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(54) **Physiologically Acceptable Heparin**

(57) 'Oxalate-free' preparations of heparin, particularly in the form of calcium salts of heparin, which can be used for the preparation of injectable solutions of heparin which have long storage life comprise from 30 to 70 ppm oxalates. The heparin preparations are obtained by selective precipitation of the heparin from aqueous solution by the addition of a non-ionic agent, for example ethanol, wherein mineral salt impurities.

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SPECIFICATION

Heparin Compositions Freed or Mineral Salts, Particularly Oxalates, and Process for Obtaining Them

The invention relates to a purification process of heparin and of heparin salts, as well as to the products obtainable by such process.

Sodium salts of heparin (also termed as "sodium heparinates") are conventionally used for the production of injectable solutions of heparin. Mixed calcium-sodium heparinates or calcium-magnesium heparinates and advantageously calcium heparinates, have more recently been brought into use, in order to do away with a number of indesirable vascular reactions, particularly in the injection area, due to the sodium ions.

Mixed heparinates, i.e. calcium-sodium heparinates or calcium-magnesium heparinates, and sodiumfree heparinates, particularly calcium heparinates are advantageously prepared from an initial heparinate, say of sodium, by the process defined in British patent 1 471 482. This process comprises contacting the initial simple salt of heparin in an aqueous medium with a salt of the desired metal to be substituted at least partially for the metal in the initial heparin salt, to form an intermediate mixed heparin salt containing the desired metal, and separating the so formed intermediate heparin salt from free metal ions contained in said medium. To the extent where a heparin salt further enriched with the metal of substitution is desired, the above said intermediate heparin salt can be recontacted in an aqueous medium with a salt of the desired metal. If desired, the conditions of operation of this process can be adjusted so as to obtain a simple heparinate from the substitution metal free of the metal contained in the initial heparin salt.

It has been found, occasionally, that on storage of solutions of heparin salts containing calcium ions, deposits or precipitates could form or a turbidity would appear in the solutions. Obviously this tendency to the formation of precipitates or turbidity, which happens to be all the greater as the said solution are more concentrated in heparin, is of particular disadvantage in the case of injectable solutions of heparin intended for therapeutical use prepared in advance industrially, notably in the form of predetermined doses, such as in the form of ampullae or dispensable syringes, and which accordingly must remain perfectly clear even after several months of storage. The formation of small deposits or even but light turbidity does of course render the said solutions inappropriate for the therapeutical administration.

This is particularly so if one bears in mind that particles or crystals are no longer visible when their sizes are below approximately 50 microns, on the one hand, and that, capillary vessels in man have internal diameters which can be as low as 1—3 microns, on the other hand. Moreover the presence, in injectable solutions, of said particles or crystals, even if no longer visible without optical equipment, may become particularly deleterious where the active principle injected is formed of heparin, owing to its anticoagulant properties. Thus the hemorrhagic risks induced by such solid particles , injected in the patient, may even be enhanced by the otherwise desired essential activity of heparin.

The fact that the solution remains limpid even does not necessarily mean that no precipitate has formed. As a matter of fact it may happen, particularly in those instances where precipitation takes place very slowly, that the minute crystals (particularly sub-visible having sizes less than 50 microns) which form tend to coalesce into very few, it not but one, relatively sizable crystals, which of course do not cause alteration of the overall limpidity of the solution. Consequently the injection of such solution may be at least as harmful as in the preceding case.

All of the risks mentioned hereabove are all the greater as the concentration of heparin in the standard pharmaceutical solutions are greater, although they may vary according to the type of injection (either subcutaneous, intramuscular, intravenous, etc. . . .). As is well known however, it is often desired to use heparin concentrations as high as possible, whereby the potentiality of precipitation or the presence of sub-visible particles in growing number are liable of becoming a very serious problem.

In an attempt to overcome such difficulties, recourse if often had to a rather long storage of the freshly formed heparin solutions, particularly of the solutions of heparin salts of physiologically acceptable metals, formed at least in part of calcium, the precipitates which might then have formed being filtered off, before the solutions are then further handled, for instance distributed into ampullae or dispensable syringes.

It will obviously be appreciated that the greater the pre-storage time, the less economical the production of the final pharmaceutical preparations.

The object of the invention is thus to essentially overcome all of the above said difficulties; thus a particular object of the invention is to provide heparins of injectable quality usable for the preparation of solutions, particularly injectable solutions, having long storage-life. Another object of the invention is to provide a process for obtaining such heparins, which enables pre-storages of the type referred to hereabove to be dispensed with.

It has in fact been found that most of the abovesaid difficulties may be attributed to the presence in the solutions of heparins of injectable quality, of mineral salts of various nature and in variable quantities (up to 2.5% by weight) depending upon the origin of the tested batch, and of the processes used for their extraction from natural sources and/or for discoloring (or whitening) the natural heparins obtained, to have them meet the colorlessness standards usually required for heparins of injectable quality, even

though such contents of mineral salts remain within the limits tolerated according to today's regulations, for instance the standards of the French CODEX.

The greater part of the residual salts is consituted by chlorates and sulfates which may respectively amount up to 1%. Other salts, particularly carbonates, sulfites, are present in smaller proportions.

The transformation of sodium salts of heparin into calcium containing salts of heparin, say according to the process recalled hereabove, should reduce the proportions of these salts, taking into account the low solubility of the calcium sulfates, sulfites and carbonates. The latter should as a matter of fact be separable as solids from the clear solution of the calcium-containing salts of heparin.

As this has already been reported in the co-pending British complete specification n° 331 70/77, 52047/77 filed on May 30th, 1978, it has been observed that, most surprisingly, at the end of the transformation of sodium heparinate into calcium heparinate, particularly in accordance with the above mentioned process, the contents of the above mentioned residual mineral salts is but little modified compared to that of the initial sodium heparinate.

Even more surprising was the finding that salts of heparin, including those which contain a metal formed at least in part of calcium, can be solubilized in form of limpid aqueous solutions, even though they may still contain minor, yet non negligible amounts of oxalic ions. This was all the more unexpected as oxalates of metals like calcium are, as is well known, highly water-insoluble.

The reason for this behaviour is not yet well understood. Nevertheless, a change in the normal solubility conditions of calcium oxalates has been ascertained, particularly when calcium chloride is in excess. Thus, it has been found that commercially available injectable heparins or heparinates may have residual salt-contents varying from 1.0 to 2.5% in weight.

Referring again to the above mentioned British complete specification, it had been noted therein that injectable preparations of heparin available in the trade have been found to have residual salt-contents varying from 1.0 to 2.5% in weight, and from about 40 to 300 ppm oxalate ions and sometimes even more. In fact, the lower limit was based on the determination, later found in error which had been made on a single sample of a commercially available sample of heparin. In fact, it was found that even in that sample, like in the other many samples which have been tested, the oxalate contents are consistently above 90 ppm.

Typically, in commercial available injectable heparins, the oxalate contents are between 90 and 300 ppm and often much higher.

As noted in the preceding British specification, it had been found that the elimination of the mineral salts, particularly of the traces of oxalates, from the treated heparins results in the disappearing of turbidity and deposits which were liable to occur in the calcium heparinate solutions after the preparation of said solutions.

The calcium or mixed calcium heparinate solutions which have undergone a purification process according to the invention as hereinafter disclosed, remain clear, even after having been stored for several months. No turbidity or deposit appears. Their contents in sub-visible particles are not increased upon long storage time, whereby the risks of crystal-coalescence referred to hereabove is reduced too.

According to the above-said specification, the most preferred heparin salts according to the invention were those which contain less than 30 ppm or even less than 20 ppm oxalate ions.

It has been found that higher amounts of oxalates—although lower than in the usual commerical heparins, particularly calcium salts of heparin—still remain compatible with the above mentioned standards of clearness and long storage stability. Particularly it has been found that oxalate contents up to 70 ppm are permissible.

Therefore heparins or heparin salts according to the invention include those, the content of which is residual mineral salts, particularly of oxalates, is of 30 to 70 ppm oxalate. Such heparin preparations are useful for the premaration of injectable solutions—and more especially calcium heparinate solutions—at concentrations normally used for this type of administration. Such solutions remain both perfectly clear and free of sub-visible particles (as detectable by conventional optical equipment, such as detecting particles having sizes ranging from 2 to 50 microns upon protracted storage which can be as long as six months, or even two years and even three to five years. If initially some particles, other than calcium salts, were present, their number will then of course remain constant.

However the aqueous solutions of calcium salt of heparin which contain above 70 ppm of oxalates are subject to high risks that precipitation or turbidity phenomena occur within the 6 month-period which follows their preparation.

Preferably, they further have a content in residual mineral salts below 0.5%, and preferably below 0.3%.

It must be understood that the expression "mineral salts" is not restricted to salts of mineral acids, such as sulfuric or hydrochloric acid, but also includes the salts of acids like oxalic or carbonic acid, or even of organic acids of low molecular weight, such as acetic acid.

It has been found that such heparin salts can be used for the production of injectable solutions which can be stored or shelved over prolonged periods of time, even when the metal of the heparin salts is, at least in part, one which, like calcium, forms oxalates which are not water-soluble.

The above-mentioned storage is understood to

be at room temperature, such as in the range of about 20—25°C.

The invention also provides a process for obtaining purified heparins according to the invention, starting from commercially available products, inclusive of strongly discolored heparin products, as obtained by conventional discoloration methods, particularly oxidative methods which, as it has been found, are liable of increasing the oxalate of the so treated heparins.

The above mentioned "mineral salts" can be considered as non heparin salts.

The process according to the invention applicable to a starting heparin preparation to be purified or, more generally, a mixture of heparin and other salts, particularly mineral salts, brings into play a selective precipitation utilizing the difference of solubility between heparin salts and residual mineral salts. It comprises adding to an aqueous solution containing the heparin-mineral salts mixture an amount of a non ionic precipitating agent such as alcohol, for instance ethanol, so adjusted as to cause a selective precipitation of the heparin or heparin salts while the mineral salt remain in the aqueous solution, recovering the heparin and, if need be, repeating this selective separation on a new aqueous solution of the heparin so recovered until the concentration of oxalate in the final heparin is less than 70 ppm, particularly from 30 to 70 ppm.

According to a preferred embodiment of the process of the invention, the heparin used is in the form of a salt of a metal, e.g. sodium, lithium, potassium, the oxalates of which are themselves water-soluble. Another metal, particularly like calcium, of which the oxalates are water-insoluble, or possibly magnesium, can be substituted, at least partially, for the metal of the purified heparin finally obtained, substantially freed from its oxalates.

The concentration of the heparin solution used for such treatment may largely vary. It is preferable, for practical reasons, that the solution should not be too diluted as the volume of ethanol used is proportional to that of the treated solution. For an equal quantity of heparin, the more diluted the solution, the larger the amount of ethanol required. To the contrary, the heparin solutions must not exceed a certain degree of concentration as their viscosity increases rapidly, and the heparin precipitation, starting from these solutions, could possibly carry down a large amount of the initial impurities which are intended to be separated.

Taking these conditions into account, the process is advantageously carried out on solutions the concentration of which is of the order of that of the solutions usually used for injections, that is containing from about 40 to about 250 g/l of sodium heparinate (corresponding to about 5000 to 30000 IU/ml).

The pH of the solution influences the result of the purification. An acid solution would rather retain the mineral salts in the solution, particularly oxalates, and consequently improve the separation.

Besides, heparin may be affected by strong acids. A solution having a pH above 3.5 and preferably comprised between 5 and 7 is advantageously used.

A sufficient amount of ethanol is added to the heparin solution thus prepared, so that practically the whole heparin precipitates while the mineral salts remain in the hydroalcoholic solution. For one volume of heparin solution, 0.5 to 1.5 volume of ethanol is preferably used.

Preferably, a practically pure neutral alcohol (99 to 100°GL) is resorted to.

The heparin precipitate will be separated from the supernatant and then kneaded and washed in order to eliminate all remaining traces of solution. The washing is preferably done with absolute alcohol. Heparin is then filtrated and dried.

Should these first separations be incomplete, this treatment of the heparin precipitate may possiby be renewed till one obtains heparin salts meeting the above indicated requirements.

Most of the initial heparin is recovered in the course of the above described precipitation; the rest remains in the hydroalcoholic solution. The latter may be treated with another alcohol quantity in order to obtain a new heparin precipitation.

The purification process of the invention has been found to be applicable in all instances for the removal of the free oxalates contained in commercial heparins, whichever be its source.

However, while this process certainly enables the removal of any measurable free oxalates until providing heparins which contain less than 30 and even than 20 ppm, when applied to a heparin salt of a metal, like sodium, of which the corresponding oxalate is water-soluble, it has occured in few instances that upon converting at least partially such heparin salt into one of a metal, like calcium, of which the oxalates are insoluble, the resulting product was finally found to contain greater amounts of free oxalates.

Though at this stage no scientific explanation can be offered for interpreting these phenenomena, it is assumed that part of the oxalates contained in the commercial heparins of some sources behaves as if it were absorbed or fixed on the heparin molecules, the latter then behaving, apparantly, as an anion-exchanger. Therefore, and as a result of these phenomena, the final heparin salts may prove again to no longer be suitable for the production of injectable solutions of the heparin which can be stored over prolonged periods of time.

It has however been further found that this difficulty can be overcome when resorting to the further improved process of this invention which comprises contacting said non-ionic heparin-precipitating-agent in the above described purification process steps with an initial aqueous solution of the heparin to be purified (or of the heparin-mineral salt mixture) which contains water-soluble mineral salts other then oxalates in

a concentration sufficient to favour a separation of the oxalates, including the apparently initially fixed or adsorbed oxalates which are then freed and remain in the aqueous solution upon the attendant precipitation of the heparin salts.

Thus some of the other salts present in the heparin solution to be purified may even, upon proper adjustment of their own concentration in the solution, whenever appropriate, participate to the more complete extraction of the oxalate ions.

Thus in preferred embodiments of the process according to the invention it will be usually required to preliminary adjust the concentration of said water-soluble mineral salts in the initial aqueous solution of heparin, prior to contacting the latter with the abovesaid non-ionic heparin precipitating agent.

As matter of fact, it has been found that a sufficient concentration, particularly of salts comprising divalent anions and preferably too, monovalent anions, have a behaviour as if they were causing the displacement from the oxalates possibly adsorbed on or fixed to the heparin.

If need be, it will then be appropriate to repeat the precipitation steps upon recontacting a solution of the heparin so recovered and of water-soluble mineral salts other then oxalates in an adjusted concentration as hereabove defined until the concentration of total oxalates in the final heparin is less than 70 ppm, particularly from 30 to 70 ppm.

Preferably, the metal (or metals) of the salts having divalent anions, other than oxalates, which are contained in or possibly added to the initial heparin solution, is (or are) selected among those containing metals the oxalates of which are water-soluble. Carbonates, particularly sodium carbonate, have been found most effective in the process according the the invention.

Preferably too the heparin in the abovesaid solution is in the form of a heparin salt of the same metal as that of the mineral salts. Any possibility of exchange of the metal contained in the latter mineral salt for the metal of the heparin salt is then avoided whereby the metal contents of the purified heparin salts is kept under close control.

Advantageously the concentration of the said mineral salts in the solution to be contacted with the non ionic precipitating agents such as alcohol, is adjusted to a value from 0.3 to 2.5, for instance of about 0.5% in weight with respect to heparin.

Advantageously too the starting solution also contains salts having monovalent anions of at least one metal, the oxalate of which is water-soluble. Sodium chloride is representative of such salts. In a preferred embodiment of the process according to the invention, the concentration of said monovalent salt has or is adjusted to a value ranging from 1 to 7%, for instance of about 2.5% in weight/volume of solution.

It has been found advantageous, though not necessary, that the pH of the solution contacted with the non-ionic precipitating agent be comprised between 7 and 10, for instance of the order of 8.5. This is actually the pH which establishes spontaneously when the salt having divalent anions used is sodium carbonate.

These salts having divalent and/or monovalent anions can then be easily eliminated in the final stage of the process, for instance in a final contacting step of the heparin solution with the non-ionic agent. Advantageously the pH is then adjusted for instance with hydrochloric acid, to a slightly acid value, sufficient for destroying the carbonates, particularly at a pH ranging from 3 to 7. The chloride ions remain in the aqueous solution, when the final heparin salt is precipitated.

Heparin compositions, particularly heparin salts are thus obtained which are substantially oxalate-free, which are

—either directly suitable for the preparation of pharmaceutical compositions, particularly injectable or perfusable solutions having long storage-life, even when metallic cations of said heparin salts are formed at least in part of metals, the oxalates of which are highly insoluble, like calcium,

—or suitable as starting heparin salts from which the metallic cations contained therein can be substituted at least in part advantageously, though not necessarily, according to the process of British patent 1,471,482 already mentioned hereabove, to provide other substantially oxalate-free heparin salts, which are then formed into pharmaceutical compositions having long storage life.

The invention thus concerns more particularly among the oxalate-free heparin preparations, the metallic salts of heparin, i.e. either simple salts of heparin, such as the sodium, potassium, calcium or magnesium salts of heparin, or mixed salts of heparin containing at least two of the abovesaid metallic cations in any relative proportions, all of these heparin salts being substantially free of oxalates, in that they contain less than a threshold of 70 ppm in a number of cases, particularly from about 30 to about 70 ppm (hereafter termed as oxalate-free heparins). Among the mixed salts one may cite the preferred series of those which contain sodium and calcium.

The invention also concerns pharmaceutical preparations in which the oxalate-free heparin is associated with a pharmaceutical vehicle, more particularly the oxalate-free, preferably colorless heparin preparations having an activity of at least 120, preferably above 150 International Units (IU)/mg, free of pyrogens, as well as the highly concentrated solutions of heparin useful for their application in therapy for the control of blood-coagulation, particularly oxalate-free, preferably colorless, solutions suitable for subcutaneous injection containing from 5000 to 35 000 IU/ml of heparin, preferably from 20 000 to 30 000, such as 25 000IU/ml, or oxalate-free solutions suitable for intra-venous injection, containing from 1 000 to 10 000, for instance 5 000 IU/ml of heparin, etc.

Advantageously the heparin of said preparation

is in the form of a physiologically acceptable metal salt of heparin containing one or several metal cations. Preferably the metal is at least in part calcium. Advantageusly calcium is the only metal of said heparin salt. The pharmaceutical preparations may be advantageously presented in dispensable syringes, ready for use at the appropriate time.

The invention also concerns a method for controlling blood coagulation in man, which method comprises administering to him an effective dose of the oxalate-free heparin of the invention, such as from 13 500 to 50 000 i.u. by the subcutaneous route, twice a day, as required by the thrombotic state of the patient, or from 20 000 to 50 000 i.u. per 24 hours by the intravenous route, evenly distributed over the day, advantageously by adjusted perfusions, of from 5 000 to 10 000 i.u., three times a week, by the intra-muscular route.

The purified heparin according to the invention may be used directly to prepare, e.g. injectable solutions under usual dosages and concentration conditions. Possibly, it may also be used as starting material for preparing other salts, such as calcium heparinates or mixed calcium-magnesium, calcium-sodium salts, for instance according to the process described in British patent No. 1 471 482.

First example of a preferred purification procedure of heparin.

In a preferred alcoholic fractionating purification process of injectable sodium heparinate, sodium heparin of bovine or porcine origin, of injectable quality is used. This heparinate is dissolved in demineralised water (having a resistivity from 300 000 to 800 000 Ω/cm, preferably 500 000 Ω/cm.

The concentration of the heparinate solution is settled between 5 000 and 30 000 IU/ml, preferably 25 000 IU/ml 0.3% metacresol is added in order to prevent from any contamination. The pH of the solution is maintained between 5 and 7, preferably 6.5, by adding either a reagent grade 5 N sodic solution, or a reagent grade 5 N hydrochloric acid solution. The solution conductivity remains between 7 000 and 15 000 μ Mhos-cm.

0.7 volume of neutral ethylic alcohol (99 to 100° GL) is added to the heparinate solution thus prepared. One leaves the precipitate to settle and possible adds a small amount of sodium chloride if necessary.

The precipitate is separated from the supernatant. It is once more solubilized in demineralised water, having the above described characteristics, so as to obtain a concentration roundabout 12 500 IU/ml. The pH is controlled to 5.5 if need be, having recourse to a NaOH 5 N or HCL 5 N solution. The solution is filtrated on a Millipore 0.3 μ filter. 1.2 volume of neutral ethylic alcohol (99—100° GL) for 1 volume of solution is added under stirring. One leaves the precipitate to settle, possible with addition of a small amount of sodium chloride.

The so obtained heparin precipitate is dehydrated by crushing in absolute ethylic alcohol, then filtrated under vacuum upon industrial Büchner, washed with absolute alcohol and dried under 1 torr vacuum at a temperature of 35/40°C.

When further precipitations are needed, they are carried out in the same way as for the second precipitation step; i.e. heparin concentration of 12 500 IU/ml, or even 25 000 IU/ml, pH 5.5, and 1.2 volume of absolute alcohol per 1 volume solution.

The heparin preparations obtained may then be converted into the corresponding calcium salts of heparin according to the process described in British patent 1.471.482.

Different batches of calcium salt of heparin containing from about 40 to about 60 ppm of oxalates and aqueous solutions thereof containing 25 000 IU/ml were so prepared. After storage for over more than one year neither any turbidity nor the formation of one or more crystals having sizes from 2 to 40 microns were observed.

The dosage of the oxalate ions in the purified heparins were effected after their extractions by the method of J.R.Helbert and M.A. Marini, Biochem.J. (1963) 2 (5) pp. 1101—6, however modified in that the oxalate ions present in said heparins were extracted therefrom in the presence of an excess sodium carbonate, prior to being adsorbed on a IRA 400 anionic resin, the use of which has been recommended by the authors. After their elution from the resin, the oxalate ions were dosed according to the fluorometric method of P. M. Zarembski and A. Hodgkinson, [Biochem. J. (1965) 96, 717—721].

Claims

1. Heparin containing from 30 to 70 ppm oxalates.

2. Heparin of claim 1 containing less than 0.5% mineral salts.

3. Heparin of claim 2 containing less than 0.3% mineral salts.

4. Heparin of claim 3 in the form of a metal salt of heparin, at least part of the metal being calcium.

5. Heparing of claim 4 wherein the metal is substantially all calcium.

6. Heparin of claim 2 having an activity of at least 120 IU/mg, preferably above 150 IU/mg.

7. A pharmaceutical composition containing a heparin according to anyone of claims 1 to 6, associated with a pharmaceutical vehicle.

8. A pharmaceutical composition according to claim 7 which is in the form of an injectable solution containing at least about 1000 IU/ml of heparin.

9. The injectable solution of claim 8 containing

from about 5,000 to about 35,000 IU/ml. of heparin.

10. The pharmaceutical solution according to anyone of claims 8 to 10 which is colorless.

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