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(54) **Device and method to aerosolize nanoparticle formulations**

(57) For delivering nano-particles in an atmosphere, a liquid formulation that comprises said nano-particles is provided and pressurized to an elevated operating pressure p. said liquid formulation is fed at said elevated operating pressure through a spray nozzle orifice of at least one spray orifice to discharge said liquid formulation as a jet of consecutive liquid droplets that contain at least one nano-particle of said nano-particles. Said nano-particles have a length λ and a maximum length λ_{max} before breakage upon elongation and said liquid formulation is subjected to a shear rate γ [per second] while passing through said spray nozzle orifice. According to the invention said liquid formulation is exposed within said spray nozzle orifice to said shear rate during a limited shear time t that is less than $\lambda_{max}/(\lambda\gamma)$ seconds.

Dit octrooi is verleend ongeacht het bijgevoegde resultaat van het onderzoek naar de stand van de techniek en schriftelijke opinie. Het octrooischrift komt overeen met de oorspronkelijk ingediende stukken.

Device and method to aerosolize nanoparticle formulations

The present invention relates to a method to aerosolize nanoparticle formulations, while aiming at maintaining their molecular integrity during the aerosolization process, including formulations containing proteins, peptides, DNA, RNA, vesicles, liposomes, antibodies, etc.

Current aqueous aerosolization devices can be classified as nebulizers and soft mist inhalers. Nebulizers generate aerosols by using either compressed air, an ultrasonic power source or a vibrating mesh/membrane to disperse the formulation. Soft mist inhalers produce a mist of fine aqueous droplets by collision of microjets at a very high velocity and may have a problem in maintaining the integrity of large molecules due to the collision forces. Nebulizers demand frequent disinfection to prevent microbial contamination, and also the nanoparticle solution itself is during an administration time of several minutes or more subject to mechanical stresses due to the ultrasonic source or vibrating membrane facilitating shear stress induced degradation of the nanoparticles. Each type of aerosolization device has its strengths and weaknesses; and certainly not all devices are suitable for aerosolization of formulations while maintaining the molecular integrity of their constituents.

Maintaining the molecular integrity of the formulation is a factor that must always be taken into account when aerosolizing delicate nanoparticles, such as formulations containing complex proteins, large biological molecules, long chain DNA & RNA, mRNA vaccines, large vesicles, large liposomes, bacteriophages, beneficial viruses, antibodies, etc.

During aerosolization the nanoparticles may be subject to substantial detrimental stresses, in particular hydrodynamic shear stresses. Shear stress on a nanoparticle originates from local velocity gradients in the formulation when the liquid is subject to high energy dispersion with air, a vibrating mesh/membrane or when it passes a small capillary during aerosolization. The hydrodynamic stress exerted on the nanoparticle will lead to a drag force, that may induce a sufficient tension and a subsequent breakage of the nanoparticle if the drag force is greater than the critical strength to maintain its integrity. This drag force is proportional to the shear rate, the latter being a velocity difference over the nanoparticle divided by the size of the nanoparticle, with dimension per second.

Induced shear rates of soft mist inhalers and nebulizers are typically in the order of 10.000 to 100.000 per second. It has been found that formulations with long chain molecules, having a molecular weight

above 100.000 g/mol, suffer from a large amount of scission during the aerosolization process. Also many formulations with vesicles or liposomes are prone to breakage when using such type of nebulizers. Normally breakage of a vesicle occurs if the vesicle, while being exposed to shear, is significantly elongated over typically about a factor 3 to 5. Most macromolecules have a globular shape in solution and a specific gyration diameter; when the total length of the macromolecule is about 1000 nm the gyration diameter λ has typically between 25-50 nm. If this globular shaped polymer is subject to shear, it may easily be stretched like vesicles by a factor 3 to 5 (in practice even a factor 20-40 when the molecule is fully stretched). Also these large macromolecules tend to break under shear at a specific length λ_{max} even before the macromolecule is fully stretched.

It is an object of the invention to maintain the molecular integrity when aerosolizing delicate nanoparticles, such as formulations containing complex proteins, peptides, long chain DNA & RNA, large vesicles, liposomes and antibodies.

To that end the present invention provides a method of the type described in the opening paragraph, comprising:

- providing a liquid formulation that comprise said nano-particles;
- pressurizing said liquid formulation to an elevated operating pressure *p*; and
- feeding said liquid at said elevated operating pressure *p* to at least one spray nozzle orifice to discharge said liquid as a jet of consecutive liquid droplets that contain said nano-particles,

wherein said nano-particles have a length λ and a maximum length λ_{max} before breakage upon elongation; wherein said liquid formulation is subjected to a shear rate γ [per second] while passing through said at least one spray nozzle orifice; and wherein said liquid formulation is exposed to said shear rate during a limited shear time t less than $\lambda_{max}/(\lambda\gamma)$ seconds.

With this mutual condition of shear rate and shear time it appears possible to maintain the molecular integrity of both long chain molecules and vesicles during the aerosolization process. Surprisingly it has been found that even by using extremely large shear rates, at least ten to hundred times higher than in conventional nebulizers, during a very short time molecular integrity of the nanoparticle formulation may still be preserved.

According to a particular embodiment, the present invention provides a method, in that the nanoparticle formulation during aerosolization is subject to a shear rate γ per second during an ultra-short shear time t of less than $\lambda_{max}/(\lambda\gamma)$ sec. In a further particular embodiment of the method

according to the invention a liquid formulation is provided that comprises nano-particles taken from a group, containing complex proteins, peptides, long chain DNA & RNA, large vesicles, liposomes and antibodies.

In a capillary the shear rate at the wall is eight times the mean velocity of the formulation through the capillary divided by the diameter of the capillary. Molecular breakage of long polymer molecules is known to be related to these hydrodynamic shear-induced forces. Long-chain polymers undergo scission in strong flows because of the coupling of fluid mechanical and atomic scale chemical processes. Molecular scission has been studied in both laminar and turbulent flow regimes and it is generally assumed that the molecular scission rate scales with an exponent between 2 and 3 of the polymer contour length. Liposomes (lipid vesicles) are capsules composed of a self-assembled lipid bilayer membrane with a liquid inside and outside the capsule. The deformation and breakage of liposomes in a strong shear flow is also related to the coupling of fluid mechanical and atomic scale chemical processes.

A preferred method according to the invention, is therefore characterized in that the nanoparticle formulation during aerosolization is subject to a shear rate γ well above 1.000.000 per second during an ultra-short shear time t less than $3x10^{-6}$ to $5x10^{-6}$ sec.

It is an insight according to the invention that large macromolecules or large vesicles have not sufficient rigidity or intrinsic resistance to withstand deformation forces due to hydrodynamic shear forces. An insight underlying the invention is that during a shear time less than $\lambda_{max}/(\lambda\gamma)$ sec on applying a shear rate of γ per second the macromolecule or vesicle has not enough time to deform sufficiently to induce breakage of the large macromolecule or vesicle. Suppose a vesicle or macromolecule in solution has a specific size or gyration diameter λ and that the maximum allowed shape deformation before breakage is of order λ_{max}. At a shear rate of γ per second the difference in velocity between the upper and lower part of the nanoparticle with size λ is then γ times λ. In a total shearing time of $\lambda_{max}/(\lambda\gamma)$ sec the nanoparticle is then elongated or deformed with a length λ about γ times λ times $\lambda_{max}/(\lambda\gamma) = \lambda_{max}$.

Another embodiment of the invention is an aerosolization device that is capable in providing the conditions to generate a shear rate γ per second during an ultra-short shear time t of less than $\lambda_{max}/(\lambda\gamma)$ sec.

To that end a device to aerosolize nanoparticle formulations of the type as described in the opening paragraph, according to the present invention, is characterized in that the device produces a jet with a velocity V coming out of an orifice with a diameter H and a length L and shearing conditions $\gamma = \frac{V}{H}$ and $t = \lambda_{max}/(\lambda\gamma)\ sec = \frac{L}{V}$.

In a pipe with a parabolic velocity distribution the shear rate at the wall is 8V/H, whereas the shear rate in the middle of the pipe is zero. However, the liquid flows in the middle of the pipe with velocity 2V, whereas there is no flow at the wall of the pipe. The volume averaged shear rate in a pipe depending on the type of flow and the length of the pipe can vary between 4V/H (long pipe with parabolic velocity distribution) and nearly zero (short pipe with entrance effects, e.g. plug flow). This implies that, in order to reduce the average shear rate, it is favourable to use very short pipes or orifices. Further it has been recognized that the flow of the liquid through an orifice does not show a parabolic velocity distribution, but is rather governed by plug flow conditions, i.e. most liquid flows with an equal velocity through the orifice at a more favourable condition that the volume averaged shear rate is significantly lower than 4V/H.

A further embodiment is therefore that the device according to the invention is characterized in that the device produces a jet with a velocity V coming out of a short pipe or an orifice, typically with a diameter H and a length L, such that L<H. A preferred method is that the formulation is fed through a nozzle orifice substantially at a velocity V, said nozzle orifice having an average diameter H and a length L, such that L is smaller than ¼.H. λ_{max}/λ.

Good results also been obtained with slightly positively tapering orifices with tapering angles between 5° and 45°, and it is believed that the tapering favours even more plug flow conditions. Increasing the velocity V of the jet well above 10-30 m/s, by increasing the operating pressure *p* exceeding 10^5 Pa, particularly exceeding 10^6 Pa, has also been found to diminish the amount of degradation, possibly due to favouring plug flow conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the breakup of a macromolecule in a shear flow.

Fig. 2 illustrates the breakup of a vesicle in a shear flow.

Fig. 3 illustrates the breakup of a vesicle in a strong shear flow.

Fig. 4 illustrates the passage of a vesicle through a long pipe.

Fig. 5 illustrates the passage of a vesicle through an orifice.

Fig. 6 illustrates the passage of a vesicle through a tapered orifice.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Fig. 1 illustrates the breakup of a macromolecule in a shear flow. Fig. 1A shows a normal globular macromolecule (1) with a gyration diameter λ without the presence of a shear flow. Fig. 1B shows the initial stretching of the globular macromolecule in the presence of a shear flow indicated by arrows (2,3). Fig. 1C shows the further stretching to a maximum length λ_{max} of the macromolecule and the upper part of the macromolecule moving with a higher velocity starts separating form the lower part with a smaller velocity. The upper part (4) and the lower part (5) are only separated by one or two remaining covalent bonds. Fig. 1D shows the macromolecule after breaking in two parts due to the hydrodynamic shear forces exerted on the upper and lower part of the macromolecule, that cause breaking of the covalent bonds in the middle of macromolecule.

Long and heavy macromolecules such as large proteins, long RNA and DNA chains, antibodies, etc. having a molecular weight above 100.000 g/mol are susceptible to shear induced degradation. In Table 1 below some results are summarized on shear degradation of immunoglobulins (protein/antibody) with varying molecular weight during aerosolization.

Macromolecule (weight percent)	Molecular Weight (g/mol)	Survival (%) Vibrating Membrane Nebulizer	Survival (%) t=300 ns Orifice 3 um	Survival (%) t=300ns + 25° tapering	Survival (%) t=100 ns Orifice 3 um
Bovine IGG (0.1%)	160.000	85	100	100	100
Bovine IGG (1%)	160.000	63	100	100	100
Bovine IGG (5%)	160.000	33	98	99	99
Bovine IGG (10%)	160.000	20	98	100	100
Bovine IGG (20%)	160.000	15	97	97	98

Bovine IGM (0.1%)	900.000	23	100	100	100
Bovine IGM (1%)	900.000	18	100	100	100
Bovine IGM (5%)	900.000	8	98	99	99
Bovine IGM (10%)	900.000	5	97	100	100

Using a vibrating membrane nebulizer with a formulation reservoir of 1 ml aerosol droplets in the range of 2-10 micron have been produced during a nebulization time of 8 minutes. Diluted formulations with protein/antibodies having a larger molecular weight (> 100.000 g/mol) clearly suffer from degradation. According to the invention aerosol droplets with an average size of 6 micron have also been made by passing the same formulations with the antibodies through an orifice having a diameter H=3 micron and a length L=3 micron at an average velocity V=10 m/s. Because of plug flow conditions the volume averaged shear rate will be significantly lower than $4V/H= 13.3x10^6$, whereas the passage time t about 300 nanosecond. Due to this short shearing time according to the invention the macromolecules present in the droplets have suffered substantially less from degradation. Using an orifice with a 25° positively tapered profile the antibodies hardly suffer from degradation even for molecular weights well above 1.000.000 g/mol. At an average velocity of V=30 m/s the passage time t through an untapered orifice is about 100 nanosecond and less degradation is found than in the case of an average velocity of 10 m/s.

Macromolecules such as DNA and RNA nucleic acids are frequently used in gene therapy, in particular non-viral gene delivery vectors with messenger RNA (mRNA) and mini DNA vectors. These macromolecules can range between 100-10.000 nucleotides or base pairs with corresponding contour lengths from 30 nanometre to 3 micron, making them susceptible to shear-induced degradation. In Table 2 below some results are summarized on shear degradation of a number of these vector molecules.

Length in Base pairs	Vector Molecule	Molecular Weight (g/mol)	Survival (%) Vibrating Membrane Nebulizer	Survival (%) Orifice 3 um t=300 ns	Survival (%) Orifice 3 um t=100 ns
336	mv281	208.000	85	100	100
1109	mv-KB4TAL-GLuc	698.000	63	100	100
1714	mv-CMV-mCherry	1.080.000	30	99	99
3000	pBLUESCRIPT	1.890.000	10	98	99
5302	pCR2.1-norE	3.340.000	2	95	97

Using a vibrating membrane nebulizer with a formulation reservoir of 1 ml aerosol droplets in the range of 2-10 micron have been produced during a nebulization time of 8 minutes. Formulations with DNA or RNA vectors (5 µg/ml) having a larger molecular weight (> 200.000 g/mol) clearly suffer from degradation. According to the invention aerosol droplets with an average size of 6 micron have also been made by passing the same formulations with the vector molecules through an orifice having a diameter H=3 micron and a length L=3 micron at an average velocity V=10 m/s. Because of plug flow conditions the volume averaged shear rate will be significantly lower than $4V/H = 13.3 \times 10^6$, whereas the passage time t about 300 nanosecond. Due to this short shearing time according to the invention the vector molecules present in the droplets have suffered substantially less from degradation. At an average velocity of V=30 m/s the passage time t through an untapered orifice is about 100 nanosecond and less degradation is found than in the case of an average velocity of 10 m/s.

Fig. 2 illustrates the breakup of a vesicle or a liposome in a shear flow. Fig. 2A shows a spherical vesicle (11) with a size λ without the presence of a shear flow. Fig. 2B shows the initial stretching of the vesicle in the presence of a shear flow indicated by arrows (12,13). Fig. 2C shows further stretching to a maximum length λ_{max} of the vesicle, because the upper part of the vesicle is moving with a higher velocity than the lower part. Fig. 2D shows the vesicle after breaking in two parts (14,15) due to the hydrodynamic shear forces exerted on the upper and lower part of the vesicle, that cause

breaking in the middle of vesicle.

Fig. 3 illustrates the breakup of a vesicle or a liposome in a strong shear flow. Fig. 3A shows the vesicle (11) with a size λ without the presence of a shear flow. Fig. 3B shows the initial stretching of the vesicle in the presence of the shear flow indicated by arrows (12,13). Fig. 3C shows further extreme stretching of the vesicle to a maximum length λ_{max}, because the upper part of the vesicle is moving with a much higher velocity than the lower part. Fig. 3D shows the vesicle after breaking in three parts (14,15,16) due to the large hydrodynamic shear forces exerted on the vesicle, that cause breaking in the vesicle in three parts.

Fig. 4 illustrates the breakup of a vesicle or a liposome (21) in a long pipe (22) with length L and diameter H. Before the entrance of the pipe the vesicle (21) is round due to the absence of a large shear flow. When the vesicle enters the pipe, it will be subject to a shear flow (23), and after the stretching stages of the vesicle it breaks in three elongated parts before it exits the pipe. The three parts travel further in the jet and due to the absence of shear the three parts attain their spherical shape.

Fig. 5 illustrates the travelling of a vesicle or a liposome (21) through an orifice with length L and diameter H. Before the entrance of the orifice the vesicle (21) is round due to the absence of a large shear flow. When the vesicle passes the orifice, it will be subject to a shear flow (23), becomes elongated, but will not break. The vesicle next enters in the jet part and will attain again its spherical shape.

Fig. 6 illustrates the travelling of a vesicle or a liposome (21) through a tapered orifice with length L and diameter H, exhibiting plug flow. Before the entrance of the tapered orifice the vesicle is round and due to the small shear in the plug flow it remains mainly spherical. The vesicle subsequently enters in the jet part and attains its spherical shape.

Vesicles and liposomes are also frequently used in gene therapy, in particular to promote the delivery of encapsulated RNA and DNA vectors to the lungs. These vesicles can typically range in size between 0.02 and 2 micron, making them potentially susceptible to shear-induced breakage during aerosolization. In Table 3 below some results are summarized on shear degradation of a number of these vesicles.

Vesicle size nanometre	Liposome (molar ratio)	Breakage (%) Vibrating Membrane Nebulizer	Breakage (%) t=300 ns Orifice 3 um	Breakage (%) t=100 ns Orifice 3 um
44	HSPC:CH (1:1)	5	0	0
78	HSPC:CH (1:1)	14	0	0
126	HSPC:CH (1:1)	26	<1	<1
254	HSPC:CH (1:1)	43	<2	<1
780	HSPC:CH (1:1)	38	<3	<3
1250	HSPC:CH (1:1)	64	<10	<5

Using a vibrating membrane nebulizer with a formulation reservoir of 1 ml aerosol droplets in the range of 2-10 micron have been produced during a nebulization time of 8 minutes. All liposome formulations (15 µg/ml) with hydrogenated soy phosphatidylcholine (HSPC) and cholesterol (CH) suffer from breakage, larger vesicles more than smaller ones. According to the invention aerosol droplets with an average size of 6 micron have also been made by passing the same formulations with the vesicles through an orifice having a diameter H=3 micron and a length L=3 micron at an average velocity V=10 m/s. Because of plug flow conditions the volume averaged shear rate will be significantly lower than $4V/H= 13.3x10^6$, whereas the passage time t about 100 nanosecond. Due to this short shearing time according to the invention the vesicles present in the droplets hardly suffer from degradation or breakage. At an average velocity of V=30 m/s the passage time t through an untapered orifice is about 100 nanosecond and less degradation is found than in the case of an average velocity of 10 m/s.

The vesicles or liposomes can be loaded with different formulations with nanoparticles, with preference a formulation is provided that comprises vesicles or liposomes with a length or size λ larger than 20 nanometre and which vesicles have a content comprising nano-particles taken from a group, containing proteins, biological molecules, DNA, RNA, bacteriophages and antibodies with a molecular weight above 100.000 Da. With this method the large nano-particles vulnerable to shear degradation are more safely protected.

Conclusies:

1. Werkwijze voor het in een atmosfeer afgeven van nanodeeltjes, omvattende:
- verschaffen van een vloeibare formulering die de genoemde nanodeeltjes omvat;
- onder druk brengen van de vloeibare formulering tot een verhoogde werkdruk p; en
- toevoeren van genoemde vloeibare formulering bij genoemde verhoogde werkdruk door een spray-opening van een sproeikop uit ten minste één spray-opening om genoemde vloeibare formulering af te voeren als een straal van opeenvolgende vloeistofdruppels die elk ten minste één nanodeeltje van genoemde nanodeeltjes bevatten,

waarbij de nanodeeltjes een lengte λ hebben en bij verlenging een maximale lengte λ_{max} voor breuk;

waarbij de genoemde vloeibare formulering wordt onderworpen aan een afschuifsnelheid $\dot{\gamma}$ [per seconde] tijdens het passeren van genoemde spray-opening; en

waarbij genoemde vloeibare formulering binnen genoemde spray-opening van de sproeikop wordt blootgesteld aan genoemde afschuifsnelheid gedurende een afschuiftijd Δt die korter is dan $\lambda_{max}/(\lambda.\dot{\gamma})$ seconde.

2. Werkwijze volgens conclusie 1, waarbij genoemde afschuifsnelheid $\dot{\gamma}$ ruim boven 100.000, in het bijzonder boven 1.000.000 per seconde ligt.

3. Werkwijze volgens conclusie 1 of 2, waarbij λ_{max}/λ typisch een waarde van tussen 3 en 5 heeft.

4. Werkwijze volgens conclusie 1, 2 of 3, waarbij genoemde vloeibare formulering nanodeeltjes omvat die uit een groep zijn genomen, omvattende complexe eiwitten, grote biologische moleculen, lange keten DNA & RNA, virussen, grote blaasjes, liposomen, bacteriofagen en antilichamen.

5. Werkwijze volgens conclusie 1, 2, 3 of 4, waarbij de vloeibare formulering macromoleculen omvat met een molecuulgewicht groter dan 100.000 g/mol.

6. Werkwijze volgens conclusie 5, waarbij genoemde vloeibare formulering DNA- of RNA-moleculen omvat met een molecuulgewicht groter dan 100.000 g/mol.

7. Werkwijze volgens conclusie 5, waarbij genoemde vloeibare formulering eiwit- of antilichaammoleculen omvat met een molecuulgewicht groter dan 100.000 g/mol.

8. Werkwijze volgens conclusie 1, 2, 3 of 4, waarbij genoemde vloeibare formulering bacteriofagen omvat met een gemiddelde grootte groter dan 20 nanometer.

9. Werkwijze volgens conclusie 1, 2, 3 of 4, waarbij de vloeibare formulering vesikels of liposomen omvat waarvan de lengte λ groter is dan 20 nanometer.

10. Werkwijze volgens conclusie 9, waarbij de vloeibare formulering vesikels omvat die een inhoud hebben, omvattende nanodeeltjes genomen uit een groep van eiwitten, biologische moleculen, DNA, RNA, vaccins, virussen, bacteriofagen en antilichamen met een molecuulgewicht boven 100.000 Da.

11. Werkwijze volgens één of meer van de voorgaande conclusies, waarbij de spray-opening is voorzien in een membraan dat wordt ondersteund door een substraat, waarbij het membraan een aantal spray-openingen met in hoofdzaak identieke afmetingen omvat, en waarbij genoemde vloeibare formulering bij genoemde werkdruk naar het aantal spray-openingen wordt toegevoerd.

12. Werkwijze volgens één of meer der voorgaande conclusies, waarbij de genoemde formulering met in hoofdzaak een snelheid V door genoemde spray-opening wordt gevoerd, waarbij genoemde spray-opening een gemiddelde diameter H heeft en een lengte L zodat de genoemde lengte L kleiner is dan $^{1}/_{4}.H.\lambda_{max}/\lambda$.

13. Werkwijze volgens conclusie 12, waarbij de diameter van de spray-opening H tussen 0,5 micron en 5 micron ligt, en de genoemde lengte L kleiner is dan de diameter H.

14. Werkwijze volgens conclusie 12 of 13, waarbij genoemde vloeibare formulering aan de genoemde spray-opening wordt toegevoerd bij een werkdruk p die groter is dan 10^5 Pa, in het bijzonder groter dan 10^6 Pa.

15. Werkwijze volgens een der voorgaande conclusies, waarbij die spray-opening is voorzien van een positieve tapsheid, die zich vernauwt van een ingang naar een uitgang onder hoofdzakelijk een taps toelopende hoek van tussen 5 ° en 45 °.

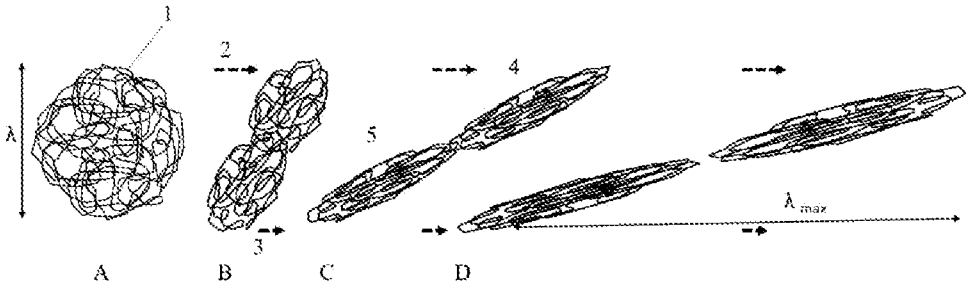


Fig. 1

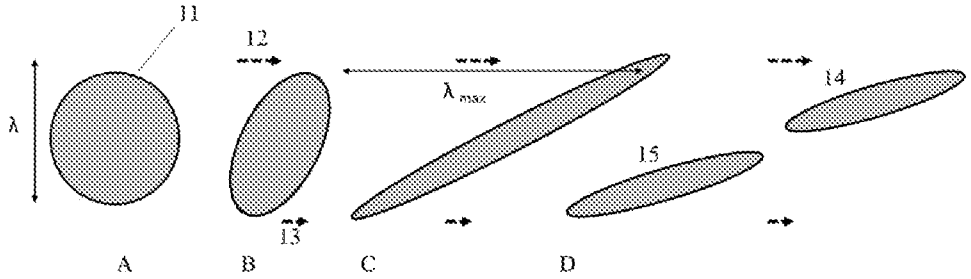


Fig. 2

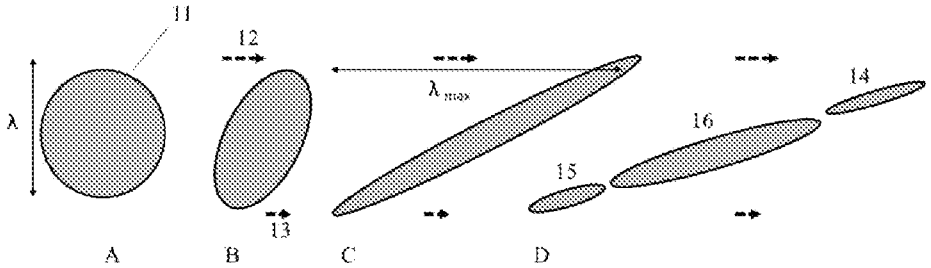


Fig. 3

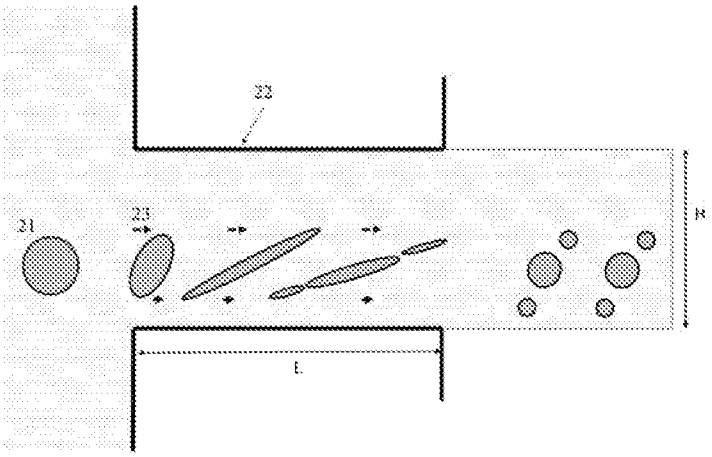


Fig. 4

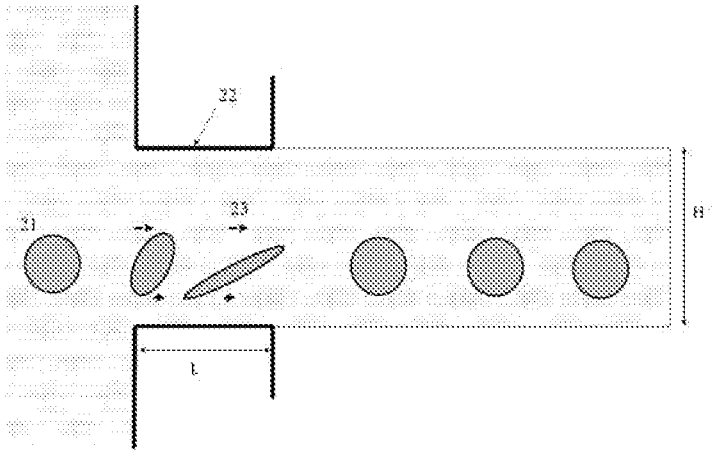


Fig. 5

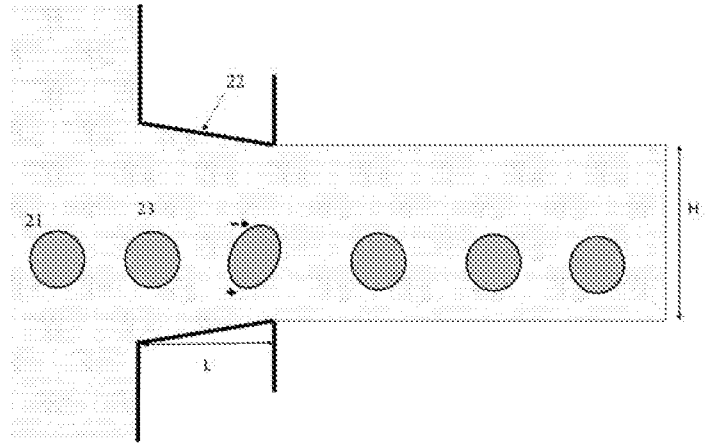


Fig. 6

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
Nederlands aanvraag nr. **2027229**	Indieningsdatum **24-12-2020**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Medspray B.V.**	
Datum van het verzoek voor een onderzoek van internationaal type **05-06-2021**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN78809**
I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)	
Volgens de internationale classificatie (IPC) **Zie onderzoeksrapport**	
II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK	
Onderzochte minimumdocumentatie	
Classificatiesysteem	Classificatiesymbolen
IPC	**Zie onderzoeksrapport**
Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen	

III.	☐	**GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐	**GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2027229

A. CLASSIFICATIE VAN HET ONDERWERP
INV. B01J13/00
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
B01J

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, WPI Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
Y	EP 3 563 894 A1 (BOEHRINGER INGELHEIM INT [DE]) 6 november 2019 (2019-11-06) * conclusies 1, 3 * * alinea [0079] * * figuur 1 *	1-15
Y	WO 02/13786 A2 (UNIV ILLINOIS [US]) 21 februari 2002 (2002-02-21) * bladzijde 5, regel 13 - regel 20 *	1-15
A	WO 2012/024595 A2 (UNIV WASHINGTON [US]; HOEMAN JOHN D [US]; HO RODNEY J Y [US]) 23 februari 2012 (2012-02-23) * het gehele document *	1-15
A	WO 2013/166542 A1 (UNIV MONASH [AU]) 14 november 2013 (2013-11-14) * het gehele document *	1-15

-/--

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[X] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
22 september 2021	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Tarallo, Anthony

1

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2027229

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
A	US 2010/179368 A1 (CONRAD MICHAEL J [US]) 15 juli 2010 (2010-07-15) * het gehele document * -----	1-15
A	WO 2017/095219 A1 (MEDSPRAY BV [NL]) 8 juni 2017 (2017-06-08) * het gehele document * -----	1-15

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2027229

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
EP 3563894	A1 06-11-2019	CN 112469393 A	09-03-2021
		EP 3563894 A1	06-11-2019
		EP 3787597 A1	10-03-2021
		JP 2021520243 A	19-08-2021
		KR 20210004955 A	13-01-2021
		US 2021228822 A1	29-07-2021
		WO 2019211424 A1	07-11-2019
WO 0213786	A2 21-02-2002	AT 449596 T	15-12-2009
		AU 8498201 A	25-02-2002
		CA 2419115 A1	21-02-2002
		EP 1355630 A2	29-10-2003
		JP 2004505761 A	26-02-2004
		KR 20030067660 A	14-08-2003
		KR 20080050540 A	05-06-2008
		US 2002054912 A1	09-05-2002
		US 2004022939 A1	05-02-2004
		US 2008175915 A1	24-07-2008
		WO 0213786 A2	21-02-2002
WO 2012024595	A2 23-02-2012	EP 2605816 A2	26-06-2013
		US 2013142868 A1	06-06-2013
		WO 2012024595 A2	23-02-2012
WO 2013166542	A1 14-11-2013	GEEN	
US 2010179368	A1 15-07-2010	US 2010179368 A1	15-07-2010
		US 2012108878 A1	03-05-2012
WO 2017095219	A1 08-06-2017	BR 112018011192 A2	21-11-2018
		CN 108602074 A	28-09-2018
		EP 3383548 A1	10-10-2018
		JP 6853822 B2	31-03-2021
		JP 2019511949 A	09-05-2019
		RU 2018123757 A	09-01-2020
		US 2018353980 A1	13-12-2018
		WO 2017095219 A1	08-06-2017
		WO 2017095227 A1	08-06-2017

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN78809	24.12.2020		NL2027229

International Patent Classification (IPC)
INV. B01J13/00

Applicant
Medspray B.V.

This opinion contains indications relating to the following items:

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

	Examiner
	Tarallo, Anthony

Application number

WRITTEN OPINION

NL2027229

Box No. I Basis of this opinion

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

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

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

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

4. Additional comments:

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

1. Statement

Novelty	Yes:	Claims	1-15
	No:	Claims	
Inventive step	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION

Box No. VIII Certain observations on the application

see separate sheet

Point V

1 Reference is made to the following documents; the numbering will be adhered to in the rest of the procedure:

D1 EP 3 563 894 A1 (BOEHRINGER INGELHEIM INT [DE]) 6 november 2019 (2019-11-06)

D2 WO 02/13786 A2 (UNIV ILLINOIS [US]) 21 februari 2002 (2002-02-21)

2 Document D1 discloses (the references in parenthesis applying to this document) a method for nebulizing a fluid by pressing said fluid through a nozzle under an operational pressure of 5 to 250 MPa (claim 1). The fluid of D1 comprises liposomes or lipid droplets or lipid particles (claim 3). The nebulized fluid of D1 is an aerosol (paragraph [0079]; fig. 1(14)) and the method of D1 involves the spraying of jets (paragraph [0079]; fig. 1(14)). It is unavoidable that the liposomes or lipid droplets or lipid particles of D1 must have a certain length λ and a certain maximum length λ_{max}.

The "operational pressure of 5 to 250 MPa" of D1 is considered to represent an "increased working pressure p" as defined in present claim 1. The "liposomes or lipid droplets or lipid particles" of D1 are considered to represent "nanoparticles" as defined in present claim 1.

3 The subject-matter of present claim 1 differs from this known from D1 in that the nanoparticles of present claim 1 are exposed to the shear rate γ of present claim 1 for a shear duration Δt of less than $\lambda_{max}/(\lambda^*\gamma)$.

The technical effect exemplified in the present application associated with this difference is that the molecular integrity of deformable nanoparticles such as e.g. proteins, large biological molecules, long DNA and RNA chains, viruses, large vesicles, liposomes, bacteriophages or antibodies is maintained in the aerosol.

The objective technical problem of the present application may therefore be regarded as how to maintain the molecular integrity of the liposomes or lipid droplets or lipid particles of D1.

The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step, because it is well known in the art that deformable compounds such as e.g. DNA may be damaged by shear forces in a nozzle and that said damage depends on the product of the shear rate and the time spent in the shear field (see e.g. page 5, lines 13-20 of D2). Confronted with the problem of how to avoid such damage, the skilled person,

knowing about D1 and D2, would therefore seriously contemplate to reduce said product of shear rate and time spent in the shear field by reducing the shear rate and/or the time spent in the shear field. The choice is therefore obvious and consequently non-inventive. Furthermore, an inventive step cannot be acknowledged over the whole scope of present claim 1 because rigid nanoparticles, such as e.g. metal nanoparticles, are not affected by the shear field in a nozzle. Their structural integrity is always maintained in the aerosol, irrespective of the time spent in the shear field.

4 Present dependent claims 2-15 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of novelty and/or inventive step, see documents D1-D2 and the corresponding passages cited in the search report.

5 Present claims 1-15 are industrially applicable.

Point VIII

1 Present claims 1, 4, 11 are not clear.

1.1 A method of and the means for measurement or calculation of the unusual parameter "$\lambda_{max}/(\lambda*\gamma)$" of present claim 1 and in particular of its components λ_{max}, λ and γ should be included in present claim 1. Failure to determine or measure said unusual parameter and its components in an unambiguous way could potentially result in a lack of disclosure of the application as a whole.

1.2 The relative terms "large", "long", "big" of present claim 4 have no well-recognized meaning and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear.

1.3 The approximative term "substantially identical" of present claim 11 must be deleted.