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(71) Applicant *(for all designated States except US)*: **TEOXANE** [CH/CH]; Les Chamilles, Rue de Lyon, 105, CH-1203 Genève (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SENTIER, Luc** [FR/FR]; 6 rue du Commerce, F-74100 Annemasse (FR). **BOURDON, François** [FR/FR]; 3 Allée des Iris, F-74240 Gaillard (FR).

(74) Agent: **LE COUPANEC, Pascale**; Nony, 3 rue de Penthièvre, F-75008 Paris (FR).

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(54) Title: PROCESS FOR PREPARING A CROSSLINKED GEL

(57) Abstract: The present invention relates to a process for preparing a crosslinked gel of at least one polysaccharide or a salt thereof, comprising at least the steps consisting in: a) providing at least one aqueous gel of said polysaccharide in a noncrosslinked form, b) bringing said aqueous gel into contact with an effective amount of at least one crosslinking agent, c) subjecting the mixture formed in step b) to conditions suitable for the crosslinking of said polysaccharide, and, if need be, d) recovering said crosslinked hydrogel, wherein said process comprises, prior to step c), at least one step e) consisting in circulating the aqueous gel of polysaccharide of step a) and/or the mixture of step b) through at least one extrusion device which has at least one filter with a porosity ranging from 2 to 100 microns and capable of retaining any particle with a diameter greater than 100 microns,

Process for preparing a crosslinked gel

The present invention aims to propose a process for preparing hydrogels based on a crosslinked polysaccharide, and preferably on hyaluronic acid or a salt thereof.

Hyaluronic acid, which is naturally present in the skin, is known for its viscoelastic properties and also its high propensity to absorb water. Its properties explain to a large extent the elasticity of the skin. Its biocompatibilities, tolerance and lack of toxicity are such that, for more than 10 years, this molecule has been used in medical and cosmetic fields, and in particular for filling wrinkles. Thus, the injection into the dermis of a crosslinked hydrogel of polysaccharide at the wrinkles to be treated makes it possible to reduce, or even eliminate the local weakening of the structure of the dermis represented by a wrinkle.

Generally, the polysaccharide, and more particularly the hyaluronic acid, is used in a crosslinked form given the increased resistance of this particular form to degradation and to heat.

These crosslinked polysaccharide gels can be prepared by various preparation processes. Generally, these processes require two main steps, the first consisting in rehydrating the polysaccharide under consideration so as to convert it into an aqueous gel and the second aimed at bringing said aqueous gel into contact with an agent capable of inducing the crosslinking thereof. Depending on the specific conditions selected for the crosslinking, it proves to be possible to adjust the viscosity or else the rheological properties of the crosslinked hydrogel formed.

By way of illustration of these processes, mention may particularly be made of the processes described in US 2006/0105022, which comprises the use of a mixture comprising at least 10% of hyaluronic acid, a crosslinking agent and water, under acidic or basic conditions, in WO 2006/056204, which comprises a step of treating the gel of hyaluronic acid crosslinked with divinylsulphone, and in US 2007/0036745, which results in a cohesive gel from a hyaluronane polymer crosslinked with divinylsulphone (DVS).

For obvious reasons, the improvement of the mechanical properties of the hydrogels for applications in the medical and cosmetic fields is a constant objective.

The present invention aims precisely to propose a process for obtaining crosslinked gels having particularly advantageous mechanical properties.

Against all expectations, the inventors have noted that one original mode of preparation of crosslinked gels makes it possible to significantly improve the properties of said gels.

Thus, according to a first of its aspects, the present invention relates to a process for preparing a crosslinked gel of at least one polysaccharide or a salt thereof, comprising at least the steps consisting in:

a) providing at least one aqueous gel of said polysaccharide in a noncrosslinked form,

b) bringing said aqueous gel into contact with an effective amount of at least one crosslinking agent,

c) subjecting the mixture formed in step b) to conditions suitable for the crosslinking of said polysaccharide, and, if need be,

d) recovering said crosslinked hydrogel,

wherein said process comprises, prior to step c), at least one step e) consisting in circulating the aqueous gel of polysaccharide of step a) and/or the mixture of step b) through at least one extrusion device which has at least one filter with a porosity ranging from 2 to 100 microns and capable of retaining any particle with a diameter greater than 100 microns.

This step e), consisting in circulating the aqueous gel of polysaccharide and/or the mixture obtained at the end of step b) through an extrusion device as mentioned above, can also be denoted, in the rest of the present description, as an "*extrusion step*".

In the rest of the present description, the device which has at least one filter that is used for carrying out extrusion step e) may also be denoted by the expression "*extrusion device*".

More specifically, the present invention results from the unexpected observation, by the inventors, that the implementation of an extrusion step makes it possible to obtain crosslinked polysaccharide gels having significantly improved viscoelastic properties, compared with the conventional crosslinking processes.

In view of the fragility of the polysaccharide chains, this observation is all the more surprising since such an extrusion step could, on the contrary, contribute to a significant alteration of the polysaccharide chains and, in this respect, of the viscoelastic properties of the crosslinked gels.

The improvement in the viscoelastic properties of the crosslinked gels obtained by implementing a process according to the invention can be explained by the fact that the extrusion step mostly contributes to a better organization of the polysaccharide chains or even, in addition, as appropriate, to protecting the aqueous gel of step a) and/or the mixture of step b) against a significant amount of residual particles or agglomerates which, for obvious

reasons, are prejudicial to good homogeneity thereof and therefore to the homogeneity of the crosslinked gels relating thereto.

According to one particular embodiment of the invention, the process can also comprise a step f) of stopping the crosslinking, consisting in exposing the crosslinked gel to conditions suitable for stopping its crosslinking, it being possible for this step to be carried out prior to, jointly with or after the recovery step d).

According to one preferred embodiment variant, step f) is carried out prior to step d).

Advantageously, the crosslinked gel obtained at the end of the process described above is a single-phase gel and more particularly a predominantly elastic viscoelastic gel, i.e. a gel with a reduced ability, or even devoid of the ability, to flow in the absence of stresses other than its own weight, like, for example, a gum.

According to another of its aspects, the present invention relates to the use of a crosslinked gel obtained by implementing a process as defined above, for the long-lasting filling of volume defects of the skin, and in particular the filling of wrinkles.

II) Process

A process according to the invention requires, firstly, providing at least one aqueous gel of at least one polysaccharide in a noncrosslinked form in accordance with the invention.

This relates more particularly to step a).

For the purpose of the present invention, the term "noncrosslinked" is intended to denote an aqueous gel of polysaccharide which is noncrosslinked or weakly crosslinked, i.e. a gel of which the phase shift angle δ, measured by dynamic rheology of 1 Hz, is greater than 40° when it is subjected to a stress greater than 1 Pa.

Such an aqueous gel can be obtained by bringing together at least one aqueous medium and polysaccharide, in an appropriate receptacle, and homogenizing jointly with and/or subsequent to the addition of said polysaccharide to said aqueous medium, or vice versa.

For obvious reasons, the homogenization step considered above should be carried out in such a way as to form homogeneous structures.

Secondly, a process according to the invention requires the addition, to said aqueous gel of polysaccharide obtained in step a), of an effective amount of crosslinking agent.

This relates more particularly to step b).

For the purpose of the present invention, the term "effective amount" is intended to mean an amount of crosslinking agent which, in relation to the amount of polysaccharide under consideration, allows satisfactory crosslinking of the aqueous gel of polysaccharide.

The determination of this effective amount of crosslinking agent is part of the general knowledge of those skilled in the art.

Advantageously, step b) is carried out over a period of greater than 5 minutes, preferably ranging from 15 to 180 minutes.

For obvious reasons, this step b) can also be accompanied by at least one further step of homogenization jointly with and/or subsequent to the addition of said crosslinking agent.

Advantageously, step b) of bringing an aqueous gel of polysaccharide in noncrosslinked form into contact with a crosslinking agent is carried out under conditions not suitable for effective implementation of the crosslinking.

Irrespective of the embodiment under consideration, steps a) and/or b) involve(s) at least one homogenization.

The objective of this operation, optionally carried out in the presence of the crosslinking agent, is more particularly to completely hydrate and homogenize the polysaccharide in the aqueous medium and, where appropriate, the crosslinking agent, and thus to contribute to the optimization of the qualities of the expected crosslinked gel.

Indeed, for obvious reasons, the homogeneity of the crosslinked gel is closely linked to the homogeneous nature of the gel before crosslinking.

The homogenization is considered to be satisfactory when the solution obtained has a homogeneous coloration, without agglomerates, and a uniform viscosity. It can advantageously be carried out under mild operating conditions in order to prevent degradation of the polysaccharide chains.

This step is all the more important when the polysaccharide has a high molecular weight. The hydration of such a compound then in fact has a tendency to generate the formation of a solution of high viscosity within which the appearance of agglomerates is commonly observed.

The duration of this homogenization step therefore depends on the nature of the polysaccharide, and more particularly on its molecular weight, on its concentration, on the operating conditions within the aqueous medium and also on the homogenization device used.

The adjustment of the duration of homogenization suitable for obtaining a sufficiently homogeneous aqueous gel of polysaccharide is part of the general knowledge of those skilled in the art.

As many homogenization cycles as are necessary are applied for as long as the dissolution of the polysaccharide or mixture of polysaccharides in the aqueous medium is not completely satisfactory.

Preferably, a homogenization step according to the present invention can be carried out over a period of less than 200 minutes, preferably less than 150 minutes, or even between 15 and 100 minutes.

As emerges from the aforementioned, the aqueous gel of step a) and/or the mixture of step b) is (are) subjected to at least one extrusion step e).

This extrusion step e) consists in circulating the aqueous gel of polysaccharide of step a) and/or the mixture obtained at the end of step b) through at least one extrusion device which has at least one filter with a porosity ranging from 2 to 100 microns and capable of retaining any particle with a diameter of greater than 100 microns.

The objective of this step is to organize the polysaccharide chains and, if need be, to protect the gel from possible residual particles prejudicial to its homogeneity, before crosslinking step c).

Moreover, this step is also advantageous in that it allows an improvement in the homogenization of the aqueous gel of step a) and/or of the mixture of step b).

It thus contributes to significantly improving the quality of the crosslinked gels obtained at the end of the process of the invention.

As mentioned above, extrusion step e) takes place prior to step c). Thus, it can take place prior to, jointly with and/or after step b) of bringing the aqueous gel of polysaccharide into contact with the crosslinking agent.

According to one preferred embodiment variant, extrusion step e) is carried out after step b) of bringing the aqueous gel of polysaccharide of step a) into contact with the crosslinking agent or, in other words, on the mixture of step b).

Advantageously, step e), in particular when it takes place after step b), is carried out under conditions suitable for keeping the gel in the noncrosslinked state, as defined above.

To do this, step e) is carried out at a temperature of less than 25°C, preferably at a temperature ranging from 15 to 25°C, and better still at ambient temperature.

The implementation of extrusion step e) is part of the general knowledge of those skilled in the art.

Those skilled in the art would be able to define the appropriate characteristics in terms of porosity, geometry, resistance and retention capacity of the filter characterizing the extrusion device in such a way that the latter allows the aqueous gel of step a) and/or the mixture obtained at the end of step b) to pass through, while at the same time retaining particles with a diameter greater than 100 microns.

In particular, the characteristics of the filter are determined from the viewpoint of the molecular weight(s) of the polysaccharide(s) used.

Advantageously, the filter consists of at least one screen or fabric, which is preferably metallic, composite or polymeric in nature.

Advantageously, extrusion step e) is carried out by means of at least one extrusion device comprising at least one filter with a porosity ranging from 5 to 50 microns, better still from 8 to 30 microns, and more particularly with a porosity of 10 microns.

Moreover, the rate at which the aqueous gel of step a) and/or the mixture obtained at the end of step b) passes through said device is also a parameter that is part of the general knowledge of those skilled in the art.

Advantageously, the linear rate of extrusion of the aqueous gel of step a) and/or of the mixture obtained at the end of step b) through the extrusion device(s) is slow so as not to degrade the polysaccharide chains during their passage therethrough.

Thus, the linear rate of extrusion of said aqueous gel of step a) and/or mixture obtained at the end of step b) through the extrusion device(s) is between 1 and 100 cm per minute, preferably between 1 and 4 cm per minute.

This rate depends on the porosity and the nature of the filter, on the polysaccharide under consideration and also on the amount of material to be treated.

By way of extrusion step e), reference may in particular be made to the following protocol.

An aqueous gel of step a) and/or of mixture of step b) can be placed in a system comprising a tank fitted with a piston and, at the outlet of said system, with at least one extrusion device as described above.

The aqueous gel of step a) and/or mixture of step b) is then passed through the extrusion device by placing the piston under pressure, it being possible for the placing under pressure to be carried out manually, mechanically, pneumatically or hydraulically.

According to one particular embodiment, extrusion step e) can result from the use of extrusion devices in a cascade, having filters with different, and preferably decreasing, porosities.

This particular embodiment is particularly advantageous in so far as it makes it possible to achieve crosslinked hydrogels having further improved viscoelastic properties. This is because this cascade system allows the polysaccharide chains to be organized and also allows an extrusion that is gradual and, consequently, less aggressive with respect to the degradation of said polysaccharide chains.

In this respect, extrusion step e) is to be distinguished from a conventional filtration which results, for purification purposes, in the separation of components initially included in the composition to be purified.

As emerges from the aforementioned, the mixture obtained at the end of step b), whether or not it is subjected to an intermediate extrusion step e), is then subjected to conditions suitable for crosslinking.

The objective of the crosslinking is to create bridges between the polysaccharide chains, and in particular hyaluronic acid chains, allowing a solid and dense three dimensional network to be obtained from a viscous solution.

The crosslinking step requires particular conditions which depend simultaneously on the nature of the polysaccharide, on its molecular weight, on the aqueous medium and on the nature of the crosslinking agent.

The term "*conditions*" is intended to denote the component which initiates the crosslinking, such as, for example, heating or UV exposure.

The choice of the initiating means suitable for obtaining a crosslinked gel is part of the general knowledge of those skilled in the art.

Advantageously, an initiating component can be implemented by:

- immersion of the receptacle comprising the "polysaccharide/crosslinking agent" mixture in a bath containing a hot fluid;

- exposure thereof to radiation of certain wavelengths of UV type, for example to microwave radiation or to infrared;

- irradiation by means of ionizing rays, like the process described in US 2008/0139796; and

- enzymatic crosslinking.

Preferably, the crosslinking step is carried out thermally.

The heating means and the intensity of said heating means are of course adjusted from the point of view of the mode of crosslinking, the degree of crosslinking and the viscosity of the gel that are desired.

A temperature particularly suitable for crosslinking step c) depends on the nature of the polysaccharide under consideration.

When the polysaccharide is hyaluronic acid, a temperature particularly suitable for crosslinking step c) is between 25 and 60°C, preferably between 45 and 55°C, and better still between 48 and 52°C.

The degree of crosslinking also depends on the duration of crosslinking to which the mixture obtained at the end of step b) is subjected, whether or not, prior to crosslinking step c), it is subjected to an extrusion step e). The longer the time, the greater the degree of crosslinking will be, with however an optimum not to be exceeded, otherwise there is a risk of the polysaccharide being degraded.

Thus, crosslinking step c) can be carried out over a period ranging from 30 to 300 minutes, preferentially from 100 to 200 minutes, and better still from 150 to 190 minutes.

Advantageously, the crosslinking conditions are adjusted so as to obtain a maximum crosslinking efficiency, i.e. the obtaining of a maximum level of actual crosslinking for a minimum amount of crosslinking agent used.

In other words, the crosslinking conditions are adjusted in order to obtain a degree of crosslinking such that the product formed is viscoelastic, or even solid.

According to one particularly preferred embodiment, crosslinking step c) is carried out in a basic medium, the receptacle containing the corresponding gel, in particular of hyaluronic acid, being placed in a thermostatic bath brought to a temperature of about from 50 to 55°C, for a period at least equal to 1 h 30, and preferably between 2 h 30 and 3 h 10.

As described above, the crosslinking can be stopped (step f)) prior to, jointly with or after step d) of recovering the gel.

Such a step, according to a process according to the invention, requires exposing the crosslinked gel or gel undergoing crosslinking, or even the receptacle containing it, to conditions suitable for stopping said crosslinking, or else to conditions capable of stopping the formation of bonds between the various polysaccharide chains.

According to one preferred embodiment variant, step f) is carried out prior to step d).

For example, in view of the thermal conditions that will have been applied in order to initiate the crosslinking process, the crosslinking can be stopped:

- by simply removing the receptacle from the thermostatic bath, and then by cooling until the temperature returns to ambient temperature;

- by placing the receptacle in a bath of cold water, preferably at a temperature below ambient temperature, until the temperature inside said receptacle is close to ambient temperature; or even

- by extracting the gel from said receptacle.

In the case of crosslinking by radiation, said crosslinking is stopped by stopping the exposure of said gel to the radiation.

According to a further particular embodiment, a process of the invention can be carried out at least partly within a specific receptacle having a deformable wall, such as, for example, a pouch.

This is because the deformability properties of such a receptacle and its hermeticity make it possible to carry out the homogenization and crosslinking steps under optimum conditions which result in an even further improved crosslinked gel being obtained, i.e. a gel having injectability properties superior to those displayed by a gel obtained according to a process using a conventional receptacle of the pot or tank type.

Advantageously, the specific receptacle can be fitted with a closable opening system or gate, suitable for introducing any compound of use in the production of a gel in accordance with the invention.

However, the extrusion step described above requires the aqueous gel of step a) and/or the mixture obtained at the end of step b) to pass through at least one extrusion device. This step consequently implies that said gel and/or mixture is (are) no longer included in the specific receptacle described above.

Thus, in order to preserve the advantages linked to the implementation of the process of the invention within a specific receptacle as described above, and in particular in order to limit any risk of contamination, the presence of the closable opening system or gate as mentioned above on the specific receptacle allows a continuous system. Such a system can, for example, be characterized by use of at least two specific receptacles, the first receptacle, devoted in particular to the preparing of the aqueous gel of polysaccharide of step a) and, where appropriate, of the mixture obtained at the end of step b), and also the homogenization thereof, being connected to the extrusion device, itself connected to the second receptacle,

devoted to the recovery of the aqueous gel of step a) and/or mixture of step b) after the extrusion step, and to the implementation of the step c) for crosslinking and, where appropriate, for stopping the crosslinking.

The crosslinked gel obtained at the end of the process as described above cannot generally be injected directly, in particular because of its polysaccharide concentration is too high and because of the possible presence of crosslinking agent residues or else because of its physiological or pH conditions.

The gel obtained at the end of the implementation of the process as described above can in particular have too great a stiffness to be injected as such into a patient.

Consequently, several additional steps, known to those skilled in the art, can be carried out.

More particularly, a step of neutralizing and expanding this gel is required in order to give it its implant qualities. The chains of the polysaccharide network are thus stretched and hydrated, while the pH is brought to that of the dermis.

A step of protecting and redensifying the gel can also be carried out in order to further improve the qualities of the implant, according to the know-how of those skilled in the art. The gel must be physiologically formulated by virtue of the presence of salts in amounts equivalent to those of the medium injected.

For even higher purity, an additional purification step may also be carried out.

The final step consists in filling syringes with the gel, which is carried out under controlled atmospheric conditions, followed by a final thermal sterilization which takes place immediately after the filling of the syringes.

II) Polysaccharide

The term "polysaccharide" in accordance with the invention is intended to mean any polymer consisting of several monosaccharides linked together by O-glycoside bonds and having the general formula: $-[C_x(H_2O)_y)]_n-$.

A polysaccharide in accordance with the invention is more particularly selected with regard to the properties that it is desired to see the crosslinked gel obtained according to the invention display. More particularly, such a polysaccharide must have good biocompatibility.

Thus, a physiologically acceptable polysaccharide or polysaccharide salt may be of natural or synthetic origin.

The polysaccharide suitable for the invention may in particular be chosen from chondroitin sulphate, keratan, keratan sulphate, heparin, heparin sulphate, xanthan, carrageenan, hyaluronic acid, chitosan, cellulose and derivatives thereof, alginate, starch, dextran, pullulan, galactomannan and biologically acceptable salts thereof.

The polysaccharide salts in accordance with the invention are more particularly chosen from physiologically acceptable salts, such as the sodium salt, the potassium salt, the zinc salt and the silver salt, and mixtures thereof, preferably the sodium salt.

Preferably, a polysaccharide or polysaccharide salt according to the invention has a high molecular weight, preferably a molecular weight greater than or equal to 100 000 Da, better still greater than or equal to 1MDa (or 1×10^6 Da), or even greater than 3 MDa (or 3×10^6 Da), depending on the application under consideration.

One particularly preferred polysaccharide is hyaluronic acid or a salt thereof, preferably sodium hyaluronate (NaHA).

Step a) of a process of the invention consists in providing an aqueous gel of polysaccharide, or of a salt thereof, in a noncrosslinked form.

Advantageously, the polysaccharide is present within the aqueous gel of said polysaccharide in a noncrosslinked form in a content of between 5 and 15% by weight, preferably in a content greater than 10% by weight, relative to the total weight of said aqueous gel.

Such an aqueous gel is obtained by formulation of said polysaccharide in an aqueous medium.

For the purpose of the present invention, the term "*aqueous medium*" is intended to mean any liquid medium containing water and which has the property of dissolving a polysaccharide or a salt thereof.

The nature of the aqueous medium is more particularly dependent on the type of crosslinking envisaged, but also on the type of polymer used.

In this respect, an aqueous medium that may be suitable may be either acidic or basic.

A particularly preferred aqueous medium is an alkaline medium, preferably sodium hydroxide (NaOH), more particularly a solution of sodium hydroxide having a pH greater than 12.

III) **Crosslinking agent**

The term "crosslinking agent" in accordance with the invention is intended to mean any compound capable of inducing crosslinking between the various polysaccharide chains.

The choice of this crosslinking agent in view of the polysaccharide to be crosslinked is clearly within the competence of those skilled in the art.

A crosslinking agent in accordance with the invention is chosen from epoxide, aldehyde, polyaziridyl, divinylsulphone (DVS) and mixtures thereof.

Preferably, a crosslinking agent in accordance with the invention is chosen from epoxy crosslinking agents which are preferably bifunctional or multifunctional, and more particularly chosen from 1,4-butanediol diglycidyl ether (BDDE), diepoxyoctane or 1,2-bis(2,3-epoxypropyl)-2,3-ethylene, 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, 1,2-bis(2,3-epoxypropoxy)ethylene, and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, and mixtures thereof.

Preferably, the crosslinking agent is 1,4-butanediol diglycidyl ether. The adjustment of the amount of crosslinking agent for carrying out the crosslinking reaction is also clearly within the competence of those skilled in the art.

Thus, for the purpose of the present invention, the expression "*effective amount*" is intended to denote an amount sufficient to obtain the expected effect.

According to one particularly preferred embodiment, the process according to the invention uses sodium hyaluronate in an alkaline medium with 1,4-butanediol diglycidyl ether as crosslinking agent.

Throughout the description, including the claims, the expression "comprising a" should be understood as being synonymous with "comprising at least one", unless specifically stated otherwise.

The expressions "between ... and ..." and "ranging from ... to ..." should be understood to mean that the limits are inclusive, unless specified otherwise.

The following example and figure are given by way of nonlimiting illustration of the invention.

FIGURE:

- Figure 1: illustrates the viscoelastic properties of hyaluronic acid gels measured by their elastic modulus (G' in Pa).

EXAMPLE

For each of the crosslinked gels described hereinafter, 10 g of hyaluronic acid (1.5 MDa), 73 g of 1% sodium hydroxide and 1.2 g of butanediol diglycidyl ether (BDDE) are used. The crosslinking is brought about by incubating for 3 hours at 52°C.

The particular conditions for each gel are the following:

Product A (control)

The preparation protocol is as follows:

1. Homogenization at ambient temperature of the "hyaluronic acid + 1% sodium hydroxide" mixture for about 1 h 30 in order to obtain a perfectly homogeneous viscous solution;

2. addition of the crosslinking agent (BDDE) and further homogenization at ambient temperature for about 20 min;

3. incubation of the hyaluronic acid/1% sodium hydroxide/BDDE viscous solution for 3 hours at 52°C so as to initiate the crosslinking step; and

4. neutralization, swelling and homogenization of the solid obtained (crosslinked hyaluronic acid solution) in an acidified phosphate buffer solution so as to obtain a hydrogel containing 20 mg/g of hyaluronic acid, with a pH close to neutrality.

Products B and B' (in accordance with the invention)

The protocol is the same as that described for product A, but carried out with, after step 2 of adding the BDDE and 15 min of homogenization, an extrusion step, at ambient temperature, consisting in circulating the mixture obtained at the end of step 2 through an extrusion device comprising a filter, respectively, of 10 microns (product B) and 50 microns (product B').

This extrusion step is carried out over a maximum period of 5 minutes.

The mixture obtained at the end of step 2 is thus placed in a tank, the outlet of which is a cylindrical section fitted with a screen filter with a porosity of, respectively, 10 and 50 µm, and a diameter of 55 mm, in particular a screen filter such as those sold by the company Rubber Fab, under the name "Platinum Screen Gasket".

The pressure applied to the mixture, produced by means of a compressed air piston, is adjusted such that the linear rate of extrusion is about 4 cm/min, which represents a flow rate of about 100 cm^3/min.

All of the products A, B and B' are packaged in syringes. The viscoelastic properties of these products are measured using a rheometer (Haake RS6000) with a cone/plate geometry (1°/ 35 mm diameter). A stress sweep is carried out, and the elastic modulus G' (in Pa) and the phase shift angle δ (°) are measured at 1 Hz for a stress of 5 Pa.

Results

Figure 1 hereinafter represents the values of the elastic moduli G' (in Pa). By way of indication, the value of the phase shift angle δ (°) is indicated at the top of each column, to the right of the G' value.

The results for products B and B' are similar.

The phase shift angle measured is extremely small, which means that these gels exhibit a mainly elastic (solid) nature.

However, in view of the results in Figure 1, a significant effect on the increase in G', of the pre-crosslinking extrusion step, is noted.

CLAIMS

1. Process for preparing a crosslinked gel of at least one polysaccharide or a salt thereof, comprising at least the steps consisting in:

a) providing at least one aqueous gel of said polysaccharide in a noncrosslinked form,

b) bringing said aqueous gel into contact with an effective amount of at least one crosslinking agent,

c) subjecting the mixture formed in step b) to conditions suitable for the crosslinking of said polysaccharide, and, if need be,

d) recovering said crosslinked hydrogel,

wherein said process comprises, prior to step c), at least one step e) consisting in circulating the aqueous gel of polysaccharide of step a) and/or the mixture of step b) through at least one extrusion device which has at least one filter with a porosity ranging from 2 to 100 microns and capable of retaining any particle with a diameter greater than 100 microns.

2. Process according to Claim 1, wherein the polysaccharide is hyaluronic acid or a salt thereof.

3. Process according to Claim 1 or 2, wherein step e) is carried out on the mixture of step b).

4. Process according to any one of the preceding claims, wherein the filter has a porosity ranging from 5 to 50 microns, better still from 8 to 30 microns, and more particularly a porosity of 10 microns.

5. Process according to any one of the preceding claims, wherein the linear rate of extrusion of the aqueous gel of step a) and/or of the mixture of step b) through the device capable of retaining any particle with a diameter greater than 100 microns is between 1 and 100 cm per minute, preferably between 1 and 4 cm per minute.

6. Process according to any one of the preceding claims, wherein the aqueous gel of polysaccharide of step a) and/or the mixture of step b) undergoes at least one homogenization, prior to step c).

7. Process according to the preceding claim, wherein the homogenization step is carried out over a period of less than 200 minutes, preferably less than 150 minutes, or even between 15 and 100 minutes.

8. Process according to any one of the preceding claims, wherein crosslinking step c) is carried out thermally at a temperature between 25 and 60°C, preferably between 45 and 55°C, and better still between 48 and 52°C.

9. Process according to any one of the preceding claims, wherein crosslinking step c) is carried out over a period ranging from 30 to 300 minutes, preferentially from 100 to 200 minutes, and better still from 150 to 190 minutes.

10. Process according to any one of the preceding claims, wherein the crosslinking agent is chosen from epoxy crosslinking agents which are preferably bifunctional or multifunctional.

11. Process according to any one of the preceding claims, wherein the crosslinking agent is chosen from 1,4-butanediol diglycidyl ether (BDDE), diepoxyoctane or 1,2-bis(2,3-epoxypropyl)-2,3-ethylene, 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, 1,2-bis(2,3-epoxypropoxy)ethylene, and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, and mixtures thereof, preferably 1,4-butanediol diglycidyl ether.

12. Process according to any one of the preceding claims, wherein the polysaccharide has a molecular weight greater than or equal to 1 MDa.

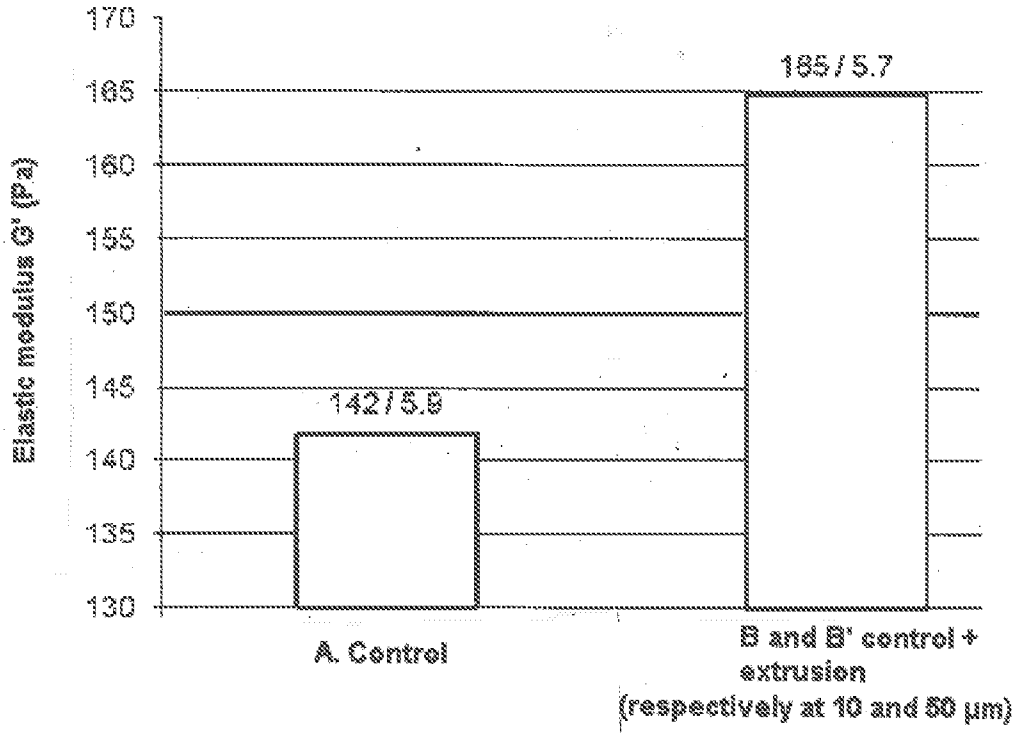
13. Process according to any one of the preceding claims, wherein the polysaccharide is present within the aqueous gel of said polysaccharide in a noncrosslinked form in a content of between 5 and 15% by weight, preferably greater than 10% by weight, relative to the total weight of said aqueous gel.

14. Process according to any one of the preceding claims, wherein it uses sodium hyaluronate in an alkaline medium, and 1,4-butanediol diglycidyl ether (BDDE) as crosslinking agent.

15. Process according to any one of the preceding claims, wherein the crosslinked gel is a predominantly elastic single-phase gel.

16. Use of a crosslinked gel obtained by implementing a process as defined according to any one of the preceding claims, for the long-lasting filling of volume defects of the skin, and in particular the filling of wrinkles.

Figure 1


170
165
160
155
150
145
140
135
130
Elastic modulus G' (Pa)
142 / 5.6
165 / 5.7
A. Control
B and B' control + extrusion (respectively at 10 and 50 µm)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/055496

A. CLASSIFICATION OF SUBJECT MATTER
INV. C08B37/00 A61K8/73 C08J3/075
ADD.

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)
C08B A61K C08J

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)
EPO-Internal, BIOSIS, COMPENDEX, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 716 224 A (SAKURAI KATUKIYO [JP] ET AL) 29 December 1987 (1987-12-29) examples column 4, line 4 - line 25	1-16
A	US 7 829 118 B1 (GRAVETT DAVID M [US] ET AL) 9 November 2010 (2010-11-09) examples	1-15
X	US 2009/143331 A1 (STROUMPOULIS DIMITRIOS [US] ET AL) 4 June 2009 (2009-06-04) examples	16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2012	02/03/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Vaccaro, Eleonora

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2011/055496

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		KR 20100117563 A	03-11-2010
		US 2009143331 A1	04-06-2009
		US 2010004198 A1	07-01-2010
		WO 2009073437 A1	11-06-2009