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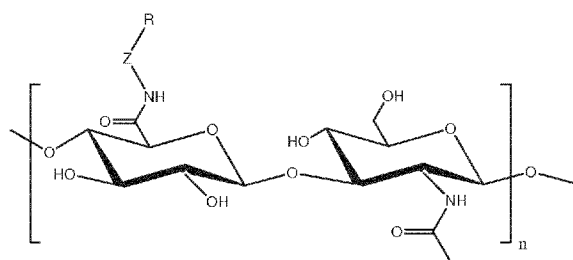
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(54) Title: USES OF HYDROPHOBICALLY-MODIFIED HYALURONIC ACID THROUGH AMIDE AND/OR HYDRAZIDE LINKAGES IN COSMETICS AND/OR DERMATOLOGY



Formule (I)

(57) Abstract: The present invention relates to the field of cosmetics and pharmaceuticals such as dermatology. Especially, the present invention relates to particles made of hyaluronic acid modified by amidation with hydrophobic groups having an amine or a hydrazine function having general formula (I), wherein Z, R and n are as defined in the claims. In particular, the particles of the invention are useful for restoring the endogenic production of hyaluronic acid and provide a lasting effect thanks to their slow degradation *in vivo*. The present invention also refers to a cosmetic care method comprising applying the particles of the invention on the skin of a subject in need thereof.

**USES OF HYDROPHOBICALLY-MODIFIED HYALURONIC ACID
THROUGH AMIDE AND/OR HYDRAZIDE LINKAGES IN COSMETICS
AND/OR DERMATOLOGY**

5 FIELD OF INVENTION

The present invention relates to the field of cosmetics and pharmaceuticals such as dermatology. Especially, the present invention relates to particles of hyaluronic acid hydrophobically modified by amidation with hydrophobic groups having an amine or a hydrazine function; and their use as a stimulating agent in the epidermis and/or in the
10 dermis. In particular, the particles of the invention are useful for restoring the endogenic production of hyaluronic acid and providing a lasting effect thanks to their slow degradation *in vivo*. The present invention also refers to a skin improvement method comprising applying the particles of the invention.

15 BACKGROUND OF INVENTION

Both the largest and the heaviest organ in the human body, the skin plays many fundamental biologic functions such as thermal regulation, barrier against the external environment or hormonal synthesis.

In addition, the skin also ensures a psychosocial role that impacts the social interactions
20 between individuals.

Its structure is organized in layers comprising the epidermis and the dermis.

The epidermis refers to the superficial layer of the skin made of keratinocyte cells, melanocytes cells or Langerhans cells. Its role is mainly directed to protection (immunity function).

25 Located under the epidermis, the dermis is the resistant part of the skin and is made of water, glycosaminoglycans (such as hyaluronic acid) and fibers of proteins (such as, elastin and collagen). These proteins, and glycosaminoglycans (and particularly

endogenic hyaluronic acid), allow ensuring good physical properties to the skin (i.e. toughness, elasticity and smoothness).

Over time, the physical properties of the skin decrease due to the lack of glycosaminoglycans, proteins and cells renewal. Consequently, the aging effects appear.

- 5 For overcoming these drawbacks, many cosmetic and/or dermo-cosmetic products comprising hyaluronic acid chains have been developed and marketed.

However, these products, (1) either contain hyaluronic acid having high molar weight that cannot diffuse through the deepest layer of the skin; or (2) contain shorter hyaluronic acid that induces inflammatory phenomenon after its application on the skin.

- 10 Second, these products only aim to be used as filling agents and/or as hydrating agents, and they do not focus on the provision of an efficient way to reactivate the production of endogenic hyaluronic acid.

- Furthermore, after the *in vivo* administration of the product in a subject in need thereof, the hyaluronic acid chains are easily degraded by the human organism over time. This
15 degradation implies a regular administration of the product by the subject that may be uncomfortable.

Thus, there still exists a need for providing efficient cosmetic, dermo-cosmetic and/or dermatological compounds for avoiding the drawbacks as described above.

- Surprisingly, the Applicant has evidenced that using hyaluronic acid modified by
20 hydrophobic groups through an amide and/or a hydrazide bond allows:

- diffusing both in the epidermis and the dermis;
- reactivating the endogenous hyaluronic acid production; and
- reducing in the administration frequency of the product by the subject in need thereof.

- 25 Preferably, the hyaluronic acid modified by hydrophobic groups through an amide and/or a hydrazide bond, is under the form of particles.

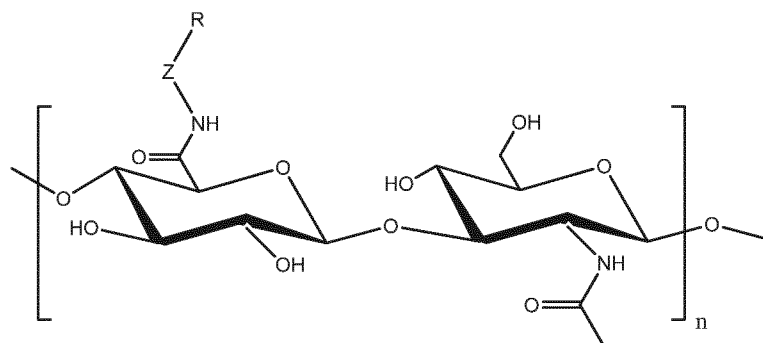
Advantageously, the hydrophobically modified hyaluronic acid particles of the invention are able to efficiently diffuse in the epidermis and the dermis; especially, in the papillary dermis, in the upper reticular dermis and in the lower reticular dermis. Furthermore, the particles of the invention do not provoke any inflammatory side-effects.

- 5 Advantageously, the particles of the invention are able to increase the expression of collagen III and/or elastin in the skin; preferably, in the epidermis and/or the dermis; more preferably, in the papillary dermis, in the upper reticular dermis and in the lower reticular dermis.

- Advantageously, the amide and the hydrazide linkages are more resistant to degradation
10 by hydrolysis of hyaluronidase enzymes in the skin compared to esters linkages. Consequently, the particles of the invention allow a lasting action, even after a sole application, contrary to the hydrophobic-modified HA having ester linkage, hyaluronic acid or any salt thereof such as sodium hyaluronate.

15 SUMMARY

The present invention thus concerns the use as a long lasting antiaging agent of a particle comprising at least one hyaluronic acid derivative of general formula (I):



Formule (I)

or any salts and solvates thereof,

- 20 wherein

Z represents a single bond or -NH-;

R represents a C1-C20 alkyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group;

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer; and

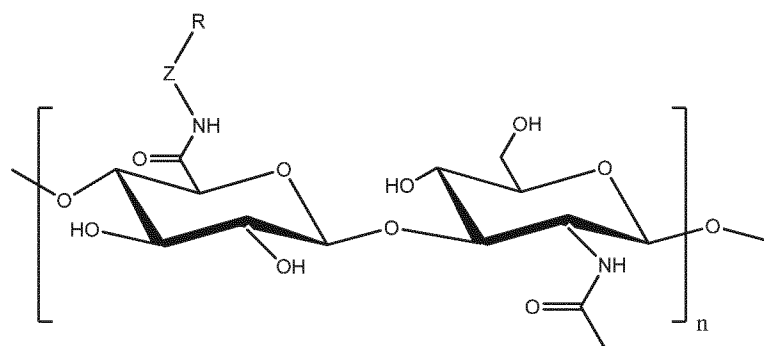
- 5 wherein the particle has a mean hydrodynamic diameter ranging from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm.

According to one embodiment, the degree of substitution (DS) of the hyaluronic acid derivatives of formula (I) ranges from 0.1 % to 55 %.

- 10 According to one embodiment, the molecular weight of the hyaluronic acid derivatives of formula (I) ranges from 100 kDa to 4 000 kDa; preferably, from 200 kDa to 3 000 kDa.

According to one embodiment, the long lasting antiaging agent is a long lasting skin plumping agent, a long lasting skin hydrating agent and/or a long lasting stimulating agent of the endogenic production of hyaluronic acid.

- 15 The present invention also concerns a particle comprising at least one hyaluronic acid derivative of general formula (I):



Formule (I)

or any salts and solvates thereof,

wherein

- 20 Z represents -NH-;

R represents a C1-C20 alkyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group;

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer; and

- 5 wherein the particle has a mean hydrodynamic diameter ranging from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm.

The present invention also concerns a particle as defined above, for use as a medicament.

- 10 According to one embodiment, the particle as defined above is for use in the treatment and/or the prevention of dermatological conditions; preferably as a healing agent.

The present invention also concerns a cosmetic and/or dermo-cosmetic composition comprising at least one particle as defined above, and a cosmetically acceptable base.

According to one embodiment, the cosmetic and/or dermo-cosmetic composition of the invention is under an injectable form.

- 15 The present invention also concerns a skin improvement method comprising using the cosmetic and/or dermo-cosmetic composition of the invention in a subject in need thereof.

According to one embodiment, the skin improvement method of the invention is for restoring the production of endogenic hyaluronic acid in the epidermis and/or the dermis.

- 20 According to one embodiment, the skin improvement method of the invention is for limiting the shortcomings of the skin; preferably, for improving the hydration of the skin.

According to one embodiment, the composition of the invention is administered to the skin of a subject in need thereof, once per week.

- 25 According to one embodiment, the composition of the invention is administered to the epidermis and/or the dermis of a subject in need thereof, by a topical application or by injection.

DEFINITIONS

In the present invention, the following terms have the following meanings:

- **“amidation reaction”**: relates to the reaction between the carboxylic function of the hyaluronic acid with (1) either the amine function of a hydrophobic compound or (2) the hydrazine function of a hydrophobic compound. In the present invention, the resulting linkage may be either an amide linkage or a hydrazide linkage;
- **“antiaging agent”**: relates to a compound that limits the effects of the age on the skin; preferably, it relates to skin plumping agent and/or skin hydrating agent;
- **“alkyl”**: relates to any saturated linear or branched hydrocarbon chain, with 1 to 20 carbon atoms, preferably 6 to 18 carbon atoms, preferably hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl; more preferably, octadecyl;
- **“alkenyl”**: relates to any unsaturated linear or branched hydrocarbon chain, with 1 to 20 carbon atoms, preferably 6 to 18 carbon atoms, preferably hexenyl, heptenyl, octenyl, nonenyl, decenyl, undecenyl, dodecenyl, tridecenyl, tetradecenyl, pentadecyl, hexadecyl, heptadecyl, octadecenyl; more preferably, tetradecenyl, octadecenyl;
- **“cycloalkyl”**: relates to a substituted or not substituted cyclic alkyl substituent such as cyclopropyl, cyclopentyl, or cyclohexyl;
- **“cosmetically acceptable base”**: relates to any compound for use in contact with the epidermis and/or the dermis, and which does not provoke any side effects such as toxicity, irritation, inflammation or allergic response. In the present invention, a cosmetically acceptable base includes base for topical administration and/or injection;
- **“cosmetic use”** refers to the use in order to improve the esthetic appearance of the body;
- **“degree of substitution (DS)”**: relates to the number of the carboxylic functions of the hyaluronic acid that have been modified after amidation with at least one hydrophobic group having an amine or a hydrazine function;

- **“dermis”**: relates to the conjunctive tissue forming with the epidermis and the hypodermis, the skin. The dermis is constituted of the papillary dermis and the reticular dermis;
- 5 - **“endogenic hyaluronic acid”**: relates to hyaluronic acid synthesized by the human body;
- **“epidermis”**: relates to the superficial layer of the skin made of keratinized dead cells;
- **“healing agent”**: relates to a compound able to favor the decrease of wounds; preferably, of superficial wounds; more preferably, of the superficial wounds of the skin or the mucous;
- 10 - **“long-lasting agent”**: relates to a compound able to provide its effects for a long time. For example, a “long-lasting antiaging agent” refers to a compound having antiaging properties, said properties being maintained or improved for a long time compared to the effects of a non-long-lasting antiaging agent;
- 15 - **“particle”**: relates to an assembly of the hyaluronic acid derivatives of the invention organized by weak bonds, i.e. an assembly of hyaluronic acid modified by amidation with at least one hydrophobic group having an amine or a hydrazine function. In the present invention, the term “particle” comprises nanoparticles and/or microparticles obtained from hydrophobically modified hyaluronic acid of the invention. According to one embodiment, the term “particles” does not comprise polymersomes or
20 liposomes. According to one embodiment, the term “particles” does not comprise aggregates;
- **“pharmaceutically acceptable base”**: relates to any ingredients of a pharmaceutical composition that are compatible with each other and not deleterious to the subject to which it is administered;
- 25 - **“hydrophobic”**: relates to a compound or a chemical group that has no affinity for water or aqueous medium;
- **“skin plumping agent”**: relates to a compound able to provide a measurable increase of the skin density; especially, of the epidermis and/or the dermis. The “skin plumping

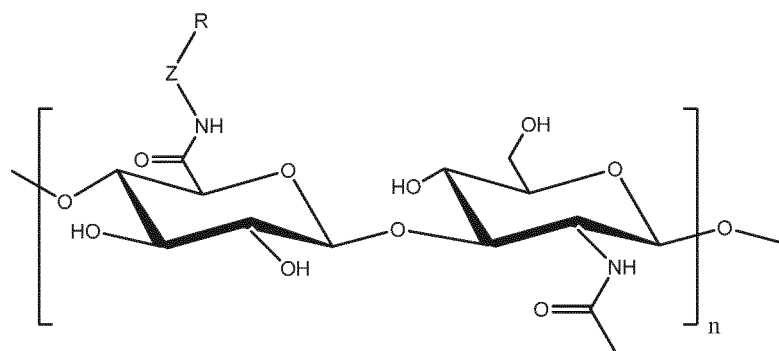
effect” may be analyzed by echography or by the implementation of techniques suitable of the human body area for which the effect is analyzed;

- “**skin hydrating agent**”: relates to a compound able to overcome the dry skin; especially, to overcome the dry of the epidermis and/or the dermis and/or the mucous.

5

DETAILED DESCRIPTION

This invention relates to a hyaluronic acid derivative; especially, a hyaluronic acid modified by amidation with at least one hydrophobic group through an amine or a hydrazine function, of general formula (I):



Formule (I)

10

or any salts and solvates thereof,

wherein

Z represents a single bond or -NH-;

R represents a C1-C20 alkyl or C1-C20 alkenyl group; preferably, a C6-C18 alkyl group;

15 more preferably, a C18 alkyl group; and

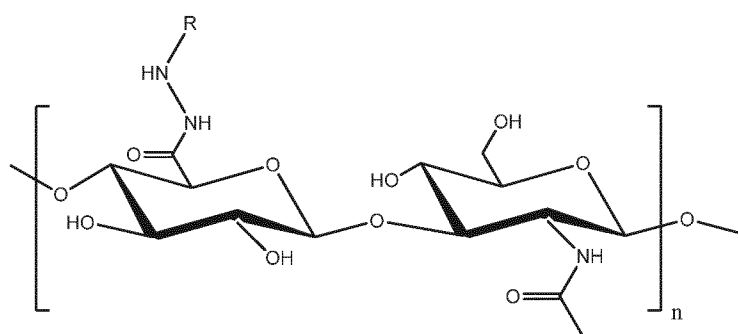
n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer.

According to one embodiment, the hyaluronic acid salt is a hyaluronate, preferably sodium hyaluronate. According to one embodiment, hydrophobic groups are grafted on

20 sodium hyaluronate.

According to one embodiment, R represents a linear C1-20 alkyl group or a linear C1-C20 alkenyl group, preferably a linear C6-C18 alkyl group, more preferably a linear C18 alkyl group. According to one embodiment, R represents a C1-C20 alkyl group. According to one embodiment, R represents a C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17 or C18 alkyl group, preferably a linear C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17 or C18 alkyl group.

According to one embodiment, preferred compounds are of formula (Ia):



Formule (Ia)

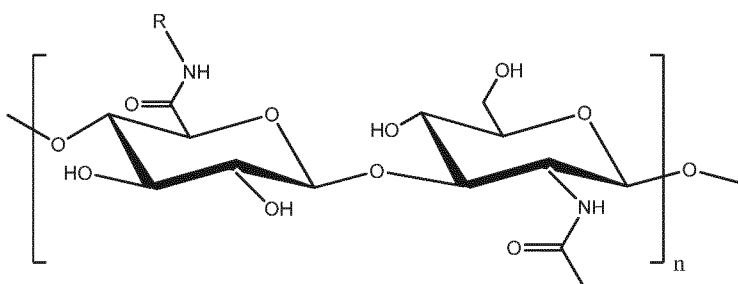
or any salts and solvates thereof,

wherein

R represents a C1-C20 alkyl or C1-C20 alkenyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group; and

n represents the number of disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine and is a positive integer.

According to another embodiment, preferred compounds are of formula (Ib):



Formule (Ib)

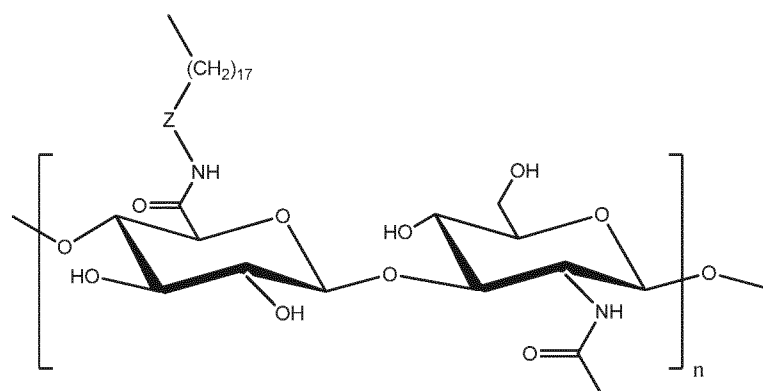
or any salts and solvates thereof,

wherein

R represents a C1-C20 alkyl or C1-C20 alkenyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group; and

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer.

Especially, the present invention relates to a hyaluronic acid modified by amidation with at least one hydrophobic group through an amine or a hydrazine function, of general formula (II):



Formule (II)

or any salts and solvates thereof,

wherein

Z represents a single bond or -NH-; and

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer.

According to one embodiment, when Z is a single bond, R is not a C1-, C15-, C16- or C20-alkyl group. According to one embodiment, when Z is a single bond, R is not a C8-, C12- or C16-alkyl group. According to one embodiment, R does not represent a C8-alkyl group. According to one embodiment, R does not represent a C12-alkyl group. According to one embodiment, R does not represent a C16-alkyl group.

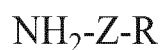
According to one embodiment, hyaluronic acid is not partially deacetylated. According to one embodiment, hyaluronic acid is not deacetylated.

According to one embodiment, hyaluronic acid derivatives of formula (I) or (II) have a molecular weight ranging from 3 kDa to 4 000 kDa; preferably from 200 kDa to 3 000 kDa. According to one embodiment, hyaluronic acid derivatives of formula (I) or (II) have a molecular weight ranging from 100 kDa to 4 000 kDa; preferably from 200 kDa to 3 000 kDa. According to one embodiment, hyaluronic acid derivatives of formula (I) or (II) have a molecular weight of about 200 kDa or of about 3000 kDa. According to one embodiment, hyaluronic acid derivatives of formula (I) or (II) have a molecular weight of about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900 or 4000 kDa.

According to one embodiment, the degree of substitution (DS) of the hyaluronic acid by the hydrophobic groups ranges from 0.01 % to 55 %; preferably, from 1 % to 15%; more preferably, from 1 to 5 %; more preferably, from 10 to 15 %. According to one embodiment, the degree of substitution (DS) of the hyaluronic acid by the hydrophobic groups ranges from 0.1 % to 55 %; preferably, from 1 % to 15 %; more preferably, from 1 to 5 %; more preferably, from 10 to 15 %. According to one embodiment, the degree of substitution (DS) is about 1.5 %. According to one embodiment, the degree of substitution (DS) is about 12 %. The degree of substitution (DS) of the hyaluronic acid by the hydrophobic groups was determined by NMR (*Nuclear Magnetic Resonance*) analysis by comparing the area of cyclic protons of the hyaluronic acid or the sodium hyaluronate (10 protons between 3.5 and 54.3 ppm), with the terminal CH₃ of the aliphatic graft (3 protons at around 1.57 ppm for 100 % of substitution). The calculation may be also done by comparing the areas of the terminal CH₃ of the aliphatic graft (3 protons around 1.57 ppm for 100 % substitution) and the CH₃ of *N*-acetyl glucosamine (3 protons around 1.47 ppm). According to one embodiment, the degree of substitution (DS) of the hyaluronic acid by the hydrophobic groups ranges from 0.1 % to 5 %; preferably from 0.1 % to 4 % preferably from 0.1 % to 3 % preferably from 0.1 % to 2 % preferably from 0.1 % to 1 %. According to one embodiment, the degree of substitution (DS) of the hyaluronic acid by the hydrophobic groups ranges from 0.1 % to 10 %. According to one embodiment, the degree of substitution (DS) of the hyaluronic acid by the hydrophobic

groups is about 1 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 % or 55 %. According to one embodiment, the degree of substitution (DS) of the hyaluronic acid by the hydrophobic groups is about 1 %, 2 %, 3 %, 4 %, 5 %, 6 %, 7 %, 8 %, 9 %, 10 %, 11 %, 12 %, 13 %, 14 % or 15 %.

- 5 According to one embodiment, the hydrophobic groups are grafted to the hyaluronic acid by a covalent linkage; especially, by an amide linkage or a hydrazide linkage. According to one embodiment, the hydrophobic groups are grafted to the hyaluronic acid or any salts and solvates thereof, by a covalent linkage; especially, by an amide linkage or a hydrazide linkage.
- 10 According to one embodiment, the hyaluronic acid is modified by an amidation between the carboxylic acid function of hyaluronic acid and the amine function of the hydrophobic compounds to be grafted. According to one embodiment, the hyaluronic acid salt which is a hyaluronate, is modified by an amidation between the carboxylate function of said hyaluronate and the amine function of the hydrophobic compounds to be grafted.
- 15 According to one embodiment, the hydrophobic compound is selected from the organic compounds having at least one amine function or having at least one hydrazine function, i.e. a function having the following structure:



wherein

- 20 Z represents a single bond or -NH-; and
- R represents a C1-C20 alkyl or C1-C20 alkenyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group.

According to one embodiment, the hydrophobic compound is not hydrazine molecule, i.e. compound of formula: $\text{NH}_2\text{-NH}_2$.

- 25 According to one embodiment, the modified hyaluronic acid does not comprise free-hydrazido groups, i.e. -NH-NH₂ or -NR'-NH₂ pendent groups wherein R' is an organic functional group as for example an alkyl, an aryl, or an alkene.

According to one embodiment, the modified hyaluronic acid does not comprise oxalic hydrazido groups.

According to one embodiment, the hyaluronic acid is modified by an alkylamine; preferably, methylamine, ethylamine, propylamine, butylamine, pentylamine, 5 hexylamine, heptylamine, octylamine, nonylamine, decylamine, undecylamine, dodecylamine, tridecylamine, tetradecylamine, pentadecylamine, hexadecylamine, heptadecylamine, octadecylamine; nonadecylamine, eicosylamine; more preferably, hexylamine, heptylamine, octylamine, nonylamine, decylamine, undecylamine, dodecylamine, tridecylamine, tetradecylamine, pentadecylamine, hexadecylamine, 10 heptadecylamine, octadecylamine. According to one embodiment, the hyaluronic acid is not modified by octylamine. According to one embodiment, the hyaluronic acid is not modified by dodecylamine. According to one embodiment, the hyaluronic acid is not modified by hexadecylamine.

According to one embodiment, the hyaluronic acid is modified by alkenylamine; 15 preferably, ethylenylamine, propylenylamine, butylenylamine, pentenylamine, hexenylamine, heptenylamine, octenylamine, nonenylamine, decenylamine, undecenylamine, dodecenylamine, tridecenylamine, tetradecenylamine, pentadecenylamine, hexadecenylamine, heptadecenylamine, octadecenylamine; nonadecenylamine, eicosenylamine; more preferably, hexenylamine, heptenylamine, 20 octenylamine, nonenylamine, decenylamine, undecenylamine, dodecenylamine, tridecenylamine, tetradecenylamine, pentadecenylamine, hexadecenylamine, heptadecenylamine, octadecenylamine.

According to one embodiment, the hyaluronic acid is modified by octadecylamine. According to one embodiment, the amine is not octylamine, hexadecylamine or 25 octadecylamine. According to one embodiment, the amine compound does not comprise more than one amine function. According to one embodiment, the amine compound does not comprise ethylamine. According to one embodiment, the amine compound does not comprise any amino-acids or derivatives. According to one embodiment, the amine compound does not comprise any amino-acid ester derivatives.

According to one embodiment, the amine compound does not comprise a hydroxyl group.
According to one embodiment, the amine compound does comprise other chemical functional groups than amino group.

According to one embodiment, the hyaluronic acid is modified by an alkylhydrazine;
5 preferably, methylhydrazine, ethylhydrazine, propylhydrazine, butylhydrazine, pentylhydrazine, hexylhydrazine, heptylhydrazine, octylhydrazine, nonylhydrazine, decylhydrazine, undecylhydrazine, dodecylhydrazine, tridecylhydrazine, tetradecylhydrazine, pentadecylhydrazine, hexadecylhydrazine, heptadecylhydrazine, octadecylhydrazine; nanodecylhydrazine, eicosylhydrazine; more preferably,
10 hexylhydrazine, heptylhydrazine, octylhydrazine, nonylhydrazine, decylhydrazine, undecylhydrazine, dodecylhydrazine, tridecylhydrazine, tetradecylhydrazine, pentadecylhydrazine, hexadecylhydrazine, heptadecylhydrazine, octadecylhydrazine.
According to one embodiment, the hyaluronic acid is modified by octadecylhydrazine.
According to one embodiment, the hyaluronic acid is modified by octadecylhydrazine
15 (also called stearylhydrazine).

According to one embodiment, the hyaluronic acid is modified by an alkenylhydrazine;
preferably, ethylenylhydrazine, propylenylhydrazine, butylenylhydrazine, pentylenylhydrazine, hexylenylhydrazine, heptylenylhydrazine, octylenylhydrazine, nonylenylhydrazine, decylenylhydrazine, undecylenylhydrazine, dodecylenylhydrazine,
20 tridecylenylhydrazine, tetradecylenylhydrazine, pentadecylenylhydrazine, hexadecylenylhydrazine, heptadecylenylhydrazine, octadecylenylhydrazine; nonadecylenylhydrazine, eicosylenylhydrazine; more preferably, hexylenylhydrazine, heptylenylhydrazine, octylenylhydrazine, nonylenylhydrazine, decylenylhydrazine, undecylenylhydrazine, dodecylenylhydrazine, tridecylenylhydrazine,
25 tetradecylenylhydrazine, pentadecylenylhydrazine, hexadecylenylhydrazine, heptadecylenylhydrazine, octadecylenylhydrazine.

According to one embodiment, the hydrazine compound does not comprise more than one hydrazine function. According to one embodiment, the hydrazine compound is a linear hydrophobic compound having one hydrazine function.

According to one embodiment, the hydrophobically-modified HA derivative is a HA of 200 kDa grafted with a C8 alkyl group via a hydrazide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 200 kDa grafted with a C6 alkyl group via an amide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 3000 kDa grafted with a C8 alkyl group via a hydrazide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 3000 kDa grafted with a C6 alkyl group via an amide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 3000 kDa grafted with a C18 alkyl group via an amide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 3000 kDa grafted with a C18 alkyl group via a hydrazide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 200 kDa grafted with a C18 alkyl group via an amide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 200 kDa grafted with a C18 alkyl group via a hydrazide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 3000 kDa grafted with a C12 alkyl group via an amide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 200 kDa grafted with a C12 alkyl group via an amide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 200 kDa grafted with a C18 alkyl group via an amide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 200 kDa grafted with a C18 alkyl group via a hydrazide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 3000 kDa grafted with a C18 alkyl group via a hydrazide linkage. According to one embodiment, the hydrophobically-modified HA derivative is a HA of 3000 kDa grafted with a C18 alkyl group via an amide linkage.

According to one embodiment, the hydrazine compound does not comprise a hydroxyl group. According to one embodiment, the hydrazine compound does comprise other chemical functional groups than hydrazine group.

According to one embodiment, the hydrophobic compound does not comprise more than one amine or hydrazine function. According to one embodiment, the hydrophobic compound does not comprise aryl compounds.

According to one embodiment, the hyaluronic acid derivative is *N*-stearylamine-modified
5 hyaluronic acid, i.e. a hyaluronic acid modified by amidation reaction with stearylamine (also called octadecylamine) by amide linkage.

According to one embodiment, the hyaluronic acid derivative is *N*-stearyl hydrazine-modified hyaluronic acid, i.e. a hyaluronic acid modified by amidation reaction with stearylhydrazine (also called octadecylhydrazine) by hydrazide linkage.

10 According to one embodiment, the hyaluronic acid is not crosslinked. According to one embodiment, the modified hyaluronic acid is not crosslinked.

The invention also relates to a particle comprising at least one hyaluronic acid derivative of formula (I) and/or (II), and salts and/or solvates thereof.

According to one embodiment, the particle of the invention has a size (i.e. a mean
15 hydrodynamic diameter) ranging from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm. The mean hydrodynamic diameter is measured in a mixture of water/phosphate buffer (50/50, v/v) by using a nanosizer apparatus. According to one embodiment, the size of the particle of the invention is about 327 nm. According to one embodiment, the size of the particle of the invention is about
20 357 nm. According to one embodiment, the particle of the invention has a size of about 200 nm, 250 nm, 300 nm, 350 nm, 400 nm, 450 nm or 500 nm. According to one embodiment, the particle of the invention has a size of about 300 nm, 310 nm, 320 nm, 330 nm, 340 nm, 350 nm or 360 nm.

According to one embodiment, the particle of the invention does not comprise
25 cyclodextrin. According to one embodiment, the particle of the invention does not comprise alpha-cyclodextrin. According to one embodiment, the particle of the invention does not comprise beta-cyclodextrin.

According to one embodiment, the particle is constituted of hyaluronic acid derivatives of formula (I) and/or (II), and salts and/or derivatives thereof. According to one embodiment, the particle of the invention does not comprise other kind of particle such as for example but not limited to, inorganic particles, hybrid particles and/or metallic particles. According to one embodiment, the particle of the invention does not comprise other polymer than hyaluronic acid. According to one embodiment, the particle of the invention does not comprise other polymer than the modified hyaluronic acid of the invention.

According to one embodiment, hyaluronic acid derivatives of formula (I) and/or (II), and salts and/or derivatives thereof are not associated with cyclodextrin. According to one embodiment, hyaluronic acid derivatives of formula (I) and/or (II), and salts and/or derivatives thereof are not associated with alpha-cyclodextrin. According to one embodiment, hyaluronic acid derivatives of formula (I) and/or (II), and salts and/or derivatives thereof are not associated with beta-cyclodextrin.

In the present invention, the hyaluronic acid derivatives of the invention have been characterized by spectroscopy and/or by Nuclear Magnetic Resonance (NMR). Especially, the hyaluronic acid derivatives of the invention have been characterized by Fourier Transform Infrared (FT-IR) spectroscopy.

The invention also relates to a composition comprising at least one hyaluronic acid derivative of formula (I) and/or (II), and salts and/or derivatives thereof; and at least one cosmetically acceptable base.

Especially, the invention also relates to a cosmetic and/or dermo-cosmetic composition comprising at least one particle of the invention as defined above, and at least one cosmetically acceptable base.

According to one embodiment, the composition of the invention comprises at least one particle having a size (i.e. a mean hydrodynamic diameter) ranging from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm. The mean hydrodynamic diameter is measured in a mixture of water/phosphate buffer (50/50, v/v) by using a nanosizer apparatus. According to one embodiment, the size of

the particle of the invention is about 327 nm. According to one embodiment, the size of the particle of the invention is about 357 nm.

The invention also relates to a pharmaceutical composition, preferably a dermatological composition, comprising at least one hyaluronic acid derivative of formula (I) and/or (II),
5 and salts and/or derivatives thereof; and at least one pharmaceutically acceptable carrier.

Especially, the invention also relates to a pharmaceutical composition comprising at least one particle of the invention as defined above, and at least one pharmaceutically acceptable carrier.

According to one embodiment, the composition does not comprise cyclodextrin.
10 According to one embodiment, the composition does not comprise alpha-cyclodextrin.
According to one embodiment, the composition does not comprise beta-cyclodextrin.

According to one embodiment, the composition comprises the particles of the invention in an amount ranging from 0.01 % to 100 % by weight to the total weight of the composition; preferably from 0.1 to 20 %; more preferably, from 0.5 % to 1 %. According
15 to one embodiment, the composition comprises the particles of the invention in an amount ranging from 1 % to 100 %, preferably from 10 % to 100 %, preferably from 20 % to 100 %, preferably from 30 % to 100 %, preferably from 40 % to 100 %, preferably from 50 % to 100 %, by weight to the total weight of the composition. According to one
20 embodiment, the composition comprises the particles of the invention in an amount ranging from 1 % to 100 %, preferably from 1 % to 20 %, preferably from 5 % to 20 %, preferably from 10 % to 20 %, preferably from 15 % to 20 % by weight to the total weight of the composition. According to one embodiment, the composition comprises the
25 particles of the invention in an amount of about 1 %, 2 %, 3 %, 4 %, 5 %, 6 %, 7 %, 8 %, 9 %, 10 %, 11 %, 12 %, 13 %, 14 %, 15 %, 16 %, 17 %, 18 %, 19 % or 20 % by weight to the total weight of the composition.

According to one embodiment, the composition comprises the particles of the invention in an amount of about 0.5 %, 0.6 %, 0.7 %, 0.8 %, 0.9 %, or 1 % by weight to the total weight of the composition.

According to one embodiment, the composition is under the form of a solid, a solution, a suspension or an emulsion. According to one embodiment, the composition is selected from oil-in-water emulsion (O/W) and water-in-oil emulsion (W/O), wherein the oil may be sourced from vegetal oil or mineral oil.

- 5 According to one embodiment, the composition further comprises a solvent; preferably, a biocompatible solvent. According to one embodiment, the composition further comprises an aqueous solution or a hydro-alcoholic solution.

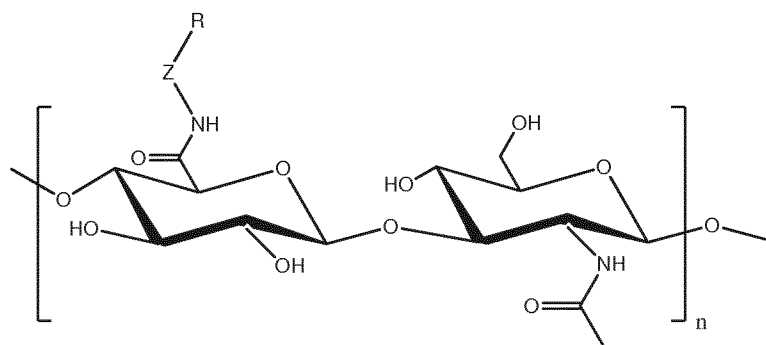
According to one embodiment, the composition further comprises at least one cosmetically acceptable base under the form of a gel, a paste, a cream, a lotion, a milk, a
10 stick, a shampoo, a powder, an aerosol, a film or a patch.

According to the present invention, the compositions of the invention may be used in combination of at least one cosmetic agent selected from fatty acids, organic solvents, gelling agents, softening agents, surfactants, detergents, gelling agents, fragrances, emulsifying agents, opacifying agents, stabilizing agents, foaming agents, chelating
15 agents, preservative agents, sunscreens, essential oils, dyes, mineral loads, or any compound used in cosmetics.

According to the present invention, the compositions of the invention may be used in combination of at least one active ingredient.

According to one embodiment, the compositions of the invention are topically
20 administrated. According to one embodiment, the compositions of the invention are administrated by a direct topical mean. According to one embodiment, the compositions of the invention are administrated by an indirect topical mean. In the present invention, "indirect topical mean" refers to the use of a textile support or a material for administering the composition of the invention. According to another embodiment, the composition of
25 the invention is injected; preferably, by transdermal injection.

The invention also relates to a process for preparing hyaluronic acid derivatives of formula (I):



Formule (I)

or any salts and solvates thereof,

wherein

Z represents a single bond or -NH-;

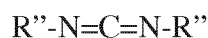
- 5 R represents a C1-C20 alkyl or C1-C20 alkenyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group; and

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer;

comprising:

- 10 (i) providing a solution comprising a carbodiimide compound, *N*-hydroxysuccinimide (NHS) and a hyaluronic acid, in a buffer;
- (ii) adding a solution comprising at least one hydrophobic compound having an amine or hydrazine function.

15 According to one embodiment, the carbodiimide compound relates to any organic compound having a carbodiimide function (i.e. a function -N=C=N-); preferably, symmetric carbodiimide. According to one embodiment, a suitable symmetric carbodiimide compound has the following formula:



20 wherein R'' is an alkyl or arylalkyl group; preferably, R'' is a cycloalkyl group; more preferably, R'' is cyclohexyl group.

According to one embodiment, the carbodiimide compound is ethyl carbodiimide.

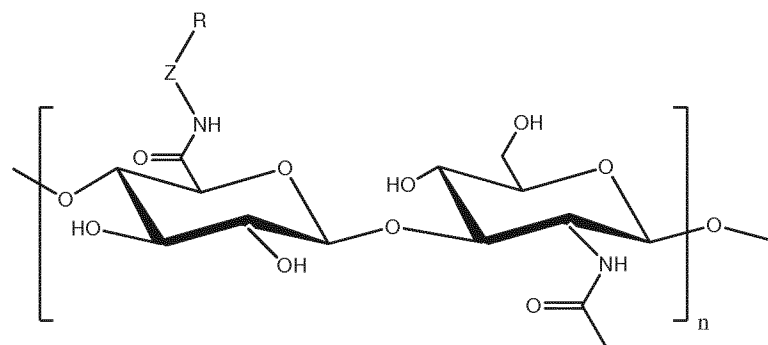
According to one embodiment, the process for preparing hyaluronic acid derivatives of formula (I) is carried out at a temperature ranging from 0°C to 40°C; preferably, from 10°C to 30°C; more preferably, at a temperature ranging from 20°C to 25°C. According to one embodiment, the process for preparing hyaluronic acid derivatives of formula (I) is carried out at room temperature.

According to one embodiment, the process for preparing hyaluronic acid derivatives of formula (I) is carried out at atmospheric pressure.

According to one embodiment, the concentration of the hyaluronic acid, ranges from 10 mg/mL to 70 mg/mL; preferably, from 20 mg/mL to 60 mg/mL; more preferably, ranges from 30 mg/mL to 50 mg/mL.

According to one embodiment, the process is carried out for a period ranging more than 0 to 12 hours; preferably, from 1 hour to 6 hours; more preferably, the time of the process is carried out about 5 hours.

The invention also relates to a process for preparing particles of hyaluronic acid derivatives of formula (I):



Formule (I)

or any salts and solvates thereof,

wherein

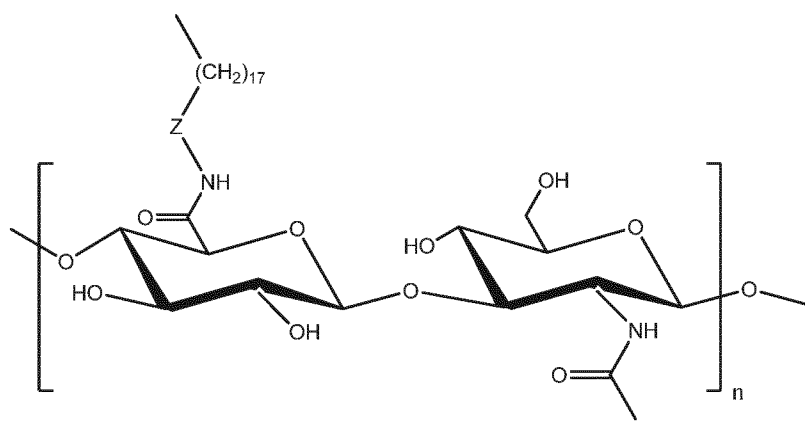
Z represents a single bond or -NH-;

R represents a C1-C20 alkyl or C1-C20 alkenyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group;

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer; and

comprising solubilizing hyaluronic acid derivatives or formula (I) in an aqueous dissolution medium at a concentration ranging from 0.25 to 2 % wt.

- 5 Especially, the invention relates to a process for preparing particles of hyaluronic acid derivatives of formula (II):



Formule (II)

or any salts and solvates thereof,

wherein

- 10 Z represents a single bond or -NH-;

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer; and

comprising solubilizing hyaluronic acid derivatives of formula (II) in an aqueous dissolution medium at a concentration ranging from 0.25 to 2 % wt.

- 15 According to one embodiment, the particle has a size (i.e. a mean hydrodynamic diameter) ranging from 100 nm to 2 000 nm; preferably, from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm. The mean hydrodynamic diameter is measured in a mixture of water/phosphate buffer (50/50, v/v) by using a nanosizer apparatus. According to one embodiment, the size of the particle of

the invention is about 327 nm. According to one embodiment, the size of the particle of the invention is about 357 nm.

According to one embodiment, the particle has a size (i.e. a mean hydrodynamic diameter) depending on the substitution degree of modified hyaluronic acid.

- 5 According to one embodiment, the aqueous dissolution medium is selected from MilliQ[®] water, phosphate buffer or mixture of MilliQ[®] water and phosphate buffer (50/50; v/v).

According to one embodiment, the phosphate buffer comprises monosodium phosphate, disodium phosphate and sodium chloride. According to one embodiment, the phosphate buffer comprises monosodium phosphate at the concentration of 0.045 g/L. According to
10 one embodiment, the phosphate buffer comprises disodium phosphate at the concentration of 0.563 g/L. According to one embodiment, the phosphate buffer comprises sodium chloride at the concentration of 8.5 g/L.

According to one embodiment, the aqueous dissolution medium has a pH ranging from 5 to 8; preferably from 5.6 to 6.9. According to one embodiment, the aqueous dissolution
15 medium has a pH ranging from about 4 to about 8; preferably is about 4.5.

According to one embodiment, the concentration in the aqueous dissolution medium of hyaluronic acid derivatives is about 0.25 %, 0.5 % or 1 %wt. According to one embodiment, the concentration in the aqueous dissolution medium of hyaluronic acid derivatives wherein Z is single bond, is about 1 %wt. According to one embodiment, the
20 concentration in the aqueous dissolution medium of hyaluronic acid derivatives wherein Z is -NH-, is about 0.25 %wt. or about 5 %wt.

According to one embodiment, the hyaluronic acid derivatives of formula (I) are completely solubilized in the aqueous dissolution medium.

In the present invention, the hyaluronic acid modified with a hydrophobic compound
25 having (1) at least one amine function or at least one hydrazine function, and (2) a C18-alkyl chain, allows improving the association of hyaluronic acid derivatives in particulate form.

The invention also relates to the use of hyaluronic acid derivatives or the particles of the invention, as described above. Especially, the invention relates to the use of hyaluronic acid derivatives of formula (I) or particles thereof, in the field of cosmetics and/or pharmaceuticals. More especially, the invention relates to the use of hyaluronic acid derivatives of formula (I) or particles thereof, as a long-lasting anti-aging agent.

According to one embodiment, the hyaluronic acid derivatives or particles thereof are useful in the field of cosmetics and/or dermocosmetics. According to one embodiment, the hyaluronic acid derivatives or particles thereof are useful as cosmetic and/or dermocosmetic agents, preferably as long lasting cosmetic and/or dermocosmetic agent; more preferably as long lasting anti-aging agents. According to one embodiment, the long lasting anti-aging effect is observed more than 3 days, preferably more than 5 days, more preferably more than 7 days after the application of the hyaluronic acid derivatives of the invention or particles thereof on the skin of a subject in need thereof.

According to one embodiment, the hyaluronic acid derivatives or particles thereof increase the surface occupied by glycosaminoglycans (GAGs) in the skin (epidermis and/or dermis). According to one embodiment, the hyaluronic acid derivatives or particles thereof increase the surface occupied by glycosaminoglycans (GAGs) in the skin (epidermis and/or dermis). According to one embodiment, the hyaluronic acid derivatives or particles thereof increase the surface occupied by glycosaminoglycans (GAGs) in the skin (epidermis and/or dermis) in papillary dermis. According to one embodiment, the hyaluronic acid derivatives or particles thereof are useful for increasing the surface occupied by glycosaminoglycans (GAGs) in the skin (epidermis and/or dermis), preferably in a range from 0.1 % to 150 %, preferably 1 % to 150 %, preferably from 10 % to 150 %, preferably from 20 % to 150 %, preferably from 30 % to 150 %, preferably from 40 % to 150 %, preferably from 50 % to 150 %, preferably from 60 % to 150 %, preferably from 70 % to 150 %, preferably from 80 % to 150 %, preferably from 90 % to 150 %, preferably from 100 % to 150 % compared to the surface occupied by glycosaminoglycans (GAGs) in an untreated skin.

According to one embodiment, the hyaluronic acid derivatives or particles thereof are useful for increasing the surface occupied by glycosaminoglycans (GAGs) in the skin

(epidermis and/or dermis) of a subject of about 3 %, 15 %, 30 % or 119 %, compared to the surface occupied by glycosaminoglycans (GAGs) in an untreated skin.

According to one embodiment, the hyaluronic acid derivatives or particles thereof are useful for increasing the surface occupied by glycosaminoglycans (GAGs) in the skin of
5 a subject (epidermis and/or dermis) of about 1 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, 10 %, 105 %, 110 %, 115 %, 120 %, 125 %, 130 %, 135 %, 140 %, 145 %, 150 %, compared to the surface occupied by glycosaminoglycans (GAGs) in an untreated skin.

According to one embodiment, the particle has a size (i.e. a mean hydrodynamic
10 diameter) ranging from 100 nm to 2 000 nm; preferably, from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm. The mean hydrodynamic diameter is measured in a mixture of water/phosphate buffer (50/50, v/v) by using a nanosizer apparatus. According to one embodiment, the size of the particle of the invention is about 327 nm. According to one embodiment, the size of the particle of
15 the invention is about 357 nm.

According to one embodiment, the hyaluronic acid derivatives of the invention and/or the particles thereof are for use as a medicament.

According to one embodiment, the present invention relates to the use of the hyaluronic acid derivatives of the invention and/or the particles thereof for the manufacture of a
20 medicament.

According to one embodiment, the hyaluronic acid derivatives and/or the particles of the invention are for use in the treatment and/or the prevention of dermatological conditions.

According to one embodiment, the hyaluronic acid derivatives and/or the particles of the invention are for use as a healing agent. According to one embodiment, the hyaluronic
25 acid derivatives and/or the particles of the invention are for use as a long-lasting healing agent.

According to one embodiment, the present invention relates to a method of treatment of dermatological diseases and/or conditions comprising the administration to a patient in

need thereof, of the hyaluronic acid derivatives of the invention and/or the particles thereof.

Especially, the invention concerns the use as a cosmetic agent of a particle comprising at least one hyaluronic acid derivative of general formula (I) as defined above. According to one embodiment, the invention concerns the use as a cosmetic agent of a particle comprising at least one hyaluronic acid derivative of general formula (Ia), (Ib) or (II) as defined above.

According to one embodiment, the hyaluronic acid derivatives or the invention and/or the particles thereof, are useful as cosmetic agents; preferably; as an anti-aging agent; more preferably, as a skin plumping agent, a skin hydrating agent and/or a stimulating agent of the endogenic production of hyaluronic acid. According to one embodiment, the hyaluronic acid derivatives or the invention and/or the particles thereof, are useful as long-lasting cosmetic agents; preferably as a long-lasting anti-aging agent; more preferably, as a long-lasting skin plumping agent, a long-lasting skin hydrating agent and/or a long-lasting stimulating agent of the endogenic production of hyaluronic acid.

According to one embodiment, the hyaluronic acid derivatives or the invention and/or the particles thereof, are useful for stimulating the expression of endogen glycosaminoglycans (GAGs), preferably for stimulating the expression of endogen elastin, collagen and/or hyaluronic acid.

According to one embodiment, the invention concerns a method for stimulating the expression of endogen glycosaminoglycans (GAGs), preferably for stimulating the expression of endogen elastin, collagen and/or hyaluronic acid, said method comprising applying at least one hyaluronic acid derivative of the invention and/or the particles thereof, on the skin of a subject in need thereof. According to one embodiment, the expression of glycosaminoglycans (GAGs) is stimulated at least during 2 days, preferably during 5 days, more preferably during 7 days, after the application of hyaluronic acid derivatives of the invention and/or the particles thereof, on the skin of a subject in need thereof.

According to one embodiment, the expression of glycosaminoglycans (GAGs) is improved in a range from 0.1 % to 150 %, preferably 1 % to 150 %, preferably from 10 % à 150 %, preferably from 20 % to 150 %, preferably from 30 % to 150 %, preferably from 40 % to 150 %, preferably from 50 % to 150 %, preferably from 60 % to 150 %, preferably from 70 % to 150 %, preferably from 80 % to 150 %, preferably from 90 % to 150 %, preferably from 100 % to 150 %, compared to the expression of glycosaminoglycans (GAGs) in an untreated skin.

According to one embodiment, the expression of glycosaminoglycans (GAGs) in the skin of a subject treated with the hyaluronic acid derivatives or particles of the invention, is improved of about 3 %, 15 %, 30 % or 119 %, compared to the expression of glycosaminoglycans in an untreated skin.

According to one embodiment, the expression of glycosaminoglycans (GAGs) in the skin of a subject treated with the hyaluronic acid derivatives or particles of the invention, is about 1 %, 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, 100 %, 105 %, 110 %, 115 %, 120 %, 125 %, 130 %, 135 %, 140 %, 145 %, 150 %, compared to the expression of glycosaminoglycans (GAGs) in an untreated skin.

The invention also relates to a skin improvement method comprising administering the composition as defined above, to the skin of a subject in need thereof. In the present invention, “administering” refers to a topical application or injection means.

According to one embodiment, the skin improvement method comprises applying the composition as defined above, on the skin of a subject in need thereof. According to one embodiment, the skin improvement method comprises applying in a sole application the composition as defined above, on the skin of a subject in need thereof. According to one embodiment, the composition is applied via a device comprising said composition; preferably, said device is a syringe, a patch, a film, a stick, a plaster, a stupe or a bandage. According to one embodiment, the skin improvement method comprises applying the composition as defined above, on the skin of a subject in need thereof, once a week. According to one embodiment, the skin improvement method comprises applying the

composition as defined above, on the skin of a subject in need thereof, once a month. According to one embodiment, the skin improvement method comprises applying the composition as defined above, on the skin of a subject in need thereof, twice a month.

According to one embodiment, the skin improvement method comprises the injection of
5 the composition as defined above to a subject in need thereof. According to one embodiment, the injection is a transdermal injection.

According to one embodiment, the skin improvement method is for restoring the production of endogenic hyaluronic acid in the epidermis and/or the dermis.

According to one embodiment, the skin improvement method is for limiting the
10 shortcomings of the skin; preferably, for improving the hydration of the skin.

According to one embodiment, the composition is applied on the skin of a subject in need thereof, in a sole application. According to one embodiment, the composition is applied on the skin of a subject in need thereof, at least once a month, preferably, twice a month; more preferably, once a week; more preferably, the composition of the invention is
15 applied only once a week.

The invention also relates to a device comprising at least one hyaluronic acid derivative of formula (I) and/or (II); or comprising at least one particle of the invention.

According to one embodiment, the device is selected from a syringe, a patch, a film, a stick, a plaster, a stupe or a bandage.
20

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is FT-IR spectra of *N*-stearylamine-modified hyaluronic acid (Figure **1A**) and *N*-stearylhydrazine-modified hyaluronic acid (Figure **1B**).

Figure 2 is a histogram showing the percentage of the surface occupied
25 glycosaminoglycans (GAGs) in papillary dermis.

EXAMPLES

The present invention is further illustrated by the following examples.

Abbreviations

- °C: Celsius degree;
- 5 DS: degree of substitution;
- HA: hyaluronic acid;
- FT-IR: Fourier-Transform Infra-Red spectroscopy;
- kDa: kiloDalton;
- M: mol/L;
- 10 MES: 2-(*N*-morpholino)ethanesulfonic acid;
- mg: milligram(s);
- ml: milliliter(s);
- Mw: molecular weight;
- NHS: *N*-hydroxysuccinimide;
- 15 N₂: dinitrogen;
- NMR: Nuclear Magnetic Resonance.

Materials and Methods

Reagents

- All chemicals and solvents have been purchased from commercial sources (Aldrich,
- 20 Acros) and were used as such. The solvents of spectroscopy quality were purchased from Aldrich.

ATR FTIR spectroscopy

- Spectra were recorded in function of time under N₂ flow using infrared spectrometer FT/IR-4100 JASCO. The principle deals with contacting a crystal (diamond) and the
- 25 sample before to be through by the infrared light.

Quasi-elastic light scattering

The mean hydrodynamic diameter of the particles of the invention was determined at 25°C using a Zetasizer Nanoseries Nano-ZS90 (Malvern Instruments, France).

Image analysis

- 5 All the microscopical observations were realized using a Leica DMLB or Olympus BX43 microscope. Pictures were digitalized using an Olympus DP72 camera and the Cell[^]D data storing software.

Collagen III immunostaining

- 10 Collagen III immunostaining has been realized on frozen sections with a polyclonal anti-collagen III antibody (SBA, Ref: 1330-01) diluted at 1:20 in PBSBSA 0.3 %-Tween 20, 0.05 % during 1h at room temperature using a Vectastain Kit Vector amplifier system avidin/biotin, and revealed by VIP (Vector laboratories, Ref: SK-4600). The immunostaining was assessed by microscopic observation.

Elastin immunostaining

- 15 Elastin immunostaining has been realized on paraffinized sections with a polyclonal anti-elastin antibody (Noviotec, Ref.:25011) diluted at 1:400 during 1h at room temperature, and revealed by AlexaFluor 488 (Lifetechnologies, Ref: A11008). Nuclei were post stained with propidium iodide. The immunostaining was assessed by microscopic observation.

20 **PART 1: CHEMISTRY**

Example 1: Synthesis of the hyaluronic acid derivatives from hydrophobic compounds having at least one amine or hydrazine function

General protocol

- 25 Typical procedures for grafting hydrophobic groups onto sodium hyaluronate are described hereinafter from an amine compound (point 1.1) or from a hydrazine compound

(point 1.2).

1.1.From an amine compound

About 1 equiv. of ethyl carbodiimide and 0.6 equiv. of *N*-hydroxysuccinimide (NHS) are dissolved in a MES buffer (pH=4.5). Then, 1 equiv. of sodium hyaluronate
5 (Mw=200 kDa) is slowly dissolved as well. After complete dissolution, 0.2 equivalent of an amine compound such as for example octadecylamine, is added so the final hyaluronic acid concentration ranges from 30 and 50 mg/mL, and the mixture is let at rest for 5 hours at room temperature. The grafted HA is diluted with a solution of sodium chloride (0.5 M) to exchange cations. Then, the grafted HA is precipitated using a water/ethanol mixture
10 (2/3; v/v) and can be further purified by washing it with more hydrophobic water/ethanol mixtures (up to 19/1; v/v). Drying is performed under vacuum between at around 30°C. A white powder is obtained.

The product was then characterized by FT-IR spectroscopy. This technique is an efficient and easy method for identifying the chemical functions of the HA derivatives by
15 comparison with native HA. For example, the spectrum of HA grafted with octadecylamine is presented Figure 1A. This analysis evidenced the presence of the bands corresponding to the grafting of alkyl chains compared to non-modified HA. The substitution degree of sodium hyaluronate (Mw=200 kDa) modified by amidation reaction with octadecylamine is 12 %.

20 1.2.From a hydrazine compound

About 0.25 equiv. of ethyl carbodiimide are dissolved in a MES buffer (pH=4.5). Then, 1 equiv. of sodium hyaluronate (Mw=3000 kDa) is slowly dissolved as well. After complete dissolution, 0.5 equivalent of a hydrazine compound such as for example octadecylhydrazine, is added so the final hyaluronic acid concentration ranges from
25 10 and 25 mg/mL, and the mixture is let at rest for 5 hours at room temperature. The grafted HA is diluted with a solution of sodium chloride (0.5 M) to exchange cations. Then, the grafted HA is precipitated using a water/ethanol mixture (2/3; v/v) and can be further purified by washing it with more hydrophobic water/ethanol mixtures (up to 19/1;

v/v). Drying is performed under vacuum between at around 30°C. A white powder is obtained.

The product was characterized by FT-IR spectroscopy. The spectrum of HA grafted with octadecylhydrazine is presented Figure 1B. This analysis evidenced the presence of the
 5 bands corresponding to the grafting of the alkyl chain compared to non-modified HA. The substitution degree of sodium hyaluronate (Mw=3000 kDa) modified by amidation reaction with octadecylhydrazide is 1.5 %.

1.3. Examples of hydrophobically-modified HA derivatives

Several hydrophobically-modified HA derivatives have been synthesized by one of the
 10 protocols described in paragraph 1.1 or 1.2. The characteristics of such compounds are presented in Table 1.

Modified HA n°	Mw of HA (kDa)	Hydrophobic alkyl group	Linkage between HA and the hydrophobic group
1	200	C8	hydrazide
2	200	C6	amide
3	3000	C8	hydrazide
4	3000	C6	amide
5	3000	C18	amide
6	3000	C18	hydrazide
7	200	C18	amide
8	200	C18	hydrazide
9	3000	C12	amide
10	200	C12	amide
11	200	C18	amide
12	200	C18	hydrazide
13	3000	C18	hydrazide
14	3000	C18	amide

Example 2: Synthesis of particles comprising hyaluronic acid derivatives

General protocol

Hydrophobically-modified hyaluronic acids of example 1 were dispersed into a mixture
 15 of MilliQ® water and phosphate buffer (50/50 %: v/v) at room temperature under magnetic stirring.

The formulation of the phosphate buffer is presented Table 2:

Phosphate buffer	Chemical formula	Concentration (g/L)
Monosodium phosphate	$\text{NaH}_2\text{PO}_4, 2\text{H}_2\text{O}$	0.045
Disodium phosphate	$\text{Na}_2\text{HPO}_4, 12\text{H}_2\text{O}$	0.563
Sodium chloride	NaCl	8.5

The final concentration of hydrophobically-modified hyaluronic acids was modified from 0.25 to 2 %wt. depending on the solubility of HA derivatives and to keep the formulation liquid for size measurement.

- The particles were characterized by quasi-elastic light scattering in order to determine their mean hydrodynamic diameter. For example, the mean hydrodynamic diameter is 327 nm for *N*-stearylamine modified HA having 200 kDa and is 357 nm for *N*-stearylamine modified HA (3000 kDa).

Examples of particles of hydrophobically-modified HA

- Several particles of hydrophobically-modified HA have been synthesized by the protocol of the invention described above. The characteristics of such compounds are presented in Table 3, including the degree of substitution calculated by NMR (DS NMR) and the molecular weight of hyaluronic acid (Mw of HA).

Particle n°	DS NMR (%)	Mw of HA (kDa)	Hydrophobic alkyl group	Linkage between HA and the hydrophobic group
P1	52	200	C8	hydrazide
P2	43	200	C6	amide
P3	15	3000	C8	hydrazide
P4	30	3000	C6	amide
P5	0.3	3000	C18	amide
P6	0.1	3000	C18	hydrazide
P7	7	200	C18	amide
P8	3	200	C18	hydrazide
P9	1.7	3000	C12	amide
P10	4	200	C12	amide
P11	12	200	C18	amide
P12	20	200	C18	hydrazide
P13	1.5	3000	C18	hydrazide
P14	1.5	3000	C18	amide

PART 2: BIOLOGY

Example 3: Lasting effect of the particles of the invention in the papillary dermis

The aim is to show that the particles of the invention are able to deliver in the papillary dermis by a sole application, a large amount of hyaluronic acid derivatives while limiting
5 the degradation of the product in the skin over time.

Three formulations were studied during 7 days on *ex vivo* human skin abdomen explants:

- P11: particles of *N*-stearylamine modified HA having 200 kDa (amide linkage);
- P14: particles of *N*-stearylamine modified HA having 3000 kDa (amide linkage);
and
- 10 - PO: particles of *O*-stearoyle modified HA which are HA modified with stearic acid
(ester linkage).

P11 and P14 are the particles of the invention whereas PO are particles for comparison, said particles PO comprising hydrophobically-modified HA through ester bond.

The skin human explants have an average diameter of 11 mm (± 1 mm) and were collected
15 after abdominoplasty from a 44-year-old woman. The explants were kept in survival in a culture medium at 37°C in a humid, 5 % CO₂ atmosphere.

For each of these formulations, the content of glycosaminoglycan (GAG) in papillary dermis has been quantified over time (day 3, day 5 and day 7) compared to a witness sample (no treated sample, noted "C"). The general morphology is observed over time
20 via histological processing.

The beginning of this experiment (day 0) corresponds to the day where a topical administration of the formulation has been carried out on the explant (4 mg/cm²).

The results are presented Figure 2.

The histogram shows that:

- 25 - After 3 days and 5 days, the amount of particles of PO (ester linkage) is slightly higher than those of P11 and P14 (amide linkage);

- After 7 days, the amount of particles of PO (ester linkage) has dramatically decreased contrary to those of P11 and P14 (amide linkage).

In conclusion, these results show that the modified HA having an amide linkage, independently of the molecular weight, is less degraded than modified HA having an ester linkage after a long time. Consequently, a sole application on the skin of the particles of the invention allows keeping the benefits of these compounds over time.

Example 4: Penetration depth of the particles of the invention in the dermis

The aim is to show that the particles of the invention are able to be diffused until the deepest layer of the skin.

For this purpose, three formulations were studied during 7 days on *ex vivo* human skin abdomen explants:

- P11: particles of *N*-stearylamine modified HA having 200 kDa (amide linkage);
- P14: particles of *N*-stearylamine modified HA having 3000 kDa (amide linkage); and
- PO: particles of *O*-stearoyl modified HA which are HA modified with stearic acid (ester linkage).

For each of these formulations, the total content of glycosaminoglycan (GAG) has been quantified over time (day 3, day 5 and day 7) through 3 different compartments of dermis: the papillary dermis, the upper reticular dermis and the lower reticular dermis. These results have been compared to a witness sample (no treated sample, noted "W").

The results show that:

- After 3 days and 5 days, the amount of particles of PO (ester linkage) is higher than those of P11 and P14 (amide linkage) in the three compartments of the dermis;
- After 7 days, the amount of particles of PO (ester linkage) has dramatically decreases in the three compartments of the dermis contrary to those of P11 and P14 (amide linkage).

In conclusion, these results evidenced that the particles of the invention are able to keep the benefits of a sole topical application until the deepest layer of the skin.

Example 5: Action of the particles of the invention on collagen III and elastin

The action of the particles on the expression of collagen III and elastin were evaluated *ex vivo* in the dermis after a sole application on human skin abdomen explants.

The studied formulations are:

- R: sodium hyaluronate;
- P11: particles of *N*-stearylamine modified HA having 200 kDa (amide linkage);
- P14: particles of *N*-stearylamine modified HA having 3000 kDa (amide linkage);
- 10 and
- PO: particles of *O*-stearoyle modified HA which are HA modified with stearic acid (ester linkage).

For each of these formulations, the collagen II and elastin contents have been quantified in the papillary dermis after 7 days compared to a witness sample (no treated sample, noted "C").

The quantification has been carried out by an image analysis of collagen II and elastin after specific immunological labelling of samples.

After 7 days, the results show that:

for elastin

- 20 - the topical application of sodium hyaluronate (R) does not induce any variations in the expression of elastin in the papillary dermis;
- the topical application of *O*-stearoyle modified HA (PO) slightly increases the expression of elastin in the papillary dermis;
- the topical application of *N*-stearylamine modified HA (P11 or P14) significantly
- 25 increases the expression of elastin in the papillary dermis.

for collagen III

- for all the formulations, the expression of collagen III is similar. These results show that the particles of the invention keep a good efficiency compared to hydrophobized HA comprising ester linkages.

5 In conclusion, these results show that the modified HA having an amide linkage, independently of the molecular weight, allows efficiently stimulating the expression of elastin compared to sodium hyaluronate and modified HA having ester linkage.

Example 6: Lasting effect of particles of modified HA having hydrazide linkage

10 The aim is to show that the particles of modified-HA comprising hydrazide linkages, are able to deliver in the papillary dermis by a sole application, a large amount of hyaluronic acid derivatives while limiting the degradation of the product in the skin over time. The aim is also to compare the efficiency as a long lasting anti-aging agent of particles made of modified-HA comprising hydrazide linkages and of particles made of modified-HA comprising amide linkages.

15 The following formulations were studied during 7 days on *ex vivo* human skin abdomen explants:

- P13: particles of *N*-stearylhydrazine modified HA having 3000 kDa (hydrazide linkage); and
- P11: particles of *N*-stearylamine modified HA having 200 kDa (amide linkage).

20 The skin human explants have an average diameter of 11 mm (± 1 mm) and were collected after abdominoplasty from a 51-year-old woman. The explants were kept in survival in a culture medium at 37°C in a humid, 5 % CO₂ atmosphere.

For each of these formulations, the content of glycosaminoglycan (GAG) in papillary dermis has been quantified over time (day 2 and day 7) compared to a witness sample (no
25 treated sample, noted "C"). The general morphology is observed over time via histological processing.

The beginning of this experiment (day 0) corresponds to the day where a topical administration of the formulation has been carried out on the explant (4 mg/cm²).

The results are presented in the following Table 4:

	Variation of the surface of GAGs in the papillary dermis vs the witness sample C	
	With particles P11 (amide linkage)	With particles P13 (hydrazide linkage)
Day 2	+ 3 %	+ 15 %
Day 7	+ 30 %	+ 119 %

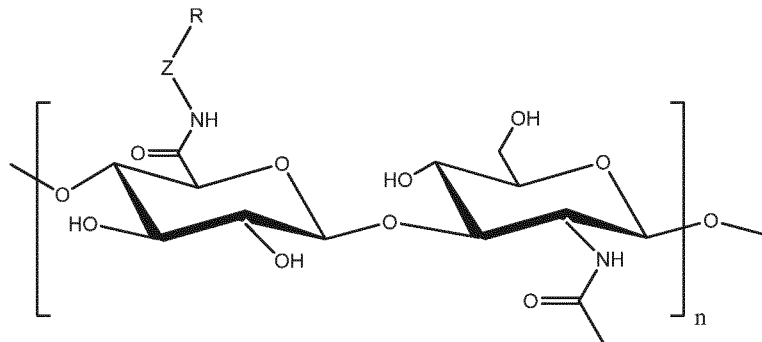
These results show that:

- 5 - a sole application of particles P11 (amide linkage) or of particles P13 (hydrazide linkage) on the skin increases the surface of GAGs in the papillary dermis even after 7 days; and
 - the application on the skin of particles P13 (hydrazide linkage) provides an improved effect compare to particles P11 (amide linkage).
- 10 In conclusion, these results show that the modified HA having a hydrazide linkage has an improved long lasting anti-aging effect than modified HA having an amide linkage.

Consequently, a sole application on the skin of the particles of modified HA having hydrazide linkages, allows achieving improved effects of these compounds over time.

CLAIMS

1. Use as a long lasting antiaging agent of a particle comprising at least one hyaluronic acid derivative of general formula (I):



Formule (I)

or any salts and solvates thereof,

wherein

Z represents a single bond or -NH-;

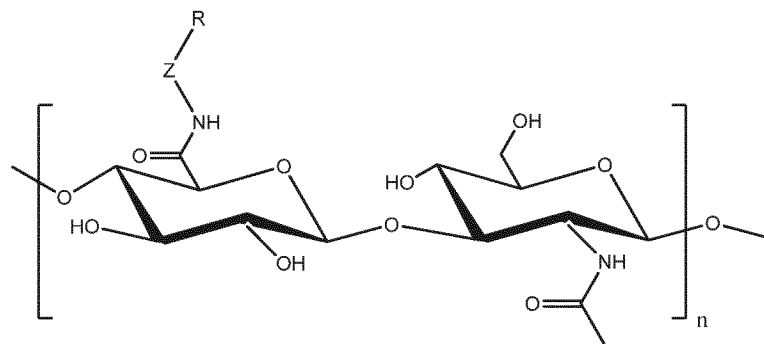
R represents a C1-C20 alkyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group;

n represents the number of disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine and is a positive integer; and

wherein the particle has a mean hydrodynamic diameter ranging from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm.

2. The use of a particle according to claim 1, wherein the degree of substitution (DS) of the hyaluronic acid derivatives of formula (I) ranges from 0.1 % to 55 %.
3. The use of a particle according to claim 1 or claim 2, wherein the molecular weight of the hyaluronic acid derivatives of formula (I) ranges from 100 kDa to 4 000 kDa; preferably, from 200 kDa to 3 000 kDa.

4. The use according to anyone of claims 1 to 3, wherein the long lasting antiaging agent is a long lasting skin plumping agent, a long lasting skin hydrating agent and/or a long lasting stimulating agent of the endogenic production of hyaluronic acid.
5. A particle comprising at least one hyaluronic acid derivative of general formula (I):



Formule (I)

or any salts and solvates thereof,

wherein

Z represents -NH-;

- 10 R represents a C1-C20 alkyl group; preferably, a C6-C18 alkyl group; more preferably, a C18 alkyl group;

n represents the number of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine and is a positive integer; and

- 15 wherein the particle has a mean hydrodynamic diameter ranging from 200 nm to 500 nm; preferably, from 250 nm to 400 nm; more preferably, from 320 nm to 360 nm.

6. A particle according to claim 5, for use as a medicament.
7. A particle for use according to claim 6, in the treatment and/or the prevention of dermatological conditions; preferably, as a healing agent.

8. A cosmetic and/or dermo-cosmetic composition comprising at least one particle according to claim 5, and a cosmetically acceptable base.
9. The cosmetic and/or dermo-cosmetic composition according to claim 8, wherein said composition is under an injectable form.
- 5 10. A skin improvement method comprising using the cosmetic and/or dermo-cosmetic composition according to claim 8 or claim 9, in a subject in need thereof.
11. The skin improvement method according to claim 10, for restoring the production of endogenic hyaluronic acid in the epidermis and/or the dermis.
12. The skin improvement method according to claim 10, for limiting the shortcomings
10 of the skin; preferably, for improving the hydration of the skin.
13. The skin improvement method according to anyone of claims 10 to 12, wherein the composition is administered to the skin of a subject in need thereof, once per week.
14. The skin improvement method according to anyone of claims 10 to 13, wherein the
15 composition is administered to the epidermis and/or the dermis of a subject in need thereof, by a topical application or by injection.

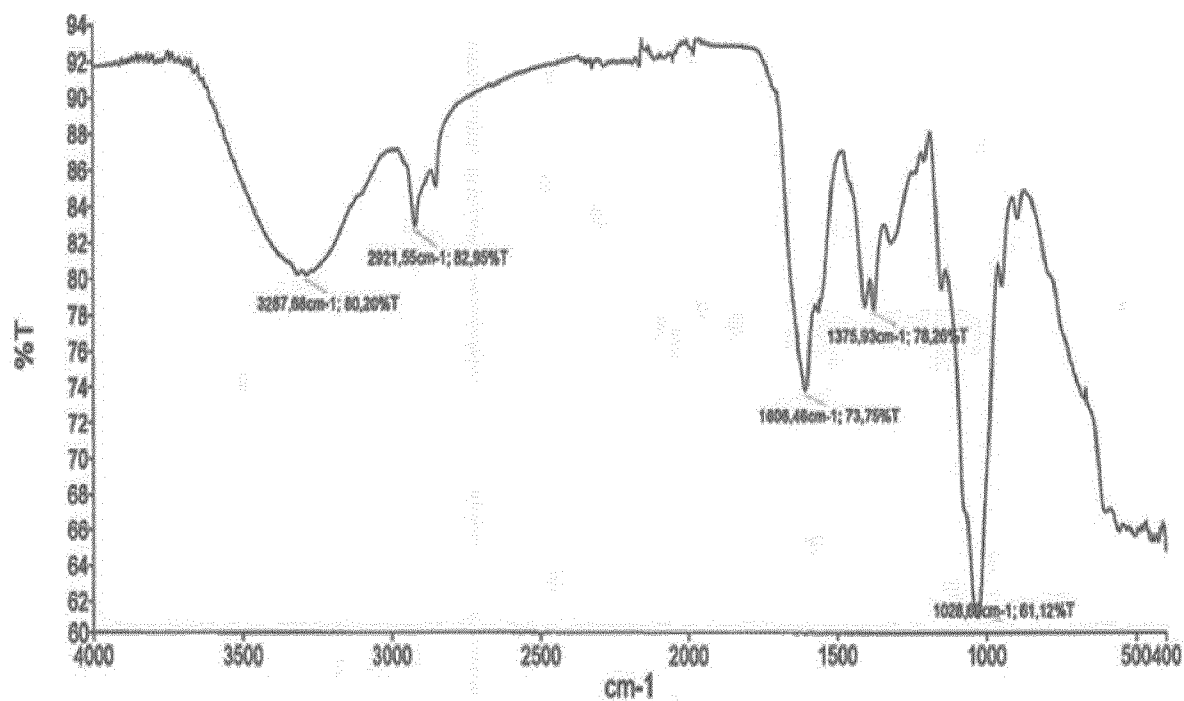


FIG. 1A

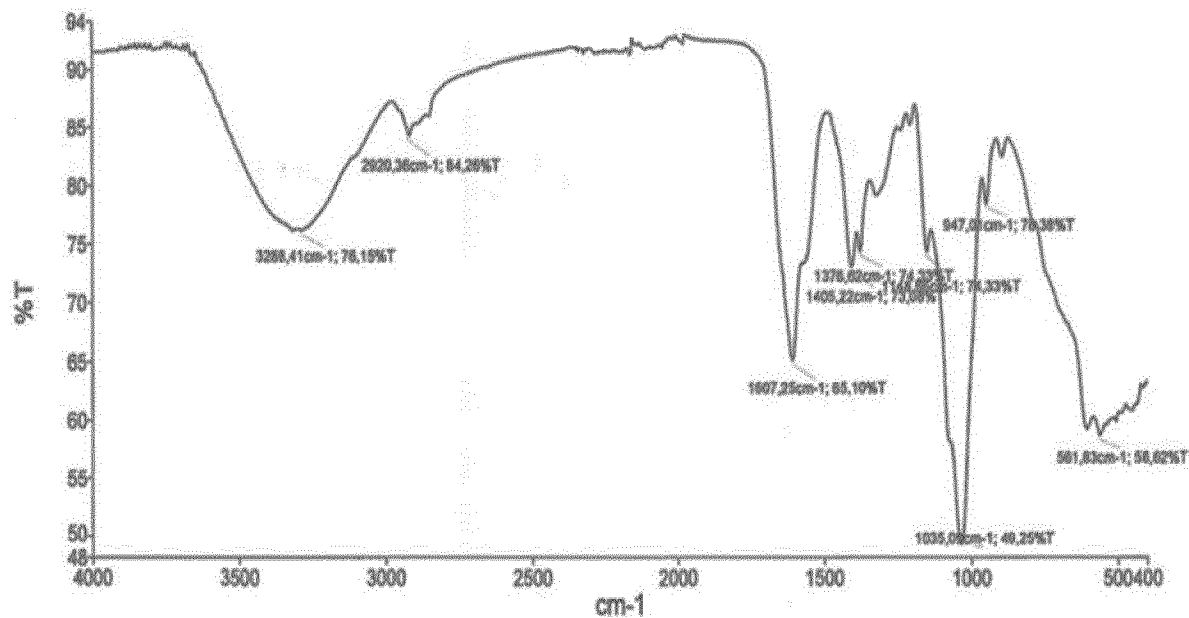


FIG. 1B

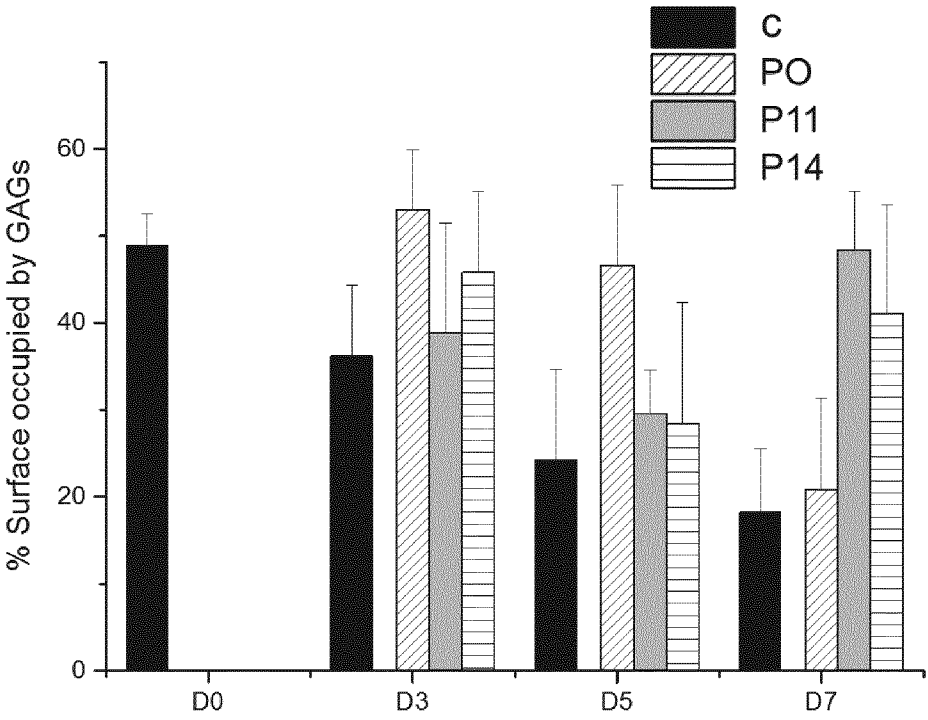


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/069844

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K8/73 A61Q19/08 A61K8/02 A61K31/728 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K A61Q		
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 practicable, search terms used) EPO-Internal, WPI Data, 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.
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 14 September 2017		Date of mailing of the international search report 11/10/2017
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Donovan-Beermann, T

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/069844

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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International application No

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