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(54) Title: FORMULATIONS OF ANIONIC POLYSACCHARIDES

(57) Abstract

A dosage formulation comprises a tablet, a pellet, a granule or a microsphere of a polysaccharide. The polysaccharide is a biocompatible anionic polysaccharide material wherein at least 5 % of the basic structural units of the polysaccharide are glucuronic acid.

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# FORMULATIONS OF ANIONIC POLYSACCHARIDES

## Introduction

The invention relates to dosage formulations of polysaccharides.

The invention in particular involves the use of polyanhydroglucuronic acids and salts thereof. The term polyanhydroglucuronic acid and salts there of as used herein also includes copolymers thereof, especially with anhydroglucose. This is hereinafter referred to as PAGA.

Co-pending patent application PCT IE98/00004 describes particular polyanhydroglucuronic acids and salts thereof and a method of preparing such compounds. In particular therefore, the term polyanhydroglucuronic acids and salts thereof includes the acids and salts referred to in this co-pending application.

## Statements of Invention

According to the invention there is provided a dosage formulation of a polysaccharide, the dosage formulation comprising a tablet, a pellet, a granule or a microsphere, the polysaccharide being a biocompatible anionic polysaccharide material wherein at least 5% of the basic structural units of the polysaccharide are glucuronic acid.

Preferably the polysaccharide is derived from a starch, cellulose or gum, or is of microbial origin.

Most preferably the polysaccharide material is polyanhydroglucuronic acid, biocompatible salts thereof, a copolymer thereof or a biocompatible intermolecular polymer complex thereof.

In a preferred embodiment the biocompatible intermolecular polymer complex is a complex of:

an anionic component comprising a linear or branched polysaccharide chain containing glucuronic acid; and

a non protein cationic component comprising a linear or branched natural, semi-synthetic or synthetic oligomer or polymer.

The cationic component may contain nitrogen that either carries a positive charge or wherein the positive charge is induced by contact with the polysaccharidic anionic component. Preferably, the cationic component is selected from derivatives of acrylamide, methacrylamide and copolymers thereof. The cationic component may be selected from polyacrylamide, copolymer of hydroxyethylmethacrylate and hydroxypropylmetacrylamide, copolymers of acrylamide, butylacrylate, maleinanhydride and/or methylmetacrylate.

In another embodiment the cationic component is a cationised natural polysaccharide.

In this case the polysaccharide may be a starch, cellulose or gum. Preferably the gum is guargumhydroxypropyltriammonium chloride.

In another embodiment the cationic component is a synthetic or semi-synthetic polyamino acid. In this case preferably the cationic component is polylysin, polyarginin, or α, β-poly-[N-(2-hydroxyethyl)-DL-aspartamide].

In a further embodiment the cationic component is a synthetic anti-fibrinolytic. The anti-fibrinolytic may be a hexadimethrindibromide (polybren).

In another embodiment the cationic component is a natural or semi-synthetic peptide. The peptide is preferably a protamine, gelatine, fibrinopeptide, or derivatives thereof.

The cationic component may also be an aminoglucane or derivatives thereof. Preferably the aminoglucane is fractionated chitin or its de-acetylated derivative chitosan.

Preferably the aminoglucane is of microbial origin or is isolated from the shells of arthropods such as crabs.

In a particularly preferred embodiment the anionic component is polyanhydroglucuronic acid and/or bicompatible salts and/or copolymers thereof.

Most preferably the polyanhydroglucuronic acid and salts thereof contain in their polymeric chain from 8 to 30 per cent by weight of carboxyl groups, at least 80 per cent by weight of these groups being of the uronic type, at most 5 per cent by weight of carbonyl groups, and at most 0.5 per cent by weight of bound nitrogen. Ideally, the polyanhydroglucuronic acid and salts thereof contain in their polymeric chain at most 0.2 per cent by weight of bound nitrogen.

In a preferred embodiment of the invention the molecular mass of the polymeric chain of the anionic component is from $1x10^3$ to $3x10^5$ Daltons. Ideally, the molecular mass of the polymeric chain of the anionic component ranges from $5x10^3$ to $1.5 \times 10^5$ Daltons.

Preferably the content of carboxyl groups is in the range of from 12 to 26 per cent by weight, at least 95 per cent of these groups being of the uronic type.

Most preferably the anionic component contains at most 1 per cent by weight of carbonyl groups.

In a preferred embodiment the carbonyl groups are intra- and intermolecular 2,6 and 3,6 hemiacetals, 2,4- hemialdals and C2-C3 aldehydes.

In one preferred embodiment the cationic component is gelatine.

In another preferred embodiment the cationic component is chitosan.

The formulation may include at least one biocompatible biologically active substance.

Alternatively or additionally the formulation includes at least one biologically acceptable adjuvant.

In one case the formulation is in the form of a tablet.

In another case the formulation is in the form of pellets.

In a further case the formulation is in the form of granules.

The granules may be prepared by granulating particles or fibers of a polysaccharide material as defined. A layer of the polysaccharide material in particulate form may be fluidised and a granulating medium applied to form agglomerated particles of a desired size.

Preferably polysaccharide material is initially in the form of particles having a size range of from 0.1 to 10μm.

The granulating medium may be a liquid such as water or a mixture of water with one or more water-miscible liquids.

Alternatively the granulating medium is a vapour which may be sprayed onto the particles.

In another case the formulation as claimed is in the form of microspheres.

In this case the microspheres may be prepared by forming a colloidal solution of particles are fibers of a polysaccharide as defined and deriving microspheres from the colloidal solution.

Preferably the microspheres are derived by dropping the colloidal solution into a water-miscible organic liquid, a solution of electrolytes or a mixture of both.

We have now found that by preparing polymeric intermolecular complexes (IMC) of glucuronoglucanes, notably microdispersed PAGA, prepared especially. according to PCT IE 98/00004 it is possible to enhance the haemostatic effect of the final products on this basis and the properties of the temporary wound cover formed after the haemostasis is achieved such as its flexibility and resistance to cracking on movable parts of the body.

It is also possible to upgrade physicomechanical properties of the final products on this basis. Such IMCs make it possible to prepare application forms whose manufacture from a pure PAGA or their simple salts is extremely difficult. Such application forms includes non-woven textile-like structures or polymeric films. To modify or upgrade the physical mechanical properties it is sufficient to use even a relatively small amount of polymeric counterion while it is possible to obtain suitable application properties within a broad concentration range of the

components. The ratio of the glucuronoglucane to polymeric counterion can be 0.99:0.01 to 0.01:0.99.

Another advantage of glucuronoglucane based IMCs is the possibility to control their biological properties such as varying the degree of haemostatis, resorption time, or immunomodulative properties, and the like.

Polymeric cations suitable to form IMCs with glucuronoglucanes prepared for example according to PCT IE 98/00004 may roughly be subdivided to the following groups:

1. Synthetic biocompatible nitrogen-containing oligomers and polymers.

   a) Derivatives of acrylamide and methacrylamide and their copolymers [such as polyacrylamide, copolymer of hydroxyethylmetacrylate and hydroxypropylmetacrylamide, copolymer of acrylamide, butylacrylate, maleinanhydride, and methylmetacrylate, and the like], or else cationised natural polysaccharides such as starches, celluloses, or gums such as guargumhydroxypropyltriammonium chloride.

   b) Synthetic or semi-synthetic polyaminoacids such as polylysin, polyarginin, α,β-poly-[N-(2-hydroxyethyl)-DL-asparamide. Synthetic antifibrinolytics hexadimethrindibromide (polybren) can also be included in this group.

2. Natural or semi-synthetic peptides such as gelatine, protamines, or fibrinopeptides, and their derivatives.

3. Natural aminoglucanes such as fractionated chitin and its de-acetylated derivative chitosan, of microbial origin or isolated from the shells of arthropods such as crabs.

In preparing IMCs on the basis of PAGA according to the invention these three groups of substances can be combined to obtain required properties of the final product.

In general it can be said that IMCs using substances from 1a and 1b would preferably be used to prepare various types of highly absorbant biocompatible dressing materials in the form of nonwovens, films, plasters, and pads.

IMCs using the substances from 2 and 3 may serve as efficient haemostatic agents for internal applications in the microfibrillar form, in the microdispersed form as dusting powders, in the form of films, granules, tablets or non-woven textile-like structures. Those preparations also display antiadhesive properties.

We have also found out that in the form of film-like cell culture matrices the latter IMCs incorporating PAGA and salts thereof as prepared according to PCT IE 98/00004 have a favourable effect on the growth of fibroblasts and keratinocytes.

While it is also possible to create IMCs using structural scleroproteins of the collagen type as disclosed in WO 9800180A, it is preferable to use the above mentioned groups of substances because of the possibility of contamination of the final product by telopeptides, viruses or pyrogens. Collagen can affect in an uncontrolled manner, the immune response of the organism because formation of antibodies can be provoked by any portion of the collagen structure even though the main determinants occur in the terminal regions of the collagen macromolecule. Removal of telopeptides only partially solves the antigenicity problem (Michaeli et al: Science, 1969, 166, 1522).

By preparing IMCs according to the invention it is possible to essentially enhance properties of the originally prepared glucoronoglucanes such as 1,4 β PAGA. For instance an intermolecular complex salt of PAGA and gelatine in one single production step can be used to prepare final products in the form of a non woven, film, microdispersed granules, or dispersions. In contrast to collagen, suitably hydrolysed gelatine is well tolerated, has no toxicity or side effects and it is a much less costly raw material. We have found out that this complex has very good haemostatic properties being about 40% higher than the original PAGA calcium sodium salt. This is despite the fact that the gelatine itself only displays a haemostatic effect after an addition of thrombin [Schwartz S.I. et al.: Principles of Surgery, St.Louis: McGraw Hill Co, 1979, p. 122-123]. In this case the absorption in the organism can be controlled by changing the composition of the complex within the range from tens of hours to several months. This complex has a higher haemostatic efficiency and can be used as an embolisation or microembolisation product. It can also be used to prepare haemostatic layers of highly absorbent multi-layer dressings or resorbable plasters, though more costly polybren or protamines could also be applied.

An important advantage of these IMCs is the fact that the compounds can be prepared within a single manufacturing operation using the hydrolytic process described in PCT IE 98/00004 which makes these products cost effective.

These IMCs can further be modified by biologically active and/or biologically acceptable substances. Because the IMCs prepared by the present procedure are either of a microdispersed or microfibrillar nature, the active substances tend to be bound uniformly and also are uniformly released in the organism without the need for other adjuvants such as microcrystalline waxes or stearates. However, the addition of such adjuvants is not excluded.

Biologically active substances which can be incorporated into the IMC may involve, for instance, antibiotics carrying at least a weak positive charge in the molecule such as cephalosporins (cephotaxin), aminoglycosides (neomycin, gentamycin, amikacin), penicillins (tikarcilin) or macrolides (erythromycin, clarithromycin) and the like.

In cases where the calcium/sodium salt of PAGA or its IMC complexes according to the invention are used as microembolisation or embolisation agents in regional chemotherapy of malign tumours, suitable types of cytostatics such as adriamycin or derivatives of 1,4-diaminoanthrachinone can be incorporated. It is also possible to use the IMCs as detaching ligands for platinum(II) based cytostatics.

Biologically acceptable substances used for modification of the IMCs include, for instance, glycerol and its polymers (polyglycerols); mono, di, and certain triglycerides; polyethyleneglycols; monopropyleneglycol; block copolymers of polyethyleneoxides and polypropyleneoxides (Pluronic); starches; cyclodextrines; polyvinylalcohols; cellulose and its derivatives; in general, substances that, in the concentrations used, are not irritating or toxic for the living organism while being capable of further optimising the physicomechanical properties of the final product based on the IMCs according to the invention.

Detailed Description

We have found that polyanhydroglucuronic acid and salts thereof particularly as described in co-pending application PCT IE98/00004 in both the powder of fibre form has an open surface with a high degree of porosity. It can therefore be pressed by conventional methods into the form of tablets or pellets without the necessity of using other auxiliary adjuvants.

The tablets may for example be used in the gastrointestinal tract for protection of abdominal and/or intestinal mucous tissue against erosion, for control of bleeding from peptic and duodenal ulcers, for control of hydrochloric acid and pepsins secretion from the abdominal wall. The tablet form is particularly suitable for these purposes compared to use of a powder, because it provides protection against partial degradation of the material by enzymes present in vivo, for example in saliva.

The tablet formulation may include other active substances to obtain a dual or multi action. Such additional active substances may, for example be compounds containing $Bi^{3+}$ $Al^{3+}$ or $Mg^{2+}$ ions which are useful in treatment of disorders in the gastric system, especially as antacids. Alternatively or additionally the formulation may include other active compounds such as histamine $H_2$ – receptor antagonists, for example ranitidine or cimetidine.

It will be appreciated that the tablets may include pharmaceutically acceptable adjuvants and/or excipients in some cases.

Polyanhydroglucuronic acid and salts thereof, particularly as described in co-pending application PCT IE98/00004 in a powder/particle form may be mixed with a suitable solvent, especially water, to form a colloid solution. The colloid solution thus formed is dropped into a water-miscible organic liquid, a solution of suitable electrolytes, or a mixture thereof. The size of the microspheres thus formed is controlled by adjusting the drop size, the concentration of the colloid solution, and/or the liquid used.

We have found that the polyanhydroglucuronic acid and salts thereof made by the oxidative hydrolysis treatment described in PCT IE98/00004 has cross linkages due to the formation of inter- and intra – molecular esters or ethers. This leads to

a larger molecular mass and a resultant modification of the viscoelastic properties of a colloidal solution which promotes the formation of microspheres.

The microspheres retain the haemostatic effect of the material and may be particularly used in application such as in a multi-layer haemostatic and/or absorbent pads and dressings. Alternatively such microspheres may be used for embolisation of larger arteries, for example in kidney treatments. The microspheres may also be used as at least part of a filter medium or filler, for example as fillers for chromatographic colums, especially those used for peptide separation.

Rigid or flexible foams may be produced by forming a colloid solution of polyanhydroglucuronic acid and salts thereof and applying conventional lyophilisation methods to the colloid solution. The rigid or flexible foams may be used by themselves or as components of dressing materials for control of bleeding in wound care, while displaying immunomodulative properties in supporting and/or accelerating the healing process.

Polyanhydroglucuronic acids and salts thereof in a power/particle form, particularly as described in our co-pending application PCT IE98/00004 may be granulated to form agglomerated particles of a desired size. A layer of the powder/particle material, preferably in a size range of 0.1 to 10μm is air fluidised in a suitable apparatus such as a mixer. A granulating medium is then added. The granulating medium may be water or a mixture of water with one or more water-miscible liquids. Alternatively the granulating medium may be in the form of a vapour, such as water vapour which may be sprayed onto the fluidised bed of particles. The granulating medium may be added in a single dose or continuously in multiple doses, depending on the type of the fluidised granulation unit used.

The granules thus formed may be fractionated and, if desired, dried to produce granules of a desired size.

The granules retain the haemostatic effect of the material and may be particularly used in applications such as in a multi-layer haemostatic and/or absorbent pads and dressings. Alternatively such granules may be used for embolisation of larger arteries, for example in kidney treatments. The granules may also be used as at least part of filter medium or filler, for example as fillers for chromatographic colums, especially those used for peptide separation.

The invention will be more clearly understood from the following description thereof given by way of example only.

Examples of Polymer Complexes of Glucuronoglucanes

Example 1:

Material: long-fibre cotton – medicinal cotton wool oxidised by $N_xO_y$ (proprietary)

$C_6OOH$ 18.8 % b/w

ash content < 0.1 % b/w

$\Sigma$ C=O 0.6 % b/w

20% solution $Na_2CO_3$ (Lachema, a.s. Neratovice)

$CaCl_2.6H_2O$ anal.grade (Lachema, a.s. Neratovice)

demineralised water 2μS

ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)

acid acetic anal.grade (Lachema, a.s. Neratovice)

$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)

N-HANCE 3000 guargumhydroxypropyltriammoniumchloride (Aqualon – Hercules)

Equipment: mixer: bottom stirring, 150 l (duplicator), stainless steel EXTRA S
vibrating screen: stainless steel, 150 mesh
rotary air pump: rotor diameter 150 mm
turbostirrer: ULTRA TURAX (Janke-Kunkel)
beaker: 5 l
pH meter PICCOLO
thermocouple thermometer

Procedure:

30g of N-HANCE 3000 were placed into and 5 l beaker and 3 l of demineralised water 2μS were added. Contents of the beaker were intensely stirred for 30 minutes. The pH value was adjusted to less than 4.5 by addition of an acetic acid solution leading to a viscosity rise.

60 l of demineralised water 2μS were introduced into a mixer. Then 3 kg of $CaCl_2.6H_2O$ anal.grade were added and the contents heated up to a temperature of 50°C under stirring. On dissolution of the calcium chloride the stirring was interrupted and 2.7 kg of the raw oxidised cotton wool were introduced. The mixer was closed and the contents were agitated for 120 seconds. Then the pH value of the contents was adjusted by addition of a 20% solution of $Na_2CO_3$ to 6 – 6.5 and 13 kg of $H_2O_2$ 30% were introduced. The fibre suspension was slowly agitated for 10 minutes. Then the pH value was readjusted to 4.5 – 5.0 and the prepared viscous solution of N-HANCE 3000 was introduced. The contents of the mixer were stirred intensely for 30 seconds. Subsequently 60 l of synthetic rectified ethanol conc. 98% were introduced into the mixer. After another 15 seconds from adding the ethanol the contents of the mixer were transferred onto a vibrating screen, and the supernatant. Liquid was filtered off. The filtration cake was redispersed in the mixer in 60 l of a mixture of 18 l of synthetic rectified ethanol conc. 98% and 42 l of demineralised water 2μS. The fibre suspension was filtered again on the vibrating screen.

The isolated material thus prepared may further serve to prepare final products of the nonwoven type via a wet or dry process.

Analysis:

| | |
|---|---|
| Ca content | 4.0 % b/w |
| Na content | 1.8 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 20.7 % b/w |

Example 2:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)
$C_6OOH$ 16.8 % b/w
ash content < 0.15 % b/w
$\Sigma$ C=O 2.6 % b/w
20% solution $Na_2CO_3$ (Lachema, a.s. Neratovice)
$CaCl_2.6H_2O$ anal.grade (Lachema, a.s. Neratovice)
redistilled water (PhBs 1997)
ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)
isopropanol 99.9% (Neuberg Bretang)
$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)
gelatine (PhBs 1997)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)
sulphonation flask 1 l
heater 1.5 kW
laboratory centrifuge: 4000 rpm
thermostated water bath
pH meter PICCOLO
glass thermometer
rotary vacuum dryer or hot-air dryer

Procedure:

Into a 1 l sulphonation flask equipped with a turbostirrer and a heater, 400 ml of redistilled $H_2O$ were placed, 15.73 g of $CaCl_2.6H_2O$ were added and on dissolution, 40.0 g of 20% $Na_2CO_3$ solution were introduced under stirring. Subsequently, 50 g of oxidised Linters were added to the white emulsion formed and the contents were heated up to 95°C and the stirring intensity set to a maximum. After 10 minutes, 30 g of 30% $H_2O_2$ were added into the flask and the hydrolysis continued for another 10 minutes. The contents were then cooled down to 60°C on a water bath and the pH of the system was adjusted to a value of 4.5 – 5.0 by addition of 20% solution of $Na_2CO_3$. Furthermore, gelatine solution (10 g of gelatine in 70 g of redistilled $H_2O$) warmed up to 50°C was added and let to react for another 20 minutes. The flask contents were then cooled down to 30°C in a water bath and 626 ml of synthetic rectified ethanol conc. 98% were added gradually under intense stirring. The suspension of IMC thus formed was isolated using a laboratory centrifuge. The supernatant liquid was filtered away and the cake was redispersed into 250 ml of 50% ethanol. The system was centrifuged again and after the separation of the supernatant liquid, the IMC was redispersed into 250 ml of synthetic rectified ethanol conc. 98% and let to stay for 4 hours. It was then centrifuged again, redispersed into 99.9 % isopropanol, and let to stay for a minimum of 10 hours at 20°C. The gel formed was centrifuged again and the product was dried in a rotary vacuum dryer or a hot-air dryer.

The product can be used, for instance, for microembolisation, for preparation of haemostatic dusting powders, for manufacture of polymer drugs, e.g. based on cytostatics, or for preparation of spheric particles for macroembolisation.

Analysis:

| | |
|---|---|
| content Ca | 4.4 % b/w |
| content Na | 2.7 % b/w |

| | |
|---|---|
| content $\Sigma$ C=O | 0.0 % b/w |
| content COOH | 20.5 % b/w |
| content N | 1.8 % b/w |

Example 3:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)

$C_6OOH$ 16.8 % b/w

ash content < 0.15 % b/w

$\Sigma$ C=O 2.6 % b/w

NaOH anal.grade (Lachema, a.s. Neratovice)

redistilled water (PhBs 1997)

ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)

isopropanol 99.9% (Neuberg Bretang)

$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)

gelatine (PhBs 1997)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)

sulphonation flask 1 l

heater 1.5 kW

laboratory centrifuge: 4000 rpm

thermostated water bath

pH meter PICCOLO

glass thermometer

rotary vacuum dryer or hot-air dryer

Procedure:

Into a 1 l sulphonation flask equipped with a turbostirrer and a heater, 400 ml of redistilled $H_2O$ were placed, and 8 g of NaOH were added. On dissolution, 50 g of oxidised Linters were added, the contents were heated up to 70°C and the stirring intensity set to a maximum. After 20 minutes, 40 g of 30% $H_2O_2$ were added into

the flask, temperature was increased to 85°C, and maintained for another 10 minutes. The contents were then cooled down to 50°C on a water bath and gelatine solution (10 g of gelatine in 70 g of redistilled $H_2O$) warmed up to 50°C was added to the hydrolysate. The temperature was decreased to 25 – 30°C and the pH of the system was checked and adjusted to a value of 6.0 – 6.5. Subsequently, 626 ml of synthetic rectified ethanol conc. 98% were added gradually under intense stirring. The suspension of IMC thus formed was isolated using a laboratory centrifuge. The supernatant liquid was filtered away and the cake was redispersed into 250 ml of 50% ethanol. The system was centrifuged again and after the separation of the supernatant liquid, the IMC was redispersed into 250 ml of synthetic rectified ethanol conc. 98% and let to stay for 4 hours. It was then centrifuged again, redispersed into 99.9 % isopropanol, and let to stay for a minimum of 10 hours at 20°C. The gel formed was centrifuged again and the product was dried in a rotary vacuum dryer or a hot-air dryer.

The product can be used, for instance, for microembolisation, for preparation of haemostatic dusting powders, for manufacture of polymer drugs, e.g. based on cytostatics, or for preparation of spheric particles for macroembolisation.

Analysis:

| | |
|---|---|
| Na content | 3.8 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 21.5 % b/w |
| N content | 2.7 % b/w |

Example 4:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)

$C_6OOH$ 16.8 % b/w

ash content < 0.15 % b/w

$\Sigma$ C=O 2.6 % b/w

20% solution $Na_2CO_3$ (Lachema, a.s. Neratovice)

$CaCl_2.6H_2O$ anal.grade (Lachema, a.s. Neratovice)

redistilled water (PhBs 1997)

ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)

isopropanol 99.9% (Neuberg Bretang)

$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)

chitosan, degree of deacetylation 92% (Henkel)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)

sulphonation flask 1 l

heater 1.5 kW

laboratory centrifuge: 4000 rpm

thermostated water bath

pH meter PICCOLO

glass thermometer

rotary vacuum dryer or hot-air dryer

Procedure:

Into a sulphonation flask, 250 ml redistilled $H_2O$ were placed, and 5 g of NaOH were added. On dissolution, 25 g of oxidised Linters were introduced under stirring, the temperature increased to 50°C and the stirring intensity set to a maximum. After hydrolysing for 15 minutes, 35 g of 30% $H_2O_2$ were gradually added to the system and the temperature was maintained at 50°C for another 20 minutes. The content were cooled down to 30°C and 400 g of highly viscous 5% solution of chitosan were added. The flask contents were then intensely stirred for another 10 minutes, and the pH of the system was adjusted, by addition of NaOH, to a value of 7.0. Subsequently 300 ml of synthetic rectified ethanol conc. 98% were added under stirring. The suspension of IMC thus formed was isolated using

a laboratory centrifuge. The supernatant liquid was filtered away and the cake was redispersed into 250 ml of 50% ethanol. The system was centrifuged again and after the separation of the supernatant liquid, the IMC was redispersed into 250 ml of synthetic rectified ethanol conc. 98% and let to stay for 4 hours. It was then centrifuged again, redispersed into 99.9 % isopropanol, and let to stay for a minimum of 10 hours at 20°C. The gel formed was centrifuged again and the product was dried in a rotary vacuum dryer or a hot-air dryer.

The product can be used, for instance, for microembolisation, for preparation of haemostatic dusting powders, for manufacture of polymer drugs, e.g. based on cytostatics, or for preparation of spheric particles for macroembolisation.

Analysis:

| | |
|---|---|
| Na content | 1.8 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 10.4 % b/w |
| N content | 2.8 % b/w |

Example 5:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)

$C_6OOH$ 16.8 % b/w

ash content < 0.15 % b/w

$\Sigma$ C=O 2.6 % b/w

NaOH anal.grade (Lachema, a.s. Neratovice)

HCl 39% anal.grade (Lachema, a.s. Neratovice)

redistilled water (PhBs 1997)

ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)

isopropanol 99.9% (Neuberg Bretang)

$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)

gelatine (PhBs 1997)
Ambroxol (H. Mack, Germany)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)
sulphonation flask 2 l
heater 1.5 kW
laboratory centrifuge: 4000 rpm
laboratory pin mill ALPINE (35 000 rpm)
thermostated water bath
pH meter PICCOLO
glass thermometer
rotary vacuum dryer or hot-air dryer

Procedure:

Into a sulphonation flask, 400 ml redistilled $H_2O$ were placed, and 8 g of NaOH were added. On dissolution, 50 g of oxidised Linters were introduced under stirring, the temperature increased to 70°C and the stirring intensity was set to a maximum. After hydrolysing for 20 minutes, 40 g of 30% $H_2O_2$ were gradually added to the system and the temperature was increased to, and maintained at, 85°C for another 10 minutes. The content were cooled down to 50°C in a water bath, and gelatine solution (2 g of gelatine in 70 g of redistilled $H_2O$) warmed up to 50°C was added to the hydrolysate. The temperature was decreased to 25 – 30°C and the pH of the system was checked and adjusted to a value of 1.6 – 1.8 by addition of 39% HCl. Under intense stirring, a solution of Ambroxol (25g of ambroxolium hydrochloride in 500 ml of redistilled $H_2O$) was added gradually. After agitating for 5 minutes the pH value was adjusted to 4.3 –4.6 by adding 5% NaOH solution, and 626 ml of synthetic rectified ethanol conc. 98% were added under intense stirring. The suspension of Ambroxol containing IMC thus formed was isolated using a laboratory centrifuge. The supernatant liquid was filtered

away and the cake was redispersed into, subsequently, 800 ml of 60% ethanol and 250 ml of 98% ethanol, wherein it was let to stay for a minimum of 10 hours. The system was centrifuged again and the product was dried at 40°C in a rotary vacuum dryer or a hot-air dryer. A white to slightly yellowish powder was obtained and further desagglomerated on an Alpine pin mill.

The product serves for the preparation of a mucoregulatory drug with a prolonged action.

Analysis:

| | |
|---|---|
| Na content | 4.6 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 14.8 % b/w |
| N content | 1.9 % b/w |

Example 6:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)

$C_6OOH$ 16.8 % b/w

ash content < 0.15 % b/w

$\Sigma$ C=O 2.6 % b/w

20% solution $Na_2CO_3$ (Lachema, a.s. Neratovice)

$CaCl_2.6H_2O$ anal.grade (Lachema, a.s. Neratovice)

redistilled water (PhBs 1997)

ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)

isopropanol 99.9% (Neuberg Bretang)

$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)

gelatine (PhBs 1997)

gentamycin sulphate (MERCK)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)
sulphonation flask 2 l
heater 1.5 kW
laboratory centrifuge: 4000 rpm
laboratory pin mill ALPINE (35 000 rpm)
thermostated water bath
pH meter PICCOLO
glass thermometer
hot-air dryer
lyophiliser (Leibold Heraus, Germany)

Procedure:

Into a 2 l sulphonation flask equipped with a turbostirrer and a heater, 400 ml of redistilled $H_2O$ were placed, 15.73 g of $CaCl_2.6H_2O$ were added and on dissolution, 40.0 g of 20% $Na_2CO_3$ solution were introduced under stirring. Subsequently, 50 g of oxidised Linters were added to the white emulsion formed and the contents were heated up to 95°C and the stirring intensity set to a maximum. After 10 minutes, 30 g of 30% $H_2O_2$ were added into the flask and the hydrolysis was continued for another 10 minutes. The contents were then cooled down to 60°C on a water bath and the pH of the system was adjusted to a value of 4.5 – 5.0 by addition of 20% solution of $Na_2CO_3$. Furthermore, gelatine solution (10 g of gelatine in 70 g of redistilled $H_2O$) warmed up to 50°C was added and let to react for another 20 minutes. The flask contents were then cooled down to 30°C in a water bath and 40 g of gentamycin sulphate in 600 ml of redistilled $H_2O$ were added gradually within 10 minutes. 626 ml of synthetic rectified ethanol conc. 98% were then added gradually under intense stirring to the antibiotic containing IMC suspension formed. The suspension of IMC thus formed was isolated using a laboratory centrifuge. The supernatant liquid was filtered away and the cake was redispersed into 250 ml of 50% ethanol. The system was centrifuged again and after the separation of the supernatant liquid,

the IMC was redispersed into 250 ml of synthetic rectified ethanol conc. 98% and let to stay for 4 hours. It was then centrifuged again, redispersed into 99.9 % isopropanol, and let to stay for a minimum of 10 hours at 20°C. The gel formed was centrifuged again and the product was dried in a rotary vacuum dryer or a hot-air dryer.

The product can be used, for instance, for the manufacture of a dusting powder or a powder spray for the treatment of infected wounds.

Analysis:

| | |
|---|---|
| Ca content | 2.4 % b/w |
| Na content | 1.6 % b/w |
| $\sum$ C=O content | 0.0 % b/w |
| COOH content | 9.6 % b/w |
| N content | 2.7 % b/w |

Example 7:

Material: long-fibre cotton – medicinal cotton wool oxidised by $N_xO_y$ (proprietary)

$C_6OOH$ 18.8 % b/w

ash content < 0.1 % b/w

$\sum$ C=O 0.6 % b/w

20% solution $Na_2CO_3$ (Lachema, a.s. Neratovice)

$CaCl_2.6H_2O$ anal.grade (Lachema, a.s. Neratovice)

demineralised water 2μS

ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)

isopropanol 99.9% (Neuberg Bretang)

acid acetic anal.grade (Lachema, a.s. Neratovice)

$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)

N-HANCE 3000 guargumhydroxypropyltriammoniumchloride
(Aqualon – Hercules)
polybren (hexadimethrindibromide) (FLUKA)
chlorhexidindigluconate

Equipment: mixer: bottom stirring, 150 l (duplicator), stainless steel EXTRA S
vibrating screen: stainless steel, 150 mesh
rotary air pump: rotor diameter 150 mm
turbostirrer: ULTRA TURAX (Janke-Kunkel)
beaker: 5 l
pH meter PICCOLO
thermocouple thermometer

Procedure:

30g of N-HANCE 3000 were placed into and 5 l beaker and 3 l of demineralised water 2μS were added. Contents of the beaker were intensely stirred for 30 minutes. The pH value was adjusted to less than 4.5 by addition of an acetic acid solution leading to a viscosity rise.

60 l of demineralised water 2μS were introduced into a mixer. Then 3 kg of $CaCl_2.6H_2O$ anal.grade were added and the contents heated up to a temperature of 50°C under stirring. On dissolution of the calcium chloride the stirring was interrupted and 2.7 kg of the raw oxidised cotton wool were introduced. The mixer was closed and the contents were agitated for 120 seconds. Then the pH value of the contents was adjusted by addition of a 20% solution of $Na_2CO_3$ to 6 – 6.5 and 13 kg of $H_2O_2$ 30% were introduced. The fibre suspension was slowly agitated for 10 minutes. Then the pH value was readjusted to 4.5 – 5.0 and the prepared viscous solution of N-HANCE 3000 was introduced. The contents of the mixer were stirred intensely for 30 seconds. A solution of 35 g of chlorhexidine digluconate in 350 ml of demineralised water 2μS was then introduced slowly

within 10 minutes. Within another 10 minutes, a solution of polybren containing 120 g of polybrenu in 1000 ml of demineralised water 2μS was added. Subsequently 60 l of synthetic rectified ethanol conc. 98% were introduced into the mixer. After another 15 seconds from adding the ethanol, the contents of the mixer were transferred onto a vibrating screen, and the supernatant. Liquid was filtered off. The filtration cake was redispersed in the mixer in 60 l of a mixture of 18 l of synthetic rectified ethanol conc. 98% and 42 l of demineralised water 2μS. The fibre suspension was filtered again on the vibrating screen.

The isolated material thus prepared may further serve to prepare, via a wet or dry process, final products of the nonwoven type having an enhanced haemostatic activity and a bactericidal effect.

Analysis:

| | |
|---|---|
| Ca content | 3.6 % b/w |
| Na content | 1.9 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 18.1 % b/w |
| N content | 0.35 % b/w |

Example 8:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)

$C_6OOH$ 16.8 % b/w

ash content < 0.15 % b/w

$\Sigma$ C=O 2.6 % b/w

20% solution $Na_2CO_3$ (Lachema, a.s. Neratovice)

$CaCl_2.6H_2O$ anal.grade (Lachema, a.s. Neratovice)

redistilled water (PhBs 1997)

ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)

isopropanol 99.9% (Neuberg Bretang)
$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)
Chitosan, degree of deacetylation 92% (Henkel)
Clarithromycin lactobionan (Abbott Laboratories, Italy)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)
sulphonation flask 1 l
heater 1.5 kW
laboratory centrifuge: 4000 rpm
thermostated water bath
pH meter PICCOLO
glass thermometer
rotary vacuum dryer or hot-air dryer
dialysing bag (regenerated cellulose)
lyophiliser (Leybold Heraus, Germany)
laboratory pin mill ALPINE (35 000 rpm)

Procedure:

Into a sulphonation flask, 250 ml redistilled $H_2O$ were placed, and 5 g of NaOH were added. On dissolution, 25 g of oxidised Linters were introduced under stirring, the temperature increased to 50°C and the stirring intensity set to a maximum. After hydrolysing for 15 minutes, 35 g of 30% $H_2O_2$ were gradually added to the system and the temperature was maintained at 50°C for another 20 minutes. The content were cooled down to 30°C and 400 g of highly viscous 2% solution of chitosan, having a pH value of 3.5, were added. The flask contents were then intensely stirred for another 10 minutes, and the pH of the system was adjusted, by addition of NaOH, to a value of 7.0. During another 10 minutes, a solution of clarithromycin (44 g of clarithromycin in 456 ml of redistilled $H_2O$) was introduced and the pH of the system was adjusted to a value of 7.0-7.5. Stirring was interrupted, the flask contents were transferred into a dialysing bag

and dialysed against water for 48 hours. Subsequently the product was isolated by centrifugation, lyophilised, and disintegrated on the laboratory pin mill ALPINE.

The product can be used, for instance, to prepare tablets or granules efficient against Helicobacter pylori occurring in the gastrointestinal tract.

Analysis:

| | |
|---|---|
| Na content | 4.8 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 18.8 % b/w |
| N content | 0.7 % b/w |

Example 9:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)
$C_6OOH$ 16.8 % b/w
ash content < 0.15 % b/w
$\Sigma$ C=O 2.6 % b/w
NaOH anal.grade (Lachema, a.s. Neratovice)
redistilled water (PhBs 1997)
ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)
isopropanol 99.9% (Neuberg Bretang)
$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)
gelatine (PhBs 1997)
$Bi(NO_3).5H_2O$ (MERCK)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)
sulphonation flask 2 l
heater 1.5 kW
laboratory centrifuge: 4000 rpm

thermostated water bath
pH meter PICCOLO
glass thermometer
rotary vacuum dryer or hot-air dryer

Procedure:

Into a sulphonation flask, 400 ml redistilled $H_2O$ were placed, and 8 g of NaOH were added. On dissolution, 50 g of oxidised Linters were introduced under stirring, the temperature increased to 70°C and the stirring intensity was set to a maximum. After hydrolysing for 20 minutes, 40 g of 30% $H_2O_2$ were gradually added to the system and the temperature was increased to, and maintained at, 85°C for another 10 minutes. The content were cooled down to 50°C in a water bath, and gelatine solution (0.5 g of gelatine in 50 ml of redistilled $H_2O$) warmed up to 50°C was added to the hydrolysate. The temperature was decreased to 25 – 30°C and the pH of the system was checked and adjusted to a value of 1.6 – 1.8 by addition of 39% HCl. A freshly prepared solution of $BiNO_3$ (54 g of $BiNO_3.5H_2O$ in 746 ml of $H_2O$) was introduced and the temperature maintained for another 15 minutes. Then the temperature was decreased to 25 – 30°C and the pH of the system was checked and readjusted to a value of 5.5 – 6.0. 626 ml of synthetic rectified ethanol conc. 98% were then added gradually under intense stirring. to the formed. The $BiO^+$ containing IMC suspension thus formed was isolated using a laboratory centrifuge. The supernatant liquid was filtered away and the cake was redispersed into 250 ml of 50% ethanol. The system was centrifuged again and after the separation of the supernatant liquid, the IMC was redispersed into 250 ml of synthetic rectified ethanol conc. 98% and let to stay for a minimum of 4 hours. It was then centrifuged again, redispersed into 99.9 % isopropanol, and let to stay for a minimum of 10 hours at 20°C. The suspension formed was then centrifuged again and the product was dried in a rotary vacuum dryer or a hot-air dryer.

The product can be used, for instance, to prepare dusting powders for wound treatment or tablets for treatment of gastrointestinal tract malfunctions.

Analysis:

| | |
|---|---|
| Na content | 1.9 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 20.0 % b/w |
| N content | < 0.3 % b/w |
| Bi content | 4.7 % b/w |

Example 10:

Material: oxidised short-fibre cotton (Linters – Temming) (proprietary)
$C_6OOH$ 16.8 % b/w
ash content < 0.15 % b/w
$\Sigma$ C=O 2.6 % b/w
20% solution $Na_2CO_3$ (Lachema, a.s. Neratovice)
$CaCl_2.6H_2O$ anal.grade (Lachema, a.s. Neratovice)
redistilled water (PhBs 1997)
ethanol, synthetic rectified conc. 98% (Chemopetrol Litvínov, a.s.)
isopropanol 99.9% (Neuberg Bretang)
$H_2O_2$ anal.grade 30% (Lachema, a.s. Neratovice)
gelatine (PhBs 1997)
cimetidine hydrochloride (SPOFA)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)
sulphonation flask 2 l
heater 1.5 kW
laboratory centrifuge: 4000 rpm
thermostated water bath

pH meter PICCOLO
glass thermometer
rotary vacuum dryer or hot-air dryer

Procedure:

Into a 1 l sulphonation flask equipped with a turbostirrer and a heater, 400 ml of redistilled $H_2O$ were placed, 15.73 g of $CaCl_2.6H_2O$ were added and on dissolution, 40.0 g of 20% $Na_2CO_3$ solution were introduced under stirring. Subsequently, 50 g of oxidised Linters were added to the white emulsion formed and the contents were heated up to 95°C and the stirring intensity set to a maximum. After 10 minutes, 30 g of 30% $H_2O_2$ were added into the flask and the hydrolysis was continued for another 10 minutes. The contents were then cooled down to 60°C on a water bath and the pH of the system was adjusted to a value of 4.5 – 5.0 by addition of 20% solution of $Na_2CO_3$. Furthermore, gelatine solution (10 g of gelatine in 70 g of redistilled $H_2O$) warmed up to 50°C was added and let to react for another 20 minutes. The flask contents were then cooled down to 30°C in a water bath and a solution of cimetidine (36 g of cimetidine hydrochloride in 400 ml of redistilled $H_2O$) were added under intens stirring. The contents were intensely agitated for 10 minutes and 800 ml of synthetic rectified ethanol conc. 98% were then added gradually. The suspension of IMC thus formed was isolated using a laboratory centrifuge. The supernatant liquid was filtered away and the cake was redispersed into 250 ml of 50% ethanol. The system was centrifuged again and after the separation of the supernatant liquid, the IMC was redispersed into 250 ml of synthetic rectified ethanol conc. 98% and let to stay for 4 hours. It was then centrifuged again, redispersed into 99.9 % isopropanol, and let to stay for a minimum of 10 hours at 20°C. The gel formed was centrifuged again and the product was dried in a rotary vacuum dryer or a hot-air dryer.

The product can be used, for instance, to manufacture tablets or granulates for the treatment of the gastrointestinal tract or other non-malignant ulcerations.

Analysis:

| | |
|---|---|
| Ca content | 4.4 % b/w |
| Na content | 2.7 % b/w |
| $\Sigma$ C=O content | 0.0 % b/w |
| COOH content | 20.5 % b/w |
| N content | 2.1 % b/w |

Example 11:

Material: IMC-MDOC complex (as per above Example 2)
[(2S;2R)-3-amino-2-hydroxy-4-phenylbutenoyl]-L-leucin (Bestatin) (Boehringer Mannheim, Germany)
redistilled water (PhBs 1997)
methanol, conc. anal.grade (Chemopetrol Litvínov, a.s.)
diethylether (Lachema, a.s. Neratovice)

Equipment: turbostirrer: ULTRA TURAX (Janke-Kunkel)
sulphonation flask 2 l
laboratory centrifuge: 4000 rpm
hot-air dryer

Procedure:

The IMC-MDOC complex as prepared in Example 2 above was redispersed into redistilled water in a sulphonation flask using a turbostirrer. A solution of Bestatin in methanol was then added to the flask in an amount sufficient to yield a 10% b/w concentration of Bestatin in the resulting Bestatin-gelatine-MDOC complex. After thorough homogenisation, the suspension formed was isolated by

centrifugation. The supernatant liquid ws filtered away and the filtration cake was redispersed into concentrated methanol again, centrifuged, redispersed in diethylether, and after being allowed to stay for 1 hour, it was dried in a hot-air dryer.

The product, a microdispersed form of a Bestatin-gelatine-MDOC complex, can be used, for instance, to prepare microembolisation agents used in regional chemotherapy of malignant tumours or flat dressing structures for wound treatment.

**Example A:** ***Preparation of granules in a fluid bed***

*MDOC = Microdispersed oxidised cellulose*

Material: MDOC, particle size 0.1 - 2.0 μm, specific surface area 86 $m^2/g$, COOH group content 22.2% b/w, Ca content 4.2 % b/w, Na content 3.8 % b/w

Equipment: set of vibrating screens with mesh size 100, 150, 200, 250, 350, 500 μm

mixer, bottom agitated, vessel size 1000 ml, 8000 rpm, equipped with a nozzle for inlet of the granulation medium

counter-flow drier BINDER

Procedure:

100 g of MDOC were placed into the mixer, the mixer was closed and the agitation begun. A water mist was gradually injected into the mixer at a rate of 10 g/45 seconds. The granulate formed was transferred to the counter-flow drier and dried at a temperature of 45°C until the humidity content was reduced to below

6% b/w. The dried granules were sieve-screened using the set of vibrating screens. The individual fractions were packaged into glass vials in amounts of 0.5 -2.0 g each as required. The preparation was sterilised by γ irradiation with a dose of 25 kGy.

Indication:

The product may be used as a) an embolisation agent, or b) an antilipemicum.

**Example B** : ***Preparation of granules from IMC-MDOC complex containing clarithromycin***

Material: IMC-MDOC complex – see Example 8
MDOC, particle size 0.1 - 2.0 μm, specific surface area 86 $m^2/g$, COOH group content 22.2% b/w, Ca content 4.2 % b/w, Na content 3.8 % b/w
IMC-MDOC complex containing $BiO^+$ - see Example 9
ethanol synthetic rectified 98%
redistilled $H_2O$

Equipment: set of vibrating screens with mesh size 100, 150, 200, 250, 350, 500μm
mixer, bottom agitated, vessel size 1000 ml, 8000 rpm, equipped with a nozzle for inlet of the granulation medium
counter-flow drier (BINDER)

Procedure:

100 g of MDOC were placed into the mixer, the mixer was closed and the agitation begun. A mist of 88% aqueous solution of ethanol was gradually injected into the mixer at a rate of 10 g/45 seconds. The granulate formed was transferred

to the counter-flow drier and dried at a temperature of 45°C until the humidity content was reduced to below 6% b/w. The dried granules were sieve-screened using the set of vibrating screens. The individual fractions were packaged into glass vials in amounts of 0.5 -2.0 g each as required. The preparation was sterilised by γ irradiation with a dose of 25 kGy.

Indication:

The granules can be used in the treatment of gastric ulcers. MDOC suppresses formation of the stomach acidity, adjusts the pH value of the environment, and protects the mucous membranes by forming a gel layer. $BiO^+$ acts as a mild astringens. Clarithromycin depresses the growth of Helicobacter pylori beyond pathologic limits.

**Example C** : ***Preparation of granules from IMC-MDOC complex***

Material: IMC-MDOC complex – see Example 2

Equipment: set of vibrating screens with mesh size 100, 150, 200, 250, 350, 500μm

mixer, bottom agitated, vessel size 1000 ml, 8000 rpm, equipped with a nozzle for inlet of the granulation medium

counter-flow drier BINDER

Procedure:

100 g of MDOC were placed into the mixer, the mixer was closed and the agitation switched on. Saturated water vapour was gradually injected into the mixer at a rate of 10 g/45 seconds. The granulate formed was transferred to the counter-flow drier and dried at a temperature of 45°C until the humidity content was reduced below 6% b/w. The dried granules were sieve-screened using the set of vibrating screens. The individual fractions were packaged into glass vials in

amounts of 0.5 -2.0 g each as required. The preparation was sterilised by γ irradiation with a dose of 25 kGy.

Indication:

The product may be used as a) an embolisation agent, or b) an antilipemicum.

**Example D:** ***Preparation of granules from IMC-MDOC complex containing antimicrobial agent***

Material: IMC-MDOC complex – see Example 2
polyvinylpyrrolidone-iodine PVP-I complex micronised (ISP-USA)
1,2-monopropyleneglycol (MERCK)
redistilled $H_2O$
ethanol synthetic rectified 98% (Chemopetrol Litvínov, a.s.)

Equipment: set of vibrating screens with mesh size 100, 150, 200, 250, 350, 500 μm
mixer, bottom agitated, vessel size 1000 ml, 8000 rpm, equipped with a nozzle for inlet of the granulation medium
counter-flow drier (BINDER)

Procedure:

90 g IMC-MDOC complex, 5 g PVP-I complex and 5 g 1,2-MPG were placed into the mixer, the mixer was closed and the agitation begun. A mist of 88% aqueous solution of ethanol was gradually injected into the mixer at a rate of 10 g/50 seconds. The granulate formed was transferred to the counter-flow drier and dried at a temperature of 45°C until the humidity content was reduced to below 6% b/w. The dried granules were sieve-screened using the set of vibrating screens. The fraction below 100 μm was used to prepare a dusting powder. The higher fractions were packaged into glass vials in amounts of 0.5 -2.0 g each as required.

Indication:

Haemostatic preparation with antimicrobial and antiviral effect (powder spray, dusting powder).

**Example E:** ***Preparation of microspheres from IMC-MDOC complex containing mitoxanthron***

Material: IMC-MDOC complex – see Example 3
1,4-bis-2-(-2-hydroxyethylamino-ethylamino-)5,8-dihydroxyantrachinon (mitoxanthron) (Aliachem a.s.)
ethanol synthetic rectified 98% (Chemopetrol Litvínov, a.s.)
redistilled $H_2O$

Equipment: turbostirrer ULTRA TURAX (Janke-Kunkel)
sulphonation flask 1 Litre
beaker 250 ml
set of vibrating screens with mesh size 100, 150, 200, 250, 350, 500 µm
counter-flow drier (BINDER)
vial 10 ml
injection syringe 25 ml

Procedure:

80 g redistilled water and 20 g IMC-MDOC complex were introduced into the beaker, and the complex was dispersed using the turbostirrer to obtain a colloidal solution.

495 ml of 98% ethanol was placed into the sulphonation flask. 1.0 g of mitoxanthron hydrochloride was placed into the 10 ml vial and dissolved in 5 g of redistilled water. The solution was then transferred into the sulphonation flask with ethanol under stirring.

The colloidal solution of IMC-MDOC complex was then gradually introduced into the mitoxanthron solution by being dropped, via the injection syringe, at a rate of 20 drops per minute into the sulphonation flask. The microspheres were isolated by filtration from the supernatant liquid, cautiously redispersed into 250 ml of 98% ethanol and allowed to stand for 4 hours. The ethanol was then removed by filtration and the microspheres were dried in the counter-flow drier at a temperature of 40°C until the humidity content was reduced to below 3 % b/w. The dry microspheres containing 50 mg of mitoxanthron per 1 g were sieve-screened using the set of vibrating screens, and packaged into glass vials in amounts of 0.5 g each.

Indication:
Intraarterial (regional) chemotherapy of malignant tumours where mitoxanthron is indicated.

**Example F** : ***Preparation of microspheres from IMC-MDOC complex containing mitoxanthron***

Material: MDOC (Ca/Na salt of PAGA), particle size 0.1 - 2.0 μm, specific surface area 86 $m^2/g$, COOH group content 22.2 % b/w, Ca content 4.2 % b/w, Na content 3.8 % b/w
ethanol synthetic rectified 98% (Chemopetrol Litvínov, a.s.)
redistilled $H_2O$
1,2-monopropyleneglycol (Sigma)
sorbitol (Sigma)

isopropanol (Sigma)

Equipment: propeller stirrer, 50 rpm
sulphonation flask 1 Litre
beaker 250 ml
set of vibrating screens with mesh size 100, 150, 200, 250, 350, 500 μm
counter-flow drier (BINDER)
injection syringe 25 ml

Procedure:

10 g MDOC Ca/Na salt and 90 g redistilled $H_2O$ were introduced into the beaker, and dispersed using the propeller stirrer to obtain a colloidal solution.

A coagulating solution was prepared by dissolving 25 g of sorbitol and 25 g of monopropyleneglycol in 450 ml of 98% ethanol and placed in the sulphonation flask. 1.0 g of mitoxanthron hydrochloride was placed into the 10 ml vial and dissolved in 5 g of redistilled water. The solution was then transferred into the sulphonation flask with ethanol under stirring.

The colloidal solution of MDOC was then gradually introduced into the sulphonation flask by being dropped, via the injection syringe, at a rate of 10 drops per minute. The microspheres were isolated from the coagulating bath by decantation, 250 ml of isopropanol were added and the microspheres were allowed to stand for 8 hours. The isopropanol was then removed by filtration and the microspheres were dried in the counter-flow drier at a temperature of 40°C until the humidity content was reduced to below 3 % b/w. The dry microspheres were sieve-screened using the set of vibrating screens, and packaged into glass vials in amounts of 0.5 g each.

Indication:

Microembolisation agent to achieve tissue necrotisation, e.g. in gynaecological treatment of non-malignant tumours.

**Example G** : ***Preparation of microspheres from IMC-MDOC complex containing platinum(II) compounds***

Material: MDOC (Ca/Na salt of PAGA), particle size 0.1 - 2.0 μm, specific surface area 86 $m^2/g$, COOH group content 22.2 % b/w, Ca content 4.2 % b/w, Na content 3.8 % b/w
ethanol synthetic rectified 98% (Chemopetrol Litvínov, a.s.)
redistilled $H_2O$
1,2-dihydroxypropane (Sigma)
polyacrylamide, 50 % aqueous solution (Aldrich)
glycerol, medicinal (PhBs 1997)

Equipment: laboratory mixer, bottom agitated, 4000 rpm
sulphonation flask 1 Litre
injection syringe 25 ml

Procedure:

A colloidal aqueous solution of an MDOC-chitosan-polyacrylamide complex containing 30% b/w of MDOC Ca/Na salt was dropped, via the injection syringe at a rate of 10 drops per minute, into an ethanol/glycerol/water system containing salts of bivalent platinum with two ammonia ($NH_3$) ligands. The microspheres formed containing $(NH_3)_2Pt(II)$ groups, were isolated from the coagulating bath by decantation, washed with concentrated ethanol, and vacuum dried at 25°C.

Indication:

Intraarterial (regional) chemotherapy of malignant tumours where diamoplatinum(II) complexes are indicated.

The invention is not limited to the embodiments hereinbefore described which may be varied in detail.

Claims

1. A dosage formulation of a polysaccharide, the dosage formulation comprising a tablet, a pellet, a granule or a microsphere and the polysaccharide being a biocompatible anionic polysaccharide material wherein at least 5% of the basic structural units of the polysaccharide are glucuronic acid.

2. A formulation as claimed in claim 1 wherein the polysaccharide is derived from a starch, cellulose or gum, or is of microbial origin.

3. A formulation as claimed in claim 1 or 2 wherein the polysaccharide material is polyanhydroglucuronic acid, biocompatible salts thereof, a copolymer thereof or a biocompatible intermolecular polymer complex thereof.

4. A formulation as claimed in claim 3 wherein the biocompatible intermolecular polymer complex is a complex of:

an anionic component comprising a linear or branched polysaccharide chain containing glucuronic acid; and

a non protein cationic component comprising a linear or branched natural, semi-synthetic or synthetic oligomer or polymer.

5. A formulation as claimed in claim 4 wherein the cationic component contains nitrogen that either carries a positive charge or wherein the positive charge is induced by contact with the polysaccharidic anionic component.

6. A formulation as claimed in claim 4 wherein the cationic component is selected from derivatives of acrylamide, methacrylamide and copolymers thereof.

7. A formulation complex as claimed in claim 6 wherein the cationic component is selected from polyacrylamide, copolymer of hydroxyethylmethacrylate and hydroxypropylmetacrylamide, copolymers of acrylamide, butylacrylate, maleinanhydride and/or methylmetacrylate.

8. A formulation as claimed in claim 4 wherein the cationic component is a cationised natural polysaccharide.

9. A formulation as claimed in claim 8 wherein the polysaccharide is a starch, cellulose or gum.

10. A formulation as claimed in claim 9 wherein the gum is guargumhydroxypropyltriammonium chloride.

11. A formulation as claimed in claim 4 wherein the cationic component is a synthetic or semi-synthetic polyamino acid.

12. A formulation as claimed in claim 11 wherein the cationic component is polylysin, polyarginin, or α, β-poly-[N-(2-hydroxyethyl)-DL-aspartamide].

13. A formulation as claimed in claim 4 wherein the cationic component is a synthetic anti-fibrinolytic.

14. A formulation as claimed in claim 12 wherein the anti-fibrinolytic is a hexadimethrindibromide (polybren).

15. A formulation as claimed in claim 4 wherein the cationic component is a natural or semi-synthetic peptide.

16. A formulation as claimed in claim 15 wherein the peptide is a protamine, gelatine, fibrinopeptide, or derivatives thereof.

17. A formulation as claimed in claim 4 wherein the cationic component is an aminoglucane or derivatives thereof.

18. A formulation as claimed in claim 17 wherein the aminoglucane is fractionated chitin or its de-acetylated derivative chitosan.

19. A formulation as claimed in claim 17 or 18 wherein the aminoglucane is of microbial origin or is isolated from the shells of arthropods such as crabs.

20. A formulation as claimed in any of claims 4 to 19 wherein the anionic component is polyanhydroglucuronic acid and/or bicompatible salts and/or copolymers thereof.

21. A formulation as claimed in any of claims 1 to 20 wherein the polyanhydroglucuronic acid and salts thereof contain in their polymeric chain from 8 to 30 per cent by weight of carboxyl groups, at least 80 per cent by weight of these groups being of the uronic type, at most 5 per cent by weight of carbonyl groups, and at most 0.5 per cent by weight of bound nitrogen.

22. A formulation as claimed in claim 21 wherein the polyanhydroglucuronic acid and salts thereof contain in their polymeric chain at most 0.2 per cent by weight of bound nitrogen.

23. A formulation as claimed in claim 21 or 22 wherein the molecular mass of the polymeric chain of the anionic component is from $1x10^3$ to $3x10^5$ Daltons.

24. A formulation as claimed in claim 23 wherein the molecular mass of the polymeric chain of the anionic component ranges from $5x10^3$ to $1.5 \times 10^5$ Daltons.

25. A formulation as claimed in any of the claims 21 to 24 wherein the content of carboxyl groups is in the range of from 12 to 26 per cent by weight, at least 95 per cent of these groups being of the uronic type.

26. A formulation as claimed in any of claims 21 to 25 wherein the anionic component contains at most 1 per cent by weight of carbonyl groups.

27. A formulation as claimed in any of claims 21 to 26 wherein the carbonyl groups are intra- and intermolecular 2,6 and 3,6 hemiacetals, 2,4-hemialdals and C2-C3 aldehydes.

28. A formulation as claimed in claim 4 wherein the cationic component is gelatine.

29. A formulation as claimed in claim 4 wherein the cationic component is chitosan.

30. A formulation as claimed in any preceding claim including at least one biocompatible biologically active substance.

31. A formulation as claimed in any preceding claim including at least one biologically acceptable adjuvant.

32. A formulation as claimed in any preceding claim in the form of a tablet.

33. A formulation as claimed in any of claims 1 to 31 in the form of pellets.

34. A formulation as claimed in any of claims 1 to 31 in the form of granules.

35. A process for preparing granules as claimed in claim 34 by granulating particles or fibers of a polysaccharide material as defined in any of claims 1 to 31.

36. A process as claimed in claim 35 wherein a layer of the polyschharide material in particulate form is fluidised and a granulating medium is applied to form agglomerated particles of a desired size.

37. A process as claimed in claim 36 wherein the polysaccharide material is initially in the form of particles having a size range of from 0.1 to 10μm.

38. A process as claimed in claim 36 or 37 wherein the granulating medium is a liquid such as water or a mixture of water with one or more water-miscible liquids.

39. A process as claimed in claim 36 or 37 wherein the granulating medium is a vapour which may be sprayed onto the particles.

40. A formulation as claimed in any of claims 1 to 31 in the form of microspheres.

41. A process for preparing microspheres as claimed in claim 40 by forming a colloidal solution of particles or fibers of a polysaccharide as defined in any of claims 1 to 31 and deriving microspheres from the colloidal solution.

42. A process as claimed in claim 41 wherein the microspheres are derived by dropping the colloidal solution into a water-miscible organic liquid, a solution of electrolytes or a mixture of both.

# INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IE 99/00072

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 A61K9/16 A61K9/20 C08B37/00 C08J9/00 //C08L5:00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| 27 October 1999 | 10/11/1999 |

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Inte ional Application No
PCT/IE 99/00072

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