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(54) A VENOUS INJECTION FOR SAFETY AND EFFECTIVE PLASMA SUBSTITUTE INCLUDING ARTIFICIAL STARCH AND THE PREPARATION THEREOF

(71) We, KYORIN SEIYAKU KABUSHIKI KAISHA, a Japanese Body Corporate, of No. 5, 2-chome, Surugadai, Kanda, Chiyoda-ku, Tokyo, Japan, 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 novel formulations useful as blood plasma substitutes, and to methods for preparing them.

Many formulations have been used in the past as blood plasma substitutes, but they have not been found to be really satisfactory because of side-effects observed in the patient when they are employed. They appear, inter alia, to cause the accumulation of colloidal particles in the tissues of the patient and the destruction and agglutination of the red cells of his blood, and thus cause chill, fever or anaphylaxis. We have observed that such reactions are most frequently encountered when polymers form part of the formulation.

It is an object of the present invention to provide a plasma substitute formulation which may be administered intravenously into the patent in relatively large amounts without chill, fever or anaphylaxis. This is partly achieved in the formulations of the present invention by ensuring the absence of pyrogens therefrom, the presence of which we have discovered largely to be the cause of these adverse side-effects, especially when polymers are also present.

Hitherto, many conventional plasma extenders and substitutes which have been used have been colloidal suspensions and these have been chosen because of their high viscosity and their consequent ability to maintain blood pressure. However, most colloidal substances are physiologically undesirable. In addition, the amount of the colloid which is excreted from the patient in his urine is very small and these undesirable substances are consequently present in the patient for long periods. In fact, the rate of excretion is so small that tissue lesions due to the accumulation of colloidal particles therein are apt to occur. Colloidal substances used hitherto, and which have this disadvantage, include arginine, gelatin, and polyvinylpyrrolidone (PVP). At the present time, dextran and artificial gelatin are preferred, but there are disadvantages associated with dextran and artificial gelatin too. Thus, dextran causes renal lesion or anaphylactic shock in 6 to 8% of the patents to which formulations containing it are administered, and, due to the gelification of artificial gelatin at room temperature, it is most inconvenient for use in a emergency. Moreover, it is found that artificial gelatin actually causes the destruction of red cells.

Generally speaking, starch (the structure of which is similar to glycogen) is more acceptable to body tissue than those colloids mentioned previously because it does not appear to act as a foreign substance in vivo. Unfortunately, soluble starch is hydrolized so rapidly by α-amylase in the blood that it cannot be used in a plasma substitute formulation.

Since Wiedersheim reported in 1957 that a hydroxyethylated starch might be employed in a plasma substitute formulation because of its low toxicity and its high resistance to attack by α-amylase, the search for a hydroxyethyl starch suitable for plasma substitute formulations has been made by numerous investigators. In general, however, the attention of the investigators, until the development of the formulation of this invention, has in the main been directed only to a way of maintaining blood pressure, and to this end they tended to use a hydroxyethyl starch having both a high molecular weight and a high degree of substi-

tution of the hydroxyethyl group. The effects of the low rate of excretion, the consequent accumulation, and the hydrolysis in the body tissues of the hydroxyethyl starch used have until now been ignored. Indeed, it was not until we began our researches that the reason why the colloidal solutions used hitherto were unsatisfactory was appreciated.

In one aspect, this invention provides an essentially pyrogen-free, isotonic blood plasma substitute formulation comprising a solution in sterile water containing from 5.8 to 6.2% (W/V) of hydroxyethyl starch having a degree of substitution of from 0.5 to 0.6 and an intrinsic viscosity of from 0.08—0.14, together with sufficient non-toxic organic and/or inorganic adjuvants to ensure both an ionic balance compatible with blood and the isotonicity of the solution, and to ensure a pH of 6.2 ± 0.5.

Preferably the solution will contain substantially 6% W/V of the hydroxyethyl starch, and the degree of substitution of the hydroxyethyl starch will most preferably be substantially 0.55.

The solutions may be ionically balanced and made isotonic in any convenient way known to those skilled in the art. Preferred adjuvants are those giving rise in solution to sodium, potassium, calcium, chloride and lactate ions, and glucose, and convenient specific adjuvants are sodium chloride, potassium chloride, calcium chloride, sodium lactate and glucose.

A particularly preferred composition contains, besides the hydroxyethyl starch, substantially 0.5% W/V sodium chloride, 0.03% W/V potassium chloride, 0.02% W/V calcium chloride, 0.224% W/V sodium lactate and 1% W/V glucose.

In another aspect this invention provides a process for the preparation of a pyrogen-free, isotonic blood plasma substitute formulation as described hereinbefore, in which hydroxyethyl starch having a degree of substitution of from 0.5 to 0.6 and an intrinsic viscosity of from 0.28 to 0.30 is treated with an acid to lower its molecular weight and to reduce its intrinsic viscosity to from 0.08 to 0.14 and adjuvants are added in sufficient quantity to ensure an ionic balance compatible with blood isotonicity, and a pH of 6.2 ± 0.5, to give the desired plasma substitute formulation.

The treatment with the acid is preferably effected in aqueous solution using a mineral acid, advantageously hydrochloric acid, in a concentration of from 0.0125 to 0.025N in the hydrolysis mixture, and the solution is preferably clarified after the acid hydrolysis by means of activated charcoal. Desirably, the pH of the solution after hydrolysis is brought to from 7.0 to 7.4 by the action of a base. The base employed is preferably an alkali-metal base, and particularly preferred bases are sodium bicarbonate and sodium hydroxide since these react with the hydrochloric acid preferably used in the hydrolysis stage to give sodium chloride, a useful adjuvant.

If the plasma substitute formulation at any stage during its preparation contains pyrogens, a depyrogenation agent must, of course, be added to remove them. However, even if there are no pyrogens present, it is still preferred to add the depyrogenation agent.

Raney nickel is the preferred depyrogenation agent and may be added either before or after the adjuvants are incorporated into the formulation.

This invention naturally extends to an isotonic, essentially pyrogen-free plasma substitute formulation whenever prepared by a process as described and claimed herein.

The plasma substitute formulations of the present invention were formulated having regard to many factors, and these will now be discussed, though only by way of illustration, with reference to the accompanying drawings in which: —

Figure 1 is a graph showing the blood polysaccharide concentration (relative to an initial value of 100) plotted against time (in hours) after infusions of HES's, soluble starch, dextrans and PVP's into rabbits.

Figure 2 is a graph showing at various times after infusion the relation between the blood polysaccharide concentration and the excretory rate in urine in rabbits and the degree of substitution of HES infused into them.

Figure 3 is a graph showing the relation between intrinsic viscosity of HES and its average molecular weight.

Figure 4 is a graph showing the relation between the half-life in the blood of HES infused and the intrinsic viscosity of the HES.

Figure 5 is a graph showing the relation between the hydrolysis rate *in vitro* of HES by pig pancreas α-amylase and the degree of substitution of HES.

Figure 6 shows the effects of several plasma substitute formulations on the red blood cells of rabbits after incubation for sixty minutes.

Figure 7 shows the effects of several plasma substitute formulations on the red blood cells of humans after incubation therewith for sixty minutes.

Figure 8 is a graph showing the variation with respect to time of the arterial blood pressure in a dog after infusion of three different plasma substitute formulations.

Figure 9 is a graph showing the variation with respect to time of the pH of arterial blood of a dog after infusion of three different plasma subitute formulations.

Figure 10 is a graph showing the blood and urinary concentration of hydroxyethyl starch in rabbits with respect to time following infusion of 30 ml/kg body weight HES formulation.

Figure 11 shows the effects of HES and dextran formulations on liver and kidney cells of a rabbit ten days after a 100 ml/kg body

ught infusion thereof, and Figure 12 shows the relation between optical density and elution during gel-filtration on Sephadex G—200 of native HES and the HES formulation produced by hydrolysis of the native HES.

Isotonic solutions respectively containing 6% of hydroxyethyl starch (HES) with varying degrees of substitution, 6% of commercial dextran, 6% of soluble starch, and 3.5% and 6% of PVP were intravenously administered separately to rabbits, and the concentrations of colloidal particles in the blood of the animals were measured every hour thereafter. As shown in Figure 1, hydroxyethyl starch generally disappeared more slowly from the blood the higher its degree of substitution. This is also borne out by Figure 2. From Figure 1 it may be seen that the persistence in the blood of hydroxyethyl starch with a degree of substitution of from 0.5 to 0.6 is nearly equal to that of dextran having an average molecular weight of 75,000. It was concluded, therefore, since the persistence of such dextran in the blood is of the correct magnitude, that hydroxyethyl starch with a degree of substitution of substantially 0.55 was preferred for use in the plasma substitute formulations of this invention.

Another factor investigated was the relationship between the molecular weight and the intrinsic viscosity of hydroxyethyl starch and its half-life period in blood. Pathological examination of the condition of main tissue was carried out the intravenous infusion of HES (DS 0.54 and molecular weight 233, 600) which showed the same concentration in the blood as control rabbits infused with dextran (average molecular weight 75,000). The infusions into rabbits were carried out daily for one month. Lesion of main tissues was not observed in 10 and 30 ml/kg body weight HES treated rabbits but a more severe lesion was observed in 90 ml/kg HES treated rabbits than in the dextran treated rabbits. After consideration of above results, it was decided to hydrolyze the HES with hydrochloric acid to obtain HES with a lower molecular weight. The relationship between the molecular weight and intrinsic viscosity, and the half-life period in blood of the hydrolysed HES, was investigated.

Figure 3 shows the relation between intrinsic viscosity and molecular weight which is determined by Archibald's method. The following equation is established from Figure 3.

$$\text{Average molecular weight} = 1.037\times10^{6}\times[\eta]-7.360\times10^{4}$$

$$\eta = 0.0103—0.300$$

Figure 4 shows the relation between intrinsic viscosity and the half-life period in blood of HES with a DS of 0.5 to 0.6 (The half-life period is the time taken from the end of infusion of the HES hydrolysates to the disappearance of 50% of the blood polysaccharides present. The half-life of HES may be seen from Figure 4 to be directly proportional to the intrinsic viscosity, and thus this latter quantity may be taken as an objective and reliable indication of the quality of the HES. It is therefore possible to select HES having the highest safety from a knowledge of its intrinsic viscosity.

A further factor which was taken into account was the hydrolysis rate of HES by pig-pancreas α-amylase. This was found to be dependent only on the degree of substitution of the hydroxyethyl group and was found to be independent of the molecular weight of the HES. Under such conditions as would hydrolyze 77% of soluble starch HES with a DS of 0.92 was not decomposed.

Figure 5 shows the resistance to α-amylase of HES with degrees of substitution between 0.4 and 0.7.

The hydrolysis was carried out in a 0.02M phosphate buffer, pH 6.9 at 30° C, and the concentrations of substrate and enzyme were respectively 10 mg/ml and 0.5 μ/ml.

The hydrolysis rate is expressed in hundredths of a milligram maltose liberated per unit enzyme in one minute.

From Figure 5 it may be seen that HES with a high DS, especially HES with a DS greater than 0.6, will remain in the body tissues for a long period because it is resistant to α-amylase and is less susceptible to hydrolysis. According to the factors already mentioned, it is clear that HES with a DS of 0.5 to 0.6 and an intrinsic viscosity of from 0.08 to 0.14 is best for use in plasma substitute formulations.

In order to obtain a highly effective and safe plasma substitute for clinical use, other factors such as the safety of red cells, the ability to maintain blood pressure, the prevention of acidosis, and the rapid disappearance from tissues in cases of intravenous infusion in large amounts had to be investigated. From the results of the following investigations it may be seen that the HES preparation formulated as in Example 7 hereinafter is most advantageous.

1) *Acute Toxicity*

An HES preparation (Example 7) was compared with both a 6% HES (DS 0.54, $=\eta 0.300$) solution untreated with acid and a solution of dextran (M.Wt. 75,000) in a determination of their acute toxicities. From the results shown in Table 1 below, it may clearly be seen that the acute toxicity figures of the HES preparation of this invention are much better than the other two, and this indicates, of course, that the plasma substitute of this invention is safer than the other formulations.

TABLE 1

LD_{50} Values (ml/kg) of HES (DS 0.55 & M.W. 230,000), HES formulation of this invention and Dextran — 75

Animals	route	sex	ex. 7 preparation	HES (DS 0.55 & M.W. 230,000) 6% in Saline Solution	Dextran-75
Rabbits	i.v.	male	416	225	—
		female	419	230	323
Mice	i.v.	male	360	210	208
		female	—	216	202
	i.p.	male	>600	>450	—
		female	>600	>450	—
Rats	i.v.	male	262	—	136
		female	193	—	125

2) *Morphological influences on red cells.*

The isotonicities of various plasma substitutes were investigated by a study of the morphological changes in red cells of rabbits and humans after they had been incubated with the various plasma substitutes. In the case of a saline solution and the HES preparation (Example 7), no morphological changes in the red cells were observed. However, red cells of rabbits incubated with low molecular weight dextran (M.W. 4,000) and clinical dextran (M.W. 7,000) were found to be agglutinative. Some artificial gelatin preparations show the worst effects on red cells as shown in Figs. 6 and 7.

3) *Maintenance of blood pressure.*

Anesthetized dogs were bled of 30ml blood/kg body weight at a bleeding rate of 10 ml/min. Immediately an equal volume of the plasma substitute (6% HES in 0.9% NaCl) was administered intravenously. The plasma substitute showed approximately the same effectiveness for maintaining arterial blood pressure in the dogs as dextran (average molecular weight 75,000) as shown in Fig. 8. The pH value of arterial blood of dogs treated with Ringer's solution decreases to a relatively low pH value as shown in Fig. 9. On the other hand, the HES preparation (Example 7), which maintains the pH may be able to prevent a tendency towards acidosis.

4) *Persistance of HES in the blood.*

The concentration of colloidal substances which remain in the blood of rabbits after intravenous infusion of the plasma substitute formulation of this invention was examined, and the results are shown in Figure 10. An infusion of 30 ml/kg body weight of the formulation was given, and the concentration of colloidal particles was plotted as a percentage of the concentration at the end of the infusion. Blood concentrations of dextran-75 (m.wt. 75,000) and dextran-40 (m.wt. 40,000) are also given, for reference.

The rate of disappearance of HES (Example 7) from the blood was found to be slightly faster than the disappearance of dextran (average molecular weight 75,000), but the persistence of HES in blood is enough to warrant its use as a plasma substitute, and is better than that of dextran of molecular weight 40,000.

5) *Accumulation of HES in the tissues.*

Accumulation of HES in the tissues of mice was not observed following a conventional dose of the Example 7 formulation administered intravenously. If a very large dose is given, however, and the presence of HES is observed in the liver and spleen, lesion of the reticuloendotherial system does not occur. When clinical dextran (average molecular weight 75,000) is infused intravenously into rabbits in large doses (100 ml/kg), histological lesions of the kidney and liver are observed. On the other hand, no lesions of the kidney and liver are observed when the same amount of HES (example 7) is administered intravenously. Fig. 11 shows the tissue of kidneys and livers ten days after a 100 ml/kg body weight infusion of dextran-75 and the formulation of this invention.

6) *Anaphylactic reaction*

The anaphylactic reaction of rats and mice to dextran has long been known as being oedema and erythema of paws, ears and snout. The occurrence of a combined anaphylactic and purpuric reaction when an injection of agar is given prior to dextran has also been demonstrated. Investigators emphasize the role of histamine release from mast cells.

The present investigation was aimed at determining whether anaphylactic reaction will occur in rats and mice injected with the HES preparation of Example 7 and agar.

An intravenous injection of dextran produced an acute, serious anaphylactoid inflammation in the ears, snout and tail of rats and mice. On the other hand, in animals injected with the HES preparation of Example 7 no such inflammation was observed. Moreover, anaphylactic purpura appeared in the paws, ears and tail of rats and mice when an injection of agar was given prior to the injection of dextran. In the animals injected with the Example 7 preparation and agar, no symptoms of anaphylactic purpura were observed. These results suggest that the Example 7 preparation does not release histamine from mast cells under the conditions employed.

7) *Immunogenicity*

Rabbits, guinea pigs and mice all received injections of dextran or the Example 7 preparation with complete Freund's adjuvant. Sera from all bleedings were analyzed by passive cutaneous anaphylaxis reaction. Arthus reaction tests in rabbits and active anaphylaxis reaction tests in guinea pigs were also performed.

No antibodies to dextran and the Example 7 preparation were detectable in rabbits and guinea pigs. This indicated that, under the conditions of immunization and testing employed, dextran and the Example 7 preparation were not immunogenic in rabbits and guinea pigs.

When mice were injected with small quantities of dextran or the Example 7 preparation, circulating antibodies were detected. However, whereas mice could be paralysed by the injection of relatively large quantities of polysaccharides, large volumes of dextran of the Example 7 preparation, given intravenously to non-immunized humans did not lead to antibody production. It is thought that such large doses in man induce a state of immunological paralysis as in mice.

It has been shown that human sera contain dextran-reacting antibodies of varying types. Moreover, these antibodies react with dextran after infusion, so anaphylactic reaction occurs.

One hundred and six normal human serum samples were investigated by passive cutaneous anaphylaxis reaction in the presence of antibodies reacting to dextran of the Example 7 preparation. Three sera gave a positive reaction to dextran. One of them reacted weakly to the Example 7 preparation. As suggested above, it is thought that the Example 7 preparation possesses less possibility of the occurrence of anaphylactic reaction than dextran.

In order that this invention may be more easily understood, the following Examples are now given, though by way of illustration, to show details of preferred formulations and techniques of this invention.

Example 1.

28 g. of hydroxethylstarch (DS 0.5—0.6, intrinsic viscosity 0.30) were dissolved in 80 ml of distilled water for injection (Japanese Pharmacopoeia VIII) at about 50° C and with stirring.

To this solution, 1.0 ml of 1N HCl and 0.1—1.0 g. of activated charcoal were added, and the mixture was gently boiled for 1 hour.

After cooling, either 1N—$NaHCO_3$ or 1N—NaOH was added to adjust the pH to 7.0—7.4. Since the reaction mixture was not

pyrogen-free, it was treated with 5—50 mg. of a depyrogen reagent, Raney nickel.

The insoluble material was removed by filtration, and the solution obtained was clarified by further filtration.

The filtrate was adjusted to 400 ml total volume with sterile distilled water suitable for injection (Japanese Pharmacopoeia VIII). The resultant solution was a viscous and clear aqueous solution which contained 5.8—6.2% of hydroxyethylstarch and sodium chloride, and showed a turbidity of 6.1 ppm and a viscosity of 1.85 cp (intrinsic viscosity 0.08—014). Isotonic plasma substitute formulations for injection were obtained by moderately adding NaCl and other salts to this solution.

Example 2.

7 g. of hydroxyethylstarch (DS 0.5—0.6, intrinsic viscosity 0.30) were dissolved in 80 ml of distilled water for injection (Japanese Pharmacopoeia VIII) at about 50° C and with stirring.

To this solution, 1.0 ml of 1N HCl and 0.1—1.0 g. of activated charcoal were added, and the mixture was gently boiled for 1 hour.

After cooling, sufficient 1N $NaHCO_3$ or 1N NaOH was added to adjust the pH to 7.0—7.4.

Since the reaction mixture was not pyrogen-free, it was treated with 5—50 mg of a depyrogenising reagent (Raney Nickel). The mixture was filtered to obtain a clear solution, and then the filtrate was adjusted to 100 ml total volume with sterile distilled water suitable for injection (Japanese Pharmacopoeia VIII). NaCl, KCl, $CaCl_2$, Na-lactate and glucose were added to this solution to form an isotonic plasma substitute injection formulation.

Example 3.

14 g of hydroxyethylstarch (DS 0.5—0.6, intrinsic viscosity 0.30) were dissolved in 140 ml of distilled water for injection (Japanese Pharmacopoeia VIII) at about 50° C and with stirring.

To the resultant solution, 3.26 ml of 0.5 NHCl and 0.1—1.0 g of activated charcoal were added and the mixture was gently boiled for 1 hour.

After cooling, 1N $NaHCO_3$ or 1N NaOH was added to adjust the pH of 7.0—7.4. Since the reaction mixture was not pyrogen-free, it was treated with 1—50 mg of Raney Nickel. This mixture was filtered to obtain a clear solution, and then the filtrate was adjusted to 200 ml total volume of isotonic solution by adding sterile distilled water suitable for injection (Japanese Pharmacopoeia VIII). NaCl, KCl, $CaCl_2$, Na-lactate and glucose were also added.

The resultant solution was able to be used as a plasma substitute after packing, sealing and sterilization by heating at 100° C for 1 hour.

Example 4.

14 g of hydroxyethylstarch (DS 0.5—0.6, intrinsic viscosity 0.30) were dissolved in 140 ml of sterile distilled water suitable for injection (Japanese Pharmacopoeia VIII) at about 50° C and with stirring.

To this solution, 3.26 ml of 0.5N HCl were added, and the mixture was kept at 100° C for 1 hour and was then cooled. If necessary, the mixture was treated with activated charcoal. After cooling the mixture was brought to pH 7.0—7.4 with 1N $NaHCO_3$ or 1N NaOH, and then 1.0 g of NaCl, 0.06 g of KCl, 0.04 g of $CaCl_2.2H_2O$, 0.448 g of Na-lactate and 2 g of glucose were added. If necessary, 1—50 mg of Raney nickel was added and the mixture was heated to 90—100° C for 30 minutes. Activated charcoal was subsequently added and the solution was completely stirred and filtered.

The resultant solution was adjusted to 200 ml total volume with sterile distilled water suitable for injection (Japanese Pharmacopoeia VIII) and the pH was brought to 6.2 + 0.5. The solution was filtered until clear, packed in a container and sealed.

It was able to be used as a plasma substitute after sterilization by heating at 100° C for 1 hour.

Example 5.

When hydroxyethylstarch was hydrolyzed by HCl in the same manner as in Example 1, the HES solution exhibited turbidity values (ppm) and viscosity (cp) dependent on the concentration of HCl in the hydrolysis medium as shown in Table II.

Table II

Relation between HCl concentration and turbidity and viscosity of HES solution.

HCl concentration (normal)	turbidity (ppm)	viscosity (cp)
0.00625	22.6	4.64
0.01250	18.0	3.11
0.01875	10.5	2.34
0.02500	8.0	1.87
0.03125	4.0	1.57
0.03750	4.3	1.57

(1) The turbidity was measured by nephelometer (TY3 Type; Made in Corp. Elmer Optical).

(2) The viscosity was measured by a rotation viscometer (BL Type; Made in Tokyo Keiki Co., Ltd.).

From these results it is clear that both the turbidity and the viscosity decrease with increasing acidity. A satisfactory balance is achieved at HCl concentrations from 0.0125 to 0.025 N.

Example 6.

HES (DS 0.54, intrinsic viscosity 0.30, MW 232000) was treated with HCl at a concentration of 0.0125N in the hydrolysis medium by the method of Example 1.

The resultant HES solution was clear and showed a DS of 0.54 an intrinsic viscosity of 0.101 and a turbidity of 6.0 ppm. Gel-filtration of this solution was carried out with Sephadex G—200 using a 2.5 cm×40 cm column and a flow adapter, and elution was by the ascending method at a flow rate per square centimetre of column area of 0.048—0.061 ml/min. The volume used for the following test was 0.25 ml (2.5 mg) and one fraction volume was 3 ml. Determination of saccharide was carried out spectrophotometrically by means of a 0.2% w/v solution of anthrone in concentrated sulphuric acid, and the results were as shown in Figure 12.

It may be seen that the treatment with the acid lowers the molecular weight and reduces the intrinsic viscosity to about 40% of its value prior to the treatment with acid. Moreover, it was concluded from the intrinsic viscosity of the hydroxyethyl starch in the last solution that the average molecular weight was about 40,000.

Example 7.

HES (DS 0.55, intrinsic viscosity 0.3) was treated in the same manner as in Examples 1—5. The resultant HES solution had a DS of 0.55 and an intrinsic viscosity of 0.08—0.14 (preferably 0.10).

It was adjusted to a pH of 6.2±0.5 and its volume was adjusted to 100 ml total volume with HES 6%. NaCl 0.5% (including NaCl which was produced by neutralization), KCl 0.03%. $CaCl_2.2H_2O$ 0.02%, Na-lactate 0.224% and glucose 1% (W/V).

This slightly turbid solution was filtered until clear, packed in bottles, and sealed. It was able to be used as a plasma substitute after sterilization by heating at 100° C for 1 hour.

WHAT WE CLAIM IS:—

1. An essentially pyrogen-free, isotonic blood plasma substitute formulation comprising a solution in sterile water containing from 5.8 to 6.2% (W/V) of hydroxyethyl starch having a degree of substitution of from 0.5 to 0.6 and an intrinsic viscosity of from 0.08—0.14, together with sufficient non-toxic organic and/or inorganic adjuvants to ensure both an ionic balance compatible with blood and the isotonicity of the solution, and to ensure a pH of 6.2±0.5.

2. A plasma substitute formulation as claimed in claim 1, in which the solution contains substantially 6% W/V of hydroxyethyl starch.

3. A plasma substitute formulation as claimed in either of the preceding claims in which the hydroxyethyl starch has a degree of substitution of substantially 0.55.

4. A plasma substitute formulation as claimed in any of the preceding claims in which the adjuvants give rise in solution to sodium, potassium, calcium, chloride and/or lactate ions.

5. A plasma substitute formulation as claimed in any of the preceding claims, in which one/or more of sodium chloride, potassium chloride, calcium chloride, sodium lactate and glucose are incorporated as adjuvant(s).

6. A plasma substitute formulation as claimed in claim 5, in which the or each adjuvant present is incorporated substantially in an amount of 0.5% W/V sodium chloride, 0.03% W/V potassium chloride, 0.02% W/V calcium chloride, 0.224% W/V sodium lactate and 1% W/V glucose.

7. A plasma substitute formulation as claimed in any of the preceding claims, and substantially as described herein with reference to the Examples.

8. A process for the preparation of a pyrogen-free isotonic plasma substitute formulation as claimed in any of the preceding claims in which hydroxyethyl starch having a degree of substitution of from 0.5 to 0.6 and an intrinsic viscosity of from 0.28 to 0.30 is treated with an acid having a physiologically-acceptable anion to lower its molecular weight and to reduce its intrinsic viscosity to from 0.08 to 0.14 and adjuvants are added in sufficient quantity to ensure an ionic balance compatible with blood isotonicity, and a pH of 6.2±0.5 to give the desired plasma substitute formulation.

9. A process as claimed in claim 8 in which the treatment with the acid is effected in aqueous solution using a mineral acid.

10. A process as claimed in claim 9, in which the mineral acid is hydrochloric acid.

11. A process as claimed in any of claims 8 to 10, in which the acid is employed in a concentration of from 0.0125 to 0.025N in the hydrolysis mixture.

12. A process as claimed in any of claims 8 to 11, in which the solution is clarified after the acid hydrolysis.

13. A process as claimed in any of claims 8 to 12, in which the pH of the solution after hydrolysis is brought to from 7.0 to 7.4 by the action of a base.

14. A process as claimed in claim 13, in which the base employed is an alkali-metal base.

15. A process as claimed in claim 14, in which the alkali-metal base is sodium bicarbonate or sodium hydroxide.

16. A process as claimed in any of claims 8 to 15, in which a depyrogenation agent is at some stage added to the plasma substitute formulation to remove pyrogens therefrom.

17. A process as claimed in any of claims 8 to 16 in which the depyrogenation agent is Raney nickel.

Example 6.

HES (DS 0.54, intrinsic viscosity 0.30, MW 232000) was treated with HCl at a concentration of 0.0125N in the hydrolysis medium by the method of Example 1.

The resultant HES solution was clear and showed a DS of 0.54 an intrinsic viscosity of 0.101 and a turbidity of 6.0 ppm. Gel-filtration of this solution was carried out with Sephadex G—200 using a 2.5 cm×40 cm column and a flow adapter, and elution was by the ascending method at a flow rate per square centimetre of column area of 0.048—0.061 ml/min. The volume used for the following test was 0.25 ml (2.5 mg) and one fraction volume was 3 ml. Determination of saccharide was carried out spectrophotometrically by means of a 0.2% w/v solution of anthrone in concentrated sulphuric acid, and the results were as shown in Figure 12.

It may be seen that the treatment with the acid lowers the molecular weight and reduces the intrinsic viscosity to about 40% of its value prior to the treatment with acid. Moreover, it was concluded from the intrinsic viscosity of the hydroxyethyl starch in the last solution that the average molecular weight was about 40,000.

Example 7.

HES (DS 0.55, intrinsic viscosity 0.3) was treated in the same manner as in Examples 1—5. The resultant HES solution had a DS of 0.55 and an intrinsic viscosity of 0.08—0.14 (preferably 0.10).

It was adjusted to a pH of 6.2±0.5 and its volume was adjusted to 100 ml total volume with HES 6%. NaCl 0.5% (including NaCl which was produced by neutralization), KCl 0.03%. $CaCl_2.2H_2O$ 0.02%, Na-lactate 0.224% and glucose 1% (W/V).

This slightly turbid solution was filtered until clear, packed in bottles, and sealed. It was able to be used as a plasma substitute after sterilization by heating at 100° C for 1 hour.

WHAT WE CLAIM IS:—

1. An essentially pyrogen-free, isotonic blood plasma substitute formulation comprising a solution in sterile water containing from 5.8 to 6.2% (W/V) of hydroxyethyl starch having a degree of substitution of from 0.5 to 0.6 and an intrinsic viscosity of from 0.08—0.14, together with sufficient non-toxic organic and/or inorganic adjuvants to ensure both an ionic balance compatible with blood and the isotonicity of the solution, and to ensure a pH of 6.2±0.5.

2. A plasma substitute formulation as claimed in claim 1, in which the solution contains substantially 6% W/V of hydroxyethyl starch.

3. A plasma substitute formulation as claimed in either of the preceding claims in which the hydroxyethyl starch has a degree of substitution of substantially 0.55.

4. A plasma substitute formulation as claimed in any of the preceding claims in which the adjuvants give rise in solution to sodium, potassium, calcium, chloride and/or lactate ions.

5. A plasma substitute formulation as claimed in any of the preceding claims, in which one/or more of sodium chloride, potassium chloride, calcium chloride, sodium lactate and glucose are incorporated as adjuvant(s).

6. A plasma substitute formulation as claimed in claim 5, in which the or each adjuvant present is incorporated substantially in an amount of 0.5% W/V sodium chloride, 0.03% W/V potassium chloride, 0.02% W/V calcium chloride, 0.224% W/V sodium lactate and 1% W/V glucose.

7. A plasma substitute formulation as claimed in any of the preceding claims, and substantially as described herein with reference to the Examples.

8. A process for the preparation of a pyrogen-free isotonic plasma substitute formulation as claimed in any of the preceding claims in which hydroxyethyl starch having a degree of substitution of from 0.5 to 0.6 and an intrinsic viscosity of from 0.28 to 0.30 is treated with an acid having a physiologically-acceptable anion to lower its molecular weight and to reduce its intrinsic viscosity to from 0.08 to 0.14 and adjuvants are added in sufficient quantity to ensure an ionic balance compatible with blood isotonicity, and a pH of 6.2±0.5 to give the desired plasma substitute formulation.

9. A process as claimed in claim 8 in which the treatment with the acid is effected in aqueous solution using a mineral acid.

10. A process as claimed in claim 9, in which the mineral acid is hydrochloric acid.

11. A process as claimed in any of claims 8 to 10, in which the acid is employed in a concentration of from 0.0125 to 0.025N in the hydrolysis mixture.

12. A process as claimed in any of claims 8 to 11, in which the solution is clarified after the acid hydrolysis.

13. A process as claimed in any of claims 8 to 12, in which the pH of the solution after hydrolysis is brought to from 7.0 to 7.4 by the action of a base.

14. A process as claimed in claim 13, in which the base employed is an alkali-metal base.

15. A process as claimed in claim 14, in which the alkali-metal base is sodium bicarbonate or sodium hydroxide.

16. A process as claimed in any of claims 8 to 15, in which a depyrogenation agent is at some stage added to the plasma substitute formulation to remove pyrogens therefrom.

17. A process as claimed in any of claims 8 to 16 in which the depyrogenation agent is Raney nickel.



18. A process as claimed in claim 16 or claim 17, in which the depyrogenation agent is added before the adjuvants are incorporated into the formulation.

19. A process as claimed in any of claims 8 to 18 and substantially as described herein, with reference to the Examples.

20. An isotonic, essentially pyrogen-free plasma substitute formulation as claimed in any of claims 1 to 7 whenever prepared by a process as claimed in any of claims 8 to 19.

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