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(54) Title: TEMPERATURE-CONTROLLED AND PH-DEPENDANT SELF-GELLING BIOPOLYMERIC AQUEOUS SOLUTION

(57) Abstract: The present invention relates a biopolymeric liquid aqueous composition for producing self-gelling systems and gels, which comprises: an acidic water-based medium, 0.1 to 10 % by weight of a pH-gelling acid-soluble biopolymer; and 0.1 to 10 % by weight of a water-soluble molecule having a basic character and a pKa between 6.0 and 8.4, or a water-soluble residue or sequence of the molecule having a basic character and a pKa between 6.0 and 8.4. The liquid composition has a final pH ranging from 5.8 and 7.4, and forms a stable solid and homogeneous gel within a temperature range from 10 to 70 °C. The present invention also relates to a method for preparing the composition and uses thereof.

TEMPERATURE-CONTROLLED AND PH-DEPENDANT SELF-GELLING BIOPOLYMERIC AQUEOUS SOLUTION

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The present invention relates to the composition of molecular assemblies in liquid solution that enables temperature-controlled pH-dependant formation of biopolymeric gels, such as polysaccharide-based, and methods of preparation thereof.

(b) Description of Prior Art

Biopolymers and macromolecules are attractive materials for the preparation and design of self-gelling and/or auto-assembling systems. Numerous attempts tend to develop such systems on the basis of polysaccharides and polypeptides.

In situ formed gels were also proposed with ionic polysaccharides. A composition can be used as a medical device for drug delivery, the application of a diagnostic agent, or the prevention of post-operative adhesions, and is composed of an aqueous liquid vehicle which is capable of being gelled *in situ*. It includes at least one ionic polysaccharide, at least one film forming polymer, and a medicament or pharmaceutical agent, water, and optionally, a counter-ion capable of gelating the ionic polysaccharide. However, the gelation is reached by interaction between the ionic polysaccharide and the film-forming polymer, or by counter-ion induced cross-linking of the ionic polysaccharide. Other *in situ* forming gels are based upon polyoxyalkylene composition or polyoxyalkylene/ polysaccharide mixture or alginate/cation mixture *in situ*.

It would be highly desirable to be provided with a biopolymeric gel that is formed while excluding any organic solvent, any organic monomers, any ionic or covalent cross-linking that may be potentially toxic or induce a reduced biological compatibility.

It would be highly desirable to be provided with a biopolymeric gel that is formed by stimulus-induced free interactions between biologically acceptable and well-recognized molecules.

It would be highly desirable to be provided with, a temperature-controlled pH-dependant formed biopolymeric gels that could be used to

encapsulate cells and cellular material while retaining their biological activity.

It would be highly desirable to be provided with such gels, which would retain its solid or gel state at the physiological temperature or 37°C.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a way allowing the preparation of a neutral clear liquid solution of a pH-controlled acid-soluble biopolymer while avoiding any unwanted precipitation or heterogeneous gelation.

A second major aim of the present invention is to provide a neutral clear liquid solution of a pH-controlled acid-soluble biopolymer that will thermally form solid homogeneous gels at a temperature close to the physiological temperature.

Another aim is to provide temperature-controlled pH-dependant formed gels, which could be used to encapsulate cells and cellular material while retaining their biological activity.

A further aim of the present invention is to provide gels that would retain its solid or gel state at the physiological temperature or 37°C.

Still one aim of the present invention is to provide a method for the preparation of such gels.

In accordance with the present invention, there is provided a biopolymeric liquid aqueous composition for producing self-gelling systems and gels, which comprises:

a) an acidic water-based medium; and

b) 0.1 to 10% by weight of a pH-gelling acid-soluble biopolymer; and

c) 0.1 to 10% by weight of a water-soluble molecule having a basic character and a pKa between 6.0 and 8.4, or a water-soluble residue or sequence of the molecule having a basic character and a pKa between 6.0 and 8.4;

wherein the liquid composition has a final pH ranging from 5.8 and 7.4, and forms a stable solid and homogeneous gel within a temperature range from 10 to 70°C.

The composition can be prepared from organic and/or inorganic acids, such as hydrochloric acid, citric acid, ascorbic acid, lactic acid, lactobionic acid, acetic acid, salicylic acid, formic acid, glutamic acid, phosphoric acid, orthophosphoric acid, or glycerophosphoric acid, or a mixture thereof.

The biopolymer preferably comprises a pH-gelling acid-soluble polysaccharide, polypeptidic or poly(amino acids), or synthetic polymer, such as a solution of chitosan, modified chitosan or chitosan derivative, the solution of chitosan being cationic and bearing amino groups.

The molecule, residue or sequence may be an organic salt selected from the group consisting of mono-phosphate salt, mono-sulfonate salt, mono-sulfate salt and mono-carboxylate salt.

Alternatively, the molecule, residue or sequence may be a salt of polyol selected from the group consisting of mono-phosphate dibasic salt, mono-sulfonate salt, mono-sulfate salt and mono-carboxylate salt of polyol, said polyol being selected from the group consisting of glycerol, histidinol, acetol, diethylstil-bestrol, indole-glycerol, sorbitol, ribitol, xylitol, arabinitol, erythritol, inositol, mannitol, glucitol, palmitoyl-glycerol, linoleoyl-glycerol, oleoyl-glycerol, and arachidonoyl-glycerol, or a mixture thereof.

The glycerol may also be selected from the group consisting of glycerol-2-phosphate, sn-glycerol 3-phosphate and L-glycerol-3-phosphate salt, or a mixture thereof.

In a further embodiment, the molecule, residue or sequence is a salt of a sugar selected from the group consisting of mono-phosphate dibasic salt, mono-sulfonate salt, mono-sulfate salt and mono-carboxylate salt of a sugar, said sugar being selected from the group consisting of fructose, galactose, ribose, glucose, xylose, rhamnulose, sorbose, erythrulose, deoxy-ribose, ketose, mannose, arabinose, fuculose, fructopyranose, ketoglucose, sedoheptulose, trehalose, tagatose, sucrose, allose, threose, xylulose, hexose, methylthio-ribose, and methylthio-deoxy-ribulose, or a mixture thereof.

The molecule, residue or sequence may be selected from the group consisting of sodium, magnesium or iron salt of glycerol-2-phosphate, sn-glycerol-3-phosphate and L-glycerol-3-phosphate, glucose-1-

phosphate, glucose-6-phosphate, fructose-1-phosphate and fructose-6-phosphate, or a mixture thereof.

The molecule, residue or sequence is preferably a sodium, magnesium or iron salt selected from the group consisting of N-[carbamoylmethyl]-2-aminoethane sulfonate (ACES), N,N-bis[2-hydroxyethyl]-2-aminoethane sulfonate (BES), 3-[N,N-bis(2-hydroxy-ethyl)amino]-2-hydroxypropanesulfonate (DIPSO), N-[2-hydroxyethyl]piperazine-N'-3-propane-sulfonate (EPPS), N-[2-hydroxyethyl]piperazine-N'-4-butane-sulfonate (HEPBS), N-[2-hydroxyethyl]piperazine-N'-3-propanesulfonate (HEPES), N-[2-hydroxyethyl] piperazine-N'-2-hydroxypropanesulfonate (HEPSO), 2-[N-morpholino]ethanesulfonate (MES), 4-[N-morpholino]butanesulfonate (MOBS), 3-[N-morpholino]butanesulfonate (MOPS), 3-[N-morpholino]-2-hydroxypropanesulfonate (MOPSO), Piperazine-N,N'-bis[2-ethanesulfonate] (PIPES), Piperazine-N,N'-bis[2-hydroxypropanesulfonate] (POPSO), 3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate (TAPSO), and N-tris [hydroxymethyl]methyl-2-minoethanesulfonate(TES), and derivatives or mixtures thereof.

The molecule, residue or sequence is preferably selected from the group consisting of N,N-bis[hydroxyethyl]glycine (BICINE), bis [2-hydroxyethyl]iminotris [hydroxymethyl]methane (BIS-TRIS), Glycyl-glycine (GLY-GLY), Triethanolamine (TEA), N-tris [hydroxymethyl]methylglycine (TRICINE), and Tris [hydroxymethyl]aminomethane (TRIZMA), and derivatives or mixtures thereof.

Still in another embodiment, the molecule, residue or sequence has either one acid group and at least one amino group, or more amino groups than acid groups. The molecule, residue or sequence may also be an amino-acid residue, an amino-acid sequence or a poly(amino acids) having a basic character and a pKa between 6.0 and 8.4.

Examples of amino acid residue can be histidine (HIS), arginine (ARG), lysine (LYS), asparagine (ASP), and glutamine (GLN), or a mixture thereof. The amino acid residue may further be modified with a radical acetyl, t-butyl, benzyl, benzoyl, ethyl, formyl, or methyl.

The molecule, residue or sequence is alternatively a sequence, derivative or polymer of at least one amino acid selected from the group

consisting of alanine (ALA), histidine (HIS), arginine (ARG), lysine (LYS), aspartic acid (ASP), glutamine (GLN), glycine (GLY), hydroxyproline (HYP), isoleucine (ILE), leucine (LEU), norleucine (NLE), phenylalanine (PHE), proline (PRO), serine (SER), threonine (THR), tyrosine (TYR), and valine (VAL).

Preferably, the composition further comprises at least one other water-soluble polymer, such as collagen, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxyethyl propylcellulose, hydroxymethyl propyl cellulose, polyethylene oxide, polypropylene oxide, poly(ethylene oxide-co-propylene oxide) copolymers, poly(ethylene oxide-co-propylene oxide-co-ethylene oxide) copolymers, polyvinyl alcohol, or polycaprolactone diols, and derivatives or mixtures thereof.

The composition of the present invention may further comprises a solid particulate or a water-soluble additive, such as a drug or a pharmaceutical agent, microorganisms, plant cells, animal cells or human cells dispersed therein.

The composition of the present invention may be used as a carrier for delivering a pharmaceutical agent *in situ*.

Still in accordance with the present invention, there is provided a method for preparing a composition as defined above. The method comprises the steps of:

a) dissolving a pH-gelling acid-soluble biopolymer within an aqueous acidic solution of a pH from about 1.0 to about 5.0 to obtain an aqueous biopolymer composition having a concentration of 0.1 to 5% by weight of the biopolymer;

b) dissolving 0.1 to 10% by weight of a water-soluble molecule having a basic character and a pKa between 6.0 and 8.4, or a water-soluble residue or sequence of the molecule having a basic character and pKa between 6.0 and 8.4, within the aqueous biopolymer composition to obtain a clear liquid formulation with a pH ranging between 5.8 and 7.4;

c) heating the liquid formulation at a temperature above 30°C to obtain a solid gel, the gel having a pH from about 5.8 to about 7.4.

The composition of the present invention may be used in cosmetics, pharmacology, medicine and/or surgery, into an implantable device or an implant for repair, reconstruction and/or replacement of

tissues and/or organs, as an implantable, transdermal or dermatological drug delivery system, as an opthalmological implant or a drug delivery system, or in cells-loaded artificial matrices for engineering and culture of bioengineered hybrid materials and tissue equivalents.

The composition may be loaded with cells selected from the group consisting of chondrocytes (articular cartilage), fibrochondrocytes (meniscus), ligament fibroblasts (ligament), skin fibroblasts (skin), tenocytes (tendons), myofibroblasts (muscle), mesenchymal stem cells and keratinocytes (skin). Such composition may be used in culturing and engineering of artificial articular cartilage and cartilageous tissues and organs, either for surgical or laboratory testing applications.

The composition of the present invention may also be used in processing and engineering of living artificial substitutes for ligaments, tendons, skin, bone muscles and any metabolic organs, either for surgical or laboratory testing applications, in living substitutes for the replacement of articular cartilages, fibrocartilages, cartilageous organs, ligaments, tendons, bone tissues or skin, to induce an ectopic formation of fibrocartilage-like or cartilage-like tissues, as an injectable or implantable gel biomaterial which acts as supports, carriers, reconstructive devices or substitutes for the formation *in situ* of bone-like, fibrocartilage-like or cartilage-like tissues, and/or in cosmetics, pharmacology, medicine and/or surgery.

For the purpose of the present invention the following terms and expressions are defined below.

The term "gelating temperature" is intended to mean any temperature ranging from about 20°C to about 80°C, preferably between 30°C and 60°C, and more preferably at about the physiological temperature or 37°C.

The term "pH-controlled acid-soluble biopolymer" refers to a biological polymer that is solubilized in an acidic aqueous medium, and precipitates or gels heterogeneously when the pH is increased. For example, chitosan is dissolved in acid/water solution at pH about 4.0, and precipitates or gels heterogeneously when the chitosan solution is neutralized at pHs above 6.2.

The expression "three-dimensional" refers herein to the fact that the polymeric solution is simultaneously gelated and shaped by the mold wherein the solution was initially poured. Gels can be produced in glass or plastic bechers, dishes, tubes or between two plates so as to obtain any expected shapes.

The expression "*in situ* gelation" refers herein to the formation of gels by injecting the liquid solution within specific sites of mammalian or human environments, e.g. any tissues (muscles, bone, ligaments, cartilages) and organs. Gelation *in situ* allows complete and precise filling of tissue defects or body cavities. The gelation of biopolymer mixture is induced by the physiological temperature.

The expression "endothermal gelation" refers herein to the thermal mechanism of the solution, which enables the solution to gelate upon standing at the desired temperature. Induction of sol to gel transitions of systems requires energy via, for example, the temperature.

The expression "residue" refers herein to a series of biochemical molecules having a common specific chemical function. Example: the amino acid residues.

The expression "sequence" refers herein to the association of two or several molecules or residues. Example: a sequence of amino acid residues (LYS-ASP-PRO-GLY-LYS).

The expression "basic character" refers herein to the ability of a chemical molecule in aqueous solution to capture protons (H^+), thus leading to an increase in pH.

The expression "cells or cellular matters" refers herein to living biologicals, such as isolated cells, cellular dispersion, cell aggregates, cell spheroids or cells adhered to solid microspheres particles, that are encapsulated within the gels.

It is assumed herein that any pH-gelling acid-soluble biopolymers or polymers would behave similarly. As a consequence, the term "biopolymer" in the present invention may be replaced by the term "polymer", "polypeptide", "poly(amino acids)".

The present invention include method of forming different gelated materials, those materials being either molded (customized

shapes, tubes, membranes, films...) or formed *in situ* within biological environments (filling of tissue defects).

In a preferred embodiment, the self-gelling biopolymer aqueous solution has a pH above that for normal precipitation, and turn into solid gel upon thermal stimulation. This biopolymer gel can be used as a carrier for drugs or as a non-living therapeutics delivery systems, as substituting materials for tissues and organs and as encapsulants for living cells or microorganisms. Gel matrices are rapidly formed at temperatures between 30 to 60°C. Such aqueous systems are used as injectable filling materials, injected and gelated *in situ* for filling and repairing tissue defects.

In a second preferred embodiment, pH-dependant gelling acid water-soluble biopolymers and derivatives having a sufficient hydrophilicity are selected for preparing temperature-stimulated gels.

Biopolymeric gels can be applied to surgical reconstructive and regeneration uses and drug delivery purposes. They provide thermally reversible or irreversible bioerodible polymeric gels with biologically well-known and compatible components for a broad range of medical/biotechnological applications.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a plot of the Elastic Modulus G' (Pa) vs. Temperature (Celsius) illustrating the thermal gelling/ungelling of chitosan (2% w/v, deacetylation 85%) solution with MOPS (2.0% w/v) upon cooling/heating;

Fig. 2 illustrates a plot of the elastic modulus G' (Pa) vs. Temperature (Celsius) illustrating the thermal gelling/ungelling of chitosan (2% w/v, deacetylation 85%) solution with MOPSO (3.0% w/v) upon cooling/heating;

Fig. 3 illustrates a plot of the Elastic Modulus G' (Pa) vs. Temperature (Celsius) illustrating the thermal gelling/ungelling of chitosan (2% w/v, deacetylation 85%) solution with BIS-TRIS (3.0% w/v) upon cooling/heating;

Fig. 4 illustrates a plot of the Elastic Modulus G' (Pa) vs. Temperature (Celsius) illustrating the thermal gelling/ungelling of chitosan (2% w/v, deacetylation 85%) solution with MES (8.0% w/v) upon

cooling/heating;

Fig. 5 illustrates a plot of the Elastic Modulus (Pa) vs. Temperature (Celsius) illustrating the thermal gelling/ungelling of chitosan (2% w/v, deacetylation 85%) solution with BES (2.0% w/v) upon cooling/heating;

Fig. 6 illustrates a plot of the Turbidimetry (NTU) vs. Time illustrating the thermal gelling of chitosan (2% w/v, deacetylation 85%) solution with GP (8.0% w/v, pH=7.2) and without GP (pH=5.4) at 37°C;

Fig. 7 illustrates a plot of the Turbidimetry (NTU) vs. Time illustrating the thermal gelling of chitosan (2% w/v, deacetylation 85%) solution with BES (2.0% w/v) and without BES (pH=5.4) at 37°C; and

Fig. 8 illustrates a plot of the Turbidimetry (NTU) vs. Time illustrating the thermal gelling of chitosan (2% w/v, deacetylation 85%) solution with BIS-TRIS, at different BIS-TRIS content, from 2.0 to 4.0% w/v, at 37°C.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention there is proposed a new gelation mechanism that combines hydrogen bonding, electrostatic interactions and hydrophilic/hydrophobic interactions. It can only be achieved through complex interactions between biological macromolecules or synthetic polymers, water molecules and specific biochemical molecules having special actions.

In accordance with the present invention, the concerned biopolymer should be insoluble in water under neutral conditions pH = 7.

A method is disclosed for preparing a composition which comprises the steps a) of dissolving a pH-gelling acid-soluble biopolymer within an aqueous acidic solution of a pH from about 1.0 to about 5.0 to obtain an aqueous biopolymer composition having a concentration of 0.1 to 5% by weight of said biopolymer, and b) dissolving 0.1 to 10% by weight of a water-soluble molecule having a moderate basic character, or any water-soluble sequence of said molecule, within said aqueous biopolymer composition to obtain a clear liquid formulation with a pH ranging between 6.5 and 7.4. The final step is the heating of liquid formulation at a temperature above 30°C to obtain a solid gel, wherein said gel has a

concentration of 0.1 to 5.0% by weight of said biopolymer, and a concentration of 0.1 to 10% by weight of said molecule, and has a pH from about 6.4 to about 7.4.

The aqueous acidic solution is prepared from organic or inorganic acids that are selected from the group consisting of acetic acid, ascorbic acid, glutamic acid, lactic acid, lactobionic acid, salicylic acid, phosphoric acid, hydrochloric acid, propionic acid, formic acid, and a mixture thereof. Solubilization of pH-controlled acid-soluble biopolymers in aqueous solution requires acidic aqueous solutions having a pH ranging from 1.0 to 5.0.

The selected biopolymer is a pH-gelling acid-soluble polysaccharide, polypeptidic or poly(amino acids), or synthetic polymer.

The preferred polysaccharide is selected from a group comprising chitosan, modified chitosan or chitosan derivative, said chitosan biopolymer being cationic and bearing amino groups.

The acid-soluble polypeptide is selected from collageneous proteins, preferentially collagen.

A second polymer, selected from a groups comprising collagen, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxyethyl propylcellulose, hydroxymethyl propyl cellulose, polyethylene oxide, polypropylene oxide, poly(ethylene oxide-co-propylene oxide) copolymers, poly(ethylene oxide-co-propylene oxide-co-ethylene oxide) copolymers, polyvinyl alcohol, polycaprolactone diols, and derivatives, and any mixture thereof, can be incorporated within the biopolymeric solution.

The admixed molecule is required to play a double role: 1) to increase the pH within the biopolymeric solution up the physiological conditions, and 2) to prevent the immediate gelation or aggregation. The required molecule, preferentially selected from organic salts and amino-acids, should have a moderate basic character and a pKa between 6.0 and 7.6. Typically, the selected molecule should have a great sensitivity in terms of hydrophilicity/hydrophobicity (hydrophobic hydration and dehydration) and thermal sensitivity. Such effects are based upon a competition for hydration between apolar and polar groups of said molecule, which enables the design of molecular machines by free energy conversion.

Other preferred molecules, residues or sequences are organic salts selected from mono-phosphate salts, mono-sulfonate salts, mono-sulfate salts or mono-carboxylate salts; said organic salts being water-soluble and having a basic character and a pKa between 6.0 and 7.6.

The organic salt is preferably a salt of polyol or sugar selected from mono-phosphate dibasic salts, mono-sulfonate salts, mono-sulfate salts or mono-carboxylate salts of polyol, said polyol being selected from the group consisting of glycerol, comprising glycerol-2-phosphate, sn-glycerol 3-phosphate and L-glycerol-3-phosphate salts, and any mixture thereof. Salt of polyol or sugar are known to greatly modify the behavior of biopolymeric acidic aqueous solutions.

The salt of polyol is preferably selected from mono-phosphate dibasic salts, mono-sulfonate salts, mono-sulfate salts or mono-carboxylate salts of polyol, said polyol being selected from a group of polyols comprising histidinol, acetol, diethylstil-bestrol, indole-glycerol, sorbitol, ribitol, xylitol, arabinitol, erythritol, inositol, mannitol, glucitol, palmitoyl-glycerol, linoleoyl-glycerol, oleoyl-glycerol, arachidonoyl-glycerol, and any mixture thereof.

The salt of sugar is preferably selected from mono-phosphate dibasic salts, mono-sulfonate salts, mono-sulfate salts or mono-carboxylate salts of sugar, said sugar being selected from a group of sugars consisting of fructose, galactose, ribose, glucose, xylose, rhamnulose, sorbose, erythrulose, deoxy-ribose, ketose, mannose, arabinose, fuculose, fructopyranose, ketoglucose, sedoheptulose, trehalose, tagatose, sucrose, allose, threose, xylulose, hexose, methylthio-ribose, methylthio-deoxy-ribulose, and any mixture thereof.

Polyols are frequently added to compositions for improving gel properties. Sorbitol and mannitol are currently used as tonicity enhancing agents. Glycerol and polyethylene glycol are proposed as plasticizers. Polyols (-ol: glycerol, sorbitol...) and sugars (-ose: fructose, glucose, galactose...) were used as thermal stabilizing agents for proteins in solutions Depending on the selected molecules, they were found to make or break structuring of water, create hydrogen bonding, electrostatic or hydrophobic interacting, and present endothermic transitions Polyols and

sugars stabilize proteins to heat denaturation through their structuring effect on water and the strengthen of hydrophobic interactions.

The molecule, residue or sequence is preferably a salt selected in a group comprising N-[carbamoylmethyl]-2-aminoethane sulfonate (ACES), N,N-bis[2-hydroxyethyl]-2-aminoethane sulfonate (BES), 3-[N,N-bis(2-hydroxy-ethyl)amino]-2-hydroxypropanesulfonate (DIPSO), N-[2-hydroxyethyl]piperazine-N'-3-propanesulfonate (HEPES), 2-[N-morpholino]ethanesulfonate (MES), 4-[N-morpholino] butanesulfonate (MOBS), 3-[N-morpholino]butanesulfonate (MOPS), 3-[N-morpholino]-2-hydroxypropanesulfonate (MOPSO), 3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate (TAPSO), N-tris [hydroxymethyl] methyl-2-minoethanesulfonate(TES), bis [2-hydroxy-ethyl]iminotris [hydroxymethyl]methane (BIS-TRIS), 3-mopholino 1-1-propane diol, and derivatives, and any mixture thereof.

The molecule, residue or sequence is preferably selected from amino-acid residues, amino-acid sequences or poly(amino acids) having a basic character and a pKa between 6.0 and 7.6, preferentially histidine (His).

The molecule, residue or sequence is preferably a sequence, derivative or oligomer of amino acids including alanine, (ALA), histidine (HIS), arginine (ARG), lysine (LYS), aspartic acid (ASP), glutamine (GLN), glycine (GLY), hydroxyproline (HYP), isoleucine (ILE), leucine (LEU), norleucine (NLE), phenylalanine (PHE), proline (PRO), serine (SER), threonine (THR), tyrosine (TYR), and valine (VAL).

A pharmaceutical or bioactive agent can be added to the liquid biopolymer containing solution of step a) or b). It can be highly soluble, sparingly soluble or non-soluble in the aqueous formulation. Solid particulate additives such as non-polymeric microspheres or nanospheres, mineral or ceramic granules or powders, can be added to the biopolymer solution of step a) or b).

The mixture can be dispensed for gelation into a desired receiver, either in a mold or within a tissue, organ or body cavity. It can be kept in a stable ungelled liquid form at a temperature ranging from about 0°C to about 20°C. The solidifying temperature is ranging from about 37°C to about 60°C, and preferably about 37°C.

Practically, the mixture is introduced within an animal or human body by injection or endoscopic administration, and gelled *in situ* at a temperature of about 37°C.

Table 1 below provides composition of some preferred examples for buffering/gelling agents with a 2% w/v chitosan solution (deacetylation 85%).

TABLE 1

Agent	pKa	Concentration (mM)	pH	Intrinsic Gelling Temperature (°C)
BES	7.1	85.03	7.1	38.5
MOPS	7.2	86.5	7.2	32
MOPSO	6.9	121.35	7.2	32.2
BIS-TRIS	6.5	191.20	7.15	25.5
MES	6.1	361.0	7.2	35.5

Formation of biopolymeric gels

A selected biopolymer in powder form is dissolved in an aqueous acidic solution until the occurrence of a clear solution is obtained. The proportion of biopolymer varies from 0.5 to 10.0% w/v, preferentially from 1.0 to 3.0% w/v. The pH of the aqueous biopolymer solution ranges from 4.0 to 5.5. Aqueous biopolymer solutions can be sterilized either by autoclaving or filtration with in-line sterile filters (0.22 micrometer). Freshly-prepared aqueous biopolymer solutions are stored preferably at low positive temperature (4°C). The added molecule with a moderate basic character is dissolved in water, then admixed to the aqueous biopolymer solution at a temperatures ranging from 4 to 15°C, preferably 10°C. When a clear homogeneous aqueous solution with a pH ranging from 5.8 to 7.0 is attained, the said solution is poured into the desired receiver, and hold to appropriate temperature to gel.

The nature of the acid that is used for the acidic biopolymer solutions does not influence fundamentally the sol to gel transition of the system. The final pH within the solution is dependent upon the pH of the water/acid solution as well as the biopolymer and molecule concentrations. As the biopolymer and molecule are two basic components, they tend to

increase the pH of the acidic solution wherein they are dissolved. Concentrations in this biopolymer and molecule can be balanced to reach the appropriate pH of the solution, while taking into consideration the solubility limit of both components, and particularly the one of biopolymer.

In situ formation of gels

The selected molecule tested to be incorporated in the polymeric solution was histidine, but similar results were obtained with other amino acids or synthetic molecules having similar functions and basic character. *In situ* gelation of the biopolymer solution can be conducted by dispensing the solution from a hypodermic syringe. If needed, the solution may be pre-gelated (initiate the thermal gelation) by keeping the syringe and biopolymer solution at desired temperature, ideally 37°C, until the first signs of gelation appear. The ready-to-gel biopolymer mixture is then administrated so as to fill tissue defects or cavities and complete *in situ* the gelation process (at 37°C). A needle having a gauge of 20 and below is ideal material for injection of such gel solution. Body cavities and tissue defects act as recipients for the solution, but the liquid materials remain in an open aqueous environment. The conformability and diffusability of the biopolymer solutions is dependent upon the solution and material properties. Increased viscosity results in formation *in situ* of more compact and less conformable gels.

Therapeutic use and other uses of biopolymeric Gels

Such a biopolymeric gel as previously described is an ideal material for drug delivery system. Such a *in situ* gel-like forming vehicle, wherein a solid particulate or water-soluble additive is incorporated prior to the gelation, can be administrated topically, directly to the body site to be treated or diagnosed. Anti-bacterial, anti-fungal, steroidal or non-steroidal anti-inflammatory, anti-cancer, anti-fibrosis, anti-viral, anti-glucoma, miotic and anti-cholinergies, anti-psychotic, anti-histaminic and decongestant, anesthesic and anti-parasitic agents may be incorporated within the composition and gel. In a similar fashion, non-living pharmaceutical agents may be incorporated within the composition or gel for restorative, re-constructive or regenerative purposes.

Living microorganisms, plant cells, animal cells or human cells may be entrapped identically within the biopolymer gel by introduction prior to the gelation. The cells or micro-organisms loaded gels may be applied to biotechnological purposes in medicine or in other industrial areas. Biopolymer *in situ* forming gels can be formed sub-cutaneously, intramuscularly, intra-peritoneally or within biological connective tissues, bone defects, fractures, articular cavities, body conduits or cavities, eye cul-de-sac, solid tumor vasculatures, etc...

The present invention will be more readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

This example shows typical experiments of acidic biopolymer solutions neutralized with an organo-phosphate, preferentially glycerophosphate (GP), and transformed to gel upon standing at 37°C.

Experiment 1: gelation of chitosan/GP

In typical experiment, 200 mg of chitosan 85% deacetylated was dissolved in 8.5 mL of aqueous HCl solution (0.1M). The chitosan solution, so obtained had a pH of about 5.0, was cooled down to around 4°C. Then 800 mg of β-glycerophosphate disodium salt pentahydrate dissolved in 1.5 mL of water were added slowly to the chitosan solution, while maintaining the cold temperature. The pH of the resulting homogeneous and clear liquid mixture become 7.1. This mixture was disposed in a glass scintillation vial in the incubator at 37°C for 2 hours, enough time to achieve bulk gelation process.

Similar results were obtained when the β-glycerophosphate disodium salt was replaced by the α- glycerophosphate disodium salt.

Experiment 2: gelation of collagen/GP

Collagen was isolated from knee joint cartilage of calf, and was made mainly of type II collagen. An aqueous solution of collagen (2% w/v) was prepared by dissolving 0.2 g of collagen in 8.5 ml of an acetic acid solution with a pH about 3.6. Once a clear solution was obtained, it was

cooled down to about 4°C, and then a cold solution of 800 mg of β-glycerophosphate disodium salt pentahydrate in 1.5 mL of water was added under continuous stirring. When the resulting neutral solution (pH ≈ 7.2) appeared quite homogeneous and clear, it was poured in a Petri dish and placed at 37°C. A homogeneous uniform gel formed within 1 hour.

Experiment 3: gelation of chitosan-Collagen/GP

Collagen (100 mg) of the same origin (Example 1, Experiment 2) was first dissolved in 10 ml of an acetic acid solution (0.1M). Then 100 mg of Chitosan was added to the resulting solution and stirred until all chitosan was completely dissolved. After the whole system was cooled down to around 4°C, and 800 mg of β-glycerophosphate disodium salt, dissolved in 1.5 ml of water, was added under continuous stirring. Once the resulting neutral solution (pH ≈ 7.2) was perfectly homogeneous and clear, it was poured in a Petri dish and placed at 37°C. The gel formed within 1 hour.

Example 2

This example shows the typical experiments of acidic biopolymer solutions neutralized with organo-sulfonate, preferentially N,N-bis[2-hydroxyethyl]-2-aminoethane sulfonate (BES), and transformed to gel upon standing at 37°C.

Experiment 1: gelation of chitosan/BES

In this experiment, 200 mg of chitosan 85% deacetylated was dissolved in 8.5 mL of aqueous HCl solution (0.1M). The chitosan solution, so obtained had a pH of about 5.0, was cooled down to around 4°C. Then 200 mg of BES in form of sodium salt was dissolved in 1.5 mL of cold water and added slowly to the cold chitosan solution under vigorous stirring. The pH of the resulting homogeneous and clear solution increases to about 7.17. This solution was disposed in a glass scintillation vial in the incubator at 37°C. Bulk gelation occurs in 10 minutes.

Experiment 2: gelation of collagen/BES

Collagen was of the same origin (Example 1, Experiment 2). An

aqueous solution of collagen (2% w/v) was prepared by dissolving 200 mg of collagen in 8.5 ml of an acetic acid solution with a pH about 3.6. Once a clear solution was obtained, it was cooled down to about 4°C, and then a cold solution of 200 mg of BES in 1.5 mL of water was added under continuous stirring. When the resulting neutral solution (pH ~ 7.2) appeared quite homogeneous and clear, it was poured in a Petri dish and placed at 37°C. A homogeneous uniform gel formed within 15 minutes.

Experiment 3: gelation of Chitosan-Collagen/BES

Collagen (100 mg) of the same origin (Example 1, Experiment 2) was first dissolved in 10 ml of an acetic acid solution (0.1M). Then 100 mg of Chitosan was added to the resulting solution and stirred until all Chitosan was completely dissolved. After the whole system was cooled down to around 4°C, and 200 mg of BES in form of sodium salt dissolved in 1.5 ml of cold water, was added under continuous stirring. Once the solution was perfectly homogeneous and clear, the liquid mixture was poured in a Petri dish and placed at 37°C. The gel formed within 5 minutes.

Example 3

This example shows the typical experiments of acidic biopolymer solutions neutralized with tertiary hydroxyalkylamine, preferentially bis-[2-hydroxyethyl]iminotris [hydroxymethyl] methane (BIS-TRIS), and transformed to gel upon standing at 37°C.

Experiment 1: gelation of chitosan/BIS-TRIS

Chitosan solution with a pH around 5.0 was prepared by dissolving 200 mg of chitosan 85% deacetylated in 8.5 mL of aqueous HCl solution (0.1M). This chitosan solution was cooled down to around 4°C, after which 400 mg of BIS-TRIS dissolved in 1.5 mL of cold water was added slowly to the cold chitosan solution under vigorous stirring. The pH of the resulting homogeneous and clear solution increases to about 7.15. This solution was disposed in a glass scintillation vial in the incubator at 37°C. Bulk gelation occurs within 10 minutes.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of

further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A biopolymeric liquid aqueous composition for producing self-gelling systems and gels, which comprises:

d) an acidic water-based medium; and

e) 0.1 to 10% by weight of a pH-gelling acid-soluble biopolymer; and

f) 0.1 to 10% by weight of a water-soluble molecule having a basic character and a pKa between 6.0 and 8.4, or a water-soluble residue or sequence of said molecule having a basic character and a pKa between 6.0 and 8.4;

wherein said liquid composition has a final pH ranging from 5.8 and 7.4, and forms a stable solid and homogeneous gel within a temperature range from 10 to 70°C.

2. A composition according to Claim 1, wherein said composition is prepared from organic and/or inorganic acid.

3. A composition according to Claim 2, wherein the organic and/or inorganic acid is selected from the group consisting of hydrochloric acid, citric acid, ascorbic acid, lactic acid, lactobionic acid, acetic acid, salicylic acid, formic acid, glutamic acid, phosphoric acid, orthophosphoric acid, and glycerophosphoric acid, or a mixture thereof.

4. A composition according to Claim 1, wherein said biopolymer comprises a pH-gelling acid-soluble polysaccharide, polypeptidic or poly(amino acids), or synthetic polymer.

5. A composition according to Claim 1, wherein said biopolymer comprises a solution of chitosan, modified chitosan or chitosan derivative, said solution of chitosan being cationic and bearing amino groups.

6. A composition according to Claim 1, wherein said molecule, residue or sequence is an organic salt selected from the group consisting

of mono-phosphate salt, mono-sulfonate salt, mono-sulfate salt and mono-carboxylate salt.

7. A composition according to Claim 1, wherein said molecule, residue or sequence is a salt of polyol selected from the group consisting of mono-phosphate dibasic salt, mono-sulfonate salt, mono-sulfate salt and mono-carboxylate salt of polyol, said polyol being selected from the group consisting of glycerol, histidinol, acetol, diethylstil-bestrol, indole-glycerol, sorbitol, ribitol, xylitol, arabinitol, erythritol, inositol, mannitol, glucitol, palmitoyl-glycerol, linoleoyl-glycerol, oleoyl-glycerol, and arachidonoyl-glycerol, or a mixture thereof.

8. A composition according to Claim 7, wherein the glycerol is selected from the group consisting of glycerol-2-phosphate, sn-glycerol 3-phosphate and L-glycerol-3-phosphate salt, or a mixture thereof.

9. A composition according to Claim 1, wherein said molecule, residue or sequence is a salt of a sugar selected from the group consisting of mono-phosphate dibasic salt, mono-sulfonate salt, mono-sulfate salt and mono-carboxylate salt of a sugar, said sugar being selected from the group consisting of fructose, galactose, ribose, glucose, xylose, rhamnulose, sorbose, erythrulose, deoxy-ribose, ketose, mannose, arabinose, fuculose, fructopyranose, ketoglucose, sedoheptulose, trehalose, tagatose, sucrose, allose, threose, xylulose, hexose, methylthio-ribose, and methylthio-deoxy-ribulose, or a mixture thereof.

10. A composition according to Claim 1, wherein said molecule, residue or sequence is selected from the group consisting of sodium, magnesium or iron salt of glycerol-2-phosphate, sn-glycerol-3-phosphate and L-glycerol-3-phosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate and fructose-6-phosphate, or a mixture thereof.

11. A composition according to Claim 1, wherein said molecule, residue or sequence is a sodium, magnesium or iron salt selected from the group consisting of N-[carbamoylmethyl]-2-aminoethane sulfonate (ACES),

N,N-bis[2-hydroxyethyl]-2-aminoethane sulfonate (BES), 3-[N,N-bis(2-hydroxy-ethyl)amino]-2-hydroxypropanesulfonate (DIPSO), N-[2-hydroxyethyl]piperazine-N'-3-propane-sulfonate (EPPS), N-[2-hydroxyethyl]piperazine-N'-4-butane-sulfonate (HEPBS), N-[2-hydroxyethyl]piperazine-N'-3-propanesulfonate (HEPES), N-[2-hydroxyethyl] piperazine-N'-2-hydroxypropanesulfonate (HEPSO), 2-[N-morpholino]ethanesulfonate (MES), 4-[N-morpholino]butanesulfonate (MOBS), 3-[N-morpholino]butanesulfonate (MOPS), 3-[N-morpholino]-2-hydroxypropanesulfonate (MOPSO), Piperazine-N,N'-bis[2-ethanesulfonate] (PIPES), Piperazine-N,N'-bis[2-hydroxypropanesulfonate] (POPSO), 3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate (TAPSO), and N-tris [hydroxymethyl]methyl-2-minoethanesulfonate(TES), and derivatives or mixtures thereof.

12. A composition according to Claim 1, wherein said molecule, residue or sequence is selected from the group consisting of N,N-bis[hydroxyethyl]glycine (BICINE), bis [2-hydroxyethyl]iminotris [hydroxymethyl]methane (BIS-TRIS), Glycyl-glycine (GLY-GLY), Triethanolamine (TEA), N-tris [hydroxymethyl]methylglycine (TRICINE), and Tris [hydroxymethyl]aminomethane (TRIZMA), and derivatives or mixtures thereof.

13. A composition according to Claim 1, wherein said molecule, residue or sequence has either one acid group and at least one amino group, or more amino groups than acid groups.

14. A composition according to Claim 1, wherein said molecule, residue or sequence is an amino-acid residue, an amino-acid sequence or a poly(amino acids) having a basic character and a pKa between 6.0 and 8.4.

15. A composition according to Claim 14, wherein said amino acid residue is selected from the group consisting of histidine (HIS), arginine (ARG), lysine (LYS), asparagine (ASP), and glutamine (GLN), or a mixture thereof.

16. A composition according to Claim 1, wherein said molecule, residue or sequence is an amino acid residue or amino acid derivative selected from the group consisting of histidine (HIS), arginine (ARG), lysine (LYS), aspartic acid (ASP), and glutamine (GLN), said amino acid residue or amino acid derivative being modified with a radical acetyl, t-butyl, benzyl, benzoyl, ethyl, formyl, or methyl.

17. A composition according to Claim 1, wherein said molecule, residue or sequence is a sequence, derivative or polymer of at least one amino acid selected from the group consisting of alanine (ALA), histidine (HIS), arginine (ARG), lysine (LYS), aspartic acid (ASP), glutamine (GLN), glycine (GLY), hydroxyproline (HYP), isoleucine (ILE), leucine (LEU), norleucine (NLE), phenylalanine (PHE), proline (PRO), serine (SER), threonine (THR), tyrosine (TYR), and valine (VAL).

18. A composition according to Claim 1, wherein said composition further comprises at least one other water-soluble polymer.

19. A composition according to Claim 1, wherein said at least one other second polymer is selected from the group consisting of collagen, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxyethyl propylcellulose, hydroxymethyl propyl cellulose, polyethylene oxide, polypropylene oxide, poly(ethylene oxide-co-propylene oxide) copolymers, poly(ethylene oxide-co-propylene oxide-co-ethylene oxide) copolymers, polyvinyl alcohol, and polycaprolactone diols, and derivatives or mixtures thereof.

20. A composition according to Claim 1, further comprising a solid particulate or a water-soluble additive.

21. A composition according to Claim 1, further comprising a drug or a pharmaceutical agent.

22. A composition according to Claim 1, further comprising microorganisms, plant cells, animal cells or human cells dispersed therein.

23. A composition according to Claim 1, for use as a carrier for delivering a pharmaceutical agent *in situ*.

24. A method for preparing a composition according to Claim 1, which comprises the steps of:

a) dissolving a pH-gelling acid-soluble biopolymer within an aqueous acidic solution of a pH from about 1.0 to about 5.0 to obtain an aqueous biopolymer composition having a concentration of 0.1 to 5% by weight of said biopolymer;

b) dissolving 0.1 to 10% by weight of a water-soluble molecule having a basic character and a pKa between 6.0 and 8.4, or a water-soluble residue or sequence of said molecule having a basic character and pKa between 6.0 and 8.4, within said aqueous biopolymer composition to obtain a clear liquid formulation with a pH ranging between 5.8 and 7.4;

c) heating said liquid formulation at a temperature above 30°C to obtain a solid gel, said gel having a pH from about 5.8 to about 7.4.

25. The method of Claim 24, wherein said aqueous acidic solution is prepared from an organic or inorganic acid selected from the group consisting of acetic acid, ascorbic acid, glutamic acid, lactic acid, lactobionic acid, salicylic acid, phosphoric acid, hydrochloric acid, propionic acid, and formic acid, or a mixture thereof.

26. The method of Claim 24, wherein said biopolymer is a chitosan or a chitosan derivative, with a degree of deactylation ranging from 35 to 99%.

27. The method of Claim 24, wherein said molecule, residue or sequence is an organic salt selected from the group consisting of a mono-

phosphate salt, a mono-sulfonate salt, a mono-sulfate salt and a mono-carboxylate salt.

28. The method of Claim 24, wherein said molecule, residue or sequence is a salt of polyol selected from the group consisting of a mono-phosphate dibasic salt, a mono-sulfonate salt, a mono-sulfate salt and a mono-carboxylate salt of polyol, said polyol being selected from the group consisting of glycerol, histidinol, acetol, diethylstil-bestrol, indole-glycerol, sorbitol, ribitol, xylitol, arabinitol, erythritol, inositol, mannitol, glucitol, palmitoyl-glycerol, linoleoyl-glycerol, oleoyl-glycerol, and arachidonoyl-glycerol, or a mixture thereof.

29. The method of Claim 25, wherein said glycerol is selected from the group consisting of glycerol-2-phosphate, sn-glycerol 3-phosphate and L-glycerol-3-phosphate salts, or a mixture thereof.

30. The method of Claim 24, wherein said molecule, residue or sequence is a salt of sugar selected from the group selected from a mono-phosphate dibasic salt, a mono-sulfonate salt, a mono-sulfate salt and a mono-carboxylate salt of sugar, said sugar being selected from the group consisting of fructose, galactose, ribose, glucose, xylose, rhamnulose, sorbose, erythrulose, deoxy-ribose, ketose, mannose, arabinose, fuculose, fructopyranose, ketoglucose, sedoheptulose, trehalose, tagatose, sucrose, allose, threose, xylulose, hexose, methylthio-ribose, and methylthio-deoxy-ribulose, or a mixture thereof.

31. The method of Claim 24, wherein said molecule, residue or sequence is a sodium, magnesium or iron salt of glycerol-2-phosphate, sn-glycerol-3-phosphate and L-glycerol-3-phosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate or fructose-6-phosphate, or a mixture thereof.

32. The method of Claim 24, wherein said molecule, residue or sequence is a sodium, magnesium or iron salt selected from the group consisting of N-[carbamoylmethyl]-2-aminoethane sulfonate (ACES), N,N-

bis[2-hydroxyethyl]-2-aminoethane sulfonate (BES), 3-[N,N-bis(2-hydroxy-ethyl)amino]-2-hydroxypropanesulfonate (DIPSO), N-[2-hydroxyethyl]piperazine-N'-3-propane-sulfonate (EPPS), N-[2-hydroxyethyl]piperazine-N'-4-butane-sulfonate (HEPBS), N-[2-hydroxyethyl]piperazine-N'-3-propanesulfonate (HEPES), N-[2-hydroxyethyl] piperazine-N'-2-hydroxypropanesulfonate (HEPSO), 2-[N-morpholino]ethanesulfonate (MES), 4-[N-morpholino]butanesulfonate (MOBS), 3-[N-morpholino]butanesulfonate (MOPS), 3-[N-morpholino]-2-hydroxypropanesulfonate (MOPSO), Piperazine-N,N'-bis[2-ethanesulfonate] (PIPES), Piperazine-N,N'-bis[2-hydroxypropanesulfonate] (POPSO), 3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate (TAPSO), and N-tris [hydroxymethyl]methyl-2-minoethanesulfonate(TES), and derivatives or mixtures thereof.

33. The method of Claim 24, wherein said molecule, residue or sequence is selected from the group consisting of N,N-bis[hydroxyethyl]glycine (BICINE), bis [2-hydroxyethyl]iminotris [hydroxymethyl]methane (BIS-TRIS), Glycyl-glycine (GLY-GLY), Triethanolamine (TEA), N-tris [hydroxymethyl]methylglycine (TRICINE), and Tris [hydroxymethyl]aminomethane (TRIZMA), and derivatives or mixtures thereof.

34. The method of Claim 24, wherein said molecule, residue or sequence has either one acid group and at least one amino group, or more amino groups than acid groups.

35. The method of Claim 24, wherein said molecule, residue or sequence is an amino-acid residue, an amino-acid sequence or a poly(amino acids) having a basic character and a pKa between 6.0 and 8.4.

36. The method of Claim 35, wherein said amino acid residue is selected from the group consisting of histidine (HIS), arginine (ARG), lysine (LYS), asparagine (ASP), glutamine (GLN), or a mixture thereof.

37. The method of Claim 35, wherein said molecule, residue or sequence is an amino acid residue or amino acid derivative selected from the group consisting of histidine (HIS), arginine (ARG), lysine (LYS), aspartic acid (ASP), and glutamine (GLN) modified with a radical acetyl, t-butyl, benzyl, benzoyl, ethyl, formyl, or methyl.

38. The method of Claim 24, wherein said molecule, residue or sequence is a sequence, derivative or polymer of at least one amino acid selected from the group consisting of alanine (ALA), histidine (HIS), arginine (ARG), lysine (LYS), aspartic acid (ASP), glutamine (GLN), glycine (GLY), hyroxyproline (HYP), isoleucine (ILE), leucine (LEU), norleucine (NLE), phenylalanine (PHE), proline (PRO), serine (SER), threonine (THR), tyrosine (TYR), and valine (VAL).

39. The method of Claim 24, wherein said aqueous biopolymer composition is a gel at a temperature above 10°C.

40. The method of Claim 24, wherein said aqueous biopolymer composition is a gel at a temperature above 37°C.

41. The method of Claim 24, wherein said biopolymer solution further comprises a solid particulate additive, said solid particulate additive being added to the biopolymer solution of step a) or b).

42. The method of Claim 24, further comprising an other water-soluble polymer is added to the biopolymer solution of step a) or b).

43. The method of Claim 24, wherein said other polymer is selected from the group consisting of collagen, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxyethyl propylcellulose, hydroxymethyl propyl cellulose, polyethylene oxide, polypropylene oxide, poly(ethylene oxide-co-propylene oxide) copolymers, poly(ethylene oxide-co-propylene oxide-co-ethylene oxide) copolymers, polyvinyl alcohol, and polycaprolactone diols, or derivatives thereof.

44. The composition of Claim 1 for use in cosmetics, pharmacology, medicine and/or surgery.

45. The composition of Claim 1 for use into an implantable device or an implant for repair, reconstruction and/or replacement of tissues and/or organs.

46. The composition of Claim 1 for use as an implantable, transdermal or dermatological drug delivery system.

47. The composition of Claim 1 for use as an opthalmological implant or a drug delivery system.

48. The composition of Claim 1 for use in cells-loaded artificial matrices for engineering and culture of bioengineered hybrid materials and tissue equivalents.

49. The composition of Claim 1, wherein said composition is loaded with cells selected from the group consisting of chondrocytes (articular cartilage), fibrochondrocytes (meniscus), ligament fibroblasts (ligament), skin fibroblasts (skin), tenocytes (tendons), myofibroblasts (muscle), mesenchymal stem cells and keratinocytes (skin).

50. The composition of Claim 49 for use in culturing and engineering of artificial articular cartilage and cartilageous tissues and organs, either for surgical or laboratory testing applications.

51. The composition of Claim 49 for use in processing and engineering of living artificial substitutes for ligaments, tendons, skin, bone muscles and metabolic organs, either for surgical or laboratory testing applications.

52. The composition of Claim 49 for use in living substitutes for the replacement of articular cartilages, fibrocartilages, cartilageous organs, ligaments, tendons, bone tissues or skin.

53. The composition of Claim 49 for use to induce an ectopic formation of fibrocartilage-like or cartilage-like tissues.

54. The composition of Claim 1 for use as an injectable or implantable gel biomaterial which acts as supports, carriers, reconstructive devices or substitutes for the formation *in situ* of bone-like, fibrocartilage-like or cartilage-like tissues.

55. Use of the composition of Claim 1 in cosmetics, pharmacology, medicine and/or surgery.

56. Use of the composition of Claim 1 into an implantable device or an implant for repair, reconstruction and/or replacement of tissues and/or organs.

57. Use of the composition of Claim 1 as an implantable, transdermal or dermatological drug delivery system.

58. Use of the composition of Claim 1 as an opthalmological implant or a drug delivery system.

59. Use of the composition of Claim 1 in cells-loaded artificial matrices for engineering and culture of bioengineered hybrid materials and tissue equivalents.

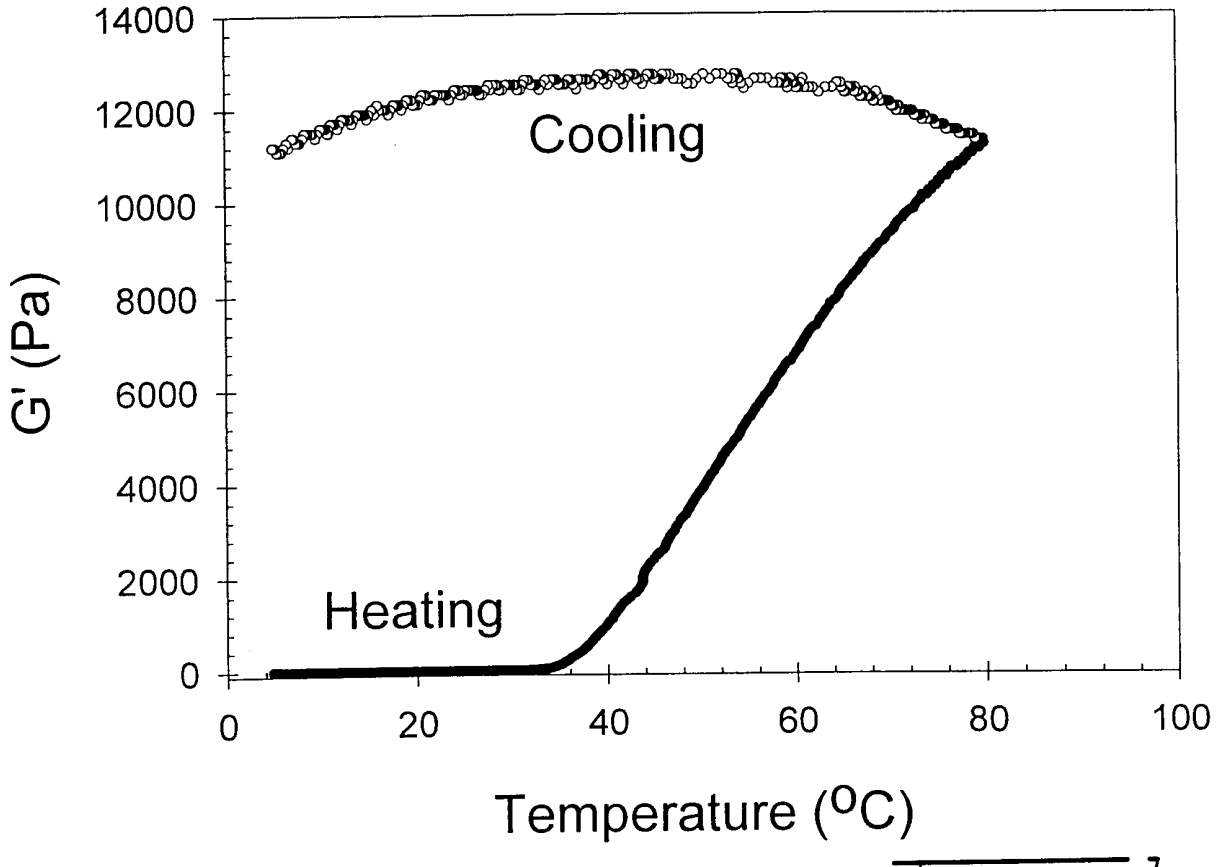
60. Use of the composition of Claim 49 in culturing and engineering of artificial articular cartilage and cartilageous tissues and organs, either for surgical or laboratory testing applications.

61. Use of the composition of Claim 49 in processing and engineering of living artificial substitutes for ligaments, tendons, skin, bone muscles and metabolic organs, either for surgical or laboratory testing applications.

62. Use of the composition of Claim 49 in living substitutes for the replacement of articular cartilages, fibrocartilages, cartilageous organs, ligaments, tendons, bone tissues or skin.

63. Use of the composition of Claim 49 to induce an ectopic formation of fibrocartilage-like or cartilage-like tissues.

64. Use of the composition of Claim 1 as an injectable or implantable gel biomaterial which acts as supports, carriers, reconstructive devices or substitutes for the formation *in situ* of bone-like, fibrocartilage-like or cartilage-like tissues.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G' (Pa)
Cooling
Heating
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20
40
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80
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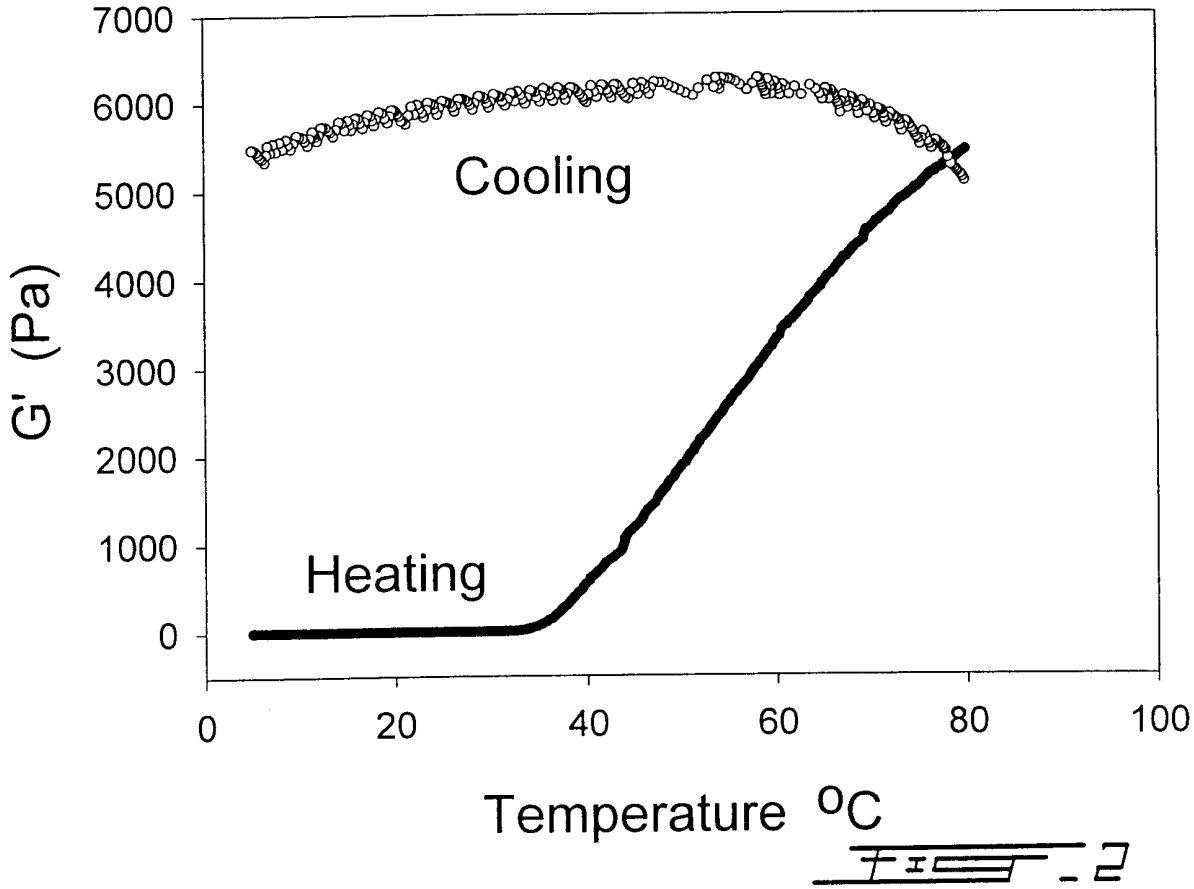
Temperature (°C)

Fig. 1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G' (Pa)
Cooling
Heating
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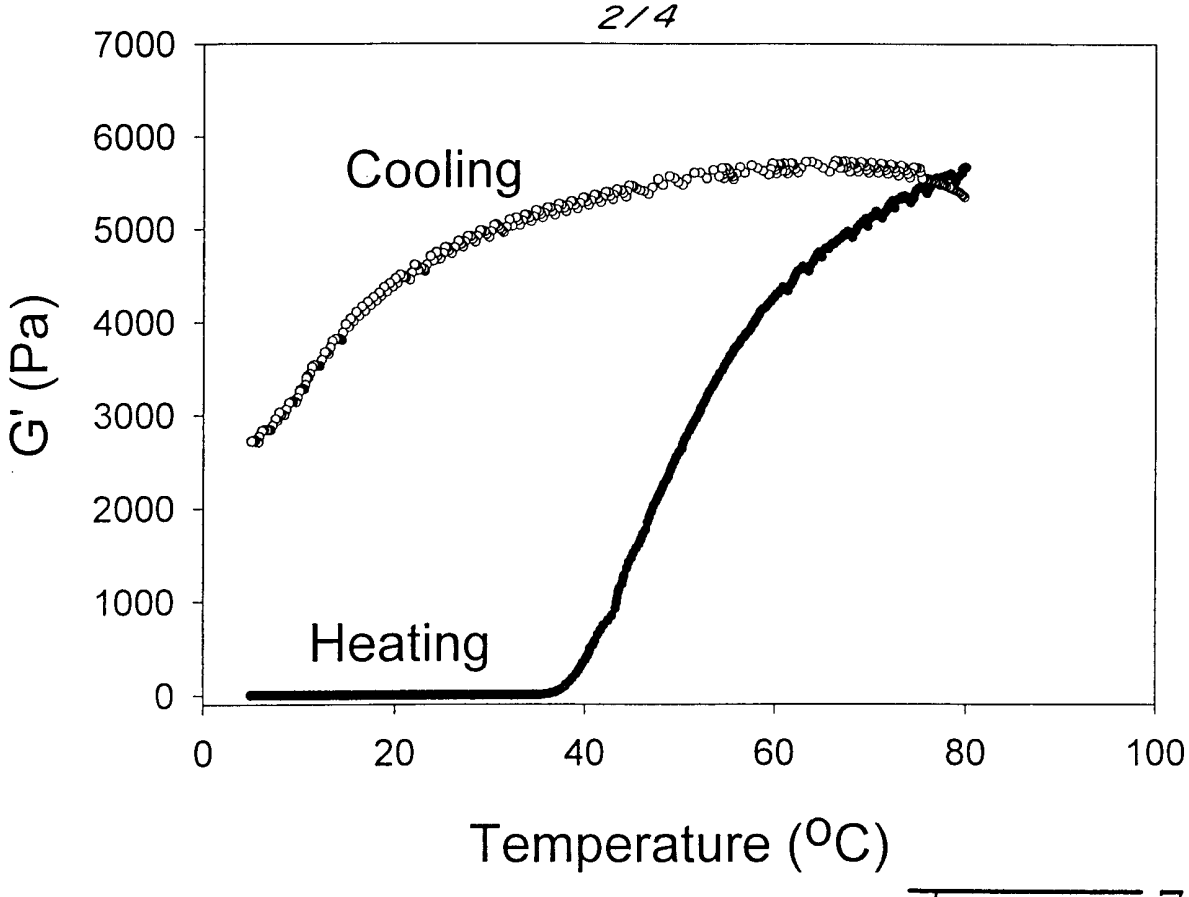
60
80
100
Temperature °C

Fig. 2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G' (Pa)
Cooling
Heating
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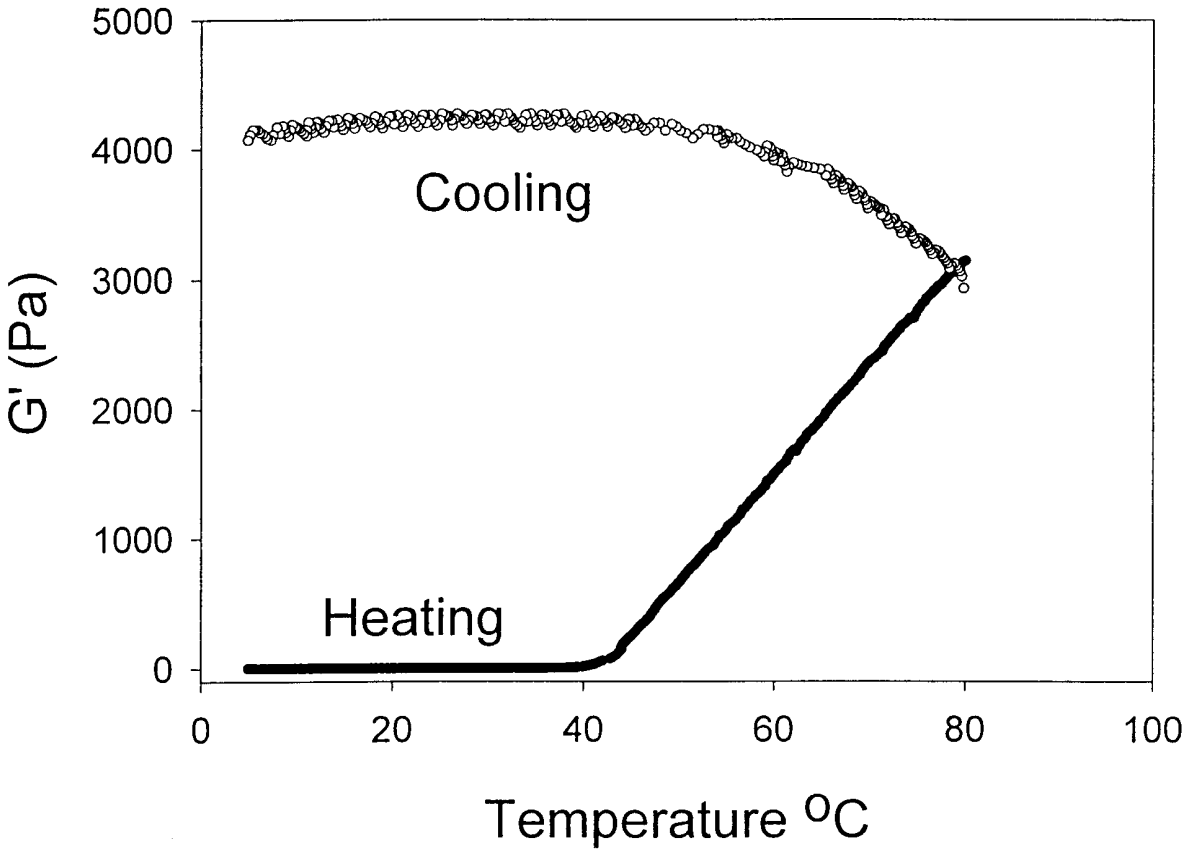
Temperature (°C)

Fig 3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0
G' (Pa)
Cooling
Heating
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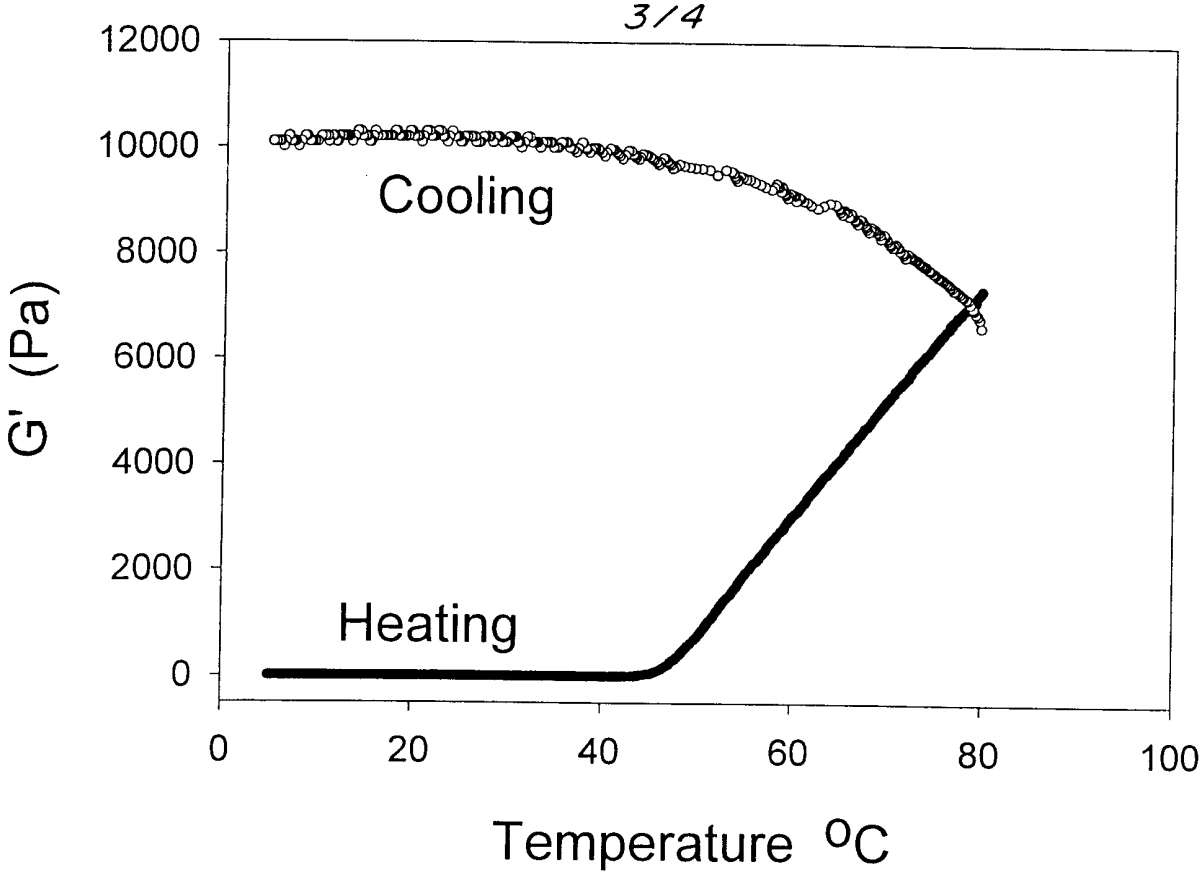
20
40
60
80
100
Temperature °C

Fig 4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10000
8000
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4000
2000
0
G' (Pa)
Cooling
Heating
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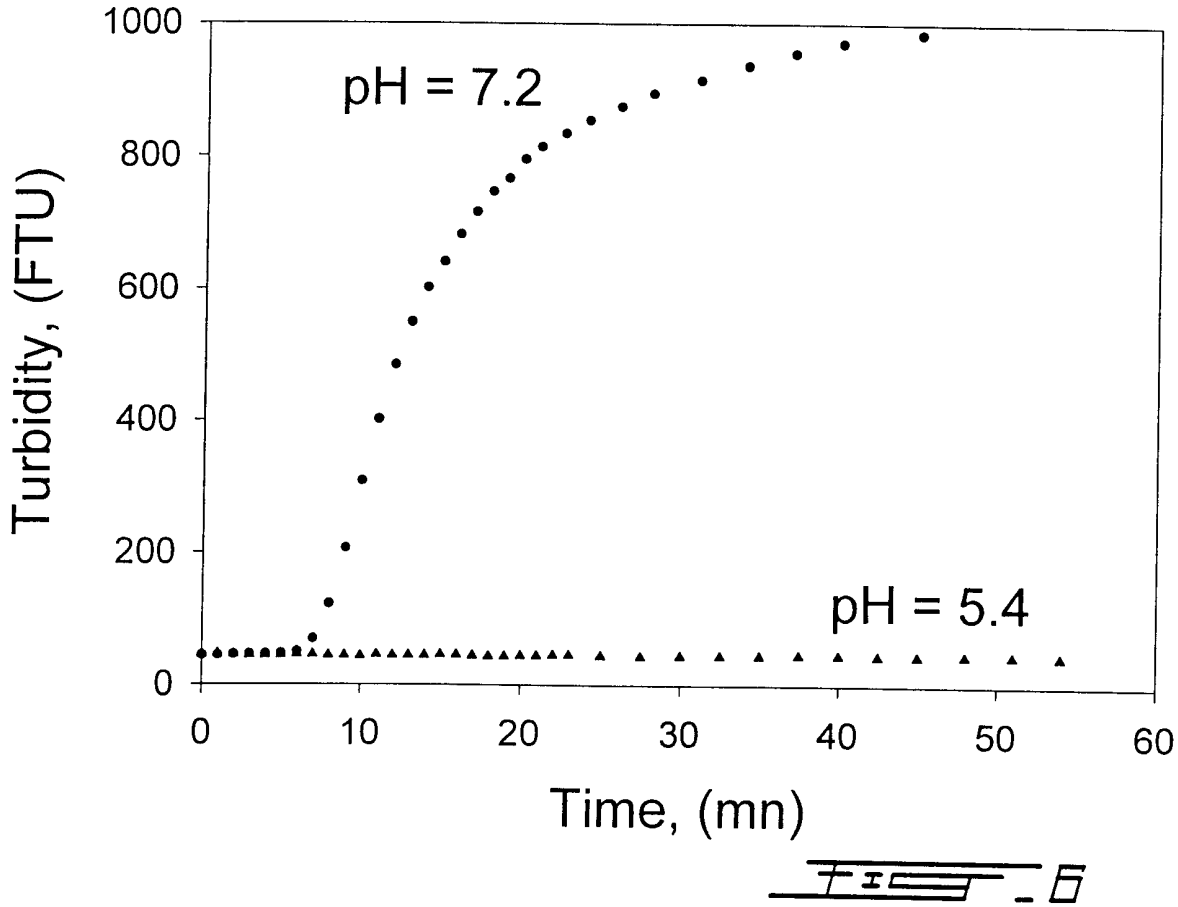
20
40
60
80
100
Temperature °C

Fig 5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800
600
400
200
0
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pH = 5.4
0
10
20
30
40
50
60
Time, (mn)

Fig 6

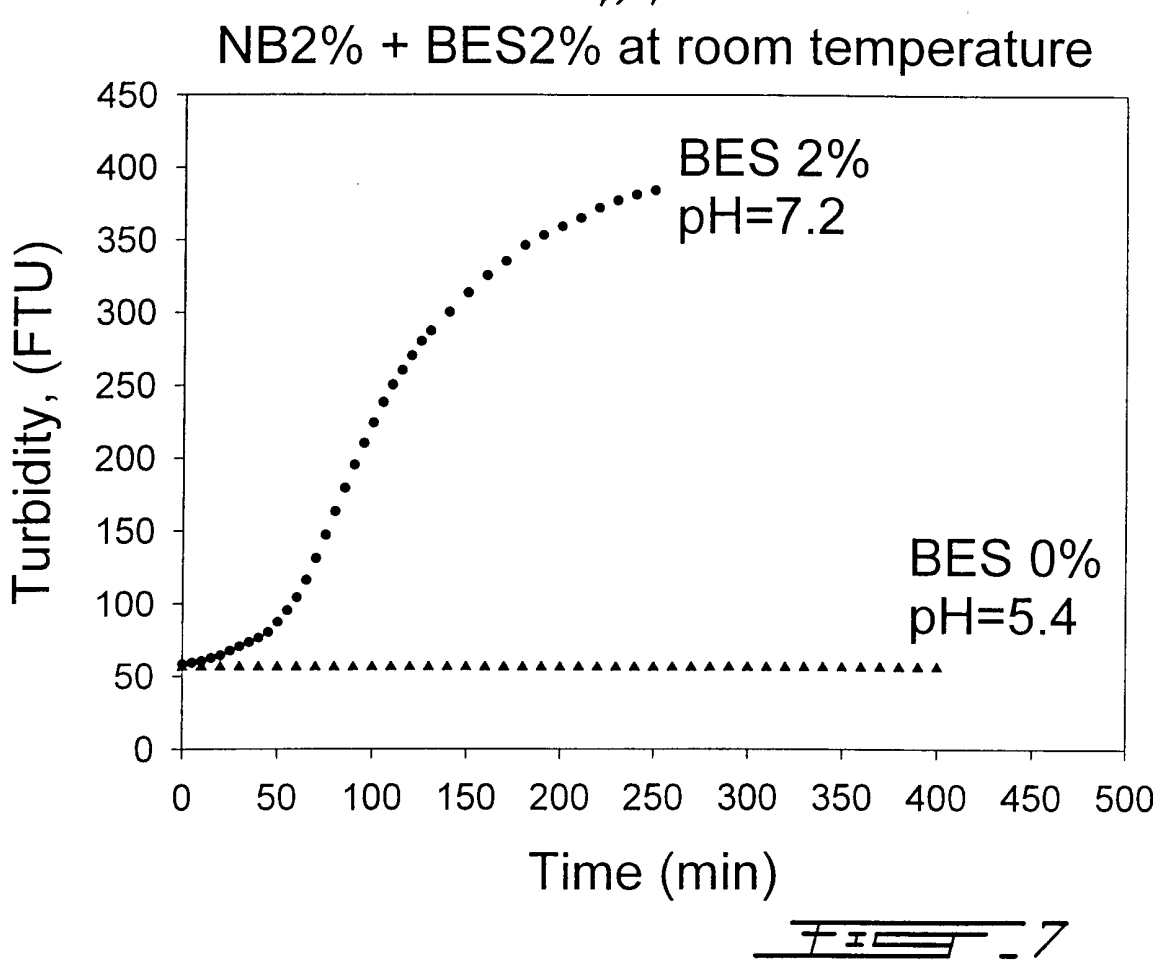
NB2% + BES2% at room temperature
Turbidity, (FTU)
Time (min)
BES 2%
pH=7.2
BES 0%
pH=5.4
0
50
100
150
200
250
300
350
400
450
500

Fig. 7

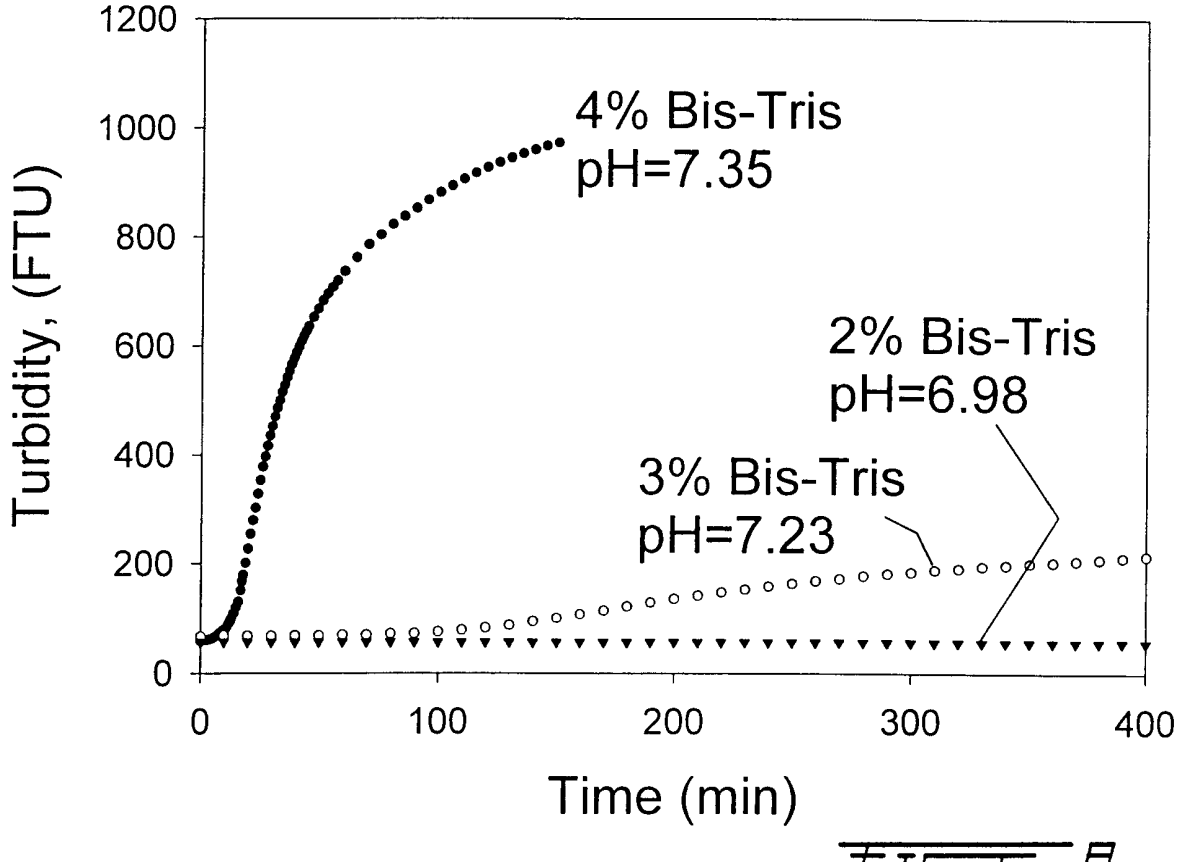
Turbidity, (FTU)
Time (min)
4% Bis-Tris
pH=7.35
2% Bis-Tris
pH=6.98
3% Bis-Tris
pH=7.23
0
200
400
600
800
1000
1200
0
100
200
300
400

Fig. 8

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/CA 00/01341

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/36 A61K47/42

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)
EPO-Internal, BIOSIS, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 07416 A (JALAL FAYROUZ ;SELMANI AMINE (CA); BIO SYNTECH LTD (CA); CHAPUT CY) 18 February 1999 (1999-02-18) page 5, line 10 -page 8, line 24 page 27 -page 28; example 1 ---	1-10, 18-31, 39-64
X	WO 97 33562 A (MERCK SHARP & DOHME CHIBRET SN ;CHASTAING GILLES (FR); ROZIER ANNO) 18 September 1997 (1997-09-18) page 9 -page 13; examples 1-3 ---	1,2,4, 23,24, 29-41
T	CHENITE A ET AL: "Novel injectable neutral solutions of chitosan form biodegradable gels in situ." BIOMATERIALS, vol. 21, no. 21, November 2000 (2000-11), pages 2155-2161, XP004216030 ISSN: 0142-9612 -----	1-64

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

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 2001	03/05/2001

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Boulois, D

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/CA 00/01341

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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