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(73) Titulaire(s):
LUXEMBOURG INSTITUTE OF SCIENCE AND TECHNOLOGY (LIST) –
4362 ESCH/ALZETTE (Luxembourg)

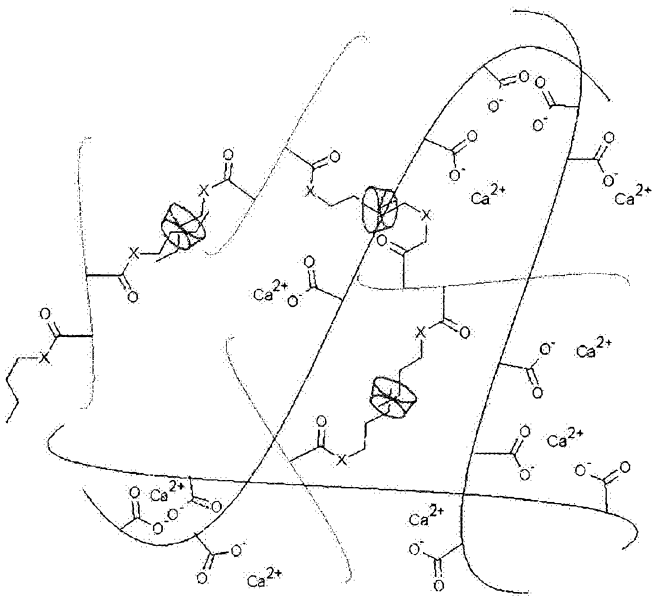
(72) Inventeur(s):
SCHLEEH THOMAS – 54338 SCHWEICH (Allemagne)

(74) Mandataire(s):
LECOMTE & PARTNERS SÀRL –
1016 LUXEMBOURG (Luxembourg)

(54) **Encapsulating agent with improved properties adapted for cell encapsulation.**

(57) Abstract : The invention is directed to a three-dimensional polymer network for encapsulating a pharmaceutical ingredient, said polymer network comprising (a) at least one first polymer; and (b) at least one cross-linking agent. Said three-dimensional polymer network is remarkable in that said at least one first polymer comprises a first polyuronate derivative, said first polyuronate derivative being modified with a hydrophobic moiety; and in that said at least one cross-linking agent is calcium chloride and/or a cyclodextrin derivative. (Fig. 1) 92895

Fig. 1



№ 92895

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Encapsulating agent with improved properties adapted for cell encapsulation

Description

Technical field

[0001] The invention is directed to the field of encapsulating agent adapted for encapsulating cell or cells.

Background art

[0002] An interpenetrating polymer network is a specific three-dimensional polymer network which comprises two or more networks that are at least partially interlaced on a molecular scale but not covalently bonded to each other and cannot be separated unless chemicals bonds are broken.

[0003] Microcapsules mean particles each comprising a matrix material having embedded therein a plurality of solid or liquid microparticles or solutes molecules. Microcapsules usually have a mean diameter of about 5 mm or smaller, *e.g.* between 1 mm and 0.05 mm, such as between 0.6 and 0.1 mm. They can also have a diameter *e.g.* between 2 mm and 0.01 mm, such as between 1.5 mm and 0.2 mm. Microcapsule can permanently or temporarily entrap substances like *e.g.* drugs, pesticides, dyes... Microcapsules can be designed in accordance with the chemical structure of a three-dimensional polymer network, and more specifically, in accordance with the chemical structure of an interpenetrating polymer network.

[0004] A hydrogel is a gel in which the swelling agent is water and may form a microcapsule. The network component of a hydrogel is usually a polymer.

[0005] A micro-sphere is a kind of microcapsule with a spherical shape without any membrane or any distinct outer layer.

[0006] A micro-bead is a polyethylene micro-sphere that is widely used in cosmetics as exfoliating agents and personal care products such as toothpaste, as well as biomedical and health science research, microscopy techniques, fluid visualization, fluid flow analysis, and process troubleshooting.

the α-CD was removed from the system to produce vesicles, and, when no α-CD was used, the system formed cross-linked micelles.

[0012] Supramolecular structures consisting in an inclusion complex of linear poly(ethyleneimine) with α-CD and γ-CD have been reported (Choi H. S. *et al.*, *Macromol.*, **2004**, *37*, 6705-6710).

[0013] Double-axle intrusion (DI) system using γ-CD and linear diblock copolymers have been reported (Joung Y.-K. *et al.*, *Adv. Mater.*, **2007**, *19* 396-400). The rheological properties in response to pH changes of those particular systems can be modulated.

[0014] A major drawback of the known system is in regard with the long-term stability of the microcapsules, micro-sphere, and/or micro-bead. Under the influence of the external conditions, such an alkaline media, those encapsulating agent collapse and disintegrate. Therefore, if cells are entrapped in such an agent, itself implanted in the human body, upon collapse of the entrapping agent, the cells are suddenly exposed to the external conditions and they died, without providing enough health benefit to the patient.

Summary of invention

Technical Problem

[0015] The invention has for technical problem to provide an interpenetrating polymer network adapted for cell encapsulation which overcomes the above mentioned drawbacks, in particular to provide such encapsulating agent which is stable in the long-term. It is indeed important for the cells present in the inner core of the microcapsule to survive and to work for long period of time in order to allow them to provide sufficient beneficial effects to the living body in which the microcapsules are located.

Technical solution

[0016] The invention has for first object a three-dimensional polymer network for encapsulating a pharmaceutical ingredient, said polymer network comprising (a) at least one first polymer; and (b) at least one cross-linking agent. Said three-dimensional polymer network is remarkable in that said at least one first polymer comprises a first polyuronate derivative, said first polyuronate derivative being modified with a hydrophobic moiety; and in that said at least one cross-linking agent is calcium chloride and/or a cyclodextrin derivative.

[0017] In a preferred embodiment, said three-dimensional polymer network further comprises a second polymer, said second polymer comprising preferentially a second polyuronate derivative, said second polyuronate derivative being preferentially unmodified.

[0018] In a preferred embodiment, said cyclodextrin derivative is selected from cyclodextrin, polymerized cyclodextrin, cyclodextrin modified with alginate with a degree of substitution equal to 1 or cyclodextrin modified with alginate with a degree of substitution inferior to 1.

[0019] In a preferred embodiment, said first polyuronate derivative has a degree of substitution equal to 1 or a degree of substitution inferior to 1.

[0020] In a preferred embodiment, said three-dimensional polymer network comprises a first polymer being a first polyuronate derivative modified with a hydrophobic moiety with a degree of substitution equal to 1, a second polymer being a second polyuronate derivative which is unmodified, and a cross-linking agent being calcium chloride and cyclodextrin, thereby forming an interpenetrating polymer network.

[0021] In a preferred embodiment, said three-dimensional polymer network comprises a first polymer being a first polyuronate derivative modified with a hydrophobic moiety with a degree of substitution equal to 1, a second polymer being a second polyuronate derivative which is unmodified, and a cross-linking agent being calcium chloride and polymerized cyclodextrin, thereby forming an interpenetrating polymer network.

[0022] In a preferred embodiment, said three-dimensional polymer network comprises a first polymer being a first polyuronate derivative modified with a hydrophobic moiety with a degree of substitution equal to 1, a second polymer being a second polyuronate derivative which is unmodified, and a cross-linking agent being calcium chloride and cyclodextrin modified with alginate with a degree of substitution equal to 1, thereby forming an interpenetrating polymer network.

[0023] In a preferred embodiment, said first polyuronate derivative and second polyuronate derivative are one of the derivative selected from mannuronate derivatives, guluronate derivatives, alginate derivatives, pectin derivatives, iduronate derivatives, galacturonate derivatives, lignin derivatives and/or any combination thereof.

[0024] In a preferred embodiment, said hydrophobic moiety modifying said first polyuronate derivative is selected from an alkyl moiety, a phenyl alkyl moiety, a fluoroalkane and/or any other hydrophobic derivatives.

[0025] In a preferred embodiment, said hydrophobic moiety modifying said first polyuronate derivative is covalently bounded to said first polyuronate derivative by an amide moiety, an ester moiety, a thioester moiety, a phosphonate moiety, an ether moiety, a thioether moiety, an imine moiety, or any other chemical group.

[0026] In a preferred embodiment, said three-dimensional polymer network encapsulates at least one pharmaceutical ingredient, said pharmaceutical ingredient being preferentially cells, more preferentially Langerhans islets.

[0027] The invention has for second object an encapsulating agent adapted for encapsulation of pharmaceutical ingredient. Said encapsulating agent is remarkable in that it comprises a three-dimensional polymer network according to the first object of the invention.

[0028] In a preferred embodiment, said encapsulating agent is under the form of a microcapsule, a micro-sphere or a micro-bead.

[0029] The invention has for third object an encapsulating agent for use as a medicament, preferentially for use in the treatment of cancer, diabetes and/or Parkinson disease, remarkable in that said encapsulating agent is

in accordance with the preferred embodiment of the second object of the invention.

[0030] The invention has for fourth object a process for making a three-dimensional polymer network, said process comprising the steps of (a) preparing an aqueous solution of at least one first polymer; (b) preparing an aqueous solution of at least one cross-linking agent; (c) mixing together under stirring the aqueous solutions so obtained in steps (a) and (b); and (d) after at least 30 minutes, filtering out the three-dimensional polymer network. Said process is remarkable in that said three-dimensional polymer network is in accordance with the three-dimensional polymer network of the first object of the invention.

Advantages of the invention

[0031] The invention is particularly interesting in that the encapsulating agents demonstrate an improved long-term stability and adjustable properties. The encapsulating agent of the present invention has a mesh size of the interpenetrating polymer network which is flexible because the hydrophobic groups present on the water-soluble polyelectrolyte are only entrapped or caged into the cyclodextrin inner cavity through supramolecular interaction. As this is not a covalent bonding between these two entities, the interpenetrating polymer network composing the encapsulating agent is flexible. Such kind of polymer network is therefore easily expandable and shrinkable.

Brief description of the drawings

[0032] Figure 1: Scheme of three-dimensional polymer network in accordance with the present invention.

[0033] Figure 2: Scheme of the crosslinking of two hydrophobic groups provided by a cyclodextrin derivative.

[0034] Figure 3: Scheme of the crosslinking of one hydrophobic group provided by a polymeric cyclodextrin derivative.

[0035] Figure 4: Generic chemical structure of the compounds used in the present invention.

Description of an embodiment

[0036] In order to provide an encapsulating agent able to encapsulate a cell, it is interesting to achieve such encapsulating agent which is not only very stable but also very flexible, *i.e.* which can easily swell and shrink. These properties will confer protection to the encapsulated cell.

[0037] The three-dimensional polymer network according to the present invention, or the specific interpenetrating polymer network, comprises a first polymer network formed by an ionic cross-linked hydrogel formed by a polyuronate derivative. A polyuronate derivative is a water-soluble polyelectrolyte with a polysaccharide structure, and can be chosen among mannuronate derivatives, guluronate derivatives, alginate derivatives, pectin derivatives, iduronate derivatives, galacturonate derivatives, lignin derivatives and/or any combinations thereof.

[0038] Preferentially, alginate derivatives are used.

[0039] Said polyuronate derivative, in particular said alginate derivative, is chemically modified with at least one hydrophobic moiety, which may be an alkyl moiety, a phenyl alkyl moiety, a fluoroalkane and/or any other hydrophobic derivatives.

[0040] Said hydrophobic moiety is covalently bounded to the polyuronate derivatives by an amide moiety. Other chemical functionalities can also be employed, such as an ester moiety, a thioester moiety, a phosphonate moiety, an ether moiety, a thioether moiety, an imine moiety, or any other.

[0041] Said polyuronate derivatives can also be chemically functionalized with 6-monodeoxy-6-monoamino-β-cyclodextrin hydrochloride.

[0042] The polyuronate derivatives are cross-linked with each other through a multivalent ion, preferentially Ca^{++}. This is achieved by adding calcium chloride during the process of making such an interpenetrating polymer network.

[0043] The three-dimensional polymer network according to the present invention, or the specific interpenetrating polymer network, comprises a

second polymer network formed by cyclodextrin moieties or by derivatives thereof such as polymerized cyclodextrins. Such polymerized cyclodextrin is formed through chemical connection of cyclodextrin moieties with epichlorhydrin.

[0044] Figure 1 schematically represents a three-dimensional polymer network in accordance with the present invention.

[0045] The cyclodextrins are a class of compound which presents an outer hydrophilic surface and an inner hydrophobic cavity. Cyclodextrins are distinguished according to the number of saccharide rings which correspond to a specific cavity diameter. For instance, the cavity diameter of α-cyclodextrin is 4.7Å-5.3Å, the cavity diameter of β-cyclodextrin is 6.0Å-6.5Å, and the cavity diameter of γ-cyclodextrin is 7.5Å-8.3Å. This hollow hydrophobic cavity can interact with hydrophobic compounds through weak interaction, *i.e.,* Van der Waals interactions.

[0046] The cyclodextrin derivatives can thus host the hydrophobic moiety which is present on the polyuronate derivatives.

[0047] These supramolecular interactions, in particular these Van der Waals interactions, which are showed either on figure 2 or on figure 3, allow a very high flexibility to the interpenetrating polymer network.

[0048] In figure 2, the cyclodextrin derivative cross-links two hydrophobic groups.

[0049] In figure 3, the cyclodextrin derivative (*i.e.* a polymeric cyclodextrin) cross-links only one hydrophobic group.

[0050] This allows to the whole structure to be kept together, and therefore, to confer protection to any objects, like cell or cells, which are entrapped or encapsulated inside the three-dimensional polymer network schematically depicted in figure 1 (these objects are not shown).

[0051] Table 1 indicates an overview of the polymers and crosslinking agents which can be combined to furnish the three-dimensional polymer network:

Crosslinking Agents	First polymer: hydrophobic-substituted polyuronate derivative Second polymer : n/a								First polymer: hydrophobic-substituted polyuronate derivative Second polymer: non-substituted polyuronate derivative							
	DS = 1				DS < 1				DS = 1				DS < 1			
$CaCl_2$					x	x		x	x	x	x	x	x		x	
CD	x				x				x				x			
Polymerized CD		x				x				x				x		
CD-Alg (DS = 1)			x				x				x				x	
CD-Alg (DS < 1)				x				x				x				x
IPN									x	x	x					
Three-dimensional polymer network	x	x	x	x	x	x	x	x				x	x	x	x	x

TABLE 1

[0052] The crosslinking agents can thus be chosen among calcium chloride ($CaCl_2$), cyclodextrin derivatives (CD), polymerized cyclodextrin (p-CD) and/or any combination thereof. Among the cyclodextrin derivatives, a preferred derivative is a cyclodextrin modified with alginate. The degree of substitution, which corresponds to the (average) number of substituent groups attached per base unit or per monomeric unit can be equal to 1 or be inferior to 1.

[0053] The three-dimensional polymer network according to the present invention, or the specific interpenetrating polymer network, may comprise a first polymer and a second polymer, or only one polymer.

[0054] Preferentially, a hydrophobic substituted polyuronic acid salt (a polyuronate derivative modified with a hydrophobic moiety) is used as first polymer, or as said one polymer in case where only one polymer is used.

[0055] In case where two polymers are used, a hydrophobic substituted polyuronic acid salt (a polyuronate derivative modified with a hydrophobic moiety) is used as first polymer and a polyuronic acid salt which is unsubstituted (a polyuronate derivative which is unmodified) is used as second polymer.

[0056] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS=1) and cyclodextrin has been designed.

[0057] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS=1) and polymerized cyclodextrin has been designed.

[0058] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS=1) and a cyclodextrin modified with alginate (DS=1) has been designed.

[0059] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS=1) and a cyclodextrin modified with alginate (DS<1) has been designed.

[0060] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS<1), calcium chloride and cyclodextrin has been designed.

[0061] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS<1), calcium chloride and polymerized cyclodextrin has been designed.

[0062] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS<1) and cyclodextrin modified with alginate (DS=1) has been designed.

[0063] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt (DS<1), calcium chloride and a cyclodextrin modified with alginate (DS<1) has been designed.

[0064] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS=1), an unsubstituted polyuronic salt as second polymer, calcium chloride and cyclodextrin has been designed. This three-dimensional polymer network has been characterized as forming an interpenetrating polymer network.

[0065] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS=1), an unsubstituted polyuronic salt as second polymer, calcium chloride and polymerized cyclodextrin has been designed. This three-dimensional polymer network has been characterized as forming an interpenetrating polymer network.

[0066] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS=1), an unsubstituted polyuronic salt as second polymer, calcium chloride and a cyclodextrin modified with alginate (DS=1) has been designed. This three-dimensional polymer network has been characterized as forming an interpenetrating polymer network.

[0067] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS=1), an unsubstituted polyuronic salt as second polymer, calcium chloride and cyclodextrin modified with alginate (DS<1) has been designed.

[0068] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS<1), an unsubstituted polyuronic salt as second polymer, calcium chloride, and cyclodextrin has been designed.

[0069] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS<1), an unsubstituted polyuronic salt as second polymer, calcium chloride and polymerized cyclodextrin has been designed.

[0070] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS<1), an unsubstituted polyuronic salt as second polymer and cyclodextrin modified with alginate (DS=1) has been designed.

[0071] The three-dimensional polymer network containing hydrophobic substituted polyuronic salt as first polymer (DS<1), an unsubstituted polyuronic salt as second polymer, calcium chloride and cyclodextrin modified with alginate (DS<1) has been designed.

[0072] Figure 4 indicates the generic structure of the compounds which have been used in the course of the present invention.

The compound of formula 1 is the sodium salt of the polyuronic acids. Alternatively, the potassium salt (not shown) can be used.

The compounds of formulas 2, 3, 4 and 5 are the hydrophobic modified polyuronic acids lacking of carboxylic acid moieties.

The compounds of formulas 6 and 7 are the hydrophobic modified sodium salt of the polyuronic acids.

The compounds of formulas 8, 9, 10 and 11 are the polyuronic acids modified with the cyclodextrin (CD).

Finally, the compound 12 schematically represents a polymerized cyclodextrin.

[0073] In order to design the three-dimensional polymer network according to the present invention, or the specific interpenetrating polymer network, the following procedure has been applied.

[0074] The preparation of an aqueous solution of the first polymer is performed.

[0075] If a second polymer has to be incorporated to the polymer network, the preparation of an aqueous solution of the second polymer is performed.

[0076] A cyclodextrin derivative must be added to the aqueous solution of the polymer.

[0077] Both said aqueous solution are then mixed upon each other and added to a hardening aqueous solution of calcium chloride. After gentle stirring, the microcapsule becomes harder and can be filtered out from the solution.

[0078] The three-dimensional polymer network according to the present invention, or the specific interpenetrating polymer network, is particular in that its building blocks, *i.e.* the cross-linking moieties, confer an improved protection to any active ingredients which are entrapped or encapsulated in the inner core of the network.

[0079] Indeed, once the three-dimensional polymer network or the interpenetrating polymer network starts, for any reason(s), to disintegrate, the cyclodextrin moieties start to cross-link with other hydrophobic moieties present in their close surroundings. Therefore, the whole system can be maintained as such, and the entrapped or encapsulated objects, *e.g.* cells, can stay protected from the external environment.

[0080] It has been in particular shown that in such a system the cells are still alive at a level of pH up to 9.5, in presence of a high concentration of sodium ion.

[0081] Examples of cells which can be encapsulated are the Langerhans islets which comprise hormone-producing cells. The size of such islets is comprised between 200 µm and 500 µm.

[0082] The three-dimensional polymer network or the interpenetrating polymer network as described above is the main component of an encapsulating agent adapted for cell encapsulation or adapted for cells encapsulation. Said encapsulating agent is either a microcapsule or a micro-sphere or a micro-bead.

[0083] The entrapped cells in the three-dimensional polymer network or in the interpenetrating polymer network (and subsequently in the encapsulating agent) is/are cell(s) able to be fed and able to secrete the proteins and/or the compounds used to treat disease, in particular burdensome diseases, such as cancer, diabetes, Parkinson's disease and/or any other.

[0084] These encapsulating agents can therefore act as a medicament and can further be implanted within the body of a living being, preferentially a

human being. They can be grafted or simply inserted under the skin. They can also be swallowed by a subject.

[0085] Example of design of three-dimensional polymer network.

[0086] A microcapsule has been designed comprising alginic acid butyl amide sodium salt as first polymer (DS_{amide} = 0.41), alginic acid sodium salt (compound of formula 1 on figure 2) as second polymer, calcium chloride and β-cyclodextrin as cross-liking agents.

50 mL of a solution comprising 1.66 % (w/v) aqueous alginic acid solution containing 0.82 % (w/v) β-cyclodextrin are prepared (solution 1).

50 mL of a solution comprising 2 % (w/v) aqueous alginic acid sodium salt solution are prepared (solution 2).

Both solutions 1 and 2 are mixed together, thereby forming a polymer aqueous solution.

Then the desired active ingredient is added in a predetermined amount, *e.g.* 1000 – 5000 cells/mL. Polysorbate as surfactant can be added in order to improve the homogeneity of the dissolved compounds.

1 L of a 0.1 M aqueous $CaCl_2$ solution is prepared as a hardening bath.

Then, the hardening bath is poured into a crystallizer dish (*e.g.* 2 - 3 cm filling height) and is stirred gently with a magnetic bar. The polymer solution is then dropped into the hardening bath with an encapsulator device or a microsyringe and the hardening of the microcapsules lasts at least 30 minutes under gentle stirring.

The microcapsules are then filtered out from the solution.

1. Réseau de polymère à trois dimensions pour l'encapsulation d'un ingrédient pharmaceutique, ledit réseau de polymères comprenant
    a. au moins un premier polymère; et
    b. au moins un agent de réticulation;

    caractérisé en ce que

    ledit au moins un premier polymère comprend un premier dérivé polyuronate, ledit premier dérivé polyuronate étant modifié avec un groupement hydrophobe; et en ce que

    ledit au moins un agent de réticulation est du chlorure de calcium et/ou un dérivé de cyclodextrine.

2. Réseau de polymère à trois dimensions selon la revendication 1, caractérisé en ce que ledit réseau de polymère à trois dimensions comprend en outre un second polymère, ledit second polymère comprenant de préférence un second dérivé polyuronate, ledit deuxième dérivé polyuronate étant préférentiellement non modifié.

3. Réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-2, caractérisé en ce que ledit dérivé de cyclodextrine est choisi parmi une cyclodextrine, une cyclodextrine polymérisée, une cyclodextrine modifiée avec de l'alginate avec un degré de substitution égal à 1 ou une cyclodextrine modifiée avec de l'alginate avec un degré de substitution inférieur à 1.

4. Réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-3, caractérisé en ce que ledit premier dérivé de polyuronate a un degré de substitution égal à 1 ou un degré de substitution inférieur à 1.

5. Réseau de polymère à trois dimensions selon la revendication 4, caractérisé en ce que ledit réseau polymère à trois dimensions comprend
    a. un premier polymère étant un premier dérivé polyuronate modifié avec un groupement hydrophobe avec un degré de substitution égal à 1,
    b. un deuxième polymère étant un second dérivé polyuronate qui est non-modifié, et
    c. un agent de réticulation étant le chlorure de calcium et une cyclodextrine,

    formant ainsi un réseau de polymère interpénétrant.

6. Réseau de polymère à trois dimensions selon la revendication 4, caractérisé en ce que ledit réseau de polymère à trois dimensions comprend
    a. un premier polymère étant un premier dérivé polyuronate modifié avec un groupement hydrophobe avec un degré de substitution égal à 1,
    b. un deuxième polymère étant second dérivé polyuronate qui est non-modifié, et
    c. un agent de réticulation étant le chlorure de calcium et une cyclodextrine polymérisée,

    formant ainsi un réseau de polymère interpénétrant.

7. Réseau de polymère à trois dimensions selon la revendication 4, caractérisé en ce que ledit réseau de polymère à trois dimensions comprend
    a. un premier polymère étant un premier dérivé polyuronate modifié avec un groupement hydrophobe avec un degré de substitution égal à 1,
    b. un deuxième polymère étant un second dérivé polyuronate qui est non-modifié, et
    c. un agent de réticulation étant le chlorure de calcium et une cyclodextrine modifiée avec de l'alginate avec un degré de substitution égal à 1,

    formant ainsi un réseau de polymère interpénétrant.

8. Réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-7, caractérisé en ce que ledit premier dérivé polyuronate et ledit deuxième dérivé polyuronate sont l'un des dérivés sélectionnés parmi des dérivés de mannuronate, des dérivés de guluronate, des dérivés d'alginate, des dérivés de pectine, des dérivés d'iduronate, des dérivés de galacturonate, des dérivés de lignine et/ou toute combinaison de ceux-ci.

9. Réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-8, caractérisé en ce que ledit groupement hydrophobe modifiant ledit premier dérivé polyuronate est sélectionné parmi un groupement alkyle, un groupement phénylalkyle, un fluoroalcane et/ou d'autres dérivés hydrophobes.

10. Réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-9, caractérisé en ce que ledit fragment modificateur hydrophobe ledit dérivé première de polyuronate est de manière covalente borné à ladite première dérivée de polyuronate par un groupement amide, un groupement ester, un groupement thioester, un fragment phosphonate, un groupement éther, un groupement thioéther, un groupement imine, ou tout autre groupe chimique.

11. Réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-10, caractérisé en ce que ledit réseau de polymère à trois dimensions encapsule au moins un ingrédient pharmaceutique, ledit ingrédient pharmaceutique étant préférentiellement des cellules plus préférentiellement des îlots de Langerhans.

12. Agent d'encapsulation adapté pour l'encapsulation d'ingrédient pharmaceutique, caractérisé en ce que ledit agent d'encapsulation comprend un réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-11.

13. Agent d'encapsulation selon la revendication 12, caractérisé en ce que ledit agent d'encapsulation est sous la forme d'une microcapsule, d'une microsphère ou d'une micro-bille.

14. Agent d'encapsulation pour une utilisation comme médicament, de préférence pour une utilisation dans le traitement du cancer, du diabète et/ou de la maladie de Parkinson, caractérisé en ce que ledit agent d'encapsulation est conforme à la revendication 13.

15. Procédé de fabrication d'un réseau de polymère à trois dimensions, ledit procédé comprenant les étapes suivantes:
    a. préparer une solution aqueuse d'au moins un premier polymère;
    b. préparer une solution aqueuse d'au moins un agent de réticulation;
    c. mélanger, sous agitation, les solutions aqueuses ainsi obtenues dans les étapes (a) et (b); et
    d. après au moins 30 minutes, filtrer le réseau de polymère à trois dimensions;

    caractérisé en ce que ledit réseau de polymère à trois dimensions est en accord avec le réseau de polymère à trois dimensions selon l'une quelconque des revendications 1-11.

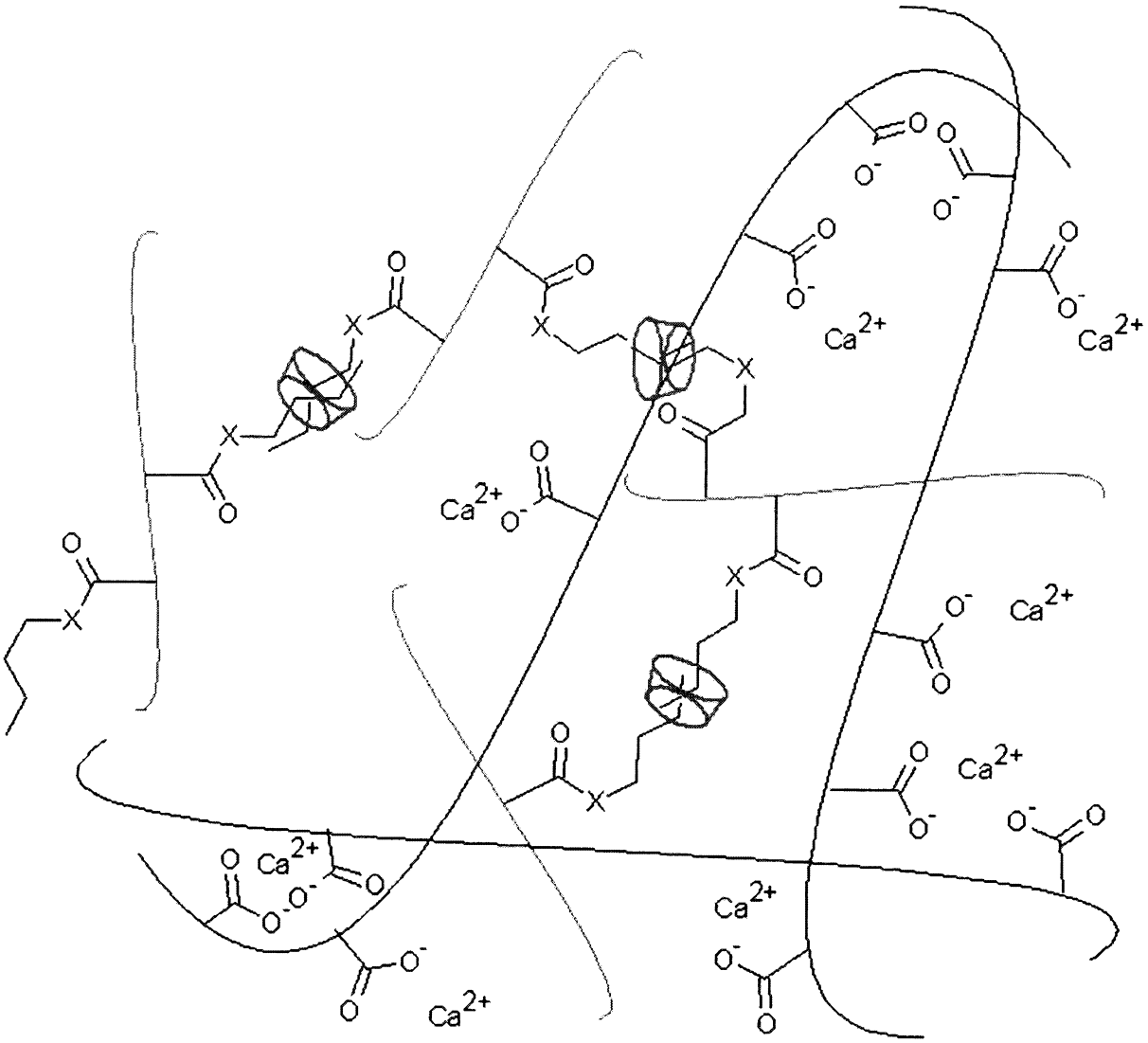


Fig. 1

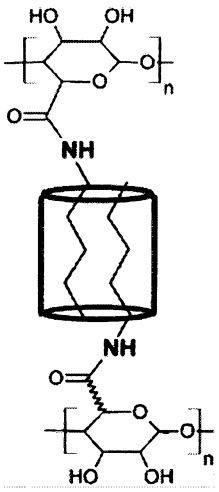


Fig. 2

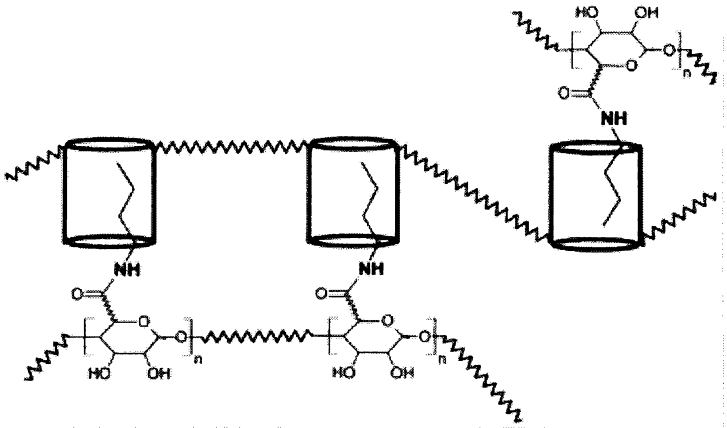
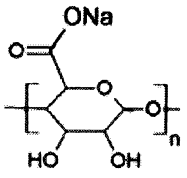
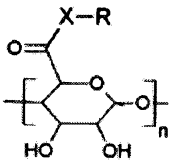


Fig. 3



(1)



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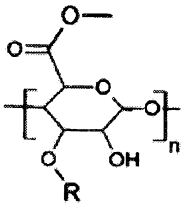

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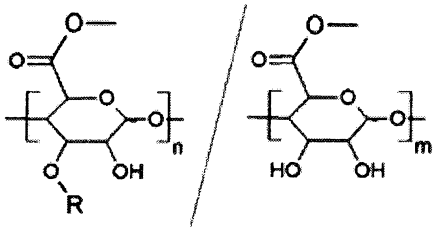

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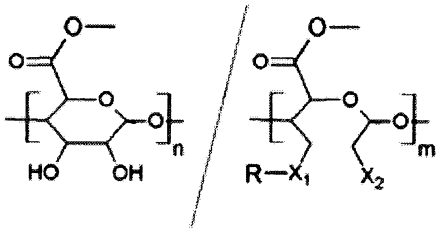

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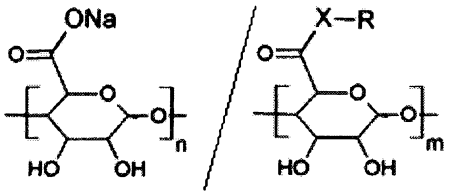

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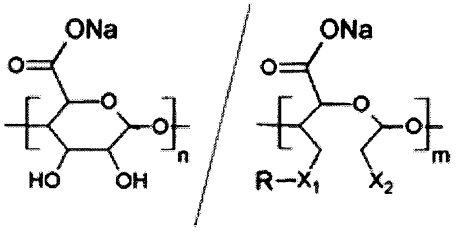

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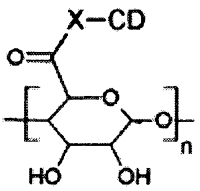

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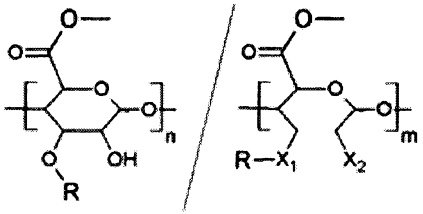

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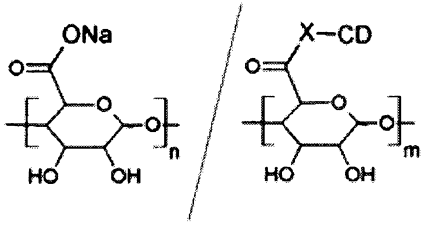

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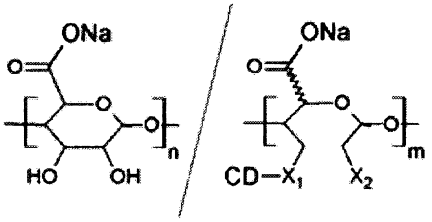

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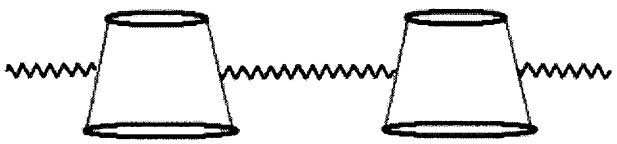

(12)

Fig. 4

Abstract

The invention is directed to a three-dimensional polymer network for encapsulating a pharmaceutical ingredient, said polymer network comprising (a) at least one first polymer; and (b) at least one cross-linking agent. Said three-dimensional polymer network is remarkable in that said at least one first polymer comprises a first polyuronate derivative, said first polyuronate derivative being modified with a hydrophobic moiety; and in that said at least one cross-linking agent is calcium chloride and/or a cyclodextrin derivative.

(Fig. 1)

LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Économie
et du Commerce extérieur

National Application Number

SEARCH REPORT

in accordance with Article 35.1 a)
of the Luxembourg law on patents
dated 20 July 1992

LO 1236
LU 92895

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	PLUEMSAB ET AL: "Synthesis and inclusion property of @a-cyclodextrin-linked alginate", POLYMER, ELSEVIER SCIENCE PUBLISHERS B.V, GB, vol. 46, no. 23, 14 November 2005 (2005-11-14), pages 9778-9783, XP005115553, ISSN: 0032-3861, DOI: 10.1016/J.POLYMER.2005.08.005 * abstract * * page 9778, column 1, line 12 - page 9780, column 2, line 21 * * page 9782 *	1-15	INV. A61K9/16 A61K35/12
X	"Cyclodextrin-linked alginate beads as supporting materials for Sphingomonas cloacae, a nonylphenol degrading bacteria", BIORESOURCE TECHNOLOGY, vol. 98, 18 October 2006 (2006-10-18), pages 2076-2081, XP002760216, * page 2077, column 1, line 31 - page 2078, column 2, line 31 * * page 2080; table 1 * * page 2080, column 1, line 14 - column 2, line 11 *	1-15	TECHNICAL FIELDS SEARCHED (IPC) A61K

-/--

The present search report has been drawn up for all claims

Date of completion of the search	Examiner
25 July 2016	Muller, Sophie

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

1

EPO FORM 1503 03.82 (P04C55)

LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Économie
et du Commerce extérieur

National Application Number

LO 1236
LU 92895

SEARCH REPORT

in accordance with Article 35.1 a)
of the Luxembourg law on patents
dated 20 July 1992

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	NAKAUMA MAKOTO ET AL: "Calcium binding and calcium-induced gelation of sodium alginate modified by low molecular-weight polyuronate", FOOD HYDROCOLLOIDS, vol. 55, 18 November 2015 (2015-11-18), pages 65-76, XP029367550, ISSN: 0268-005X, DOI: 10.1016/J.FOODHYD.2015.10.021 * abstract * * page 66 - page 67 * * page 75 * -----	1-15	

TECHNICAL FIELDS SEARCHED (IPC)

The present search report has been drawn up for all claims

Date of completion of the search	Examiner
25 July 2016	Muller, Sophie

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EPO FORM 1503 03.82 (P04C55)

LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Économie
et du Commerce extérieur

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
LO1236	08.12.2015		LU92895

International Patent Classification (IPC)
INV. A61K9/16 A61K35/12

Applicant
Luxembourg Institute of Science and Technology (LIST)

This report contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☐ Box No. VIII Certain observations on the application

Examiner
Muller, Sophie

Application No.
LU92895

WRITTEN OPINION

Box No. I Basis of the opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	2, 5-7
	No:	Claims	1, 3, 4, 8-15
Inventive step	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Cited Documents

The following documents are referred to in this communication:

D1 PLUEMSAB ET AL: "Synthesis and inclusion property of @a-cyclodextrin-linked alginate",
POLYMER, ELSEVIER SCIENCE PUBLISHERS B.V, GB,
vol. 46, no. 23, 14 November 2005 (2005-11-14), pages 9778-9783,
XP005115553,
ISSN: 0032-3861, DOI: 10.1016/J.POLYMER.2005.08.005

D2 "Cyclodextrin-linked alginate beads as supporting materials for Sphingomonas cloacae, a nonylphenol degrading bacteria",
BIORESOURCE TECHNOLOGY,
vol. 98, 18 October 2006 (2006-10-18), pages 2076-2081,

D3 NAKAUMA MAKOTO ET AL: "Calcium binding and calcium-induced gelation of sodium alginate modified by low molecular-weight polyuronate",
FOOD HYDROCOLLOIDS,
vol. 55 , pages 65-76, XP029367550,
ISSN: 0268-005X, DOI: 10.1016/J.FOODHYD.2015.10.021

2. Novelty

D1 discloses (see the abstract; page 9778, column 1, line 12 - page 9780, column 2, line 21 and page 9782) stable and spherical beads which are obtained by dropping an aqueous solution of modified cyclodextrin-alginate (DS=0,24) as first polymer into a calcium chloride solution as cross-linking agent. These cyclodextrin-alginate beads would provide a suitable structure for bacteria cell encapsulation in addition to the inclusion ability for guest compound.

The subject-matter of claims 1,3,4,8-15 is therefore not new in view of D1.

D2 discloses (see page 2077, column 1, line 31 - page 2078, column 2, line 31; table 1 on page 2080 and page 2080, column 1, line 14 - column 2, line 11) beads comprising a three-dimensional microporous network structure comprising alpha-cyclodextrin alginate (first polymer) and calcium chloride as a supporting matrix for bacterial degradation of nonylphenol, an endocrine disruptor.

The subject-matter of claims 1,3,4,8-15 is therefore not new in view of D2.

3. Inventive Step

The subject-matter of claims 1,3,4,8-15 is not new and can therefore not involve an inventive step.

Present claims 2,5-7 are not considered to be inventive with respect to D1-D3, insofar as they do not appear to comprise any technical features which lead to a non-obvious solution of a technical problem.

4. Industrial applicability

Claims 1-15 satisfy the criterion of industrial applicability.