PATENT SPECIFICATION

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The inventor of this invention in the sense of being the actual deviser thereof within the meaning of Section 16 of the Patents Act, 1949, is HAROLD EUGENE FREDERICK NOTTON, a British Subject, of 62, Bowrons Avenue, Wembley, Middlesex.

COMPLETE SPECIFICATION

Improvements in and relating to Injectable Iron Preparations

We, THE CROOKES LABORATORIES LIMITED, a British Company, of Gorst Road, Park Royal, London, N.W.10, do hereby declare the invention, for which we pray that a Patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the manufacture of colloidal pharmaceutical preparations containing iron and suitable for injection purposes, such preparations being hereinafter called "colloidal injectable iron preparations."

It is already well known that the preparation commercially called saccharated iron oxide can be used for injection purposes, for example it has been stated by Nissim (Lancet, July 12th, 1947, at page 49) and subsequently by many other authors that saccharated iron oxide can be administered intravenously in human beings but that different samples have different toxicities. Hitherto a recommended method of preparing the saccharated iron oxide used for therapy has been by a two stage process, although the stages have not necessarily been performed separately. In accordance with previous methods hydrated ferric oxide, to take an example, is in stage one rendered alkaline in the presence of a substantial concentration of cane sugar and is gently warmed to produce a clear colloidal solution containing electronegative saccharated iron oxide. For convenience we shall refer to this as Solution A. In stage two, this Solution A is evaporated to a syrup or even to a dry residue and subsequently heated, for example, in an oven and finally redissolved in water.

We shall refer to this solution as Solution B.

It has been stated that solution B as compared with Solution A has the following improved properties for a given iron dosage:—

(a) a reduced toxicity to animals
(b) a decreased number of toxic reactions in human beings.
(c) a decreased tendency to precipitate human serum according to the test of Slack and Wilkinson (Lancet, January 1st, 1949, page 11).
(d) a lower pH of precipitation (hereinafter abbreviated to P.P) according to the test of Nissim and Robson (Lancet, April 23rd, 1949, page 686).

It should be here mentioned that where the test of Nissim and Robson is referred to it will be understood to be carried out as follows:—

Dilute 2 ml. of colloid to 500 ml. Take several lots of 30 ml. of this solution and adjust these to pH values in the desired range by the addition of N/50 solutions of hydrochloric acid or sodium hydroxide. A positive reaction is shown by the formation of precipitate or turbidity in one or more of the samples and the highest pH at which turbidity develops after a few hours is noted as the P.P. Should no turbidity be readily detectable, the test is extended to cover the pH range of 2.5 to 9.5 with intervals not greater than 0.5 pH unit between individual lots. If all these solutions remain clear the colloid is said to give a negative result in the P.P. test. We consider the above pH range to be significant from the point of view of determining the therapeutic value of the colloid, although in all cases which we

have tested we have found that colloids which are negative over the above range are also negative over the pH range of 1 to 2.5.

It should also be noted that for convenience of description the colloidal injectable iron preparations will be taken to contain 20 mg. of iron element in each ml., except where otherwise stated, though clearly the invention is not limited to solutions of this strength.

It has also been stated that up to a point the improvement in properties of Solution B over Solution A is greater the greater the amount of heating to which it is subjected in stage two above, but on the other hand it has been stated that if the heating is continued beyond a certain point, namely that at which the P.P. has fallen to 3.7, the resulting Solution B is rendered unsuitable for use, since it becomes more toxic, being partially charred and giving a precipitate on standing.

It is thus well known that when ferric compounds are rendered alkaline in the presence of sugars there are formed under suitable conditions of temperature and concentration electronegative colloids of iron. Nissim and Robson in the report referred to above (Lancet, April 23rd, 1949) prepared from hydrated ferric oxide, cane sugar and alkali a "saccharated iron" colloid which at the moment of formation had a P.P. of 8. This freshly formed colloid is also known to have a marked tendency to precipitate with human serum according to the test of Slack and Wilkinson. We have confirmed this observation and extended it to the other sugars mentioned in this specification and we find that in every case the P.P. of the freshly prepared colloid is on the alkaline side of neutrality, i.e. in the range of 7—10.

Our experiments have shown that the known processes for the preparation of injectable iron preparations of the type of saccharated iron oxide, for example the process outlined above for making Solution A and for converting it into Solution B are somewhat laborious and more important are difficult to control accurately. For example, owing to the risk of passing the point at which toxicity develops due to local uneven heating, temperature variations and so on, the beneficial effect of heating cannot be obtained to the full extent under manufacturing conditions. Furthermore we have found that the products obtained by carrying out the above process have a relatively high osmotic pressure, which renders them unsuitable for administration except by the intravenous route, severe reactions being caused if the products are accidentally injected elsewhere.

To sum up therefore it may be stated that it has been the aim of the authors referred to above and of others to improve the therapeutic properties of the first formed colloid by a heat treatment and it has been noticed that this causes a reduction of P.P for example to 5.0. On the other hand it has been stated that if the P.P. is reduced below a certain value, namely 3.7 there is an increase in toxicity. Thus in the prior methods all the colloids described show a positive reaction in the test of Nissim and Robson.

We have found that the changes in properties which take place in the conversion of Solution A in to Solution B are due mainly to chemical changes in the sugar component of the solution and that these changes are similar to those taking place in the known process of caramelisation of sugars.

The term "caramel" is applied in the art to a variety of dark coloured products obtained by heating sugars and carbohydrate mixtures, but for the purpose of the present invention we define "caramel" as any product of heat treatment of a sugar or sugar mixture which differs in chemical composition from the starting product and which has lost at least 10% of its original dry weight. The process of caramelisation is also known to be progressive in the sense that the product tends to become darker in colour as heating continues; there is also a progressive loss of volatile matter and a corresponding change in the chemical composition of the residue, although the product at any given time is usually a mixture containing substances whose chemical nature is not fully understood at present. The position today with regard to caramelisation is for example not significantly different from that stated by O. Wohryzek in Chemie der Zuckerarten 2nd Edition, 1928, page 78 *et seq.*

We have found that injectable iron-containing colloids having a reduced P.P. as compared with those prepared by stage one of the above known process can be more readily prepared if the sugar is subjected to a caramelisation process as defined above before it is used as a component in the iron-containing colloid, and furthermore that since the separate caramelisation of sugar tends more to the formation of the desired product and less to the occurrence of undesirable side reactions, the process can be carried further, leading to the production of a hitherto unknown type of colloid, namely one which gives a negative reaction in the

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have tested we have found that colloids which are negative over the above range are also negative over the pH range of 1 to 2.5.

It should also be noted that for convenience of description the colloidal injectable iron preparations will be taken to contain 20 mg. of iron element in each ml., except where otherwise stated, though clearly the invention is not limited to solutions of this strength.

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Our experiments have shown that the known processes for the preparation of injectable iron preparations of the type of saccharated iron oxide, for example the process outlined above for making Solution A and for converting it into Solution B are somewhat laborious and more important are difficult to control accurately. For example, owing to the risk of passing the point at which toxicity develops due to local uneven heating, temperature variations and so on, the beneficial effect of heating cannot be obtained to the full extent under manufacturing conditions. Furthermore we have found that the products obtained by carrying out the above process have a relatively high osmotic pressure which renders them unsuitable for administration except by the intravenous route, severe reactions being caused if the products are accidentally injected elsewhere.

To sum up therefore it may be stated that it has been the aim of the authors referred to above and of others to improve the therapeutic properties of the first formed colloid by a heat treatment and it has been noticed that this causes a reduction of P.P for example to 5.0. On the other hand it has been stated that if the P.P. is reduced below a certain value, namely 3.7 there is an increase in toxicity. Thus in the prior methods all the colloids described show a positive reaction in the test of Nissim and Robson.

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P.P. test of Nissim and Robson.

The new negative-reacting colloids are particularly suitable for injection purposes since they show all or some of the following advantages over those prepared by earlier methods, viz.

(a) other things being equal, a lower osmotic pressure;

(b) a pH value more nearly neutral;

(c) a serum reaction (Slack & Wilkinson) showing substantially complete freedom from precipitation after several hours.

Thus it is one object of the present invention to produce injectable iron-containing colloidal preparations which give a negative result in the P.P. test of Nissim and Robson. A further object is to produce such iron-containing colloids without having to heat the colloid after its formation except insofar as such heating is necessary for bacteriological sterilisation. It will also be readily understood that from what follows that our invention also provides an improved process of preparing injectable iron colloids of a type similar to those already known, namely those having a reduced P.P. as compared with a colloid prepared by rendering alkaline a ferric compound in presence of an unheated sugar.

According to the present invention there is provided a colloidal injectable iron preparation comprising the product produced by mixing a precaramelised sugar or sugar mixture, a solution or suspension of a ferric compound as specified and a suitable alkali to ensure that the pH value of the preparation is 7.5 or over. We shall call this preparation the "primary colloid."

The invention also includes a method of making a colloidal injectable iron preparation characterised in this that the method comprises subjecting a sugar or sugar mixture to a process of caramelisation then dissolving the precaramelised product in a solution or suspension of a ferric compound as specified, rendering the preparation alkaline by the addition of a suitable alkali, for example sodium hydroxide, in order to ensure that the pH value of the preparation is 7.5 or over and warming if necessary for the purpose of forming the colloidal solution.

The present invention, quite apart from the properties of the preparation, has several distinct advantages over the known art. For example in the known process a colloid has to be evaporated before the heating takes effect. This is a time-consuming operation and is wasteful in heat. On the other hand it is a simple matter to heat the sugar as in the present invention. There is another aspect of this problem, by heating only the sugar it is clear that the bulk of the material being heated is less than heretofore which is an advantage and further, should the heating stage miscarry the value of the material wasted is the value of the sugar only and not of the other ingredients as well. It may also be observed that chemical control tests are easier in the heating of the sugar alone because of its generally simpler composition, also owing to the fact that the heated sugar is less viscous than the sugar iron mixture in the former heating process the heating stage is easier to control.

In carrying the method according to the present invention into effect we find that commercial specimens of caramel are rarely suitable because they are usually processed to have a maximum tinctorial power, whereas for therapeutic use it is advantageous to have the product as light in colour as possible. Thus, when subjecting the sugar or sugar mixture to caramelisation we prefer to avoid conditions which lead to excessive darkening or decomposition, as for example too high a temperature, or the use of excessive amounts of known basic materials and we prefer to carry out the desired reaction (i.e. caramelisation to the extent required) in such a way that it takes place or is completed in a reasonable time, say 2—3 hours. We also prefer to operate on the sugar or sugars without other additions under a reduced pressure of, say 1 to 60 mm. of mercury. With regard to the exact procedure necessary to secure the required degree of caramelisation our experiments have shown that it is impossible to specify generally applicable conditions in terms of temperatures, pressures and times since different batches of materials tend to give different results up to a point. In carrying the invention into effect therefore it is necessary to decide on the required P.P. and then during caramelisation of the sugar or sugar mixture to withdraw small portions or samples of the heated sugar at various stages, and having converted each into an electronegative colloidal iron to determine the P.P. by the Nissim and Robson test. It will be found that the P.P. of successive samples tends to decrease and when the desired P.P. has been attained the heating is stopped and the cooled batch of caramelised sugar is ready for the preparation of an iron-containing colloid for injection purposes according to the invention. Caramelisation is therefore carried on until the sugar has lost at least 10% of its original dry weight and until the required product is produced, testing being carried out in much the same way

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that the test for pectin and gel formation is made from time to time in jam making. Clearly when this testing technique has been carried out once or twice under a given set of conditions further batches of material may if desired be prepared without testing.

In making the periodic tests during caramelisation we employ the following routine procedure.

The weight loss is determined and a weight of caramel equivalent to 10 g. of original sugar is withdrawn and dissolved by stirring in liquor ferri dialysati B.P.C. (0·6 g. Fe). The solution is poured into 10 ml. water containing about 0.3 g. sodium hydroxide and well mixed and the resulting colloid, having a pH of about 10, is used for the P.P. test.

When our object is to produce a colloid which reacts negative in the P.P. test we may prefer to continue the caramelisation process for a further period after a negative reaction has been attained in the periodic test, but not long enough to cause any of the following effects, viz. (a) excessive darkening, (b) gelatinisation when mixed with the dialysed iron solution, (c) the final alkaline colloid contains particles of insoluble carbonaceous matter, is excessively viscous, or contains non-colloidal iron compounds. Thus experience has shown that in general under given experimental conditions colloids with negative P.P. and otherwise satisfactory chemical properties can be obtained over a range of heating times and that all these are suitable for injection purposes, although they may differ among themselves in their toxicity to animals.

As the raw material for the caramelisation process we may use any one or any mixture of the following sugars:—

Sucrose, dextrose, fructose, mannose, arabinose, lactose, galactose, raffinose, rhamnose, sorbose, xylose.

The term sugar or sugar mixture as employed in the specification is therefore to be construed as covering any one of the above sugars or any mixture thereof. We prefer however to use sucrose.

Further details will be given hereinafter of processes for producing caramels for use in the preparation of colloidal injectable iron preparations according to the invention, but it is particularly important to remember that the requisite time of heating varies with the type of sugar or sugar mixture employed, with the size of batch and with other factors so that the figures given in the examples are for general guidance only and it is essential to employ the periodic testing technique described above if the best results are to be achieved. Some examples of actual experiments in producing injectable iron preparations will now be given with particular reference to the caramelisation step. The results in table I were obtained with batches of caramel produced from sugars heated alone and converted into colloid by the above routine procedure.

TABLE I.

Sugar	Amount	Time of Heating	Bath Temp.	Pressure	P.P. of Preparation
Arabinose	10 g.	3 h.	185° C.	40 mm. Hg.	Negative
Lactose	,,	12 h.	,,	,,	5.4
,,	,,	2 h.	205° C.	,,	Negative
,,	,,	3 h.	,,	,,	3.2
Rhamnose	,,	2 h.	175° C.	,,	Negative
Mannose	,,	3 h.	185° C.	,,	,,
Raffinose	,,	2¾ h.	150° C.	,,	4.5
Invert Sugar	50 g.	4 h.	185° C.	Atmospheric	Negative
Dextrose	,,	2½ h.	203° C.	1—3 mm.	,,
Sucrose	1 Kg.	3 h.	175° C.	42 mm.	,,
Galactose	10 g.				

In order to produce a colloid of negative P.P. it is not necessary that the caramel and iron should be present in the relative proportions given in our routine procedure. For any given specimen of caramel there is however a certain minimum caramel/iron ratio below which a colloid of negative P.P. cannot be obtained. We have found that, other things being equal, this ratio tends to decrease, up to a point, as the time of caramelisation increases. Examples for general guidance are given in tables II and III, and it is obvious that in the case of any given caramel it is easily possible to ascertain by experiment the minimum practicable caramel/iron ratio.

In these tables the colloids all contain

2% Fe and are prepared by the routine procedure apart from variations in the caramel/iron ratio.

TABLE II.

Sucrose heated at 185° and 48 mm. pressure

Time of Heating	Caramel Content of Preparation	P.P. of Preparation
1¾ h.	38.1%	5.5
1¾ h.	30.2%	6.0
2¼ h.	29.5%	Negative
,,	24.0%	,,
,,	17.7%	6.2
2¾ h.	22.0%	Negative
,,	20.0%	5.4
3¼ h.	17.5%	Negative
,,	18.1%	5.4

TABLE III.

Dextrose heated at 175° and 40 mm. pressure

Time of Heating	Caramel Content of Preparation	P.P. of Preparation
1½ h.	45.2%	6.2
2 ,, h.	30.1%	7.0
,, h.	35.5%	Negative
2½ h.	29.5%	6.3
2½ h.	22.2%	Negative
	17.7%	6.2

In carrying the method according to our invention into effect we found it convenient to use liquor ferri dialysati B.P.C. as a source of iron. When this is used it should be of recent preparation and free from brownish or reddish reflex in reflected light. Many other ferric compounds may be used for carrying out the invention and we wish it to be understood that where a ferric compound is specified we include only those ferric compounds which give, according to the invention, a preparation suitable for injection purposes.

Suitable ferric salts are (a) soluble ferric salts such as the chloride, nitrate, sulphate or acetate and double salts such as ferric ammonium sulphate (but excluding ferric salts which contain iron in the form of a complex anion, or those which are capable of forming alkali-soluble complex salts, or which when treated according to our invention yield a solution containing non-colloidal iron, for example, ferric citrate, ferric ammonium citrate, ferric potassium tartrate, ferric glycerophosphate, ferric malate and ferric potassium oxalate).

(b) ferric oxy-salts, such as may be prepared by dissolving ferric hydroxide in a solution of one of the above specified ferric salts; dialysed iron solution B.P.C. and freshly prepared and washed hydrated ferric oxide (ferric hydroxide).

(c) any ferric compound which is capable of yielding ferric hydroxide when rendered alkaline in presence of caramel, provided no chemically or therapeutically disadvantageous substance is thereby simultaneously produced or introduced.

As an alkali to render the preparation alkaline we prefer to use sodium hydroxide but clearly any suitable hydroxide may be used provided that the hydroxide selected does not introduce any properties that would be harmful to the patient. Obviously the effect of a given hydroxide might be beneficial or the reverse, according to the nature of the malady being treated. Alkalis chemically suitable for completing the colloidal solution include:—hydroxides of lithium, potassium, ammonium, magnesium and calcium; lithium, sodium and potassium carbonates and relatively non-toxic water-soluble aliphatic amines, such as mono-ethanolamine. Some alkaline-reacting salts such as sodium acetate may also be used, but not others such as tri-sodium phosphate or borax.

It will be apparent to those skilled in the art that when an iron-containing colloid is formed according to our invention there is usually formed at the same time a larger or smaller amount of an electrolyte. When the amount of this electrolyte is so great as to be chemically or therapeutically disadvantageous we may prefer to form the primary colloid in a more dilute solution than usual and if a more concentrated solution is desired, to purify the resulting product by one of the hereinafter specified methods.

In a preferred method of carrying our invention into effect we caramelise sucrose by heating under a reduced pressure, of say, 1 to 60 mm. of mercury. Heating is carried out at a controlled temperature in the range 180° to 205° C. (bath temperature) and is continued until the sucrose has lost 10—15% of its original dry weight and gives a negative result in the P.P. test. The product can be shown by chromatographic methods to be substantially free from unchanged sucrose, it is a brittle brownish-black glassy solid, transparent in thin layers, soluble in water and almost free from sweet taste. This caramel is dissolved in Liquor Ferri Dialysati B.P.C. in the proportion of at least 10 and preferably about 15 parts by weight of caramel to 1 part of elementary iron and the mixture is rendered alkaline by the addition of sodium hydroxide.

If desired purified colloidal injectable iron preparations may be prepared, which are practically iso-osmotic with human blood serum and have satisfactory qualities for injection by one of the following methods:—

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(1) by subjecting a preparation according to the invention to dialysis against distilled water until the desired osmotic pressure has been attained;

(2) a solution of heat-caramelised sugar may be dialysed and the product used in the preparation of a colloidal ferric compound solution of low osmotic pressure;

(3) by mixing a preparation according to the invention with a suitable water-miscible solvent such as methyl alcohol, ethyl alcohol or acetone in sufficient quantity to precipitate the colloid, separating the latter from the solution, e.g. by filtration and re-dissolving it in distilled water to the required strength. If the final solution is hypotonic a suitable amount of a physiologically inactive and alkali-stable substance such as cane sugar may be added to adjust the osmotic pressure.

When the pH of a colloidal injectable iron preparation prepared according to the invention is allowed to fall below 7.5 there is a tendency for the toxicity to increase due to the presence of non-colloidal iron. When preparing the preparation according to the invention therefore we add if necessary, as a final step, and subsequent to any process of purification, sufficient of a suitable alkali such as sodium hydroxide to ensure that the final pH after any heat-treatment necessary for sterilisation is not lower than this value.

Solidified preparations suitable for the extempore production of colloidal injectable iron preparations by the addition of distilled water may be obtained by the dehydration of colloidal solutions prepared according to this invention for example by evaporation under reduced pressure or alternatively by the addition of a suitable water miscible solvent in quantity sufficient to precipitate the colloid with subsequent separation and desiccation of the precipitate.

Examples.

Example 1.

Cane sugar (50 g.) was caramelised by heating in a glass container at a reduced pressure of 30 mm. of mercury for 3¼ h. in an oil bath maintained at 190°. The product, cooled to room temperature, was dissolved in 83.5 ml. of liquor ferri dialysati containing 3.59 g. Fe per 100 ml., the resulting solution poured slowly into 5 ml. of 19% w/v sodium hydroxide and water added to 150 ml. The pH of the solution was 9.57 but fell during heat sterilisation to about 7.5.

The resulting solution has a freezing point depression of 1.77° C. as compared with pure water and an iron content of approximately 2%. When tested for pH of precipitation according to Nissim and Robson it showed no precipitation over the pH range 2.7—10.5.

This material (a) and a commercial saccharated iron preparation (b) simultaneously tested for intravenous toxicity in mice gave LD 50 values of (a) over 500 mg./kilo (b) 60 mg./kilo.

A product made from cane sugar by an identical process, but omitting the caramelisation had a freezing point depression of about 3° C.

Example 2.

The colloidal ferric compound solution produced in Example 1 (50 ml.) was dialysed for 72 h. in a bag made of material known under the Registered Trade Mark Cellophane against distilled water. The remaining colloid, after evaporation on the water bath to 50 ml. gave the following analytical results:—

Fe —1.99%
pH —approximately 7.0
Dry Residue —17.1%
Freezing point depression 0.28° C. compared with pure water.
Serum Test (Slack & Wilkinson)—
No precipitation after several hours at 37.5° C.

This preparation must be treated immediately before sterilisation with sufficient alkali to ensure that the final pH value is not lower than 7.5.

Example 3.

Cane sugar (30 g.) was heated as in Example 1. The product was dissolved in water to 90 ml. and dialysed against distilled water for 48 h. The residue in the dialyser (210 ml.) gave a freezing point depression of 0.12° C. and a dry residue of 6.64%. 168 ml. of this solution evaporated on the water bath to 25 ml., was mixed with 40 ml. of liquor ferri dialysati containing 3.65% Fe and the mixture added slowly to 2 ml. of 19% sodium hydroxide. Water was added to 72 ml.

The solution had a pH of 10.2 and gave a negative result in the P.P. test. 50 ml. of this solution evaporated to 20 ml. gave a colloidal solution containing 5% Fe (approximately).

Example 4.

Cane sugar (500 g.) was heated at 190° C. for 3¼ h. at 20 mm. pressure, the caramel weighing 445 g. The caramel (29.4 g.) was dissolved in 40 ml. of solution containing 10 g. $FeCl_3.6H_2O$ and the solution poured in to 23.5 ml. of 20% w/v sodium hydroxide, thoroughly stirred and warmed, to 60° C., with

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stirring, resulting in the formation of a clear colloidal solution. After dilution to 100 ml. the pH was 10.2 and the P.P. test was negative. The product was then subjected to dialysis against distilled water, whereby substantially no iron was found to pass through the membrane.

EXAMPLE 5.

Caramel (267 g.) prepared as in example 4 was dissolved in 473 ml. of liquor ferri dialysati (3.9% Fe) poured into 48 ml. of sodium hydroxide (20% w/v) and diluted to 900 ml. To this colloidal solution was added 3600 ml. of ethyl alcohol 90% with stirring and the precipitate was separated as completely as possible by filtration. It was redissolved in about 1200 ml. of distilled water at 60° C. and evaporated under reduced pressure at 35° C. to remove traces of solvent. The volume was adjusted to 900 ml. and the pH brought to 9.9 by addition of sodium hydroxide. It was filled into containers and sterilised at 10 lbs. excess pressure for 30 minutes. The pH was then 8.0 and the freezing point depression of the solution as compared with distilled water was 0.28° C. This material was administered intravenously to several patients in 10—15 ml. doses without the appearance of toxic symptoms due to iron.

EXAMPLE 6.

Caramel (89 g.) prepared as in Example 4 was dissolved in approximately 400 ml. of a suspension of freshly precipitated and washed hydrated ferric oxide (containing 6 g. Fe) with 4 g. of sodium hydroxide added. The suspension was warmed at 75° C. with stirring until solution ensued, after which the volume was reduced to 300 ml. by evaporation on a water bath. The final pH was 9.4.

EXAMPLE 7.

Sucrose (1 kg.) was heated for 2 hrs. at 202—205° and 3—5 mm. pressure to give 852 g. of caramel. This caramel (14.1 g.) was dissolved in 26 ml. of liquor ferri dialysati B.P.C. (3.9% Fe) and lithium carbonate (1 g.) added. The resulting colloid diluted to 50 ml. has a pH of 9.1, a negative P.P. and was free from non-colloidal iron compounds.

What we claim is:—

1. A colloidal injectable iron preparation comprising the product produced by mixing a precaramelised sugar or sugar mixture, a solution or suspension of a ferric compound as specified and a suitable alkali to ensure that the pH value of the preparation is 7.5 or over.

2. A colloidal injectable iron preparation comprising a precaramelised sugar or sugar mixture dissolved in a solution or suspension of one or a mixture of the following ferric compounds, liquor ferri dialysati B.P.C., ferric chloride, nitrate, sulphate or acetate, ferric ammonium sulphate or freshly precipitated and washed hydrated ferric oxide, and a suitable alkali to ensure that the pH value of the preparation is 7.5 or over.

3. A colloidal injectable iron preparation according to claim 1 or 2 characterised in this that the precaramelised sugar or sugar mixture consists of or comprises precaramelised sucrose.

4. A colloidal injectable iron preparation according to any of the preceding claims, characterised in this that the alkali is a hydroxide.

5. A colloidal injectable iron preparation according to Claim 4 characterised in this that the alkali is sodium hydroxide.

6. A method of manufacturing a colloidal injectable iron preparation characterised in this that the method comprises subjecting a sugar or sugar mixture to a process of caramelisation, then dissolving the pre-caramelised product in a solution or suspension of a ferric compound as specified and rendering the preparation alkaline by the addition of a suitable alkali to ensure that the pH value of the preparation is 7.5 or over with subsequent warming if necessary to form a colloidal solution.

7. A method of manufacturing a colloidal injectable iron preparation characterised in this that the method comprises subjecting a sugar or sugar mixture to a process of caramelisation, then dissolving the precaramelised product in a solution or suspension of one or a mixture of the ferric compounds, liquor ferri dialysati B.P.C., ferric chloride, nitrate, sulphate or acetate, ferric ammonium sulphate, or freshly precipitated and washed hydrated ferric oxide and rendering the preparation alkaline by the addition of a suitable alkali to ensure that the pH value of the preparation is 7.5 or over.

8. A method according to claim 6 or 7 of manufacturing a colloidal injectable iron preparation and characterised in this that the sugar or sugar mixture consists of or comprises sucrose.

9. A method of manufacturing a colloidal injectable iron preparation characterised in this that the method comprises subjecting sucrose to a process of caramelisation, dissolving the caramel in a solution or suspension of one or a mixture of the following ferric compounds, liquor ferri dialysati B.P.C., ferric chloride, nitrate, sulphate or acetate, ferric ammonium sulphate, or freshly precipitated and washed hydrated

ferric oxide and rendering the preparation alkaline by the addition of a suitable alkali to ensure that the pH value of the preparation is 7.5 or over.

10. A method according to any of the preceding claims 6 to 9 characterised in this that the preparation is rendered alkaline by the addition of an hydroxide, and preferably sodium hydroxide.

11. A method of manufacturing a colloidal injectable iron preparation comprising subjecting sugar or a sugar mixture to a process of caramelisation until the sugar has lost between 10—15% of its original dry weight by heating in the range 180° to 205° C. at a reduced pressure of 1—60 mm. of mercury, then dissolving the caramelised product in a solution or suspension of a ferric compound as specified and rendering the preparation alkaline by the addition of a suitable alkali to ensure that the pH value of the preparation is 7.5 or over.

12. A method according to any of the preceding claims 6 to 11 characterised in this that the preparation is rendered substantially iso-osmotic with human blood serum by subjecting the preparation to dialysis against distilled water until the desired osmotic pressure has been attained.

13. A method according to any of the preceding claims 6 to 11 characterised in this that a solution of the caramelised sugar is dialysed and the product of dialysis used in the preparation of a colloidal ferric compound solution of low osmotic pressure.

14. A method according to any of the preceding claims 6 to 11 characterised by the steps of mixing the preparation (i.e. the primary colloid) with a suitable water-miscible solvent such as methyl alcohol, ethyl alcohol or acetone in sufficient quantity to precipitate the colloid, separating the precipitate from the solution and redissolving the precipitate in distilled water.

15. A method according to any of the preceding claims 6 to 14 characterised in this that in cases in which the injectable iron preparation is hypotonic a suitable amount of a physiologically inactive and alkali-stable substance such as sucrose may be added to adjust the osmotic pressure.

16. A solidified preparation suitable for the extempore production of an injectable iron preparation according to any of claims 1 to 5 by the addition of distilled water.

17. A method of preparing the solidified preparation as claimed in claim 7 characterised by the steps of dehydrating a colloidal solution prepared by the method claimed in any of claims 6 to 9 for example by evaporation under a reduced pressure or by the addition of a suitable water miscible solvent in quantity sufficient to precipitate the colloid with subsequent separation and desiccation of the precipitate.

18. A colloidal injectable iron preparation substantially as hereinbefore described.

19. A method of preparing a colloidal injectable iron preparation substantially as hereinbefore described.

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PROVISIONAL SPECIFICATION

Improvements in and relating to Injectable Iron Preparations

We, THE CROOKES LABORATORIES LIMITED, a British Company, of Gorst Road, Park Royal, London, N.W.10, do hereby declare this invention to be described in the following statement:—

This invention relates to the manufacture of injectable iron preparations.

It is already well known that the preparation known in commerce as saccharated iron oxide can be used for injection purposes, for example it is stated by Nissim (Lancet, July 12th, 1947, page 49) and subsequently by many other authors that saccharated iron oxide can be administered intravenously in man but that different samples have different toxicities. Hitherto a recommended method of preparing the saccharated iron oxide used for therapy has been by a two stage process although the stages have not necessarily been performed separately. In accordance with previous methods hydrated ferric oxide or a suitable ferric salt is, in stage one, rendered alkaline in the presence of a substantial concentration of cane sugar and gently warmed to produce a clear colloidal solution containing electronegative saccharated iron oxide. For convenience we shall call this solution, solution A. In stage two, solution A is either (1) heated in an autoclave or (2) evaporated to a syrup or even a dry residue and subsequently heated in an oven and finally redissolved in water, thus, by (1) or (2) producing what we shall call solution B. It has been stated that solution B as compared with solution

A has the following improved properties for a given iron dosage:—

(a) a reduced toxicity to animals;
(b) a decreased number of toxic reactions in human beings;
(c) a decreased tendency to precipitate human serum according to the test of Slack and Wilkinson (Lancet, January 1st, 1949, page 11); and
(d) a lower pH of precipitation according to Nissim and Robson (Lancet, April 23rd. 1949, page 686).

It has been found up to a point that the improvement in properties of solution B over solution A is greater, the greater the amount of heating to which solution A is subjected. On the other hand it has been stated that if the heating of solution A is prolonged beyond a certain point, namely that at which the pH of precipitation has fallen to 3.7 the resulting solution B is rendered unsuitable for use since it becomes toxic due to the occurrence of such reactions as precipitation of the colloid, carbonisation and formation of non-colloidal iron compounds. Thus, in the prior methods, all the colloids described show some degree of precipitation in the test of Nissim and Robson.

Our experiments have shown that the processes outlined above for the preparation of solution A and for the conversion of solution A into solution B are laborious and more important are difficult to control accurately. For example, owing to the risk of passing the point at which toxicity develops, due to local uneven heating, temperature variations and so on, the beneficial effect of heating cannot be obtained to the full extent.

Furthermore we have found that the products obtained by carrying out the above process have a relatively high pH value and a relatively high osmotic pressure which renders them unsuitable for administration except by the intravenous route, severe reactions being caused if the products are accidentally injected elsewhere.

It should be mentioned that for convenience of description the colloidal solutions of iron compounds referred to in this specification will be taken to contain 20 m.g. of iron element in each ml., except where otherwise stated, though clearly the invention is not limited to solutions of this strength which is given merely by way of example.

It is an object of the present invention to provide injectable iron preparations having improved properties as compared with the known iron preparations described above.

According to a feature of the present invention there is provided a method of manufacturing an injectable iron preparation comprising subjecting a sugar or sugar mixture to a caramelisation process, dissolving the product in a suitable solution or suspension of a ferric compound and rendering the resulting mixed solution alkaline for example by mixing with a suitable hydroxide with warming if necessary. The invention also includes the new injectable iron preparation comprising a colloidal solution containing a ferric compound and a caramelised sugar and a suitable alkali such as sodium hydroxide or a product from such a colloidal solution by adjustment to a suitable pH. The sugar for caramelisation may be cane sugar, dextrose, fructrose or a mixture of one or more of these.

Therefore it will be understood that our experiments have shown that a further series of colloidal ferric compounds similar to those whose preparation has been outlined above but having improved properties for injection purposes can be more readily obtained by using instead of simply cane sugar in stage one of the known process the material obtained by caramelisation of a sugar. The product thus formed is suitable for use, without treatment according to stage two described above, apart from any heating necessary for sterilisation.

As in the known processes, so in the new method according to the present invention, the characteristics of the colloidal preparations formed undergo a progressive improvement, up to a point, as the time and temperature of the heating stage increases. However in accordance with the present invention the heating process may be carried further than heretofore resulting in the preparation of colloidal solutions containing iron and having the following definite advantages over those preparations produced by the earlier methods:—

(a) a lower osmotic pressure;
(b) a pH value more nearly neutral;
(c) a serum reaction (Slack and Wilkinson) showing substantially complete freedom from precipitation after several hours;
(d) substantially no precipitation as a result of the precipitation point test (Nissim and Robson).

According to a further feature of the present invention we may subject a

colloidal ferric compound (e.g. ferric oxide) solution prepared with a caramelised sugar or mixture of sugars to dialysis against distilled water whereby our experiments have shown that the osmotic pressure of the final product may be reduced to a value equal to or lower than that of blood serum. The dialysis product thus obtained retains its satisfactory qualities under (b), (c) and (d) above. As an alternative the caramelised sugar solution may be dialysed and the product used in the preparation of a colloidal ferric compound solution of low osmotic pressure.

In the preparation of colloidal solutions containing iron in accordance with the present invention it is desirable to keep the colour of the final solution as light as possible and for this reason in our preferred process we carry out the caramelisation of cane sugar by heating the sugar under reduced pressure.

We also find that "liquor ferri dialysatum" B.P.C., or its obvious chemical equivalent is a convenient source of iron for this preparation.

In our preferred process we heat cane sugar under reduced pressure under such conditions of time and temperature that the sugar is substantially totally converted into suitable products of caramelisation, yet without any greater amount of heating than will effect this end.

The chemical changes in the process known as "caramelisation" are not fully understood but are believed to be very complex, the product being mixtures of compounds varying with the conditions of heating. It is therefore only possible to define the desired product in empirical terms. It is formed in our preferred process by heating as above until the sugar has lost approximately 10—12% of its original weight and has the following properties:—a brittle, blackish, glassy solid, transparent in thin layers, completely soluble in water, substantially free from sweet taste, and substantially failing to exhibit the known colour reaction of sucrose with diethyl-*p*-phenylenediamine hydrochloride. When used as a component of an electronegative colloidal ferric compound solution as described below, the resulting solution does not show turbidity when tested for precipitation point by the method of Nissim and Robson.

In order that the invention may be more clearly understood one or two typical examples of methods and preparations in accordance with the present invention will now be given:—

EXAMPLE 1.

Cane sugar (50 g.) was caramelised by heating for 3¼ hours in an oil bath maintained at 190°, in a glass container, at a reduced pressure of 30 mm. of mercury. The product, cooled to room temperature, was dissolved in 83.5 ml. of liquor ferri dialysatum containing 3.59 g. Fe per 100 ml., the resulting solution poured slowly into 5 ml. of 19% sodium hydroxide and water added to 150 ml. The pH of the solution was 9.57, but fell during heat sterilisation to about 7.5.

The resulting solution has a freezing point depression of 1.77° C. as compared with pure water and an iron content of approximately 2%. When tested for pH of precipitation according to Nissim and Robson it showed no precipitation over the pH range 2.7—4.0.

A product made from cane sugar by an identical process, but omitting the caramelisation has a freezing point depression of about 3° C.

EXAMPLE 2.

The colloidal ferric compound solution produced in Example 1 (50 ml.) was dialysed for 72 hours in a "cellophane" bag against distilled water. The remaining colloid, after evaporation on the water bath to 50 ml. gave the following analytical results:—

Fe	—1.99%
pH	—approximately 7.0
Dry Residue	—17.1%

Freezing point depression 0.28° C. compared with pure water.

Serum Test (Slack and Wilkinson) —No precipitation after several hours at 37.5° C.

EXAMPLE 3.

Cane sugar (30 g.) was heated as in Example 1. The product was dissolved in water to 90 ml. and dialysed against distilled water for 48 hours. The residue in the dialyser (210 ml) gave a freezing point depression of 0.12° C. and a dry residue of 6.64%. 168 ml. of this solution evaporated on the water bath to 25 ml. was mixed with 40 ml. of liquor ferri dialysatum containing 3.65% Fe and the mixture added slowly to 2 ml. of 19% sodium hydroxide. Water was added to 72 ml.

The solution had a pH of 10.2 and showed no precipitation in the test of Nissim and Robson over the pH range 2.7—6.0. 50 ml. of this solution evaporated to 20 ml. gave a colloidal solution containing 5% Fe. (approximately).

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