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 Jørgen Schlichtkrull, a Danish Subject, of 28 B, Ved Bellahøj, Brønshøj, Denmark.

COMPLETE SPECIFICATION

Improvements in or relating to Insulin Preparations

We, NOVO TERAPEUTISK LABORATORIUM A/S, a Danish Body Corporate, of 115, Fuglebakkevej, Copenhagen, Denmark, 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:—

The present invention relates to a quick-acting and stable insulin preparation and to a process for producing such preparation.

After the appearance of clinically useful insulin preparations having a prolonged insulin action, insulin solutions that are known to be quick-acting have gradually been replaced by these more suitable insulin preparations. Insulin solutions, however, at present have three applications, each of which renders these solutions indispensable in therapy, that is, in the shock treatment of psychiatry, in the treatment of acute emergencies in diabetes therapy and the employment in combination with the insulin preparations having a prolonged action in the daily treatment of diabetics. In numerous cases in which use is made of aqueous suspensions of amorphously precipitated zinc-protamine-insulin having a highly protracted effect, it is advantageous to add thereto an insulin solution which is admixed in the injection syringe. It will also often be advisable to make use of such admixture when employing an aqueous suspension of crystalline zinc-protamine-insulin.

In all cases in which it is desired to use an addition of insulin in solution or in which such addition will be used, it should, however, be taken into consideration that the insulin solutions used up to now are incompatible with the insulin preparations having a protracted effect so far as the acidity is concerned. While most frequently the preparations having a protracted effect are neutral insulin suspensions, the insulin solutions used up to now are acid and have a pH of about 3, as they have been produced by dissolving amorphous or particularly crystalline insulin in a dilute acid, usually hydrochloric acid, to which has been added a preserving agent and a substance making the solution isotonic and, if desired, a water-soluble zinc salt, such as zinc chloride. The said suspensions having a protracted effect, however, go into solution at such a low pH-value and are more or less unstable at the pH-value that will result after admixing the insulin in solution. As a consequence thereof, the admixture of dissolved insulin creates an uncertainty in the treatment of diabetes and necessitates a quick application of the produced mixtures.

The present invention aims at the production of a quick-acting insulin preparation containing dissolved insulin as active constituent and being compatible with the insulin preparations having a protracted effect because it has neutral reaction, in contradistinction to the insulin solutions used hitherto.

The invention is based on the discovery that insulin produced from pig pancreas, in what follows called pig insulin, has such a high solubility at a pH of between 7 and 8 that no insulin precipitates will be formed at this pH-value, so that it is ensured that, even when ions of zinc, that promote crystallization of the insulin, are present in the solution at a concentration just sufficiently high for the crystallization of the insulin from other pancreas glands, and even after prolonged storage, no precipitation of crystalline insulin will take place. If the insulin instead of being present in dissolved form, is present in the form of crystals suspended in an aqueous medium containing ions of added zinc a very highly prolonged insulin activity can be obtained by parcutical injection.

It has been found that, when employing pig insulin, it is possible to arrive at stable insulin solutions having a pH-value of between 7 and 8.

The process according to the invention is characterised in that pig insulin is dissolved in an aqueous injectable medium being given a pH-value of between 7 and 8.

As mentioned in the foregoing, in the production of the acid insulin solutions hitherto, use has sometimes been made of an addition of zinc chloride in order to stabilize the solutions. It is known that zinc ions and ions of the other crystallization-promoting metals cobalt, nickel and cadmium, reduce the solubility of the insulin at neutral reaction and may cause precipitation of the dissolved insulin. Therefore, if in the process according to the invention use is made of an addition of a water-soluble zinc salt or a salt of one of the other crystallization -promoting metals, or if use is made of insulin having a high content of these metals, care should be taken that in the insulin solution produced the content of ions of the crystallization-promoting metals is less than about $13 \times A \times 10^{-3}$ milli-equivalents per litre, A indicating the number of international units of insulin per millilitre.

In the production of preparations with neutral reaction and having a protracted effect and consisting of an aqueous suspension of an insulin precipitate, it is known to make use of a buffering substance in order to maintain the neutral reaction. Example of such buffering substances are phosphate, citrate, acetate, borate, maleate, diethylbarbiturate and glycerol buffers.

It is also known that certain of these buffering substances bind ions of the said metals in such a way that the solubility of the insulin at neutral reaction is not reduced when together with the metal ions such buffering substances are present. Thus ions of all the said metals are complexed by citrate buffer.

The process according to the invention is therefore further characterised in that, if there is added a solution of a compound of one or more of the metals zinc, cobalt, nickel and cadmium, that are prerequisite to the crystallization of insulin, or if pig insulin having a high content of these metals is used, in the absence of substances, such as buffering substances forming slightly soluble or complex compounds with the said metals, the solution is given a content of the metals of less than about $13 \times A \times 10^{-3}$ milli-equivalents per litre, A being the number of international units of insulin per millilitre. With zinc as an example and an insulin concentration of 40 international units per millilitre, the produced solution should in the absence of, for example, phosphate and citrate buffers contain less than about 1.7 mg.% of zinc. If, however, phsophate or citrate buffers are present, the produced preparation may contain considerably more zinc if only the amount of zinc ions being at the disposal of the insulin and consequently not bound by the buffering substances does not exceed the above-mentioned upper limit.

Further, in view of the stability of the solution when stored for a long period of time, the upper limit for the content of the said metals in the insulin solution is dependent on the pH value and the storage temperature of the solution. The higher the metal content and the lower the storage temperature, the higher the pH value of the solution should be in order that the latter should be stable on prolonged storage.

The insulin solutions according to the present invention are excellently appropriate for being admixed with the insulin preparations consisting of aqueous insulin crystal suspensions having a content of ions of one or more of the metals zinc, cobalt, nickel and cadmium and showing a prolonged insulin action. The admixture gives these preparations a desired initial effect. The mixing may even take place on an industrial scale before the preparations are marketed, as the produced mixtures are completely stable even after prolonged storage under the conditions prevailing in practice.

The invention will now be further illustrated by the following specific examples.

EXAMPLE 1

1.74 grams (40,000 international units) of amorphous zinc-free pig insulin is dissolved in 500 millilitres of water containing 2 millilitres of N hydrochloric acid, 50 grams of glucose and 2 grams of phenol. The solution is sterile-filtered, neutralized with N sodium hydroxide to pH 7.6 and diluted with sterile water to 1 litre.

EXAMPLE 2

1.74 grams (40,000 international units) of crystalline pig insulin containing about 0.4 per cent of zinc is dissolved in 500 millilitres of water containing 2 millilitres of N hydrochloric acid, 50 grams of glucose and 1-gram of methyl *p* hydroxybenzoate. The solution is sterile-filtered and neutralized with 500 millilitres of a sterile solution of 0.02 M sodium phosphate (pH 7.5). If necessary, the pH of the mixture is re-adjusted with N sodium hydroxide to between pH 7.3 and 7.5

EXAMPLE 3

1.74 grams (40,000 units) of crystalline pig insulin containing about 0.7% of zinc is dissolved in 400 millilitres of water containing 2 millilitres of N hydrochloric acid and I gram of methyl *p*-hydroxybenzoate. The solution is sterile-filtered and admixed with 500 millilitres of a sterile solution containing 1.4 per cent by weight of sodium chloride and 0.02 mole sodium acetate per litre. The pH is adjusted to be tween 7.3 and 7.5 with N sodium hydroxide, after which the solution is diluted with sterile water to 1 litre.

In all the above examples the produced

solutions have an insulin content of 40 international units per ml. It is, however, according to the invention also possible to produce more concentrated solutions having, for example, an insulin content of 80 international units per ml. With this higher insulin concentration, the composition of the solutions being otherwise identical, a pH-value more close to 8 should be used in order to ensure that no insulin is precipitated from the solutions when stored. As stressed above, the content of zinc and the other metals of the solutions and the kind of the used buffer, if any, will, however, influence the solubility of the pig insulin, which should also be taken into consideration when using higher insulin concentrations.

EXAMPLE 4

0.2 g. of phenol and 5 g. of glucose are dissolved in 50 ml. of water and the pH-value of the solution is adjusted to 7.8 by addition of about 0.1 ml. of 0.1 N NaOH. To this solution there is added 0.174 g. of amorphous zinc-free pig insulin. The addition of the pig insulin causes the pH-value of the mixture to decrease to about 5.0, and while stirring about 0.9 ml. of N NaOH is added to raise the pH-value to 7.8, which causes the amorphous insulin to dissolve completely. The solution is sterile-filtered and diluted with sterile water to a volume of 100 ml. The pH-value of the sterilized solution is 7.7.

WHAT WE CLAIM IS:—

1. A quick-acting stable insulin preparation consisting of a solution of pig insulin as hereinbefore defined, in an aqueous injectable medium having a pH-value between 7 and 8.

2. An insulin preparation as claimed in Claim 1, which shows a content of ions of one or more of the metals zinc, cobalt, nickel and cadmium, preferably zinc, of less than about $13 \times A \times 10^{-3}$ milli-equivalents per litre, A being the number of international units of insulin per millilitre.

3. A process for producing a quick-acting stable insulin preparation, as defined in Claim 1 or 2, wherein pig insulin as hereinbefore defined is dissolved in an aqueous injectable medium being given a pH-value of between 7 and 8.

4. A process as claimed in Claim 3, wherein there is added a solution of a compound of one or more of the metals zinc, cobalt, nickel or cadmium, that are prerequisite to the crystallization of the insulin or use is made of pig insulin with a high content of these metals, which process is characterised in that in the absence of substances, such as buffering substances forming slightly soluble or complex compounds with such metals, the solution is given a content of the metals of less than about $13 \times A \times 10^{-3}$ milli-equivalents per litre, A being the number of international units of insulin per millilitre.

5. A process for producing a quick-acting stable insulin preparation substantially as hereinbefore set forth, with particular reference to the Examples.

6. Quick-acting stable insulin preparations whenever prepared by a process as claimed in any preceding claim.

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