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HEPARIN-DERIVED POLYSACCHARIDE MIXTURES, PREPARATION
METHOD AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAME

The present invention relates to mixtures of
5 polysaccharides derived from heparin, their method of
preparation and the pharmaceutical compositions
containing them.

Heparin is a mixture of sulphated mucopoly-
saccharides of animal origin which is used in
10 particular for its anticoagulant and antithrombotic
properties.

Heparin nevertheless has disadvantages which
limit the conditions for its use. In particular, its
high anticoagulant activity (anti-IIa activity) can
15 cause haemorrhages.

Low-molecular-weight heparins obtained by
basic depolymerization of heparin esters have been
proposed (EP40144); however, these products still have
a high anti-IIa activity.

20 The invention relates to mixtures of
polysaccharides derived from heparin possessing a more
selective activity towards activated factor X (factor
Xa) and activated factor II (factor IIa) than heparin.

The subject of the present invention is more
25 particularly the mixtures of sulphated polysaccharides
possessing the general structure of the constituent



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polysaccharides of heparin and exhibiting the following characteristics:

- they have a mean molecular weight of 1500 to 3000 daltons, an anti-Xa activity of 100 to 150 IU/mg, an anti-IIa activity of 0 to 10 IU/mg and an anti-Xa activity/anti-IIa activity ratio greater than 10,

- the constituent polysaccharides of the mixtures contain 2 to 26 saccharide units and have a 4,5-unsaturated glucuronic acid 2-O-sulphate unit at one of their ends, in the form of an alkali or alkaline-earth metal salt.

As alkali or alkaline-earth metal salt, the sodium, potassium, calcium and magnesium salts are preferred.

The mean molecular weight is determined by high-pressure liquid chromatography using two columns in series, for example those marketed under the name TSK G3000 XL and TSK G2000 XL. The detection is carried out by refractometry. The eluent used is lithium nitrate and the flow rate is 0.6 ml/min. The system is calibrated with standards prepared by fractionation of enoxaparin (AVENTIS) by chromatography on agarose-polyacrylamide gel (IBF). This preparation is carried out according to the technique described by Barrowcliffe et al., Thromb. Res., 12, 27-36 (1977-78) or D.A. Lane et al., Thromb. Res., 12, 257-271

(1977-78). The results are calculated with the GPC6 software (Perkin Elmer).

The anti-Xa activity is measured by the amidolytic method on a chromogenic substrate described
5 by Teien et al., Thromb. Res., 10, 399-410 (1977), with, as standard, the first international standard for low-molecular weight heparins.

The anti-IIa activity is measured by the technique described by Anderson L.O. et al., Thromb.
10 Res., 15, 531-541 (1979), with, as standard, the first international standard for low-molecular weight heparins.

Preferably, the mixtures as described above exhibit an anti-Xa activity of between 125 and
15 150 IU/mg.

Most particularly, the mixtures as described above exhibit an anti-Xa activity of between 140 and 150 IU/mg and have a mean molecular weight of between 2000 and 3000 daltons.

20 Preferably, the mixtures according to the invention have an anti-IIa activity of 0 to 5 IU/mg.

Still more preferably, the mixtures have an anti-Xa activity/anti-IIa activity ratio greater than 25.

25 The mixtures of oligosaccharides according to the invention may be prepared by depolymerization of a quaternary ammonium salt of the benzyl ester of heparin in organic medium, by means of a strong organic base

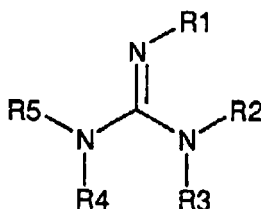
with a pka greater than 20 or of sodium imidazolate,
 conversion of the quaternary ammonium salt of the
 benzyl ester of the depolymerized heparin to a sodium
 salt, saponification of the ester and optionally
 5 purification.

The quaternary ammonium salt of the benzyl
 ester of heparin is preferably the benzethonium,
 cetylpyridinium or cetyltrimethylammonium salt.

The depolymerization is generally carried out
 10 in an inert organic solvent such as a chlorinated
 solvent (for example dichloromethane), tetrahydrofuran,
 anisole, at a temperature of -20°C to 40°C.

As strong organic base with a pka greater
 than 20, it is possible to use 1,5,7-triaza-
 15 bicyclo[4.4.0]dec-5-ene, 2-tert-butylimino-2-
 diethylamino-1,3-dimethylperhydro-1,3,2-diaza-
 phosphorine, the bases of the family of guanidine and
 phosphazenes.

The bases of the guanidine family are
 20 preferably those of formula:



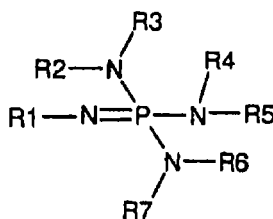
in which R₁ is hydrogen or alkyl, R₂, R₃, R₄ and R₅,
 which are identical or different, each represent an
 25 alkyl radical.

More particularly, R_1 is hydrogen and R_2 , R_3 , R_4 and R_5 are methyl radicals.

The strong organic bases related to the phosphazene family are defined for example according to
 5 R. Schwesinger et al., Angew. Chem. Int. Ed. Engl. 26, 1167-1169 (1987), R. Schwesinger et al., Angew. Chem. 105, 1420 (1993).

Among the bases of the phosphazene family, those of formula:

10



in which the radicals R_1 to R_7 are identical or different and represent alkyl radicals are preferably used.

15 In the preceding formulae, the alkyl radicals contain 1 to 6 carbon atoms in the form of a straight or branched chain.

Advantageously, the strong organic base with a pK_a greater than 20 or sodium imidazolate/quaternary
 20 ammonium salt of the benzyl ester of heparin mol ratio is between 0.2 and 5 and preferably between 1 and 4.

More particularly, the degree of esterification of the quaternary ammonium salt of the benzyl ester of heparin is between 50 and 100% and
 25 preferably between 70 and 90%. This degree of

esterification corresponds to the molar percentage of esterification of the uronic acids of the heparin.

The conversion of the quaternary ammonium salt of the benzyl ester of the depolymerized heparin to a sodium salt is generally carried out by treating the reaction medium with an alcoholic solution of sodium acetate and preferably with a 10% solution of sodium acetate in methanol (weight/volume), at a temperature of between 15 and 25°C. The equivalent by weight of acetate added is preferably 3 times greater than the mass of quaternary ammonium salt of the benzyl ester of heparin used in the depolymerization reaction.

The saponification is generally carried out by means of an alkali metal hydroxide such as sodium hydroxide, potassium hydroxide or lithium hydroxide, in an aqueous medium, at a temperature of between 0 and 20°C and preferably between 0 and 10°C. 1 to 5 molar equivalents of alkali metal hydroxide will be generally used. Preferably, the saponification will be carried out in the presence of 2 to 3 molar equivalents of alkali metal hydroxide.

The final product may be optionally purified by any known method of purifying depolymerized heparins and in particular according to the method described in patent EP 0037319. Preferably, the purification is carried out by means of hydrogen peroxide, in an aqueous medium, at a temperature of 10 to 50°C.

Preferably, this operation is carried out between 20 and 40°C.

The quaternary ammonium salt of the benzyl ester of heparin may be prepared according to the following reaction scheme:

a) conversion of the heparin in the form of a sodium salt by means of benzethonium chloride in order to obtain benzethonium heparinate,

b) esterification of the benzethonium salt obtained above by means of benzyl chloride and treatment with an alcoholic solution of sodium acetate in order to obtain the sodium salt of the benzyl ester of heparin,

c) transalification of the sodium salt of the benzyl ester of heparin to a quaternary ammonium salt and preferably to a benzethonium, cetylpyridinium or cetyltrimethylammonium salt.

The reaction of step a) is carried out in an aqueous medium, by the action of benzethonium chloride in excess, on heparin in the form of a sodium salt, at a temperature in the region of 15 to 25°C.

Advantageously, the benzethonium chloride/heparin in the form of a sodium salt molar ratio is between 2 and 3 and more particularly 2.5.

The starting heparin in the form of a sodium salt used is preferably a pig heparin. The latter may be purified beforehand in order to reduce its dermatan

sulphate level according to the method described in patent FR2663639.

The esterification of step b) is preferably carried out in a chlorinated organic solvent (for
5 example chloroform or methylene chloride), at a temperature of between 25 and 45°C and preferably between 30 and 40°C. The ester in the form of a sodium salt is then recovered by precipitation by means of sodium acetate at 10% by weight/volume in an alcohol
10 such as methanol. 1 to 1.2 volumes of alcohol are generally used per volume of reaction medium. The quantity of benzyl chloride and the reaction time are adjusted in order to obtain a degree of esterification of between 50 and 100% and preferably between 70 and
15 90%. Preferably, 0.5 to 1.5 parts by weight of benzyl chloride are used for 1 part by weight of benzethonium salt of heparin. Likewise, preferably the reaction time will be between 10 and 35 hours.

The transalification step c) is carried out
20 by means of a quaternary ammonium chloride and preferably by means of benzethonium chloride, cetylpyridinium chloride or cetyltrimethylammonium chloride, in an aqueous medium, at a temperature of between 10 and 25°C. Advantageously, the quaternary
25 ammonium chloride/sodium salt of the benzyl ester of heparin mol ratio is between 2 and 3.

The mixtures according to the invention, in the form of a sodium salt, may be converted to another

salt of an alkali or alkaline-earth metal salt. The passage from one salt to another is optionally achieved using the method described in patent US4168377.

The mixtures according to the invention are
5 not toxic and may thus be used as medicaments.

The mixtures of the present invention may be used as antithrombotic agents. In particular, they are useful for the prevention of venous thromboses, arterial thrombotic accidents, in particular in the
10 case of myocardial infarction. They are also useful in the prevention and treatment of the proliferation of the smooth muscle cells, angiogenesis, and as neuroprotective agents for atherosclerosis and for arteriosclerosis.

15 The present invention also relates to the pharmaceutical compositions containing, as active ingredient, a mixture of polysaccharides according to the invention, optionally in combination with one or more inert excipients.

20 The pharmaceutical compositions are for example solutions which can be injected by the subcutaneous or intravenous route. They are also useful by the pulmonary route (inhalation).

The dosage may vary according to the age,
25 weight and state of health of the patient. For an adult, it is in general between 20 and 100 mg per day by the intramuscular or subcutaneous route.

The following examples illustrate the invention.

EXAMPLE A: PREPARATION OF THE BENZETHONIUM SALT OF THE BENZYL ESTER OF HEPARIN

5 Benzethonium heparinate

A solution of 25 g of benzethonium chloride in 125 ml of water is added to a solution of 10 g of heparin in the form of a sodium salt in 100 ml of water at a temperature in the region of 20°C. The product is
10 filtered, washed with water and dried.

Benzyl ester of heparin (sodium salt)

16 ml of benzyl chloride are added to a solution of 20 g of benzethonium heparinate in 80 ml of methylene chloride. The solution is heated at a
15 temperature of 30°C for 12 hours. 108 ml of a 10% solution of sodium acetate in methanol are then added, the mixture is filtered, washed with methanol and dried. 7.6 g of benzyl ester of heparin are thus obtained in the form of a sodium salt whose degree of
20 esterification is 77%.

Benzyl ester of heparin (benzethonium salt)

36 g (0.0549 mol) of benzyl ester of heparin (sodium salt) and 540 ml of distilled water are introduced into a 2-litre Erlenmeyer flask A. After
25 homogenization at a temperature of about 20°C, a pale yellow solution is obtained. A solution of 64.45 g (0.1438 mol) of benzethonium chloride and 450 ml of water is prepared, with magnetic stirring, in a 1-litre

Erlenmeyer flask B. The solution in Erlenmeyer B is poured over about 35 minutes into the solution in Erlenmeyer A, with stirring. The formation of an abundant white precipitate is observed. The Erlenmeyer B is rinsed with 200 ml of distilled water and the wash water is introduced into the Erlenmeyer A. The stirring is then stopped and the suspension is allowed to settle for 12 hours. The clear portion of the supernatant is removed and discarded. 560 ml of water are added to the sedimented precipitate (slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 30 minutes. The supernatant is removed and discarded (560 ml). This operation of washing with about 560 ml of distilled water is repeated twice on the sedimented precipitate. In the last washing operation, the precipitate is left in suspension and filtered on Sintered Glass 3. The cake is then washed with 4 times 200 ml of distilled water. The wet white solid is drained and then dried under reduced pressure (2.7 kPa) at a temperature in the region of 60°C. After drying for 12 hours, 87.5 g of benzyl ester of heparin, benzethonium salt, are obtained. The yield obtained is 94.9%.

EXAMPLE 1

Depolymerization and conversion to a sodium salt:

28 ml of dichloromethane are introduced into a 50 ml Erlenmeyer flask A. 4 g (0.00238 mol) of benzyl ester of heparin (degree of esterification: 77%,

benzethonium salt) obtained as described in Example A are slowly loaded, with stirring. After complete dissolution, 1.32 g (0.00948 mol) of 1,5,7-triazabicyclo[4.4.0]dec-5-ene are added. The mixture is stirred at a temperature in the region of 20°C for 3 hours and 30 minutes. During this time, a solution of 12 g of sodium acetate is prepared at 4°C in an Erlenmeyer flask B in 120 ml of methanol. The reaction mixture in Erlenmeyer flask A is poured into the methanolic solution of sodium acetate, with magnetic stirring. A practically translucent gelatinous yellow precipitate appears. The stirring is then stopped and the suspension is allowed to separate by settling for one hour. The clear portion of the supernatant is removed and discarded (62 ml). 50 ml of methanol are added to the sedimented precipitate (yellow slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 30 minutes. The supernatant is removed and discarded (49 ml). 50 ml of methanol are added to the sedimented precipitate and the mixture is stirred for 20 minutes. The precipitate in suspension is then filtered on Sintered Glass 4. The golden yellow cake obtained is then washed with twice 25 ml of methanol. The wet solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 1.21 g of

depolymerized heparin are obtained (benzyl ester, sodium salt). The yield obtained is 77.2%.

Saponification:

1.21 g (0.0018 mol) of the depolymerized
5 heparin (benzyl ester, sodium salt) obtained above and
11 ml of water are introduced into a 25 ml Erlenmeyer
flask. 0.18 ml (0.0018 mol) of 30% caustic soda is
introduced, with magnetic stirring. After addition, the
mixture is cooled to 4°C and stirred for 2 hours.
10 1.43 g of NaCl are added and the solution is
neutralized by addition of HCl at 1 mol/l (14 ml). The
mixture is transferred to a 100 ml Erlenmeyer flask and
52 ml of methanol are added. The formation of a yellow
precipitate is obtained. The stirring is then stopped
15 and the suspension is allowed to sediment for 12 hours
at a temperature in the region of 20°C. The supernatant
is then removed and then discarded (44 ml). 25 ml of
methanol are added to the sedimented precipitate
(yellow slurry appearance) and the mixture is stirred
20 for 20 minutes. The precipitate is allowed to
resediment for about 30 minutes. The supernatant is
removed and discarded (21 ml). 25 ml of methanol are
added to the sedimented precipitate and the mixture is
stirred for 20 minutes. The precipitate in suspension
25 is then filtered on Sintered Glass 3. The light yellow
cake obtained is then washed with twice 10 ml of
methanol. The wet solid is drained and then dried under
reduced pressure (2.7 kPa), at a temperature in the

region of 60°C. After drying for 12 hours, 0.66 g of crude depolymerized heparin is obtained (sodium salt). The yield obtained is 60%.

Purification:

5 0.66 g of crude depolymerized heparin
obtained above and 5.9 ml of distilled water are
introduced into a 10 ml Erlenmeyer flask. The mixture
is heated to 40°C, with magnetic stirring. The pH is
brought to between 9 and 10 by addition of sodium
10 hydroxide at 0.1 mol/l and 33 microlitres of an aqueous
solution of hydrogen peroxide at 30% are added. After
stirring for about 2 hours, 0.65 g of sodium chloride
is added. The mixture is then neutralized by addition
of HCl at 0.1 ml/l. The solution is then filtered and
15 transferred to a 25 ml Erlenmeyer flask. 23.3 ml of
methanol are poured in. The formation of a white
precipitate is observed. The stirring is then stopped
and the suspension is allowed to sediment for 12 hours
at a temperature in the region of 20°C. The supernatant
20 is then removed and then discarded (5 ml). 5 ml of
methanol are added to the sedimented precipitate
(slurry appearance) and the mixture is stirred for 20
minutes. The precipitate is allowed to resediment for
about 30 minutes. The supernatant is removed and
25 discarded (5 ml). 5 ml of methanol are added to the
sedimented precipitate and the mixture is stirred for
20 minutes. The precipitate in suspension is then
filtered on Sintered Glass 3. The white cake obtained

is then washed with twice 5 ml of methanol. The wet solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 0.51 g of a purified mixture of polysaccharides (sodium salt) is obtained. The yield obtained is 77.2%.

The characteristics of this mixture are the following:

Mean molecular weight: 1600 daltons

10 Anti-Xa activity: 94 IU/mg

Anti-IIa activity: < 0.1 IU/mg

Anti-Xa activity/anti-IIa activity ratio: > 100

EXAMPLE 2

Depolymerization and conversion to a sodium salt:

15 70 ml of dichloromethane are introduced into a 100 ml Erlenmeyer flask A. 10 g (0.00595 mol) of benzyl ester of heparin (degree of esterification: 77%, benzethonium salt) obtained as described in Example A are slowly loaded, with stirring. After complete
20 dissolution, 1.7 ml (0.00587 mol) of 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine are added. The reaction is allowed to continue for about 3 hours and 30 minutes at a temperature in the region of 20°C. During this time, a
25 solution of 30 g of sodium acetate in 300 ml of methanol is prepared at 4°C in an Erlenmeyer flask B. The reaction mixture in Erlenmeyer A is poured into the methanolic solution of sodium acetate, with magnetic

stirring. A practically translucent gelatinous yellow precipitate appears. The stirring is then stopped and the suspension is allowed to separate by settling for one hour. The clear portion of the supernatant is
5 removed and discarded (204 ml). 125 ml of methanol are added to the sedimented precipitate (yellow slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 30 minutes. The supernatant is then removed and discarded
10 (162 ml). 125 ml of methanol are added to the sedimented precipitate and the mixture is stirred for 20 minutes. The gelatinous precipitate in suspension is then filtered on Sintered Glass 3. The yellow gelatinous cake obtained is then washed with 2 portions
15 of 63 ml of methanol. The gelatinous solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 3.34 g of depolymerized heparin (benzyl ester, sodium salt) are obtained. The yield obtained is 85.3%.

20 Saponification:

1.67 g of depolymerized heparin (benzyl ester, sodium salt) obtained above are saponified according to the saponification method described in Example 1. 0.94 g of a light yellow powder is obtained.
25 The yield of crude depolymerized heparin (sodium salt) is 61%.

Purification:

0.94 g of crude depolymerized heparin (sodium salt) obtained above is purified according to the method of purification described in Example 1. 0.71 g of a white powder is obtained. The yield is 75.5%.

5 The purified mixture of polysaccharides (sodium salt) obtained has the following characteristics:

Mean molecular weight: 2500 daltons

Anti-Xa activity: 146.6 IU/mg

10 Anti-IIa activity: 2.15 IU/mg

Anti-Xa activity/anti-IIa activity ratio: 68

EXAMPLE 3

Depolymerization and conversion to a sodium salt:

28 ml of dichloromethane are introduced into
15 a 50 ml Erlenmeyer flask A. 4 g (0.00238 mol) of benzyl ester of heparin (degree of esterification: 77%, benzethonium salt) obtained according to Example A are slowly loaded, with stirring. After complete dissolution and cooling to 2°C, 0.333 g (0.00239 mol)
20 of 1,5,7-triazabicyclo[4.4.0]dec-5-ene is added. The reaction is allowed to continue for about 3 hours and 30 minutes at a temperature in the region of 20°C. During this time, a solution of 12 g of sodium acetate in 120 ml of methanol is prepared at 4°C in an
25 Erlenmeyer flask B. The reaction mixture in Erlenmeyer A is poured into the methanolic solution of sodium acetate, with magnetic stirring. A yellow precipitate appears. The stirring is then stopped and the

suspension is allowed to separate by settling for one hour. The clear portion of the supernatant is removed and discarded (90 ml). 50 ml of methanol are added to the sedimented precipitate (yellow slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 30 minutes. The supernatant is removed and discarded (61 ml). 50 ml of methanol are added to the sedimented precipitate and the mixture is stirred for 20 minutes. The precipitate in suspension is then filtered on Sintered Glass 4. The cake obtained is then washed with twice 25 ml of methanol. The wet solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 1.19 g of depolymerized heparin (benzyl ester, sodium salt) are obtained. The solid is dark yellow. The yield obtained is 75.9%.

Saponification:

1.19 g of depolymerized heparin (benzyl ester, sodium salt) obtained above are saponified according to the saponification method described in Example 1. 0.78 g of a light yellow powder is obtained. The yield of crude depolymerized heparin (sodium salt) is 71.5%.

Purification:

0.78 g of crude depolymerized heparin (sodium salt) obtained above is purified according to the method of purification described in Example 1. 0.58 g of a white powder is obtained. The yield is 72.5%.

The purified mixture of polysaccharides (sodium salt) obtained have the following characteristics:

Mean molecular weight: 2700 daltons

5 Anti-Xa activity: 100.1 IU/mg

Anti-IIa activity: 3.3 IU/mg

Anti-Xa activity/anti-IIa activity ratio: 27.3

EXAMPLE 4

Depolymerization and conversion to a sodium salt:

10 28 ml of dichloromethane are introduced into
a 50 ml Erlenmeyer flask A. 4 g (0.00238 mol) of benzyl
ester of heparin (degree of esterification: 77%,
benzethonium salt) obtained as described in Example A
are slowly loaded, with stirring. After complete
15 dissolution and at 2°C, 0.6 ml (0.00222 mol) of 2-tert-
butylimino-tris(dimethylamino)phosphorane is added. The
reaction is allowed to continue for about 3 hours and
30 minutes at a temperature in the region of 0°C.
During this time, a solution of 12 g of sodium acetate
20 in 120 ml of methanol is prepared at 4°C in an
Erlenmeyer flask B. The reaction mixture in Erlenmeyer
flask A is poured into the methanolic solution of
sodium acetate, with magnetic stirring. A practically
translucent gelatinous yellow precipitate appears. The
25 stirring is then stopped and the suspension is allowed
to separate by settling for one hour. The clear portion
of the supernatant is removed and discarded (108 ml).
50 ml of methanol are added to the sedimented

precipitate (yellowish slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 30 minutes. The supernatant is removed and discarded (60 ml). 50 ml of methanol are added to the sedimented precipitate and the mixture is stirred for 20 minutes. The yellowish white precipitate in suspension is then filtered on Sintered Glass 4. The cake obtained is then washed with twice 25 ml of methanol. The solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 1.22 g of depolymerized heparin (benzyl ester, sodium salt) are obtained. The yield obtained is 77.8%.

Saponification:

1.22 g of depolymerized heparin (benzyl ester, sodium salt) obtained above are saponified according to the saponification protocol described in Example 1. 0.69 g of a very light yellow powder is obtained. The yield of crude depolymerized heparin (sodium salt) is 61.6%.

Purification:

0.69 g of crude depolymerized heparin (sodium salt) obtained above is purified according to the purification protocol described in Example 1. 0.67 g of a white powder is obtained. The yield is 97.1%.

The purified mixture of polysaccharides (sodium salt) obtained has the following characteristics:

Mean molecular weight: 2900 daltons

Anti-Xa activity: 145.2 IU/mg

Anti-IIa activity: 4.5 IU/mg

Anti-Xa activity/anti-IIa activity ratio: 32.6

5 EXAMPLE 5

Depolymerization and conversion to a sodium salt:

28 ml of dichloromethane are introduced into a 50 ml round-bottomed flask A. 4 g (0.00238 mol) of benzyl heparinate (degree of esterification: 77%, benzethonium salt) are slowly loaded, with stirring. After complete dissolution and at 40°C, 0.95 g (0.00949 mol) of sodium imidazolate is added. The reaction is allowed to continue for about 4 hours at the reflux temperature of dichloromethane. During this time, a solution of 12 g of sodium acetate in 120 ml of methanol is prepared at 4°C in an Erlenmeyer flask B. The reaction mixture in Erlenmeyer flask A is poured into the methanolic solution of sodium acetate, with magnetic stirring. A practically translucent gelatinous yellow precipitate appears. The stirring is then stopped and the suspension is allowed to separate by settling for one hour. The clear portion of the orange-coloured supernatant is removed and discarded (88 ml). 50 ml of methanol are added to the sedimented precipitate (orange-coloured slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 30 minutes. The supernatant is removed and discarded (51 ml). 50 ml of

methanol are added to the sedimented precipitate and the mixture is stirred for 20 minutes. The orange-coloured precipitate in suspension is then filtered on Sintered Glass 4. The cake obtained is then washed with
5 twice 25 ml of methanol. The solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 1.34 g of depolymerized heparin (benzyl ester, sodium salt) are obtained. The yield obtained is 76.6%.

10 Saponification:

1.2 g of depolymerized heparin (benzyl ester, sodium salt) obtained above are saponified according to the saponification protocol described in Example 1. 0.63 g of a beige powder is obtained. The yield of
15 crude depolymerized heparin (sodium salt) is 52.5%.

Purification:

0.63 g of crude depolymerized heparin (sodium salt) obtained above is purified according to the purification method described in Example 1. 0.42 g of a
20 beige-white powder is obtained. The yield is 66.7%.

The purified mixture of polysaccharides (sodium salt) obtained has the following characteristics:

Mean molecular weight: 2250 daltons

25 Anti-Xa activity: 134.5 IU/mg

Anti-IIa activity: 1.5 IU/mg

Anti-Xa activity/anti-IIa activity ratio: 90.5

EXAMPLE 6

Depolymerization and conversion to a sodium salt:

28 ml of dichloromethane are introduced into a 50 ml Erlenmeyer flask A. 4 g (0.00238 mol) of benzyl ester of heparin (degree of esterification: 77%, benzethonium salt) obtained as described in Example A are slowly loaded, with stirring. After complete dissolution, 1.33 g (0.00956 mol) of 1,5,7-triazabicyclo[4.4.0]dec-5-ene are added. The mixture is stirred at a temperature in the region of 20°C for 3 hours and 30 minutes. During this time, a solution of 12 g of sodium acetate is prepared at 4°C in an Erlenmeyer flask B in 120 ml of methanol. The reaction mixture in Erlenmeyer flask A is poured into the methanolic solution of sodium acetate, with magnetic stirring. A practically translucent gelatinous yellow precipitate appears. The stirring is then stopped and the suspension is allowed to separate by settling for one hour. The clear portion of the supernatant is removed and discarded (56 ml). 60 ml of methanol are added to the sedimented precipitate (yellow slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 30 minutes. The supernatant is removed and discarded (70 ml). 50 ml of methanol are added to the sedimented precipitate and the mixture is stirred for 15 minutes. The precipitate in suspension is then filtered on Sintered Glass 4. The golden yellow cake obtained is then washed with twice 50 ml of

methanol. The wet solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 0.92 g of depolymerized heparin are obtained (benzyl ester, sodium salt). The yield obtained is 64%.

Saponification:

0.92 g (0.0014 mol) of the depolymerized heparin (benzyl ester, sodium salt) obtained above and 17.5 ml of water are introduced into a 25 ml Erlenmeyer flask. 0.38 ml (0.00379 mol) of 30% caustic soda is introduced, with magnetic stirring. After addition, the mixture is kept at a temperature in the region of 20°C and stirred for 5 hours. 1.8 g of NaCl are added and the solution is neutralized by addition of concentrated HCl (final volume of the solution about 18 ml). The mixture is transferred to a 100 ml Erlenmeyer flask and 46 ml of methanol are added. The formation of a yellow precipitate is observed. The stirring is then stopped and the suspension is allowed to sediment for 12 hours at a temperature in the region of 20°C. The supernatant is then removed and then discarded (52 ml). 25 ml of methanol are added to the sedimented precipitate (yellow slurry appearance) and the mixture is stirred for 20 minutes. The precipitate is allowed to resediment for about 1 hour. The supernatant is removed and discarded (27 ml). 25 ml of methanol are added to the sedimented precipitate and the mixture is stirred for about 1 hour. The precipitate in suspension is then

filtered on Sintered Glass 4. The cake obtained is then washed with twice 10 ml of methanol. The wet solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After
5 drying for 3 hours, 0.42 g of crude depolymerized heparin is obtained (sodium salt). The yield obtained is 45.6%.

Purification:

0.42 g of crude depolymerized heparin
10 obtained above and 3.8 ml of distilled water are introduced into a 10 ml Erlenmeyer flask. The mixture is heated to 38°C, with magnetic stirring. The pH is brought to between 9 and 10 by addition of sodium hydroxide at 0.1 mol/l and 25 microlitres of an aqueous
15 solution of hydrogen peroxide at 30% are added. After stirring for about 2 hours 30 minutes, 0.5 g of sodium chloride is added. The mixture is then neutralized by addition of HCl at 0.1 mol/l. The solution is then filtered and transferred to a 25 ml Erlenmeyer flask.
20 11.3 ml of methanol are poured in. The formation of a white precipitate is observed. The stirring is then stopped and the suspension is allowed to sediment for 12 hours at a temperature in the region of 20°C. The supernatant is then removed and then discarded
25 (9.8 ml). 5 ml of methanol are added to the sedimented precipitate (slurry appearance) and the mixture is stirred for 15 minutes. The precipitate is allowed to resediment for about 3 hours. The supernatant is

removed and discarded (6.2 ml). 5 ml of methanol are added to the sedimented precipitate and the mixture is stirred for 20 minutes. The precipitate in suspension is then filtered on Sintered Glass 3. The white cake
5 obtained is then washed with twice 5 ml of methanol. The wet solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for about 2 hours 20 minutes, 0.39 g of pure depolymerized heparin (sodium salt) is
10 obtained. The yield obtained is 92.8%.

The characteristics of the depolymerized heparin thus obtained are the following:

Mean molecular weight: 1950 daltons

Anti-Xa activity: 115.6 IU/mg

15 Anti-IIa activity: < 2 IU/mg

Anti-Xa activity/anti-IIa activity ratio: > 57

EXAMPLE 7

Depolymerization and conversion to a sodium salt:

140 ml of dichloromethane are introduced into
20 a 400 ml reactor A. 20 g (0.0119 mol) of benzyl ester of heparin (degree of esterification: 77%, benzethonium salt) obtained as described in example A are slowly loaded, with stirring. After complete dissolution, the water content of the reaction medium is measured by the
25 Karl Fisher method. The value obtained is 0.1% water. 3.5 ml (0.0121 mol) of 2-tert-butylimino-2-diethylamino-1-3-dimethylperhydro-1,3,2-diazaphosphorine are then added. The reaction is allowed to proceed for

about 24 hours at a temperature in the region of 25°C. During this time, a solution of 30 g of sodium acetate is prepared at 4°C in an Erlenmeyer flask B in 300 ml of methanol. Half of the reaction mixture of reactor A
5 is poured into the methanolic solution of sodium acetate, with magnetic stirring. A practically translucent gelatinous yellow precipitate appears. The stirring is maintained for one hour and the suspension is allowed to separate by settling for about 12 hours
10 at 4°C. The clear portion of the supernatant is removed and discarded (220 ml). 220 ml of methanol are added to the sedimented precipitate (yellow slurry appearance) and the mixture is stirred for 50 minutes. The precipitate is allowed to resediment for about 40
15 minutes. The supernatant is removed and discarded (204 ml). 204 ml of methanol are added to the sedimented precipitate and the mixture is stirred for 40 minutes. The gelatinous precipitate in suspension is then filtered on Sintered Glass 3. The yellow
20 gelatinous cake obtained is then washed with 2 portions of 100 ml of methanol. The gelatinous solid is drained and then dried under reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for about 12 hours, 2.6 g of depolymerized heparin (benzyl ester, sodium salt) are obtained. The yield obtained is
25 70.6% (calculated on the basis of half of the reaction medium treated).

Saponification:

2.6 g of depolymerized heparin (benzyl ester, sodium salt) obtained above are saponified according to the saponification method described in Example 1.

1.48 g of a light yellow powder are obtained. The yield
5 of crude depolymerized heparin (sodium salt) is 62.9%.

Purification:

1.48 g of crude depolymerized heparin obtained above and 15 ml of distilled water are introduced into a 50 ml Erlenmeyer flask. The mixture
10 is heated to 40°C, with magnetic stirring. The pH is brought to between 9 and 10 by addition of sodium hydroxide at 1 mol/l. The solution is filtered on a filter membrane having a porosity of 1 μ m. 76 micro-
litres of a 30% aqueous hydrogen peroxide solution are
15 then added. After stirring for about 2 hours, 1.5 g of sodium chloride are added. The mixture is then neutralized by addition of HCl at 1 mol/l. The solution is filtered on a filter membrane having a porosity of 1 μ m. 38 ml of methanol are poured in. The formation of
20 a white precipitate is observed. The stirring is then stopped and the suspension is allowed to sediment for 1 hour at a temperature in the region of 20°C. The supernatant is then removed and then discarded (37 ml). 37 ml of methanol are added to the precipitate and the
25 mixture is stirred for 45 minutes. The precipitate is allowed to resediment for about 45 minutes. The supernatant is removed and discarded (34 ml). 34 ml of methanol are added to the sedimented precipitate and

the mixture is stirred for 15 minutes. The precipitate in suspension is then filtered on Sintered Glass 3. The white cake obtained is then washed with twice 25 ml of methanol. The wet solid is drained and then dried under
5 reduced pressure (2.7 kPa), at a temperature in the region of 60°C. After drying for 12 hours, 1.29 g of pure depolymerized heparin (sodium salt) are obtained. The yield obtained is 87.2%.

The purified depolymerized heparin (sodium
10 salt) obtained has the following characteristics:

Mean molecular weight: 2250 daltons

Anti-Xa activity: 149.6 IU/mg

Anti-IIa activity: < 0.85 IU/mg

Anti-Xa activity/anti-IIa activity ratio: 176

CLAIMS

1. Mixtures of sulphated polysaccharides possessing the general structure of the constituent polysaccharides of heparin and exhibiting the following characteristics:

- they have a mean molecular weight of 1500 to 3000 daltons, an anti-Xa activity of 100 to 150 IU/mg, an anti-IIa activity of 0 to 10 IU/mg and an anti-Xa activity/anti-IIa activity ratio greater than 10,

- the constituent polysaccharides of the mixtures contain 2 to 26 saccharide units and have a 4,5-unsaturated glucuronic acid 2-O-sulphate unit at one of their ends, in the form of an alkali or alkaline-earth metal salt.

2. Mixtures according to Claim 1, characterized in that the anti-Xa activity is between 125 and 150 IU/mg.

3. Mixtures according to Claim 1 or 2, characterized in that the anti-Xa activity is between 140 and 150 IU/mg and the mean molecular weight is between 2000 and 3000 daltons.

4. Mixtures according to any one of Claims 1 to 3, in the form of the sodium, potassium, calcium or magnesium salt.

5. Mixtures according to any one of Claims 1 to 4, having an anti-IIa activity of 0 to 5 IU/mg.

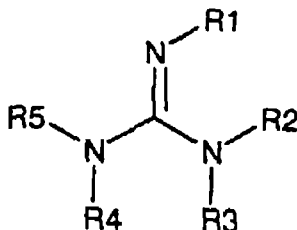
6. Mixtures according to any one of Claims 1 to 5, having an anti-Xa activity/anti-IIa activity ratio greater than 25.

7. Method of preparing the mixtures of Claim 1, characterized in that a quaternary ammonium salt of the benzyl ester of heparin is depolymerized in an organic medium by means of a strong organic base with a pka greater than 20 or of sodium imidazolate, the quaternary ammonium salt of the benzyl ester of the depolymerized heparin is converted to a sodium salt, the ester is saponified and the product is optionally purified.

8. Method according to Claim 7, for which the quaternary ammonium salt of the benzyl ester of heparin is the benzethonium, cetylpyridinium or cetyltrimethylammonium salt.

9. Method according to Claim 7, for which the strong organic base with a pka greater than 20 is chosen from 1,5,7-triazabicyclo[4.4.0]dec-5-ene, 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine, the bases of the family of guanidine and phosphazenes.

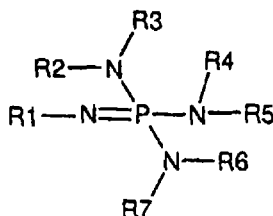
10. Method according to Claim 9, for which the bases of the guanidine family are those of formula:



in which R_1 is hydrogen or alkyl, R_2 , R_3 , R_4 and R_5 , which are identical or different, each represent an alkyl radical, the alkyl radicals having 1 to 6 carbon atoms in the form of a straight or branched chain.

11. Method according to Claim 10, for which R_1 is hydrogen and R_2 , R_3 , R_4 and R_5 are methyl radicals.

12. Method according to Claim 9, for which the bases of the phosphazene family are those of formula:



in which the radicals R_1 to R_7 are identical or different and represent alkyl radicals containing 1 to 6 carbon atoms in the form of a straight or branched chain.

13. Method according to one of Claims 7 to 12, for which the strong organic base with a pK_a greater than 20 or sodium imidazolate/quaternary

ammonium salt of the benzyl ester of heparin mol ratio is between 0.2 and 5.

14. Method according to one of Claims 7 to 13, for which the quaternary ammonium salt of the benzyl ester of heparin has a degree of esterification of between 50 and 100%.

15. Method according to one of Claims 7 to 14, for which the conversion of the quaternary ammonium salt of the benzyl ester of the depolymerized heparin to a sodium salt is carried out by treating the reaction medium with an alcoholic solution of sodium acetate.

16. Method according to one of Claims 7 to 15, for which the saponification is carried out by means of an alkali metal hydroxide.

17. Method according to one of Claims 7 to 16, for which the purification is carried out by means of hydrogen peroxide.

18. Mixtures as defined in any one of Claims 1 to 6, as medicaments.

19. Mixtures as defined in any one of Claims 1 to 6, as antithrombotic medicaments.

20. Pharmaceutical compositions comprising, as active ingredient, a mixture according to one of Claims 1 to 6.