



US 20060194758A1

(19) **United States**  
 (12) **Patent Application Publication** (10) **Pub. No.: US 2006/0194758 A1**  
**Lebreton** (43) **Pub. Date: Aug. 31, 2006**

(54) **CROSS-LINKING OF LOW AND HIGH MOLECULAR WEIGHT POLYSACCHARIDES PREPARATION OF INJECTABLE MONOPHASE HYDROGELS AND POLYSACCHARIDES AND HYDROGELS THUS OBTAINED**

(76) Inventor: **Pierre Lebreton**, Annecy Le Vieux (FR)

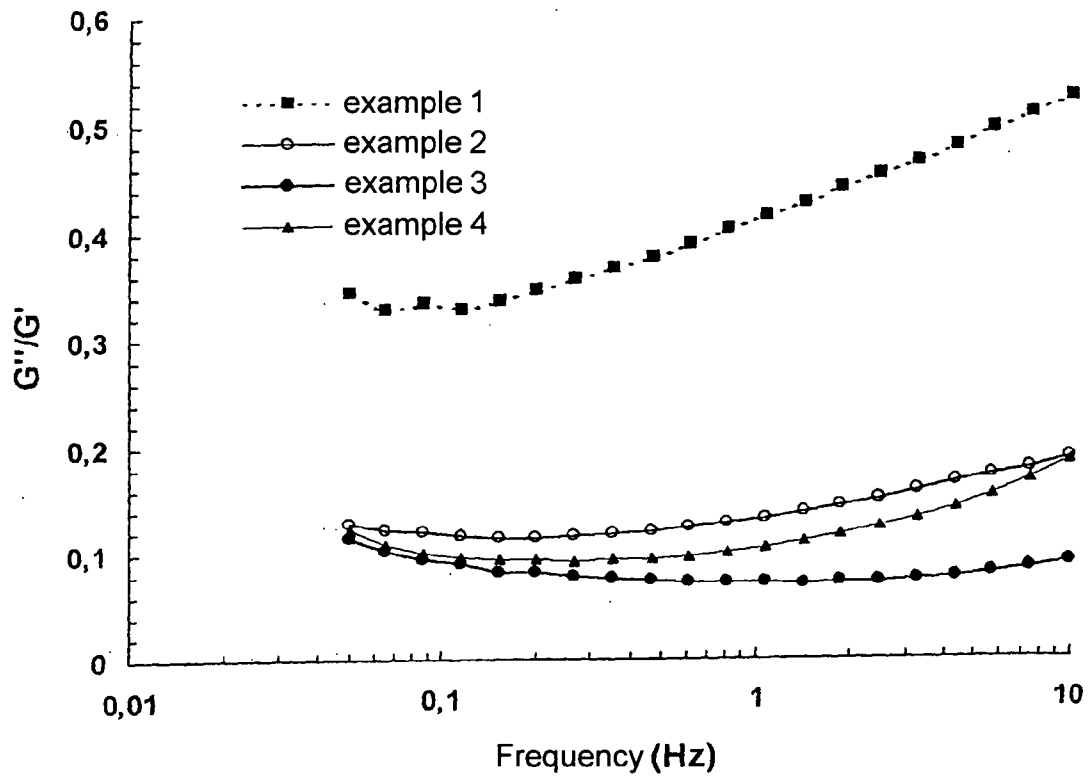
Correspondence Address:  
**COHEN, PONTANI, LIEBERMAN & PAVANE**  
**551 FIFTH AVENUE**  
**SUITE 1210**  
**NEW YORK, NY 10176 (US)**

(21) Appl. No.: **10/552,309**  
 (22) PCT Filed: **Apr. 8, 2004**  
 (86) PCT No.: **PCT/FR04/00870**

(30) **Foreign Application Priority Data**  
 Apr. 10, 2003 (FR)..... 0304444

**Publication Classification**  
 (51) **Int. Cl.**  
*A61K 31/728* (2006.01)  
*C08B 37/00* (2006.01)  
 (52) **U.S. Cl.** ..... **514/54; 536/53**

(57) **ABSTRACT**  
 A process for the crosslinking of at least one polymer selected from polysaccharides and derivatives thereof, which is carried out in an aqueous solvent by the action of an effective and non-excessive amount of at least one crosslinking agent, characterized in that it is carried out on a mixture containing at least one low-molecular weight polymer and at least one high-molecular weight polymer. A process for the preparation of an injectable monophasic hydrogel of at least one crosslinked polymer selected from polysaccharides and derivatives thereof is also disclosed. Crosslinked polymers and injectable monophasic hydrogels, respectively, are obtainable by each of said processes.



**CROSS-LINKING OF LOW AND HIGH  
MOLECULAR WEIGHT POLYSACCHARIDES  
PREPARATION OF INJECTABLE MONOPHASE  
HYDROGELS AND POLYSACCHARIDES AND  
HYDROGELS THUS OBTAINED**

[0001] The present invention relates to:

[0002] a novel process for the crosslinking of at least one polymer selected from polysaccharides and derivatives thereof;

[0003] a process for the preparation of an injectable monophasic hydrogel of at least one such polymer; and

[0004] the crosslinked polymers and injectable monophasic hydrogels respectively obtainable by each of said processes.

[0005] The hydrogels in question, based on said crosslinked polymers, have numerous outlets, especially as filling materials in plastic, cosmetic and dental surgery, in ophthalmology, in orthopedics, etc., as products for preventing tissue adhesions, in general surgery, in urology, etc. Said hydrogels are particularly suitable for repairing vocal cords. The outlets indicated above for products of this type, without implying any limitation, are familiar to those skilled in the art.

[0006] The invention is the result of a genuine effort to optimize the operation of crosslinking the polymers in question with a view to obtaining injectable monophasic hydrogels that are of particular value in respect of the following compromise: on the one hand mechanical properties and remanence, and on the other hand injectability (with acceptable injection forces and injection needle diameters).

[0007] It is pointed out here that the term “injectable” employed in the present text, with reference to both the hydrogels of the prior art and the hydrogels of the invention, denotes manual injectability by means of syringes equipped with conventional needles (having a diameter of between 0.1 and 0.5 mm). Within the framework of the present invention, it is possible in particular to formulate hydrogels that can be injected through hypodermic needles of 30 G $\frac{1}{2}$ , 27 G $\frac{1}{2}$ , 26 G $\frac{1}{2}$  and 25 G.

[0008] According to the prior art, hydrogels, especially injectable hydrogels, have already been prepared from polysaccharides and derivatives thereof—especially hyaluronic acid salts—having a zero, low or high degree of crosslinking.

[0009] With reference to the specific problem of injectability, biphasic compositions have been proposed whose continuous phase, in particular, is based on such hydrogels. The continuous phase serves as a plasticizer, injection vehicle for a disperse phase. This disperse phase is more or less solid and more or less differentiated from the continuous phase. Thus:

[0010] the biphasic compositions described in patent application EP-A-0 466 300 consist of two bioabsorbable phases—continuous and disperse—and take the form of slurries. Said two phases are advantageously prepared from

[0011] the biphasic compositions described in patent application WO-A-96 337 51 also have two bioabsorbable phases with a better separation, the disperse phase consisting of insoluble fragments of a highly crosslinked polymer hydrogel (selected from hyaluronic acid and its salts);

[0012] the biphasic compositions described in patent application WO-A-00 014 28 contain a non-bioabsorbable disperse phase (particles of at least one hydrogel of a (co)polymer obtained by the polymerization and crosslinking of acrylic acid and/or methacrylic acid and/or at least one derivative of said acids) suspended in an aqueous solution of a crosslinked or non-crosslinked polymer selected from proteins, polysaccharides and derivatives thereof.

[0013] These biphasic systems are not fully satisfactory insofar as they are associated with justifiable fears of uneven flow during injection and particularly after injection, a more rapid disappearance of the continuous phase (having a zero or low degree of crosslinking) and hence an at least partial loss of the desired effect, especially filling effect.

[0014] Monophasic hydrogels, developed from the same types of polymers, were therefore also proposed in parallel.

[0015] In patent applications WO-A-98 356 39 and WO-A-98 356 40, the product in question is not an injectable hydrogel but a product of solid consistency. Said patent applications in fact describe ocular implants used to temporarily fill a surgically created void. The hydrogel developed in U.S. Pat. No. 4,716,154 is proposed as a substitute for the vitreous body. The polymer in question (sodium hyaluronate) has a very low degree of crosslinking in order to obtain an injectable hydrogel. The monophasic hydrogel described in patent application WO-A-02 057 53 is laden with an antiseptic that is effective in protecting it from free radicals after implantation. Patent application WO-A-02 063 50 describes a process capable of generating this type of hydrogel that is very homogeneous throughout.

[0016] All these monophasic hydrogels were obtained from high-molecular weight polymers crosslinked using an effective and non-excessive amount of at least one crosslinking agent, in an aqueous solvent.

[0017] In the light of this prior art, the inventors wished to improve the efficacy of crosslinking of the polymer in question, especially in order to improve the degradation resistance (remanence) of the implanted hydrogel while at the same time preserving the possibility of injecting said hydrogel under acceptable conditions.

[0018] To improve the crosslinking efficacy, the inventors initially considered using more crosslinking agent. This approach was quickly discarded on the grounds that it inescapably causes denaturation of the polymer in question and chemical contamination of the crosslinked product obtained.

[0019] Said inventors then considered increasing the concentration of polymer in the reaction mixture. In the same way, this second approach had to be discarded, a priori, because of the polymers conventionally used hitherto, namely high-molecular weight polymers. Thus sodium hyaluronate is always used with high molecular weights (Mw > 10<sup>6</sup> Da,  $\approx$  2.10<sup>6</sup> Da, 3.10<sup>6</sup> Da) at concentrations close to

the reaction mixture becomes too high) and inescapably causes problems of solubility, poor homogeneity, etc.

[0020] Concentrating the reaction medium, on the other hand, is found to be possible with low-molecular weight polymers (sodium hyaluronate of molecular weight 300,000 Da, having an intrinsic viscosity of 600 ml/g (those skilled in the art are perfectly familiar with the relationship between these two parameters: molecular weight (M) and intrinsic viscosity ( $\eta$ ), which is given by the Mark-Houwink formula:  $M=k\eta^\alpha$ , the values of k and  $\alpha$  depending on the nature of the polymer in question), can be concentrated from 110 to 200 mg/g). Unfortunately the crosslinked polymer obtained generates an inhomogeneous, injectable biphasic hydrogel under these conditions.

[0021] In such a context, the inventors surprisingly established that associating low-molecular weight polymer(s) with high-molecular weight polymer(s) affords an excellent compromise, namely the possibility of generating, for a non-excessive degree of crosslinking (equivalent to that of the prior art), an injectable monophasic hydrogel which has improved mechanical and remanence properties. This low-molecular weight/high-molecular weight association makes it possible to obtain a hydrogel that more than satisfies the following specifications:

[0022] monophasic;

[0023] better mechanical properties and remanence than the equivalent products of the prior art;

[0024] unaffected or even improved injectability that is still possible with conventional injection forces using conventional injection devices.

[0025] The key factor of the crosslinking process of the invention therefore lies in the concentration of the reactants (which is greater than that of the reaction mixtures of the prior art due to the use of low-molecular weight polymer(s)), although the crosslinking of said concentrated reactants is "governed" by the use of high-molecular weight polymer(s), which guarantee the homogeneity of the crosslinked product obtained and then of the hydrogel obtained.

[0026] According to its first subject, the present invention therefore relates to a process for the crosslinking of at least one polymer selected from polysaccharides and derivatives thereof, which is carried out in an aqueous solvent by the action of an effective and non-excessive amount of at least one crosslinking agent, said process being improved in that it is carried out on a mixture containing at least one low-molecular weight polymer and at least one high-molecular weight polymer.

[0027] Said mixture of course contains said low-molecular weight polymer(s) in a sufficient amount to guarantee a relatively high concentration of polymer(s) in the reaction medium, and said high-molecular weight polymer(s) in a sufficient amount to guarantee that said crosslinked polymer obtained has a homogeneous consistency.

[0028] The crosslinking process of the invention is a process for the crosslinking of polymers selected from polysaccharides and derivatives thereof. The polymer(s) in question can therefore be natural or synthetic. Examples of natural polymers are hyaluronic acid and its salts, other

biologically acceptable salts, starch, amylose, dextran, xanthan, pullulan, etc. Examples of synthetic derivatives of natural polysaccharides are carboxy cellulose, carboxymethyl cellulose, alkyl celluloses such as hydroxyethyl cellulose and hydroxypropyl methyl cellulose (HPMC), oxidized starch, etc.

[0029] The process of the invention is suitable for the crosslinking of any one of these polymers insofar as said polymer is used with low and high molecular weights.

[0030] The process of the invention is suitable for the crosslinking of mixtures of such polymers, said mixtures containing at least one low-molecular weight polymer and at least one high-molecular weight polymer.

[0031] The terms "low" and "high" applied to the molecular weights in question obviously cannot be defined more precisely at this stage of the description of the invention since they depend on the mixture in question and the nature of the polymer(s) present. Likewise, it is not generally possible to indicate the relative proportions in which the polymer(s) present is(are) used. However, those skilled in the art have a perfect understanding of the spirit of the invention, which is to concentrate the reaction medium containing the low-molecular weight polymer(s), but to introduce at least one high-molecular weight polymer to moderate and control the crosslinking in question. The aim is to obtain a coherent crosslinked product that is the precursor of a monophasic hydrogel. It is desirable to avoid the formation of lumps that may be coherent when crosslinking has ended, but capable of losing their coherence when the injectable hydrogel is prepared.

[0032] The above explanations are given a posteriori. The result obtained was in no way predictable.

[0033] Within the framework of one advantageous variant, the reaction medium contains a single polymer which is used with at least two differentiated molecular weights, at least one being low and at least one being high. Within the framework of this advantageous variant, the same polymer is preferably used with a single low molecular weight and a single high molecular weight.

[0034] The polymer in question is advantageously a hyaluronic acid salt. It is very advantageously selected from the sodium salt, the potassium salt and mixtures thereof. It preferably consists of the sodium salt (NaHA).

[0035] In the context of the crosslinking of this type of polymer, those skilled in the art understand that said crosslinking is carried out in a basic aqueous solvent. In general, said crosslinking is obviously carried out under pH conditions that favor the dissolution of the polymer in question.

[0036] In the context of the crosslinking of this type of polymer (hyaluronic acid salt(s)), in one preferred variant of carrying out the crosslinking, the reaction mixture contains:

[0037] at least one hyaluronic acid salt of low molecular weight m, where  $m \leq 9.9 \cdot 10^5$  Da, advantageously  $10^4$  Da  $\leq m \leq 9.9 \cdot 10^5$  Da; and

[0038] at least one hyaluronic acid salt of high molecular weight M, where  $M \geq 10^6$  Da, advantageously  $10^6$

said low-molecular weight and high-molecular weight salts advantageously being of the same nature and very advantageously consisting of sodium hyaluronate (NaHA).

[0039] In such a context, said reaction mixture advantageously has an intrinsic viscosity of less than 1900 ml/g, i.e.  $\sum \omega_i [\eta]_{i,0} \leq 1900$  ml/g, where  $\omega_i$  is the mass fraction of polymer fraction  $i$ , having an intrinsic viscosity  $[\eta]_{i,0}$ , in the reaction mixture. Those skilled in the art are familiar with the intrinsic viscosity parameter and are aware of the laws of additivity of said parameter.

[0040] The condition stated above makes it possible to obtain a monophasic hydrogel that is optimized in respect of its remanence and injectability properties. It fixes the relative proportions of the salts of low molecular weight ( $m$ ) and high molecular weight ( $M$ ).

[0041] In the context referred to here (NaHA of molecular weights  $m$  and  $M$ ), the reaction mixture advantageously contains more than 50% by weight, very advantageously more than 70% by weight, of at least one hyaluronic acid salt of low molecular weight  $m$ , and hence, logically, advantageously less than 50% by weight, very advantageously less than 30% by weight, of at least one hyaluronic acid salt of high molecular weight  $M$ .

[0042] In general, to obtain the expected effect, there is at least 5% by weight of at least one hyaluronic acid salt of high molecular weight  $M$  in the reaction mixture.

[0043] The crosslinking process of the invention is advantageously carried out with the sodium salt of hyaluronic acid used with one low molecular weight  $m$  and one high molecular weight  $M$ , said parameters then very advantageously being as follows:  $m \approx 3 \cdot 10^5$  Da and  $M \approx 3 \cdot 10^6$  Da.

[0044] Any agent known for crosslinking polysaccharides and derivatives thereof via its hydroxyl groups can be used as the crosslinking agent with all types of polymer, said crosslinking agent being at least bifunctional in order to ensure crosslinking, an epoxy compound or derivatives thereof being used in particular.

[0045] It is recommended to use bifunctional crosslinking agents, by themselves or in a mixture. It is particularly recommended to use epichlorohydrin, divinyl sulfone, 1,4-bis(2,3-epoxypropoxy)butane (or 1,4-bisglycidoxybutane or 1,4-butanediol diglycidyl ether (BDDE)), 1,2-bis(2,3-epoxypropoxy)ethylene, 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, and aldehydes such as formaldehyde, glutaraldehyde and crotonaldehyde, taken by themselves or in a mixture. It is very particularly recommended to use 1,4-bis(2,3-epoxypropoxy)butane (BDDE).

[0046] Those skilled in the art will know how to determine the effective and non-excessive amount of crosslinking agent(s) to use. It is recommended to use an effective and non-excessive amount such that the degree of crosslinking ( $\tau$ ), defined by the following ratio:

$$\tau = \frac{\text{Total number of reactive groups in said crosslinking agent}}{\text{Total number of disaccharide units in the polymer molecules}} \times 100,$$

[0047] The crosslinking process of the invention is novel by virtue of the forms in which the polymers in question are used. In other respects it is carried out in conventional manner with at least one crosslinking agent. It is noted that said crosslinking agent is generally reacted with the dissolved polymer(s), but reacting it with said polymer(s) during hydration, by the process described in WO-A-02 06 350, is in no way ruled out.

[0048] The crosslinked product obtained after carrying out the crosslinking process of the invention is generally formulated for generating the desired injectable monophasic hydrogel. If necessary, it is neutralized beforehand. It has been seen that the hyaluronic acid salts are actually crosslinked in a basic medium. The formulation is carried out in a solution buffered to a pH compatible with the human body (since the hydrogel in question is generally intended for injection into the human body), said pH being between 6.5 and 7.5, advantageously between 7 and 7.4 and very advantageously between 7.1 and 7.3. The crosslinked polymer is in equilibrium in said solution. It also acquires an osmolarity compatible with that of the human body. Surprisingly, after this formulation step, the diluted crosslinked polymers of the invention are monophasic hydrogels.

[0049] In one preferred variant of carrying out the invention, an injectable hydrogel of the invention is prepared by crosslinking a mixture of at least one polymer consisting of hyaluronic acid salt(s) (see above), neutralizing the crosslinked product obtained, and then formulating it into a solution buffered to a pH of between 7.1 and 7.3, at a concentration of between 10 and 40 mg/g, advantageously of between 20 and 30 mg/g.

[0050] The process for the preparation of the injectable monophasic hydrogel from the crosslinked polymer (obtained by the crosslinking process constituting the first subject of the present invention) constitutes the second subject of the present invention.

[0051] We now come to the third and fourth subjects, which respectively consist of the crosslinked polymer obtainable after carrying out the crosslinking process (first subject), and the injectable monophasic hydrogel obtainable by the formulation (second subject) of said crosslinked polymer, as stated above.

[0052] Said polymer and hydrogel advantageously contain low-molecular weight sodium hyaluronate and high-molecular weight sodium hyaluronate, the proportion of said low-molecular weight sodium hyaluronate very advantageously being more than 50% by weight.

[0053] The structure of the injectable monophasic hydrogel—fourth subject of the present invention—is novel. Its consistency is resistant to degradation. This resistance of the hydrogel is far greater than that of the equivalent products of the prior art.

[0054] Those skilled in the art are aware that one of the methods of estimating the consistency of a hydrogel, especially of this type, is to measure the following parameter:

$$\tan \delta = \frac{G''}{G'} = f(\text{stressing frequency}).$$

[0055] The hydrogels of the invention have the outlets

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.