oligomeres is reduced by membrane processes having a cut-off value of 340-800 Da. After this 25 process, the content of dextran is determined by optical rotation ($\alpha_D^{20} \sim 200$) to be 1,976 kg, and the amount of reducing sugar is determined by use of Somogyi's reagent to be 32.0% b.w.

The reducing capability is first decreased by 30 treatment with sodium borohydride. For 939 kg dextran 18,4 kg sodium borohydride is added at basic pH. By this partial hydrogenation it is expected that among the aldehyde groups which are hydrogenated, those dextrans with relatively low molecular weight 35 preponderate.

After the sodium borohydride treatment, the reducing capability is determined to 6.53% b.w.

Hereafter the solution is neutralized to pH < 7.0, and subsequently de-ionized. The average molecular weights and the molecular weight distribution is determined chromatographically.

The chromatography also reveals that 90% by weight of the dextran has molecular weights less than 2,700 Da and that the weight average molecular weight (Mw) of the 10% by weight fraction of the dextran having the highest molecular weights is below 3,200 Da.

Mw is found to be 1,200 and the number average molecular weight (Mn) is 800 Da.

Thereafter oxidation is performed using sodium hypochlorite at pH 9.5 and at 50°C. 1075 l of an aqueous 15% w/v NaOCl solution is added.

After the termination of the oxidation, reducing sugar is determined as 0.9% b.w.

After the oxidation diafiltration is performed against pure water to obtain a specific conductivity of 3 mS/cm. The amount of dextran was at this stage 635 kg. NMR-analysis showed that all double-bonded oxygen atoms were present as carboxylic acid groups.

25 (ii) **Synthesis of iron-dextran**

300 kg dextran, produced as above, is as an 15% solution mixed with 300 kg FeCl₃ · 6H₂O.

To the agitated mixture, 250 kg Na₂CO₃ as a saturated aqueous solution is added to obtain pH 3.5, and, thereafter, the pH is raised to 11.5 using 50 litres concentrated aqueous NaOH (27% w/v).

The mixture thus obtained is heated above 100°C until it turns to a black or dark brown colloidal solution that can be filtered through a 0.45 µm filter.
35 The solution is cooled, neutralized to pH 5.00 using

concentrated hydrochloric acid, and filtered. The solution is purified using membrane processes until the chloride content in the solution is less than 0.68% calculated on basis of a solution containing 5% w/v iron.

If the chloride content of the solution is less than desired to obtain an isotonic solution, sodium chloride is added and pH is finally adjusted to 5.6 and the solution is filtered through a 0.45 μm (or alternatively a 0.2 μm) membrane filter.

The solution is spray dried and the iron-dextran powder is ready for marketing or for further processing.

As alternative to spray drying, the solution can be used for direct production of injection liquids having an iron content of e.g. 5%, as described above.

When using the iron-dextran powder for producing injection or infusion liquids the powder is re-dissolved in an aqueous medium, the pH is checked, and, if necessary, adjusted, and the solution is filled into ampoules or vials after being sterilized by filtration. Alternatively, the sterilization can take place by autoclaving after filling into ampoules or vials.

25 EXAMPLE 2

(i) **Hydrolysis, hydrogenation and oxidation of dextran**

This portion of the synthesis is performed as described under (i) in Example 1 above.

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(ii) **Synthesis of iron-dextran**

240 kg of the above mentioned dextran as an 12% solution is mixed with 300 kg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$.

To the agitated mixture is added 250 kg Na_2CO_3 as a saturated aqueous solution to obtain a pH-value of

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3.5, and thereafter the pH of the mixture is raised to pH 11.6 using 50 litres concentrated aqueous NaOH (27% w/v).

The mixture thus obtained is heated above 100°C until it turns to a black or dark brown colloidal solution that can be filtered through a 0.45 µm filter. The solution is cooled, neutralized to pH of 5.3 using concentrated hydrochloric acid and filtered. The solution is purified using membrane processes until the chloride content is less than 0.68% calculated on basis of a solution containing 5% w/v iron.

If the solution is at this stage heated to above 100°C for 2 hours the apparent peak molecular weight (Mp) is found to be 104898 Da after cooling. The solution is stable.

The solution is spray dried and the iron-dextran powder is thus finished.

This powder is suitable for producing a liquid iron-dextran preparation containing approximately 5% w/v iron.

In both examples, the yield of iron-dextran powder is above 95%, calculated on basis of the iron used in the process.

25 EXAMPLE 3

Further iron-dextran preparations were produced using the procedures similar to the one described in Example 1 and 2. The characteristics of the starting materials, the intermediates and the results are shown in the below table.

Table

Synthesis No.	1	2	3	4	5	
5	Mw of hydrolyzed dextran (Da)	6200	2566	1212	1212	922
10	Reducing sugars after reduction step	4.4% b.w.	14.4% b.w.	6.5% b.w.	6.5% b.w.	8.9% b.w.
15	Reducing sugars after oxidation step	1.2% b.w.	3.0% b.w.	0.9% b.w.	0.9% b.w.	1.8% b.w.
	Used amount of reduced and oxidized dextran	240 kg	240 kg	300 kg	240 kg	240 kg
20	Used amount of FeCl ₃ , 6H ₂ O	300 kg	300 kg	300 kg	300 kg	300 kg
25	Mp of iron-dextran (Da)	126,350	102,653	88,146	96,875	88,326
	Stable	Yes	Yes	Yes	Yes	Yes*

*) : Stability test at 70°C for 10 min.

30 It is thus possible to produce stable low molecular weight iron-dextran preparations using dextrans hydrogenated and oxidized to various extents within the scope of the invention.

35 EXAMPLE 4 (Comparison Example)

604 kg of a dextran with a Mw of 1209 Da and a content of reducing sugars of 26.6% b.w. was, without previous hydrogenation, oxidized by treatment with 1780
40 l of a 15% (w/v) solution of NaOCl in water at pH 9.5,

temperature 50°C. After the oxidation the content of reducing sugars was determined to 0.54%.

Preliminary attempt to synthesize iron-dextran compounds using this oxidized dextran failed because the
5 mixture containing iron and dextran formed a gel even before all the Na₂CO₃ was added. Heating such a gelling solution does not lead to formation of a stable colloidal and filterable solution.

This Example shows that it is essential to decrease
10 the proportion of reducing groups in the dextran by hydrogenation before performing the oxidation.

EXAMPLE 5

15 An iron-dextran solution was prepared as in Example 2.

After the chloride removing membrane process, the pH was adjusted to 8.5 using 10.5 kg citric acid dissolved in an aqueous sodium hydroxide solution. The solution was
20 then heated to above 100°C for 2 hours. After cooling, the pH is adjusted to 5.6 using concentrated hydrochloric acid. The solution is adjusted to a concentration corresponding to 5.0 w/v% iron. The apparent peak molecular weight is determined to 111,666 and the compound
25 is stable.

By comparing this Example with the Example 2 it appears that the addition of citrate does not significantly alter the molecular weight of the iron-dextran product.
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It is to be understood that a reference herein to a prior art document does not constitute an admission that the document forms part of the common general knowledge in the art in Australia or in any other country.

35 In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary



implication, the word "comprising", or a grammatical variation thereof, is used in the sense of "including", i.e. the features specified may be associated with further features in various embodiments of the invention.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for producing an iron-dextran compound, in which the molecular weight of a dextran is
5 reduced by hydrolysis, and functional aldehyde terminal groups thereof converted into alcohol groups by hydrogenation; said dextran as an aqueous solution is combined with at least one water-soluble ferric salt; base is added to the resulting solution to form ferric
10 hydroxide, and the resulting mixture is heated to transform the ferric hydroxide into ferric oxyhydroxide as an association compound with the dextran, characterized in that the hydrogenation is only partial, leaving, however, at the most 15% by weight reducing sugar, calculated on
15 the total amount of carbon hydrates, and said dextran before being combined with the ferric salt, and after being subjected to hydrogenation is subjected to an oxidation, said hydrogenation and oxidation being performed to obtain dextran having substantially all
20 aldehyde groups converted into alcohol and carboxylic groups.

2. A process according to claim 1, characterized in that the dextran before being combined with the at least one ferric salt has a weight mean molecular weight
25 less than 7,000 Da.

3. A process according to claim 1 or 2, characterized in that after the hydrolysis, but before being combined with the water-soluble ferric salt, the dextran is purified by one or more membrane separations
30 having a cut-off value suitable for holding back dextran molecules above 2,700 Da, possibly followed by further hydrolysis and one or more membrane separations having a cut-off value between 340 and 800 Da removing the smaller molecules.

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4. A process according to any of claims 1-3, characterized in that the dextran molecules have a reducing sugar content not above 4% b.w. after the oxidation.

5 5. A process according to any of claims 1-4, characterized in that the hydrogenation is performed by means of sodium borohydride in aqueous solution.

6. A process according to any of claims 1-5, characterized in that the oxidation is performed by means
10 of a hypochlorite, preferably sodium hypochlorite in basic aqueous solution.

7. A process according to any of the preceding claims, characterized in the following steps:

15 preparing an aqueous solution comprising the hydrogenated and oxidized dextran and at least one water-soluble ferric salt;

adjusting the pH of said aqueous solution to a value above 10 by addition of a base;

20 heating the mixture to a temperature above 100°C until it turns into a black or dark brown colloidal solution and is filterable through a 0.45 μ m filter;

purification and stabilization of the solution using filtration, heating and membrane separations and addition of one or more stabilizers, and

25 optionally drying the solution to obtain the desired iron-dextran compound as a stable powder.

8. A process according to claim 7, characterized in that the stabilisation comprises addition of at least one salt of an organic hydroxy acid, preferably selected
30 from citrates and gluconates.

9. A process for producing a dextran preparation, in which process the molecular weight of a dextran is reduced by hydrolysis, and functional aldehyde terminal groups thereof converted into alcohol groups by
35 hydrogenation; characterized in that the hydrogenation is only partial, leaving, however, at the most 15% by weight reducing sugar, calculated on the total amount of carbon



hydrates, and said dextran is subsequently subjected to oxidation, said hydrogenation and oxidation being performed to obtain dextran having substantially all aldehyde groups converted into alcohol and carboxylic

5 groups.

10. Iron-dextran compound produced according to claims 1-8, characterized in that its apparent peak molecular weight (Mp) is 50,000-150,000 Da, and its iron content is 15-45% b.w.

10 11. Iron-dextran compound according to claim 10, characterized in that its apparent peak molecular weight (Mp) is 70,000-130,000 Da.

15 12. Iron-dextran compound according to claim 10 or 11 characterized in that its apparent peak molecular weight (Mp) is 80,000-120,000 Da.

13. Dextran preparation obtainable by a process according to claim 9.

14. Dextran preparation according to claim 13, obtained by a process according to claim 9.

20 15. A pharmaceutical composition for prophylaxis or treatment of iron-deficiency by parenteral administration comprising a compound according to any of claims 10-12.

25 16. A pharmaceutical composition according to claim 15, characterized in that it comprises a salt of an organic hydroxy acid, preferably selected from citrates and gluconates as stabilizer.

30 17. Use of an iron-dextran compound according to any of claims 10-12, for preparation of a parenterally administrable therapeutical composition for prophylaxis or treatment of iron-deficiency by parenteral administration.

18. Use of a dextran preparation obtainable by a process according to claim 9, for the production of an iron-dextran compound.

35 19. A method for prophylaxis or treatment of iron-deficiency in a human or animal organism comprising parenterally administering an iron-dextran compound according to any of claims 10-12.



20. Use of an iron-dextran compound according to any of claims 10-12 for prophylaxis or treatment of iron-deficiency by parenteral administration.

21. A process for producing an iron-dextran
5 compound substantially as herein described with reference to Example 1 or Example 2.

22. A process for producing a dextran preparation substantially as herein described with reference to any one of Examples 1-3 or 5.

10 23. A method for prophylaxis or treatment of iron deficiency in a human or animal organism substantially as herein described.

Dated this 8th day of November 2002

15 PHARMACOSMOS HOLDING A/S

By their Patent Attorneys
GRIFFITH HACK

