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**FIELD OF THE INVENTION**

The invention relates to a process for manufacturing a tablet comprising a nucleic acid vector.

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**TECHNOLOGICAL BACKGROUND OF THE INVENTION**

Gene therapy uses nucleic acids (in particular DNA or RNA) to treat or prevent diseases. Depending on the pathology, the nucleic acid is administered to regulate, repair, replace, add or suppress a genetic sequence. The therapeutic, prophylactic or diagnostic effect depends directly on the nucleic acid sequence or the product of the gene expression of this sequence. Therapeutic nucleic acids include, for example, antisense oligonucleotides, siRNAs or else shRNAs. siRNAs or small interfering RNAs, in particular, coupled to vectors form active substances with a high therapeutic potential due to their ability to specifically extinguish the expression of pathological proteins. Therapeutic nucleic acids could be injected directly into cells, in the form of bare nucleic acid, but they are most often transported into the cells of the patient thanks to a nucleic acid vector. The nucleic acid vectors may be viral vectors, or else synthetic vectors based on polymeric or lipid systems.

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At the time being, nucleic acid vectors are in the form of colloidal suspensions administered primarily parenterally. This formulation type as well as the mode of administration impose strong constraints such as the dimension of the administered vectors or the obtainment of sterile preparations. These constraints hinder the development of nucleic acid vectors as new drug candidates.

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Hence, there is a need for new formulations of nucleic acid vectors allowing avoiding the mentioned constraints while preserving the biological activity of the therapeutic nucleic acids.

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**SUMMARY OF THE INVENTION**

According to a first aspect, the invention relates to a process for the manufacture of a tablet comprising a complex between:

(i) a nucleic acid; and

(ii) a lipid particle comprising a cationic lipid, the lipid particle being preferably a liposome or a micelle, preferably a liposome;

said process comprising the following steps of:

a) drying, in particular by freeze-drying, an aqueous mixture comprising:

- a complex between (i) a nucleic acid and (ii) a lipid particle comprising a cationic lipid, the lipid particle being preferably a liposome or a micelle, preferably a liposome;

- one or several excipient(s) suitable for drying, in particular for freeze-drying;

b) mixing one or several compression excipient(s) with the dry mixture obtained in step a); and

c) compressing the mixture obtained in step b).

In a particular embodiment, said cationic lipid is selected from lipopolyamines, quaternary ammoniums, and lipids having a cationic head of the guanidine or imidazole type, preferably the cationic lipid is selected from Di-Myristylaminopropylaminopropyl (DMAPAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), [1,2-bis(oleoyloxy)-3-(trimethylammonio)propane] (DOTAP), 3• [N-(N',N'-dimethylaminoethane)-carbonyl]cholesterol (DCCHol) and dioctadecylamidoglycylspermine (DOGS), preferably Di-Myristylaminopropylaminopropyl (DMAPAP). Said cationic lipid may optionally be mixed with a neutral lipid, in particular selected from 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, dioleoyl-sn-glycero-3-phosphocholine (DOPC), dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and N'-(rac-1-[1-(F-octyl)undec-10-enyl]-2-(hexadecyl)glycero-3-phosphoethanol)-sperminocarboxamide, preferably the neutral lipid is 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). In particular, the mixture between the cationic lipid and the neutral lipid may comprise between 50 and 99 mol% cationic lipid in the mixture, preferably about 50 mol% and between 1 and 50 mol% neutral lipid in the mixture, preferably about 50 mol%.

According to a particular embodiment, the complex comprises a therapeutic nucleic acid, in particular an siRNA, an miRNA, an shRNA, a plasmid or an mRNA.

According to a particular embodiment, the charge ratio (+/-) between the positive charges of the cationic lipid and the negative charges of the nucleic acid is comprised between 0.5 and 10, preferably between 4 and 10, preferably about 8.

5           According to the process of the invention, the excipient(s) suitable for drying (in particular freeze-drying) may be protective agents (cryoprotectants or lyoprotectants) and/or fillers, in particular selected from trehalose, mannitol, sucrose, sorbitol, lactose, glucose, glycerol, glycine, alanine, lysine, polyethylene glycol, polyvinyl pyrrolidone and dextran, optionally in mixture. In particular, the excipient suitable for freeze-drying is  
10    trehalose, in particular in a proportion comprised between 10 and 100 mass% of the excipient(s) suitable for freeze-drying, optionally mixed with another excipient suitable for freeze-drying, preferably mannitol, in particular in a proportion comprised between 0 and 90 mass% of the excipient(s) suitable for freeze-drying.

15           According to the process of the invention, the compression excipient(s) protect the complex between the nucleic acid and the lipid particle comprising the cationic lipid during the compression step c), said compression excipients being in particular a lubricant (for example magnesium stearate, stearic acid and talc, preferably magnesium stearate), optionally mixed with another compression excipient, preferably a diluent (for  
20    example lactose, starch, calcium phosphate and derivatives thereof and cellulose and derivatives thereof, preferably lactose). In particular, the lubricant may represent from 0.25 to 5 mass% of the finished tablet, preferably 0.5%.

          In a particular embodiment, the process according to the invention comprises a  
25    compression step (step c) carried out at a pressure between 10 and 400 MPa, preferably between 50 and 250 MPa.

          According to another aspect, the invention relates to tablet obtainable by the previously-described process. The tablet of the invention may comprise:

30           - a complex between a nucleic acid and a liposome or a micelle comprising a cationic lipid;

- one or several suitable freeze-drying excipients to protect said complex during the freeze-drying step a) and/or to obtain a freeze-dried product of a texture suitable for the compression step, preferably trehalose and mannitol.

5       - one or several suitable compression excipients to protect said complex during the compression step c) and to preserve the biological activity of the complex.

According to a particular embodiment, the tablet of the invention is intended for oral, vaginal, rectal, sublingual or transmucosal administration, preferably for oral administration.

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The present invention also relates to the previously described tablet for use in the treatment of gastrointestinal tract or vaginal disorders.

#### LEGENDS OF THE FIGURES

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**Figure 1: Freeze-drying cycle:** Representation of the temperature of the product during the three steps of the cycle; (a) freezing, primary drying and start of the secondary drying; (b) zoom on freezing in liquid nitrogen.

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**Figure 2: Effectiveness of the freeze-dried products (=lyoph) and of the tablets containing lipoplexes as a function of the compressive stress applied during the compression process (CX with X = 50, 100, 150, 200 or 250 MPa):** Transfection on B16Luc cells for 48h, in triplicate, 0.5 µg of luciferase siRNA per well. Assay of luciferase and expression of the inhibition percentage compared to non-transfected cells. Positive control (T+): cells transfected with lipoplexes prepared extemporaneously (not freeze-dried and not compressed), negative control (siCtrl): cells transfected with lipoplexes containing a control siRNA, resuspended from a tablet.

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**Figure 3:** SAXS profile of siRNA lipoplexes (DMAPAP/DOPE + Alginate), prepared at a +/- charge ratio of 8 in a 150 mM NaCl buffer. The analysis of the peaks allows identifying two phases, a cubic majority ( $C_1$ ,  $q=0.084 \text{ nm}^{-1}$ ) and a lamellar minority ( $L_1$ ,  $q=0.101 \text{ nm}^{-1}$ ).

**Figure 4:** SAXS profile of siRNA lipoplexes in a dry form, in powder, having undergone a freeze-drying step then a compression step. The siRNA lipoplexes (DMAPAP/DOPE + Alginate), prepared at a +/- charge ratio of 8 in a 150 mM NaCl buffer, are supplemented with trehalose and maltose then freeze-dried for 30h. The freeze-dried product is weighed, supplemented with lactose and compressed. The SAXS analysis is carried out on freeze-dried product or tablet powder placed in a capillary. A unique and wide peak ( $q=0.096 \text{ nm}^{-1}$ ) is detected.

**Figure 5:** SAXS profile of siRNA lipoplexes in suspension in NaCl/sugar. The lipoplexes prepared as indicated in the legend of Figure 3 are supplemented with a first Trehalose/Mannitol mixture or a second Trehalose/Mannitol/Lactose mixture under the same conditions as the freeze-dried or compressed samples respectively. The analysis of the peaks allows identifying a single cubic phase the position of the first peak of which is slightly offset between the plot of the first mixture ( $C_1$ ,  $q=0.095 \text{ nm}^{-1}$ ) and the plot of the second mixture ( $C_1$ ,  $q=0.097 \text{ nm}^{-1}$ ).

**Figure 6:** SAXS profile of siRNA lipoplexes in suspension. The first curve corresponds to the samples having undergone (A) freeze-drying then (B) compression (cf. the legend in Figure 4) then resuspension by addition of water. A second curve corresponds to the sample in suspension which has not undergone freeze-drying or compression but supplemented with the same amount of (A) trehalose/mannitol as the resuspended freeze-dried sample or (B) trehalose/mannitol/lactose as the resuspended compressed sample. The analysis of the peaks allows identifying a single phase in the plot corresponding to the second curve, of the cubic type ( $C_1$ , (A)  $q=0.094 \text{ nm}^{-1}$ ; (B)  $q=0.096 \text{ nm}^{-1}$ ), a cubic phase in the plot of the first curve in (A) ( $C_1$ ,  $q=0.093 \text{ nm}^{-1}$ ) and two phases in the plot of the first curve in (B), a cubic one ( $C_1$ ,  $q=0.086 \text{ nm}^{-1}$ ) and a lamellar one ( $L_1$ ,  $q=0.101 \text{ nm}^{-1}$ ).

#### **DETAILED DESCRIPTION OF THE INVENTION**

The inventors suggest shaping nucleic acid vectors in the form of tablets for oral, vaginal, rectal, sublingual or transmucosal administration thereof. The "tablet" form allows having access to easy administration, while getting rid of the above-mentioned

formulation and administration constraints. Moreover, the tablet provides access to numerous formulation possibilities allowing controlling or modulating the release of the nucleic acid vectors according to the targeted therapeutic targets. The “tablet” form also represents a breakthrough in the stability of nucleic acid vectors. In particular, it allows overcoming their extemporaneous preparation. In addition, the “tablet” form improves patients' comfort and compliance with treatments.

Yet, before the implementation of the present invention, no nucleic acid vector tablet has been available or even conceivable, a person skilled in the art fearing a loss of activity of the nucleic acids during the steps necessary for the formation of a tablet. Nonetheless, against this prejudice, the inventors suggest an innovative means for producing tablets of nucleic acid vectors.

The invention relates to a method for manufacturing a tablet comprising at least one nucleic acid vector. More particularly, the proposed invention allows setting nucleic acid vectors in a “tablet” form, while preserving their therapeutic properties despite the mechanical stresses associated with this shaping operation.

#### Definitions

By “tablet”, it should be understood any homogeneous solid formulation without determined shape or dimension, obtained from compressed powder, the powder could be agglomerated or not.

By “nucleic acid vector” (or also the “complex”, hereinbelow), it should be understood any structure known to be suitable to vectorize a nucleic acid; in particular, by these terms, it should be understood a complex between (i) a nucleic acid and (ii) a lipid particle comprising a cationic lipid, the lipid particle preferably being a liposome or a micelle, preferably a liposome.

By “lipoplex”, it should be understood a complex between (i) a nucleic acid and (ii) a liposome comprising a cationic lipid.

By “cationic lipid”, it should be understood a lipid having a positive overall charge. A cationic lipid comprises a cationic polar head and one or several hydrophobic chain(s).

5 By “neutral lipid”, it should be understood a lipid whose overall charge is neutral, more particularly a zwitterionic lipid.

By “micelle”, it should be understood a spheroidal aggregate of amphiphilic molecules having a hydrophilic polar head and a hydrophobic chain.

10 By “liposome”, it should be understood an artificial vesicle formed by concentric lipid bilayers, trapping aqueous compartments therebetween.

For example, the nucleic acid vector may be selected from liposomes, lipids, for example cationic lipids, anionic lipids, amphoteric lipids or uncharged lipids, cationic  
15 polymers, polymers, hydrogels, micro- or nano-capsules (biodegradable), microspheres (possibly bioadhesive), cyclodextrins, protein carriers, or any combination of such elements. Preferably, the vector may be selected from delivery systems based on lipids, delivery systems based on polyimines, dendrimers, particles of PLGA (poly(lactide-co-glycolide)), the systems described in WO15023775, and similar systems.

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By “about”, it should be understood the value + or - 10%, preferably + or - 5% of the latter. For example, about 50 means from 45 to 55, and preferably from 47.5 to 52.5

#### Preparation of the nucleic acid vector

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In particular, the nucleic acid may be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), of any size, single-stranded or double-stranded, linear or circular, unmodified or chemically modified (such as the phosphorothioate, phosphoroamidate and/or 2'-O-methyl forms). The size of the nucleic acid could vary to a large extent, for  
30 example from 10 base pairs to several tens of thousands of base pairs. For example, the size of the nucleic acid could reach about 10000 base pairs, and even more, in the case of a plasmid, about 19-25 nucleotides in the case of a double-stranded siRNA or about 20-30 nucleotides in the case of a single-stranded microRNA. These may be natural,

hybrid or synthetic sequences, of any origin (prokaryote, eukaryote, virus, parasite, plant, etc.). Preferably, the nucleic acid is a therapeutic nucleic acid, i.e. a nucleic acid which has a therapeutic effect through the regulation, repair, replacement, addition or suppression of a genetic sequence. Preferably, the nucleic acid could modulate the expression of a protein and may be selected in particular from among antisense oligonucleotides, oligonucleotides for exon skipping, oligonucleotides for alternative splicing modification, interfering RNAs, messenger RNA or plasmid DNA. Preferably, the nucleic acid is an interfering RNA. In the context of the invention, an "interfering RNA" is an RNA which allows inhibiting gene expression. More particularly, an interfering RNA is a small ribonucleic acid (especially between 20 and 25 base pairs, in particular between 21 and 23 base pairs) which interferes with a specific messenger RNA, leading to degradation thereof and to inhibition of the transcription thereof into protein. For example, siRNA (small interfering RNA) or miRNA (micro RNA) are interfering RNAs.

In particular, the nucleic acid is selected from a plasmid, a siRNA, a miRNA, an mRNA and a shRNA, preferably the nucleic acid is a siRNA or a miRNA. Preferably, the nucleic acid is a siRNA.

The tablet manufactured according to the invention comprises one or several nucleic acid vector(s) (or complex(es)), as defined hereinabove. According to a particular embodiment, the tablet comprises a combination of several different nucleic acid vectors, in particular a combination of at least 2, at least 3, at least 4, or else at least 5 different nucleic acid vectors. According to another particular embodiment, the tablet comprises a combination of one or several nucleic acid vector(s) (or complex(es)) with at least one probiotic.

By "probiotic", it should be understood a living microorganism which, when administered in a sufficient amount, exerts positive effects on health, beyond traditional nutritional effects. The positive effects on health include, for example, an improvement in digestion or immune defences.

According to the present invention, a complex is formed by electrostatic interactions between a negatively-charged nucleic acid and a lipid particle comprising a cationic lipid.

5 Preferably, the lipid particle comprising a cationic lipid is a micelle or a liposome.

Even more preferably, the lipid particle is a liposome. According to this embodiment, the complex formed between the nucleic acid and the liposome is called "lipoplex".

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According to a particular embodiment, the invention relates to a process for manufacturing a tablet comprising a complex between:

(i) a nucleic acid; and

(ii) a lipid particle comprising a cationic lipid, said lipid particle preferably being a liposome or a micelle, even more preferably a liposome.

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According to a particular embodiment, the process of the invention may comprise, before the drying step described in details hereinbelow, a step of forming a complex between the nucleic acid and the lipid particle comprising a cationic lipid, the lipid particle preferably being a liposome or a micelle, preferably a liposome.

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According to a particular embodiment, the nucleic acid may be premixed with an anionic polymer, prior to the step of forming the complex between the nucleic acid and the lipid particle. Said anionic polymer is an adjuvant allowing improving structuring of the complex between the nucleic acid and the lipid particle, and could thus increase the effectiveness of said complex. The anionic polymer may be selected from anionic polysaccharides and anionic polypeptides, preferably the anionic polymer is selected from sodium alginate and sodium polyglutamate. According to a particular embodiment, the anionic polymer is an alginate, in particular sodium alginate. Thus, the manufacturing process according to the invention may further comprise a step of mixing the nucleic acid and an anionic polymer, prior to the step of forming the complex between the nucleic acid and the lipid particle.

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Furthermore, the process according to the invention may comprise a prior step of forming the lipid particle comprising a cationic lipid, the lipid particle preferably being a liposome or a micelle, preferably a liposome.

5 Preferably, the cationic lipid is selected from cationic lipids capable of forming a particle by association of the hydrophobic chains of several lipids. The cationic lipid according to the invention may be selected from lipopolyamines, quaternary ammoniums, and lipids having a cationic head of the guanidine or imidazole type. Preferably, the cationic lipid is selected from Di-Myristylaminopropylaminopropyl (or  
10 DMAPAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (or DOTMA), [1,2-bis(oleoyloxy)-3-(trimethylammonio)propane] (or DOTAP), 3• [N-(N',N'-dimethylaminoethane)-carbonyl]cholesterol (DOChol), and dioctadecylamidoglycylspermine (or DOGS). Preferably, the cationic lipid is Di-Myristylaminopropylaminopropyl (or DMAPAP).

15 According to a particular embodiment, the cationic lipid is mixed with a neutral lipid. Preferably, the neutral lipid is selected from 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, dioleoyl-sn-glycero-3-phosphocholine (DOPC), dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and N'-(rac-1-[1-(F-octyl)undec-10-enyl]-2-(hexadecyl)glycero-3-phosphoethanol)-sperminocarboxamide.  
20 Preferably, the neutral lipid is 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE).

Thus, the tablet according to the invention may comprise a complex between (i) a nucleic acid and (ii) a lipid particle comprising a cationic lipid possibly mixed with a  
25 neutral lipid.

According to a preferred embodiment, the tablet according to the invention comprises a complex between (i) a nucleic acid and (ii) a lipid particle comprising a mixture between a cationic lipid and a neutral lipid. Preferably, the lipid particle is a  
30 micelle or a liposome, more preferably a liposome.

The mixture between the cationic lipid and the neutral lipid comprises in particular a defined proportion of cationic lipid and of neutral lipid. Preferably, the

proportion of cationic lipid is comprised between 50 and 99 mol% lipid in the mixture. In particular, the proportion of cationic lipid may be about 50 mol%, about 60 mol%, about 70 mol%, about 80 mol% or about 90 mol% lipid in the mixture. Preferably, the proportion of cationic lipid is about 50 mol% lipid in the mixture.

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Preferably, the proportion of neutral lipid is comprised between 1 and 50 mol% lipid in the mixture. In particular, the proportion of neutral lipid may be about 10 mol%, about 20 mol%, about 30 mol%, about 40 mol% or about 50 mol% lipid in the mixture. Preferably, the proportion of neutral lipid is about 50 mol% lipid in the mixture.

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According to a preferred embodiment, the cationic lipid is mixed with the neutral lipid according to a 1:1 equimolar ratio.

The process according to the invention may comprise a mixing step between the cationic lipid and the neutral lipid, prior to the step of forming the lipid particle, the lipid particle then being used during the step of forming the complex with the nucleic acid.

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According to a particular embodiment, the mixture between a cationic lipid and a neutral lipid is a mixture between DMAPAP and DOPE.

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According to a particular embodiment, the nucleic acid of the invention is mixed with the lipid particle of the invention according to a defined charge ratio. The "charge ratio" corresponds to the +/- charge ratio between the number of positive charges of the cationic lipid and the number of negative charges of the nucleic acid. This charge ratio could affect the effectiveness of the formed complex by acting on the physico-chemical properties (for example the size or the surface charge). Said charge ratio could vary and be adapted by a person skilled in the art, according to the selected lipid and nucleic acid as well as the desired application (targeted organ or cell type). Preferably, the +/- charge ratio is comprised between 0.5 and 10, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Preferably the +/- charge ratio is comprised between 4 and 10, for example about 4, 5, 6, 7, 8, 9 or 10. Even more preferably, the charge ratio is about 8.

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According to a particular embodiment, the mixture between the nucleic acid and the lipid particle is an aqueous mixture. Thus, the process according to the invention may comprise a step of preparing an aqueous mixture between the nucleic acid and the lipid particle comprising a cationic lipid.

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Preferably, the aqueous mixture between the nucleic acid and the lipid particle is carried out in a buffer solution. In particular, the pH of said buffer solution is about 4 to 8. Preferably, the selected buffer solution is a solution comprising NaCl. In particular, said buffer solution may comprise a NaCl concentration of about 150 mM. For example, a person skilled in the art can use a buffer solution of the HEPES or opti-MEM type, or a solution comprising glucose.

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Thus, according to a particular embodiment, the process for manufacturing a tablet according to the invention may comprise:

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- a step of forming the lipid particle, preferably the liposome, in a buffer solution; and/or
- a step of preparing the nucleic acid in a buffer solution; and or
- a step of mixing the nucleic acid and the lipid particle in a buffer solution.

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According to a particular embodiment, the mixing time between the nucleic acid and the lipid particle, preferably a liposome, is comprised between 10 seconds and several days, for example between 10 seconds and 10 hours, in particular between 5 minutes and 5 hours, in particular between 10 min and 1 hour. Preferably, the mixing time is greater than or equal to 30 minutes, in particular equal to about 30 minutes. Mixing may be carried out in a wide temperature range. Nonetheless, according to a preferred embodiment, mixing is carried out at a temperature comprised between 10°C and 30°C, in particular between 15°C and 25°C.

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After formation of the complex, the latter may be subjected directly to the next steps of the process according to the invention, or stored, in particular for at least 1 day, before said next steps.

"Tablet" Formulation

The process according to the invention comprises a phase of forming a tablet from the aqueous mixture comprising the nucleic acid and the lipid particle as described hereinabove. The inventors of the present application have succeeded in setting nucleic acid vectors (or complexes) in a "tablet" form, for possible oral, vaginal, rectal, sublingual or transmucosal administration, while preserving the biological properties of said nucleic acid vectors.

More particularly, the process according to the invention comprises the steps of:

- drying the aqueous mixture comprising a complex as defined hereinabove; and
- compressing the dry mixture thus obtained.

Preferably, the process according to the invention comprises the following steps of:

a) drying of an aqueous mixture comprising:

- the complex as defined hereinabove; and
- one or several excipient(s) suitable for drying

b) mixing one or several compression excipient(s) with the dry mixture obtained in step a); and

c) compressing the mixture obtained in step b).

- **Drying step a):**

By drying, it should be understood any dehydration process capable of eliminating water contained in a substance or body. For example, drying may be carried out by spray drying, by convective drying or by freeze-drying. According to a preferred embodiment, drying is carried out by freeze-drying. According to this embodiment, the excipient(s) suitable for drying is/are excipients suitable for freeze-drying. Freeze-drying is a process of drying under vacuum, at low temperature, allowing the elimination by sublimation of water contained in a product frozen beforehand.

According to a preferred embodiment, the drying step a) consists of freeze-drying. According to a particular embodiment, the implemented freeze-drying comprises the following steps of:

- freezing the aqueous mixture comprising a complex as defined hereinabove and one or several excipient(s) suitable for freeze-drying;
- primary drying (or sublimation); and
- secondary drying.

Preferably, the process according to the invention comprises, prior to the drying step, a step of mixing the complex as defined hereinabove with the excipient(s) suitable for drying.

The excipient(s) suitable for drying are excipients capable of preserving the structure of the nucleic acid vectors during the drying step, thus allowing preserving the biological activity of the nucleic acid. The excipient(s) suitable for drying also allow(s) obtaining a dry mixture suitable for the compression step. More particularly, the excipient(s) allow(s) obtaining a dry product whose texture (affected by the residual moisture) is suitable for the compression step. The excipient(s) also allow(s) obtaining a dry product whose mass and/or mechanical strength are adapted for the compression step, said excipients allowing, for example, preventing a collapse of the structure of the dry product. Preferably, said excipient(s) suitable for drying, preferably freeze-drying, facilitate(s) grinding and then homogeneous mixing of the dry product obtained with the compression excipient(s). The optimisation of the parameters such as the texture, the mass and/or the mechanical strength of the dry product through the selection of the nature and the amount of the excipients suitable for drying is conventionally carried out by a person skilled in the art, in particular according to the physico-chemical characteristics of each of the excipients (existence of eutectics, glass-transition temperatures, etc.).

According to the preferred embodiment of the invention, implementing freeze-drying in step a), the excipient(s) suitable for drying are suitable for freeze-drying. Thus, the excipient(s) suitable for freeze-drying allow(s) protecting the complex during the

freeze-drying step and/or allow(s) obtaining a freeze-dried product with a texture suitable for the compression step.

According to a particular embodiment, the excipient(s) suitable for freeze-drying are protective agents (cryoprotectors or lyoprotectors) and/or charge agents (or weighting agents). Cryoprotective excipients are excipients that protect the structures of nucleic acid vectors during the freezing step. Lyoprotective excipients are excipients that protect the structures of the nucleic acid vectors during the dehydration step. During freeze-drying, these agents substitute for water molecules to interact with the polar heads of the phospholipids (water replacement assumption) and form a glassy matrix which contributes to preserving the structure of the lipoplexes (vitrification assumption). Charge agents (or weighting agents) are excipients that allow preserving the structure and the rigidity of the freeze-dried product (in contrast with a collapsed state).

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According to a particular embodiment, the excipient(s) suitable for freeze-drying are selected from trehalose, mannitol, sucrose, sorbitol, lactose, glucose, glycerol, glycine, alanine, lysine, polyethylene glycol, poly vinyl pyrrolidone (PVP) and dextran, possibly as a mixture.

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According to a particular embodiment, the excipient suitable for freeze-drying is trehalose, in a proportion comprised between 1 and 100%, in particular between 10 and 100%, by mass with respect to the mass of excipient(s) suitable for freeze-drying. For example, the proportion of trehalose is about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or about 100% by mass with respect to the mass of excipient(s) suitable for freeze-drying.

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According to a particular embodiment, the excipient suitable for freeze-drying is mannitol, in a proportion comprised between 1 and 90% by mass with respect to the mass of excipient(s) suitable for freeze-drying. For example, the proportion of mannitol is about 0%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80% or about 90% by mass with respect to the mass of excipient(s) suitable for freeze-drying.

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Preferably, the excipient suitable for freeze-drying is trehalose, possibly mixed with another excipient suitable for freeze-drying selected from mannitol, sucrose, sorbitol, lactose, glucose, glycerol, glycine, alanine, lysine, polyethylene glycol, poly vinyl pyrrolidone (PVP) and dextran, preferably mannitol.

According to a particular embodiment, the trehalose is mixed with the mannitol according to defined proportions. In particular, the proportion of trehalose may be comprised between 10 and 99% by mass with respect to the trehalose/mannitol mixture. For example, the proportion of trehalose is about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80% or about 90% by mass with respect to the trehalose/mannitol mixture, preferably about 71% by mass relative to the trehalose/mannitol mixture.

According to a particular embodiment, the proportion of mannitol may be comprised between 1 and 90% by weight with respect to the trehalose/mannitol mixture. For example, the proportion of mannitol is about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80% or about 90% by mass with respect to the trehalose/mannitol mixture, preferably about 29% by mass with respect to the trehalose/mannitol mixture.

According to a particular embodiment, the process according to the invention may comprise the following steps of:

- preparing excipients suitable for drying, said excipients being preferably prepared in aqueous media;
- mixing the excipients suitable for drying with the complex defined hereinabove;
- and
- drying, in particular by freeze-drying, the mixture thus obtained.

According to a particular embodiment, the freezing step implemented during the freeze-drying corresponds to freezing of the mixture comprising the excipients suitable for drying and the complex defined hereinabove. The freezing is carried out so as to preserve the biological activity of the nucleic acid vectors, preferably so as to preserve

the supramolecular assembly of the nucleic acid vectors. The freezing parameters are controlled to promote amorphisation and/or limit the crystalline growth of the constituents of the mixture.

5            Preferably, the freezing kinetics are rapid, the latter depending in particular on the freezing temperature. Preferably, the freezing rate is comprised between  $-40^{\circ}\text{C}/\text{min}$  and  $-0.5^{\circ}\text{C}/\text{min}$ .

10           Preferably, in order to promote amorphisation, freezing is carried out in liquid nitrogen, whose temperature is in particular about  $-190^{\circ}\text{C}$ . Preferably, the duration of freezing in liquid nitrogen is comprised between 1 minute and 24 hours, in particular between 1 minute and 1 hour, said freezing duration being in particular about 10 minutes.

15           According to a particular embodiment, the parameters of the freeze-drying process allow preserving the biological activity of said complexes. In particular, the operating parameters that control the freeze-drying cycle (room temperature or shelf temperature, pressure in the drying chamber and duration of the cycle) are properly selected so as to preserve the biological activity of the complexes, and could be adapted  
20           according to the volume of the aqueous mixture comprising said complexes (more particularly according to the volume of water to be sublimated).

              According to a particular embodiment, the freeze-drying duration including the primary drying and the secondary drying is comprised between 10 and 80 hours, preferably between 15 and 72 hours, more preferably between 20 and 48 hours, preferably around 30 hours. The freeze-drying duration could be adapted by a person  
25           skilled in the art according to the volume and the formulation of the aqueous mixture to be freeze-dried.

30           According to a particular embodiment, drying, in particular by freeze-drying, is carried out at atmospheric pressure.

- **Mixing step b):**

The process according to the invention may comprise a step intended to break the structure of the product obtained after drying. This step may be a step of (low energy) light grinding or of redispersion of the product. It may be carried out for example by using a vibration or calibration mesh, or in a paddle mixer for example during mixing with the diluent.

Before compression, the dry mixture, possibly ground, is mixed with one or several compression excipient(s). By "compression excipient", it should be understood any excipient:

- allowing protecting the complex between the nucleic acid and the lipid particle during the compression step c); and/or
- allowing reducing friction phenomena during the compression step c); and/or
- allowing a regular feeding of the die used for the compression, the die delimiting the compartment where the compression of the powder is performed
- and/or
- allowing obtaining a tablet with a sufficient mass and adequate mechanical strength,
- and/or
- allowing controlling or delaying the release of the nucleic acid vector,
- and/or
- allowing the disintegration of the tablet.

According to a particular embodiment, the compression excipient(s) is/are selected from the excipients used in granular mixtures for compression.

In a preferred embodiment, the compression excipient is a lubricant, possibly mixed with at least one other compression excipient well known to a person skilled in the art, preferably a diluent.

By "lubricant", it should be understood any excipient allowing reducing the adhesion of the powder to the punch and to the die of the compression machine, and/or

causing the reduction of frictions between the particles and the die during compression and ejection of the tablet.

5 According to a particular embodiment, the lubricant represents from 0.25 to 5% by mass of finished tablet. Preferably, the lubricant represents about 0.5% by weight of the finished tablet. According to a preferred embodiment, the lubricant is selected from magnesium stearate, talc, sodium stearyl fumarate and stearic acid, possibly as a mixture. Preferably, the lubricant is magnesium stearate.

10 By "diluent", it should be understood any excipient having a filling role when the amount of active principle and of the other excipients is not enough to make a tablet with a suitable size, thus allowing setting the mass and the dimensions of the tablet.

15 According to a preferred embodiment, the diluent is selected from lactose, starch and derivatives thereof, calcium phosphate and derivatives thereof, in particular dicalcium phosphate, and cellulose and derivatives thereof, in particular microcrystalline cellulose, possibly as a mixture. Preferably, the diluent is lactose.

20 The tablet may further contain pharmaceutical excipients well known to a person skilled in the art such as flow agents, disintegrants, binders, excipients allowing controlling or delaying the release of the nucleic acid vectors, excipients conferring muco-adhesion properties on the tablet and combination thereof. For example, the tablet may contain one or more of the following excipients: sucrose, glucose, mannitol, trehalose and silica.

25 Preferably, the compression excipient(s) are dry excipients, preferably in a powder form.

30 The method of the invention may comprise a step of mixing the different compression excipients prior to the step of mixing with the dry mixture obtained upon completion of the drying step a).

- **Compression step c):**

The process according to the invention further comprises a step c) of compressing the mixture obtained upon completion of step b). The compression step is carried out under conditions allowing preserving the biological function of the nucleic acid vectors.

5 In particular, the compression step is carried out so as to preserve the therapeutic activity of the nucleic acid vectors, for example the inhibition of the expression of a target gene, in the case of siRNAs.

10 The mechanical stresses applied during the compression stage could be controlled and monitored. More particularly, the pressure applied to the mixture comprising the nucleic acid vectors according to the invention is controlled and monitored during the compression step. Preferably, the compression of step c) is carried out at a pressure comprised between 10 and 400 MPa, preferably between 50 and 250 MPa.

15 According to a preferred embodiment, the compression of step c) is carried out by means of a tablet press, for example a rotary press or a reciprocating press.

20 In a particular embodiment, a wet granulation or dry granulation step is carried out upstream of the compression step c). The "wet granulation" or "dry granulation" steps consist in increasing the size of the particles in order to obtain agglomerates, in order to facilitate the compression. More particularly, the wet granulation step comprises the addition of a binding agent allowing obtaining granules in the form of agglomerates which are then dried and possibly sieved before the compression step. The dry granulation consists in strongly compressing the particles to obtain an  
25 agglomerate before grinding them to form grains which are possibly calibrated before the final step of shaping by compression.

30 According to a preferred embodiment, the compression step c) is direct compression, the direct compression requiring no prior granulation step. According to this embodiment, the dry mixture obtained upon completion of the drying step (step a)), possibly mixed with compression excipients (step b)) has properties suitable for direct compression.

In a particular embodiment, the process according to the invention comprises, after the compression step, a step of laminating (or film-coating) the obtained tablet. The interest of this step is the production of a tablet which is better protected, whose stability is improved, and/or whose release kinetics are adapted. The nature of the film-coating and/or coating agents and the process used to implement this step are well known to a person skilled in the formulation art.

According to a second aspect, the invention relates to a tablet that could be obtained by means of the method as described hereinabove.

In particular, the invention relates to a tablet comprising:

- one or several nucleic acid vector(s) (or complex(es)) as described hereinabove;
- one or several excipient(s) suitable for drying;
- one or several compression excipient(s).

In particular, the tablet of the invention may comprise:

- a complex between a nucleic acid and a liposome or a micelle comprising a cationic lipid;

- one or several suitable freeze-drying excipient(s) allowing protecting said complex during the freeze-drying step a) and/or allowing obtaining a freeze-dried product with a texture suitable for the compression step, preferably trehalose and mannitol;

- one or several suitable compression excipient(s) allowing protecting said complex during the compression step c) and preserving the biological activity of the complex.

In particular, the finished tablet comprises from 1  $\mu\text{g}$  to 1 mg of nucleic acid, preferably about 10  $\mu\text{g}$ .

The mass of cationic lipid in the finished tablet may be comprised between 18.7  $\mu\text{g}$  and 18.7 mg, preferably about 170  $\mu\text{g}$ .

The mass of neutral lipid in the finished tablet may be comprised between 16.5 µg and 16.5 mg, preferably about 150 µg.

5 The mass of polymer in the finished tablet may be comprised between 1 µ and 1 mg, preferably about 10 µg.

10 The mass of the excipients suitable for drying may be comprised in the finished tablet between 36 mg and 720 mg, preferably about 126 mg. In particular, the mass of trehalose in the finished tablet may be comprised between 3.6 mg and 720 mg, preferably about 90 mg. In particular, the mass of mannitol in the finished tablet may be comprised between 0 mg and 648 mg, preferably about 36 mg.

15 The proportion of the excipients suitable for compression may be comprised between 0.25% and 95%, in mass percentage with respect to the finished tablet. In particular, the proportion of lubricant, preferably being magnesium stearate, may be comprised between 0.25% and 5%, in mass percentage with respect to the finished tablet, preferably about 0.5%. In particular, the proportion of the other compression excipients may be comprised between 0% and 94.75%, in mass percentage with respect to the finished tablet.

20

According to a particular embodiment, the tablet according to the invention comprises:

- 25
- a complex between a nucleic acid and a liposome or a micelle comprising DMAPAP and DOPE, the complex further comprising alginate, in particular sodium alginate;
  - trehalose and mannitol; and
  - lactose.

30 According to a variant of this embodiment, the tablet thus composed has, after return back in suspension in water, a SAXS (Small Angle X Ray Scattering) profile in a synchrotron radiation comprising a cubic phase and a lamellar phase, and possibly a liquid crystalline phase. The SAXS profile may be determined according to the protocol presented in the examples. The SAXS profile of the tablet according to the invention

after return back in suspension in water features in particular peaks at  $q=0.086 \text{ nm}^{-1}$  and  $q=0.101 \text{ nm}^{-1}$ . According to a particular embodiment, the lamellar phase prevails over the cubic phase.

5           According to a particular embodiment, the tablet according to the invention is intended for oral, vaginal, rectal, sublingual or transmucosal administration. Preferably, the tablet according to the invention is intended for oral administration.

10           According to another aspect, the present invention relates to the tablet of the invention for use in the treatment of local, local-regional pathologies or in the treatment of systemic pathologies. Preferably, the tablet according to the invention is used in the treatment of gastrointestinal tract pathologies. For example, the gastrointestinal tract pathologies may be Crohn's disease (the nucleic acid being for example a siRNA inhibiting inflammatory proteins such as TNFalpha), ulcerative colitis (the nucleic acid  
15           being for example an anti-ICAM-1 oligonucleotide, a plasmid encoding IL10 or an anti-TNFalpha siRNA), or a gastric cancer (the nucleic acid being for example an anti Her2 siRNA or oligonucleotide). The tablet according to the invention could also be used in the treatment of vaginal pathologies, in particular vulvovaginal candidiasis or an infection with HSV, HPV or HIV (via the administration of antiviral siRNAs for example).

20

          Furthermore, the tablet could be used in the treatment of pathologies such as spinal amyotrophy (the nucleic acid being for example the antisense oligonucleotide Nusinersen), familial hypercholesterolemia (the nucleic acid being for example the antisense oligonucleotide Mipomersen), Duchenne myopathy, or age-related macular  
25           degeneration (the nucleic acid being for example the aptamer Macugen).

25

          The tablet according to the invention is stable, i.e. it substantially preserves its biological activity, preferably over a time period that could reach 2 weeks, 1 month, 3 months, 1 year, at room temperature (between the formulation of the tablet and the  
30           administration).

30

#### EXAM PLES

**Example 1: Preparation of the tablet***1. Formation of the lipoplexes*

5           The first step consists in the formation of the lipoplex, i.e. the complex between the nucleic acid and the liposome. The nucleic acid used in this example is a specific siRNA of luciferase. The liposome used herein comprises a cationic lipid (the DMAPAP) and a neutral lipid (the DOPE). The formation of the lipoplexes has been carried out using the following constituents:

10

|                                   |                        |
|-----------------------------------|------------------------|
| luciferase siRNA or control siRNA | 1.3 µg/µL              |
| liposome (DMAPAP, DOPE)           | 20 mM                  |
| polymer (sodium alginate)         | 1.5 µg/µL              |
| NaCl                              | 150 mM in milliQ water |

The siRNA, liposome and polymer preparations have been vortexed.

- The liposome has been mixed with the NaCl solution to obtain a final volume of 200 µL (10.7 µL of liposome with 189 µL of NaCl solution).

15

- On the other hand, the siRNA has been mixed with the polymer in a NaCl solution to obtain a final volume of 200 µL (7.5 µL of siRNA + 6.5 µL of polymer + 186 µL of NaCl solution). Hence, the obtained mixture comprises a ratio between the siRNA and the polymer of 1:1 by mass.

20

- The two obtained preparations, each 200 µL, have been mixed, vortexed, then left for 30 min at room temperature to enable the formation of the complex between the siRNA and the liposome.

The constituents hereinabove have been mixed so as to form a lipoplex whose +/- charge ratio is 8.

25

*2. Freeze-drying*

In this example, mannitol and trehalose have been used as excipients suitable for freeze-drying.

The previously-obtained lipoplex suspension has been transferred into a 50 mL centrifuge tube. A solution comprising 2.5% w/v trehalose and 1% w/v mannitol has been prepared. Afterwards, 3.6 mL of this trehalose/mannitol solution have been added to the 400  $\mu$ L of the lipoplex suspension. Afterwards, the tube has been immersed in liquid nitrogen for 10 minutes.

The freeze-drying has been carried out on a freeze-dryer comprising a hedgehog equipped with 12 taps on which the tubes of frozen samples are connected. The freeze-drying has been carried out for 30 hours, at a pressure in the freeze-drying chamber lower than 0.2 mbar (more particularly between 0.1 and 0.18 mbar). The energy input necessary for compensating for the energy absorbed during sublimation is supplied by ambient air (room temperature, more particularly between 20 and 25°C).

Temperature probes have been placed in the samples to monitor the temperature of the product during the freezing (cf. figure 1b) and freeze-drying (cf. figure 1a) process.

### *3. Compression*

In this example, magnesium stearate (lubricant) and lactose (diluent) have been used as excipients suitable for the compression.

The freeze-dried product obtained before has been first reduced to powder by light grinding in a mortar. Then, 36.2 mg of lactose and 0.8 mg of magnesium stearate have been added in the mortar and mixed with the freeze-dried product.

The compression has been carried out using a StylOne Evolution compression simulator according to the following parameters:

- Chamfered flat punches with a 6mm diameter
- Compression cycle = cycle 1 compression (default cycle), speed 2%
- Force control
- Assay (die fill-up height) = 18mm
- Manual fill-up

- Compression force = 1.4 to 7.1 kN (namely 50 to 250 MPa)

### **Example 2: Examples of compositions of Tablets containing lipoplexes**

5           The following Table 1 is presented in order to illustrate the composition of the tablets containing lipoplexes. The different constituents are expressed in mg.

|              | Tablet 1 | Tablet 2 | Tablet 3  |
|--------------|----------|----------|-----------|
| Nucleic acid | 0.01 mg  | 0.10 mg  | 0.01 mg   |
| DMAPAP       | 0.17 mg  | 1.70 mg  | 0.17 mg   |
| DOPE         | 0.15 mg  | 1.50 mg  | 0.15 mg   |
| Alginate     | 0.01 mg  | 0.10 mg  | 0.01 mg   |
| Trehalose    | 90.00 mg | 90.00 mg | 90.00 mg  |
| Mannitol     | 36.00 mg | 36.00 mg | 36.00 mg  |
| Mg stearate  | 0.75 mg  | 0.75 mg  | 2.25 mg   |
| Lactose      | 22.91 mg | 19.85 mg | 126.34 mg |

Table 1. Examples of compositions of tablets containing lipoplexes

### **Example 3: Testing the effectiveness of the tablet**

10           Afterwards, the effectiveness of the tablets is assessed on cultured cells expressing the luciferase gene. When these cells are set in the presence of vectors containing specific siRNAs of luciferase, the luciferase activity is inhibited. The test  
 15           consists in transfecting mouse melanoma cells expressing luciferase (B16-Luc) and assaying the residual luciferase activity in the cells 48 hours later. Compared to the activity of cells that are untreated or treated with vectors containing control siRNAs (without any inhibitory effect), this measurement allows obtaining the percentage of inhibition induced by the studied siRNA vector.

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To assess the maintenance of this activity after compression, the tablets are dissolved before being brought into contact with the cells to be transfected. The freeze-dried products (obtained before the compression step) have also been rehydrated to analyse the effectiveness of the dry mixture before compression.

Hence, the test has consisted in treating the B161uc cells with:

- A negative control: i.e. lipoplexes containing a control siRNA (whose sequence has been selected so that it does not inhibit any protein target), set again in suspension from a compressed form;

5       - A positive transfection control: i.e. the lipoplexes in suspension (not freeze-dried, not compressed);

- A rehydrated freeze-dried product; or

- A rehydrated tablet.

10       More specifically, the lipoplexes in suspension (corresponding to the positive transfection control) have been prepared as described hereinabove, then mixed with culture medium. The culture medium corresponds to complete medium (DMEM + Glutamax + fetal calf serum + penicillin/streptomycin) supplemented with Geneticin (G418).

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The freeze-dried product and the tablet have been prepared as described hereinabove, then dissolved in distilled water or milliQ and mixed with culture medium.

The manipulation is performed over 4 days (D0, D1, D2 and D3):

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On D0, the cells are prepared on a 24-well plate (40,000 cells/well).

On D1, the cell culture medium is replaced with 1 mL of transfection medium (so as to add 0.5 µg of siRNA per well). The transfection medium corresponds to the medium comprising the negative control, the positive transfection control, the freeze-dried product or the tablet.

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On D2, the transfection medium is replaced with culture medium (complete medium + G418).

30

On D3, the cells are lysed and the luciferase activity assayed.

The test is performed in triplicate (3 repetitions per condition).

**Results:****Test of the effectiveness of the tablet**

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The cell transfection tests have demonstrated a good effectiveness of the tablets containing the lipoplexes (effectiveness expressed by the inhibition percentage of the tablets compared to non-transfected cells). In addition, over the entire range of applied compressive stresses, no effect of the compressive stress on the inhibition percentage of the luciferase gene has been observed (Figure 2).

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**Example 4: supramolecular structure**

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The SAXS (Small Angle X Ray Scattering) technique has been used to identify the supramolecular structure of the siRNA vectors, both on suspended particles or on dry forms, such as a freeze-dried product or a tablet. The used siRNAs are control siRNAs, and each sample comprises 100 micrograms of siRNA. This identification is made necessary by the fact that the structure of this type of particles is directly related to their effectiveness in the nucleic acid administration. The SAXS analysis in a synchrotron radiation is the only technology providing access to this structure identification in various dosages, in suspension in an aqueous medium or in a powder.

20

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Firstly, the structure of siRNA lipoplexes (DAPAP/DOPE + alginate) prepared at a +/- charge ratio of 8 has been determined. The study carried out on the lipoplexes in suspension in a saline buffer (150 mM NaCl) has allowed showing that these lipoplexes were in the form of two coexisting structures, a lamellar phase and a cubic phase (Figure 3).

30

Afterwards, we have studied the fate of this structure when the vectors in suspension have undergone a freeze-drying step, a necessary prior step before being able to perform the compression step, then after the compression step. Figure 4 shows that a signal is detected for both sample types (freeze-dried product and tablet), with a substantially wide single peak at  $q=0.096 \text{ nm}^{-1}$ . Its position, intermediate between the

values of the peaks  $L_1$  and  $C_1$  of the lipoplex in suspension (Figure 3), and its uniqueness do not allow deducing the phase corresponding to this peak. The peak observed for the freeze-dried sample is more intense than that of the compressed sample, but the latter nevertheless remains perfectly detectable.

5

The freeze-drying and compression steps require the addition of excipients. Hence, we have assessed the signals obtained for lipoplexes diluted in a NaCl/excipient mixture under the same concentration conditions as the freeze-dried and compressed samples (Figure 5). The obtained profiles indicate the presence of a single cubic phase, the first peak of which is slightly offset between the “freeze-dried” and “compressed” conditions. The position of this peak is close to the peak detected during the analysis of the powders, which might indicate that the phase present in the dry forms is a cubic phase. The peak corresponding to the lamellar phase is no longer detectable, either in the dry forms or in the suspensions, in the presence of excipients.

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Afterwards, we have analysed the SAXS profile of the resuspended freeze-dried or compressed samples. The previous analysis having shown the influence of the presence of the excipients on the structure of the particles (Figure 5), we have compared each resuspended sample with lipoplexes in suspension under the same conditions of concentration of excipients. The obtained results are shown in Figure 6. It is noticed that the SAXS profile of the resuspended freeze-dried sample is close to the profile of lipoplexes in suspension under the same conditions (Figure 6A), which suggests that freeze-drying does not modify the structure of the lipoplexes. In the presence of an excipient, only a cubic phase is detected. However, for the resuspended compressed sample, the obtained profile allows clearly detecting two phases, a lamellar one and a cubic one, whose main peaks have positions close to those obtained for the initial suspension of lipoplexes (Figure 3). The profile of this sample is clearly different from that of a suspension of lipoplexes placed under identical conditions of excipient concentration. It is also different from that of the initial suspension of lipoplexes, since the relative proportion of the cubic and lamellar phases is reversed. In the initial suspension, the cubic phase prevails, in the resuspended compressed sample, it is the lamellar phase which prevails. This result suggests that compression has an influence on

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the structure by modifying the relative proportion of one phase with respect to the other, yet without making a new phase appear or making it disappear.

**Example 5: Assessment of the effectiveness in vivo**

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**Model:** induction of ulcerative colitis in mice by administration of dextran sulphate (SD) in drinking water for 7 days.

10        **Assessment of the effectiveness of the vectors:** oral administration of tablets containing siRNA lipoplexes. The tablets are given on D1, D3 and D5 to mice under SD treatment (started on D0). Each mouse receives a tablet containing 50 µg of siRNA formulated in lipoplexes.

Three groups of 10 mice are used:

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- Group 1: drink water, no administration of lipoplexes
- Group 2: drink SD, on D1, 3 and 5, tablet containing siRNA lipoplexes directed against TNF alpha
- Group 3: drink SD, on D1, 3 and 5, tablet containing control siRNA lipoplexes (without inhibitory effect)
- 20        • Group 4: drink SD, no administration of lipoplexes

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During the treatment, monitoring of the weight and appearance of the faeces (presence of blood). On D7, euthanasia, removal of the colon, measurement of the size of the colon, assay of inflammatory cytokines on a colon lysate, histological analysis of the inflammation and of the tissue damage on sections of the colon.

**Expected result:**

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Decrease and even disappearance of the clinical and biochemical signs of the disease after the treatment. This decrease is specific to the sequence of the siRNA, i.e. it is observed in mice treated with the lipoplexes containing an anti-TNF alpha siRNA and not in those treated with lipoplexes containing a control siRNA.

**Patentkrav**

1. Fremgangsmåde til fremstilling af en tablet omfattende et kompleks mellem:

(i) en nucleinsyre; og

(ii) en lipidpartikel omfattende et kationisk lipid, idet lipidpartiklen fortrinsvis

5 er et liposom eller en micelle, fortrinsvis et liposom;

hvilken fremgangsmåde omfatter følgende trin:

a) tørring, især ved lyofilisering, af en vandig blanding omfattende:

- et kompleks mellem (i) en nucleinsyre og (ii) en lipidpartikel omfattende et kationisk lipid, idet lipidpartiklen fortrinsvis er et liposom eller en micelle,

10 fortrinsvis et liposom;

- et eller flere excipienser, der er egnede til tørring, især egnede til lyofilisering;

b) blanding af et eller flere tableteringshjælpemidler med den i trin a) opnåede tørre blanding; og

15 c) presning af den i trin b) opnåede blanding.

2. Fremgangsmåde ifølge krav 1, **kendetegnet ved, at** det kationiske lipid er valgt

blandt lipopolyaminer, kvaternære ammoniumforbindelser og lipider med et

kationisk hoved af guanidin- eller imidazoltypen, idet det kationiske lipid fortrinsvis

20 er valgt blandt di-myristylaminopropylaminopropyl (DMPAP), N-[1-(2,3-

dioleoyloxy)propyl]-N,N,N-trimethylammoniumchlorid (DOTMA), [1,2-bis(oleoyloxy)-

3-(trimethylammonio)propan] (DOTAP), 3•-[N-(N',N'-dimethylaminoethan)-

carbamoylester]cholesterol (DCChol) og dioctadecylamidoglycylspermin (DOGS),

fortrinsvis di-myristylaminopropylaminopropyl (DMPAP);

25 idet det kationiske lipid eventuelt er blandet med et neutralt lipid, især valgt blandt

1,2-dioleoyl-sn-glycero-3-phosphoethanolamin (DOPE), kolesterol, dioleoyl-sn-

glycero-3-phosphocholin (DOPC), dipalmitoyl-sn-glycero-3-phosphocholin (DPPC)

og N'-(rac-1-[11-(F-octyl)undec-10-enyl]-2-(hexadecyl)glycero-3-

phosphoethanoylester)-spermincarboxamid, idet det neutrale lipid fortrinsvis er 1,2-

30 dioleoyl-sn-glycero-3-phosphoethanolamin (DOPE).

3. Fremgangsmåde ifølge krav 2, ved hvilken blandingen af det kationiske lipid og det neutrale lipid omfatter:

- mellem 50 og 99 mol-% kationisk lipid i blandingen, fortrinsvis omkring 50 mol-%;
- 5       - mellem 1% og 50 mol-% neutralt lipid i blandingen, fortrinsvis omkring 50 mol-%.

4. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, ved hvilken nucleinsyren er en terapeutisk nucleinsyre, især en siRNA, en miRNA, en shRNA,  
10 et plasmid eller en mRNA.

5. Fremgangsmåde ifølge et hvilket som helst af de foregående krav,  
**kendetegnet ved, at** ladningsforholdet (+/-) mellem det kationiske lipids positive ladninger og nucleinsyrens negative ladninger ligger mellem 0,5 og 10, fortrinsvis  
15 mellem 4 og 10, fortrinsvis omkring 8.

6. Fremgangsmåde ifølge et hvilket som helst af de foregående krav,  
**kendetegnet ved, at** det eller de til lyofilisering egnede ekscipients er beskyttelsesmidler (kryoprotektorer eller lyoprotektorer) og/eller fyldstoffer, især  
20 valgt blandt trehalose, mannitol, saccharose, sorbitol, lactose, glucose, glycerol, glycin, alanin, lysin, polyethylenglycol, polyvinylpyrrolidon og dextran, eventuelt i blanding.

7. Fremgangsmåde ifølge krav 6, **kendetegnet ved, at** det til lyofilisering egnede  
25 ekscipiens er trehalose, især i et forhold mellem 10 og 100 masse-% i forhold til massen af til lyofilisering egnede ekscipiens(er), eventuelt i blanding med et andet til lyofilisering egnet ekscipiens, fortrinsvis mannitol, især i et forhold mellem 0 og 90 masse-% i forhold til massen af til lyofilisering egnede ekscipiens(er).

30 8. Fremgangsmåde ifølge et hvilket som helst af de foregående krav,  
**kendetegnet ved, at** tabletteringshjælpemidlet eller -midlerne tillader beskyttelse af komplekset mellem nucleinsyren og lipidpartiklen omfattende det kationiske lipid

under kompressionstrinnet c), idet tabletteringshjælpemidlet især er et glidemiddel (for eksempel magnesiumstearat, stearinsyre og talkum, fortrinsvis magnesiumstearat), eventuelt i blanding med et andet tabletteringshjælpemiddel, fortrinsvis et fortyndingsmiddel (for eksempel lactose, stivelse, calciumphosphat og derivater deraf og cellulose og derivater deraf, fortrinsvis lactose).

**9.** Fremgangsmåde ifølge krav 8, **kendetegnet ved, at** glidemidlet udgør fra 0,25 til 5 masse-% af den færdige tablet, fortrinsvis 0,5%.

**10.** Fremgangsmåde ifølge et hvilket som helst af de foregående krav, **kendetegnet ved, at** presningen i trin c) udføres ved et tryk mellem 10 og 400 MPa, fortrinsvis mellem 50 og 250 MPa.

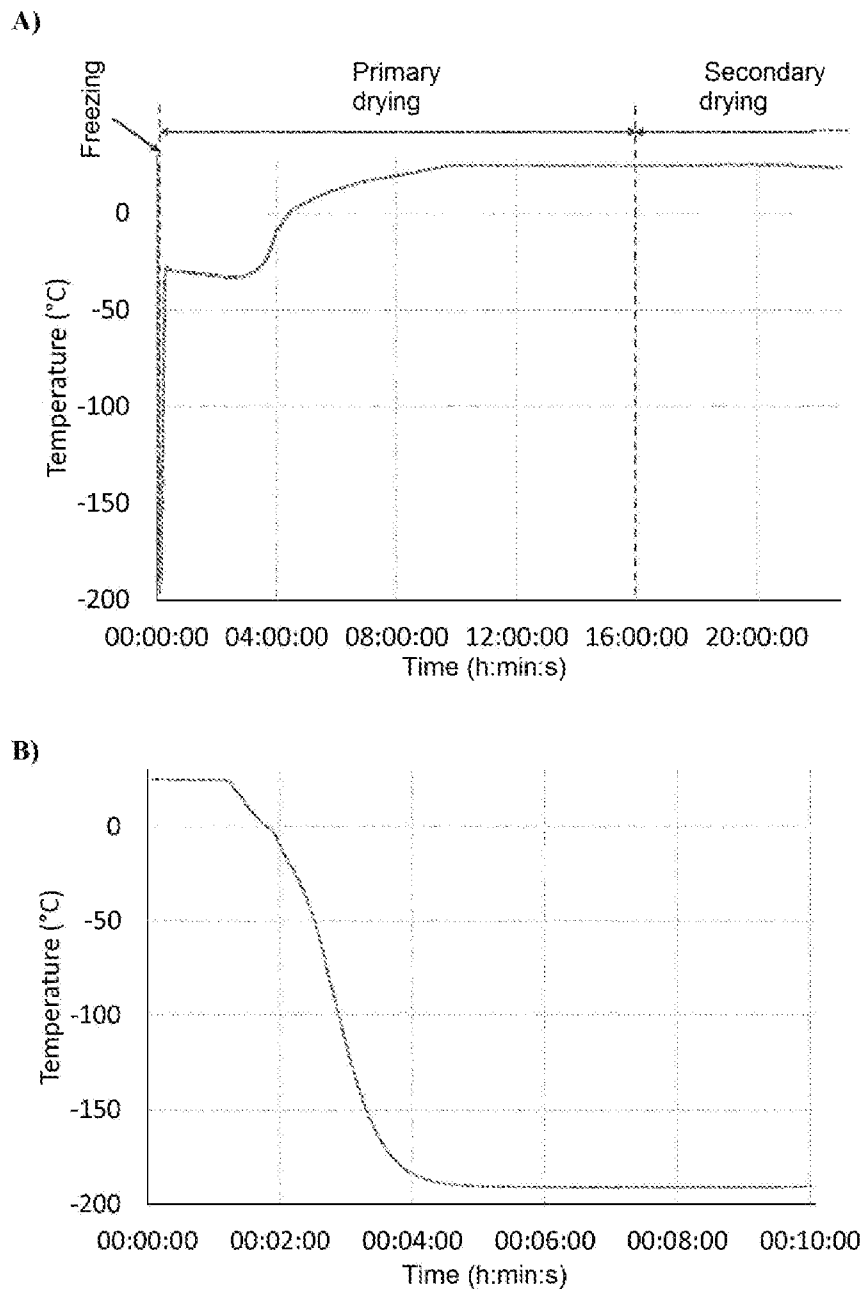
**11.** Tablet, som kan fås ved fremgangsmåden ifølge et hvilket som helst af kravene 1 til 10.

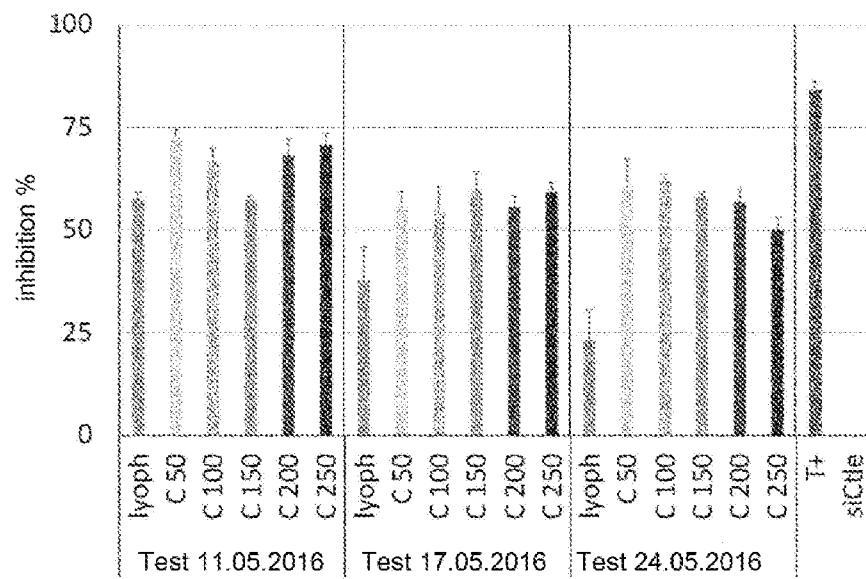
**12.** Tablet omfattende:

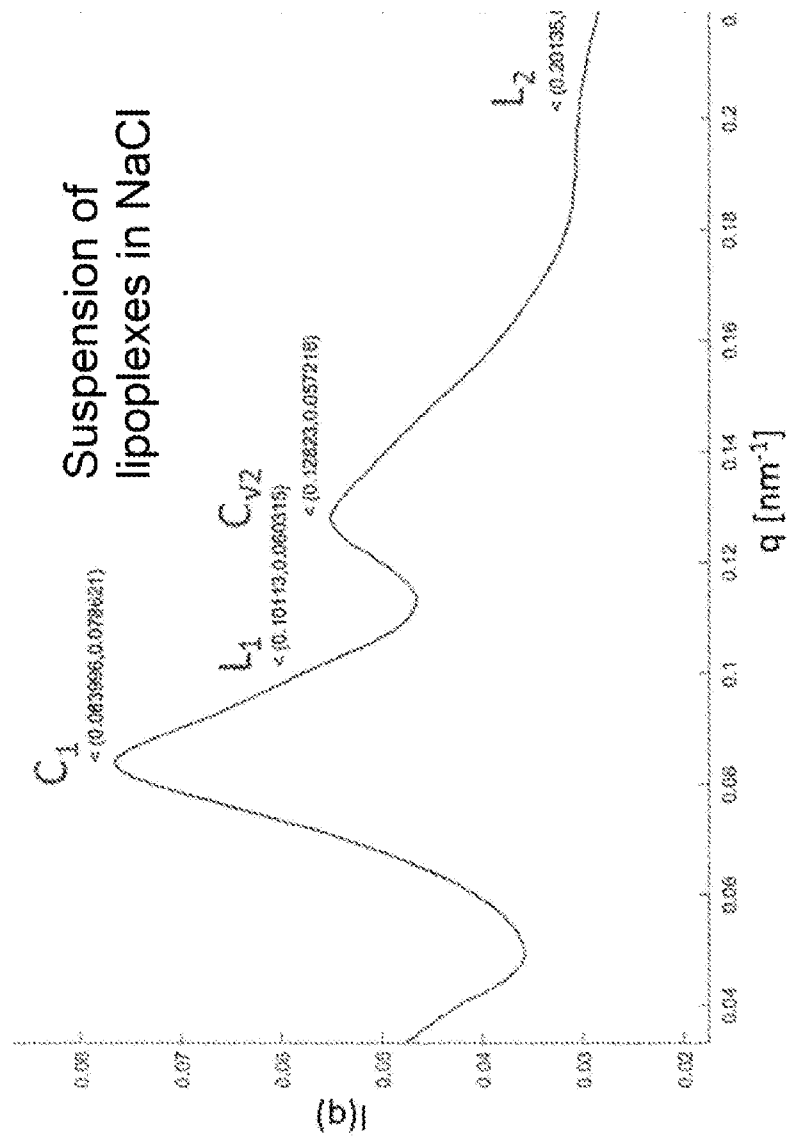
- et kompleks mellem en nucleinsyre og et liposom eller en micelle omfattende et kationisk lipid;
- et eller flere excipients, der er egnede til lyofilisering og tillader at beskytte komplekset under lyofiliseringstrinnet a) og/eller tillader at opnå et lyophilisat med en tekstur, der er egnet til presningstrinnet, fortrinsvis trehalose og mannitol.
- et eller flere excipients, der er egnede til presning og tillader at beskytte komplekset under presningstrinnet c) og bevare kompleksets biologiske aktivitet.

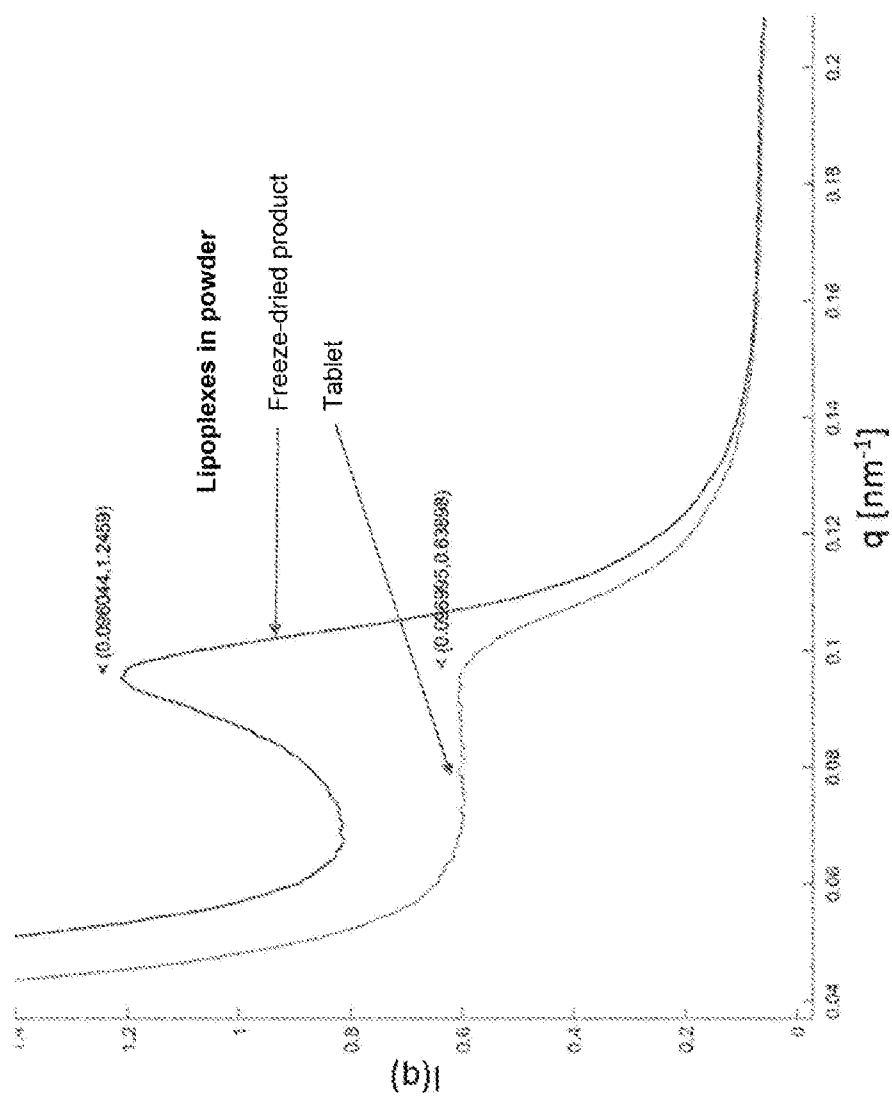
**13.** Tablet ifølge krav 11 eller 12, som er bestemt til oral, vaginal, rektal, sublingual eller transmucosal indgivelse, fortrinsvis oral indgivelse.

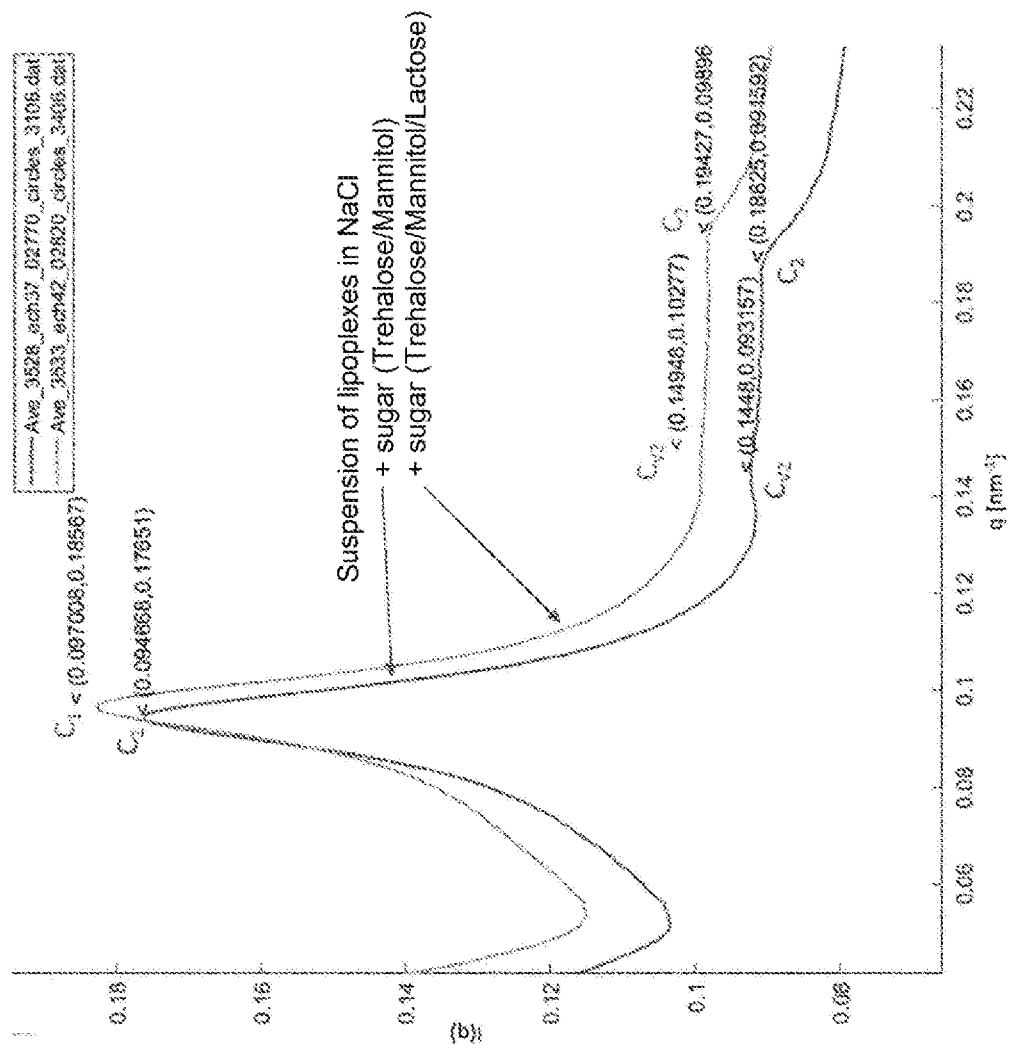
**14.** Tablet ifølge et hvilket som helst af kravene 11 til 13 til anvendelse ved behandling af lidelser i mavetarmkanalen eller vaginale lidelser.

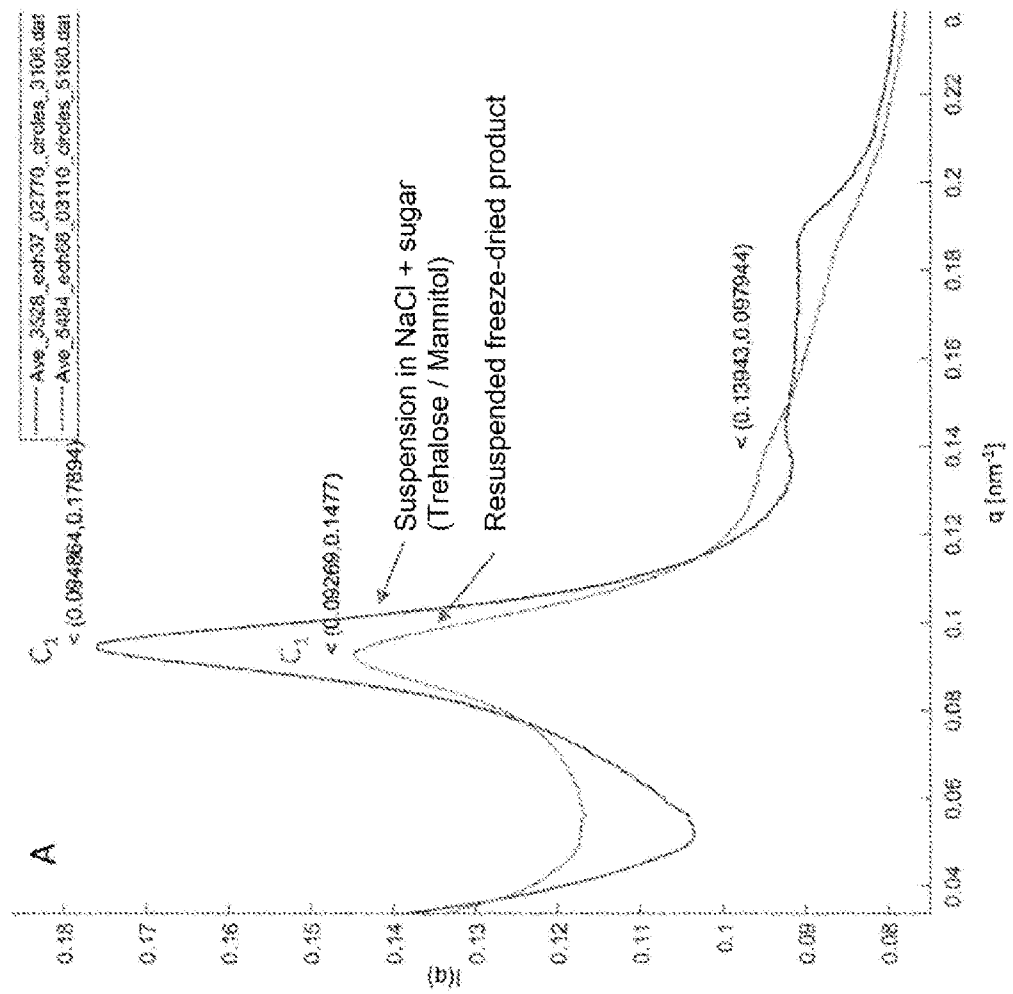
**Figure 1**

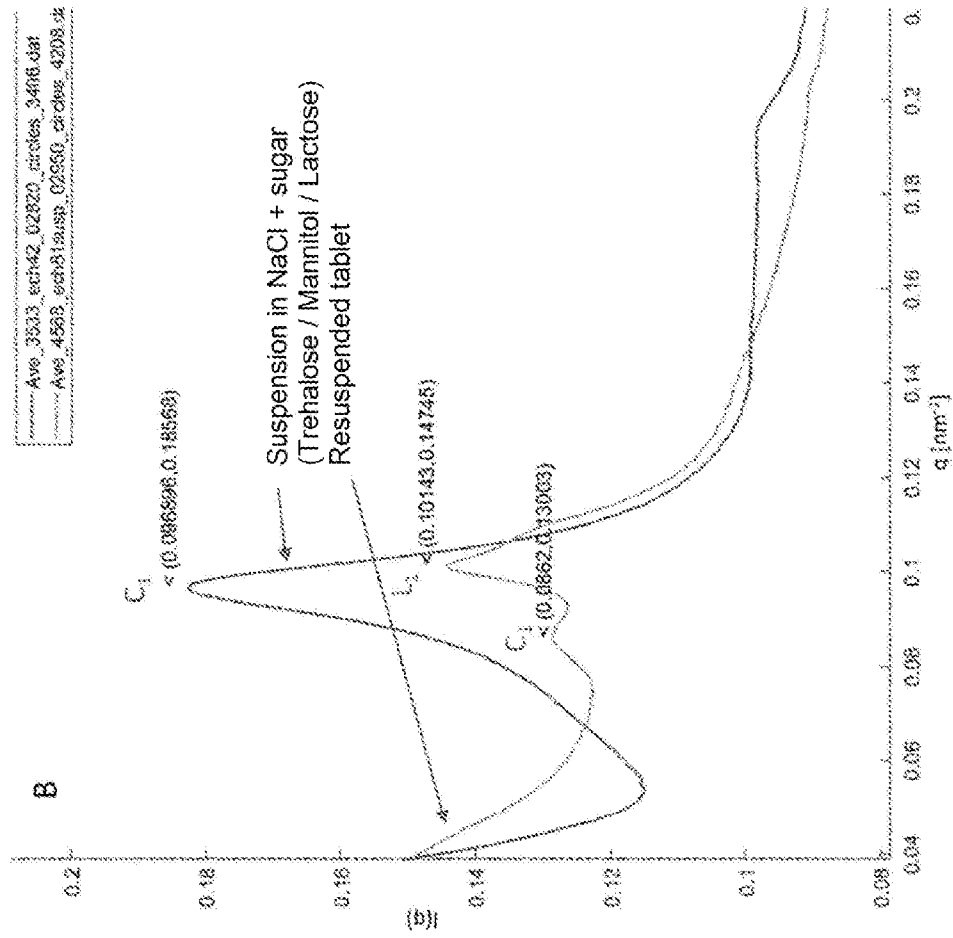
**Figure 2**

**FIGURE 3**

**FIGURE 4**

**FIGURE 5**

**FIGURE 6**

**FIGURE 6 (CONT'D)**