



Excipient for biotherapeutics

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(57) Abstract: The present invention relates to excipients for stabilizing active agents, in particular peptides, polypeptides, nucleic acids, viruses, virus-like particles, proton pump inhibitors and antibiotics. The excipient reduces aggregate and/or particle formation in preparations comprising said agents. The excipient is a diamide of a dicarboxylic acid comprising at least one N-H amido group, at least one unsubstituted or substituted N-hydroxyethylamido group and/or at least one unsubstituted N-hydroxymethylamido group. In particular, the excipient is N,N,N',N'-tetrakis-(2-hydroxyethyl) adipic acid amide.



WO 2021/009020 A1

Excipient for Biotherapeutics

Description

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The present invention relates to new excipients for stabilizing biomolecules, in particular peptides, polypeptides, nucleic acids, viruses, virus-like particles, and other types of agents, e.g. antibiotics. The excipients reduce aggregate and/or particle formation in preparations comprising said biomolecules and agents.

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Background

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Peptides, polypeptides, nucleic acids, viruses, virus-like particles and other sensitive biomolecules are used frequently, e.g. as active agents in medicine, for detecting biomarkers in diagnostics [1] or as enzymes in multiple technical fields [2]. Examples of biomolecules for use in medicine include antibodies and antibody derivatives, interferons, coagulation factors such as Factor VIII, erythropoietin, interleukins, Vascular Endothelial Growth Factor, adeno-associated viruses and oncolytic herpes viruses. Examples of biomolecules for use in diagnostics include antibodies [3]. Examples of proteins and (poly)peptides for use as industrial enzymes include lipases [4] or cellulases [5].

25

When biomolecules are subjected to certain stress conditions like freezing/thawing, shaking, heat, shear forces and/or light, they tend to form particles or aggregates [6]. The presence of such particles or aggregates is undesired for different reasons. On the one hand, aggregation often

30

deactivates the biomolecule, so that it does no longer fulfill its desired function [7]. On the other hand, the presence of particles or aggregates in medicaments is frequently associated with the occurrence of hazardous immune responses. In the case of pharmaceutical products, there are strict regulatory limits regarding the number of aggregates per dose [8].

Thus, it is desirable to formulate active agents, e.g. biomolecules in a way that they are stable at room temperature. In particular, they need to be resistant to shaking stress, which can occur during production, handling or transport.

The stability of active agents, e.g. biomolecules, can be influenced by different parameters, e.g. the pH, the buffer substance and the ionic strength of the formulation. Further, it is possible to add excipients, which help to stabilize the active agent via different mechanisms.

In principle, different types of excipients are suitable as stabilizers of biomolecules and other types of agents [9]. For example, proteins such as human serum albumin have been used frequently as stabilizers. They have, however, found to be undesirable for different reasons, since they may hamper analytics of the biomolecules to be stabilized. They are also expensive and - unless produced recombinantly - entail biological risks, for they are produced from blood. Surfactants like polysorbates or poloxamers are also often present in formulations of biomolecules. They are chemically heterogeneous, tend to have a volatile impurity profile and can oxidatively denature biomolecules [10,11]. Furthermore, it was shown in long-term studies that polysorbates disintegrate into free fatty acids, which precipitate as particles [12]. As a result, surfactants are very often used as stabilizers, when they are needed to ensure stability, because no adequate alternatives could be found, but there is a very strong desire in the community of formulations scientists to get rid of polysorbates and/or to replace them by

alternative stabilizers. Other commonly used excipients include amino acids, sugars and salts.

Extensive prior art regarding stabilization of biomolecules is available. However, in practice, only a few types of excipients are used. These excipients are almost exclusively selected from amino acids, sugars and sugar alcohols and surfactants. Despite decades of experience in stabilization and hundreds of publications and patents, however, stabilization of biomolecules in liquid preparations is still fraught with difficulties. Certain biomolecules cannot be sufficiently stabilized at all or they only exhibit limited stability even after freeze-drying, in particular, they are only stable for a short time after reconstitution. Numerous preparations additionally exhibit particular impurities, which can only be eliminated by "*bedside filtration*". Other preparations exhibit impurities, e.g. particles, nanoparticles and/or aggregates, which do not exceed the limits set by the authorities and the pharmacopoeias and are therefore being tolerated. In view of the known immunogenicity of such impurities, their quality is not optimal and should be improved.

Thus, there is an urgent need for additional new stabilizers, in particular in the field of stabilization against aggregation and/or particle formation. This is mainly because many of the available excipients have disadvantages when used alone or in combinations. For example, the effects of sugars and amino acids are mostly linked to high concentrations of such excipients. As described above, surfactants, in particular polysorbates, which are most widely used, exhibit self-decomposition and catalyze decomposition of active agents and other excipients due to the presence of impurities. Dosing them, however, is difficult, since it is hard to deplete or enrich them in working steps like dialysis or filtration. They lead to foaming of solutions and intensify dissolution of leachable and extractable components from surfaces.

Summary of the Invention

The present inventors have found that compounds from the class of dicarboxylic acid diamides are capable of stabilizing biomolecules such as antibodies or interferons in liquid preparations. In particular it was shown that when subjected to e.g. freezing/thawing stress, shaking stress or stirring stress, the presence of these substances in formulations of biomolecules leads to a reduced amount of particles compared to formulations without excipients or formulations with known excipients L-arginine or D(+)-trehalose in the same concentration. Stabilizing effects were also observed in preparations that are free from surfactants such as polysorbates.

A first aspect of the invention is the use of a diamide of a dicarboxylic acid (in the following "*diamide*"), wherein said diamide comprises at least one N-H amido group, i.e. a C(=O)-N-H group, at least one unsubstituted or substituted N-hydroxyethyl amido group, and/or at least one unsubstituted or substituted N-hydroxymethyl amido group as a stabilizer of an active agent, particularly of a biomolecule, in a liquid or dried preparation to reduce the formation of aggregates and/or particles.

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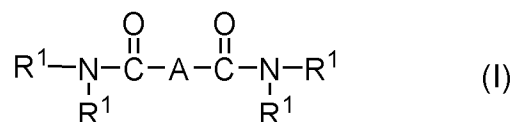
A further aspect of the invention is a preparation, in particular an aqueous liquid preparation comprising an active agent, particularly a biomolecule, and a diamide of a dicarboxylic acid wherein said diamide comprises at least one N-H amido group, i.e. a C(=O)-N-H group, at least one unsubstituted or substituted N-hydroxyethyl amido group, and/or at least one unsubstituted or substituted N-hydroxymethyl amido group.

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In certain embodiments, the diamide is a hydroxyalkyl diamide, i.e. a diamide comprising at least one unsubstituted or substituted N-hydroxyethyl amido group, and/or at least one unsubstituted or substituted N-hydroxymethyl amido group.

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In certain embodiments, the diamide has the structure of Formula (I):

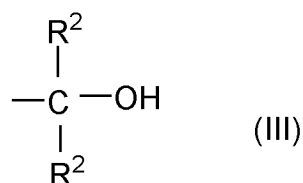
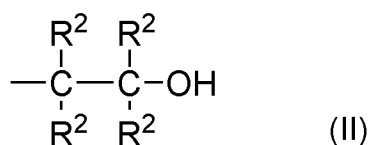


wherein each R¹ is independently selected from H and C₁-C₁₀ hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom,

and wherein two R₁ together may form a ring,

with the proviso that at least one R¹ is H, a group of Formula (II) or a group of Formula (III):

10



wherein each R² is independently selected from H and C₁₋₈ hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom,

15 and

wherein A is selected from linear, branched or cyclic C₁-C₂₄ hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom.

20 Detailed Description of the Invention

The present invention enables the production of stable preparations of active agents, particularly biomolecules, in liquid or dried form by adding diamides as described above. The preparations can be used, for example, in industrial enzyme catalysis, in diagnostics, in cosmetics, in analytics or in medicine

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including human medicine and veterinary medicine. There are many more possible applications.

The terms "*stable*" and "*stabilized*" mean that preparation comprising a
5 diamide as described above are less likely to form aggregates and/or
particles when subjected to stress than formulations without an excipient.
The present invention particularly stabilizes in case of freezing/thawing
stress, shaking stress and/or stirring stress. It must be mentioned that such
well-defined, yet somehow artificial stresses represent -as a surrogate- what
10 a biomolecule encounters as part of the manufacturing process, regular
storage time and handling and are therefore highly relevant for the quality
and stability of such biomolecule and products containing such biomolecules.

"*Aggregates*" and "*particles*" as referred to in the present invention are
15 typically in the size range of about 1 nm to about 1 mm as determined by size
exclusion chromatography (SEC) and FlowCam images.

The term "*active agent*" as used herein particularly relates to biomolecules
such as peptides including modified or cyclic peptides, polypeptides including
20 unglycosylated and glycosylated, monomeric or multimeric polypeptides,
nucleic acids including oligonucleotides, DNA, RNA and nucleic acid
analogues, viruses, virus-like particles and other types of agents, like e.g.
proton-pump inhibitors and antibiotics.

25 The term "*peptide*" refers to a compound comprising at least one chain of up
to 50 natural or non-natural amino acids that are linked via peptide bonds.
The term "*polypeptide*" refers to a compound comprising at least one chain of
51 or more natural or non-natural amino acids that are linked via peptide
bonds. Peptide or polypeptide chains can be associated or linked with each
30 other by covalent bonds and/or non-covalent interactions.

The biomolecule described herein can be, for example, a therapeutically or enzymatically active substance, or a virus vector. Non-limiting examples of biomolecules include antibodies and antibody derivatives, interferons such as interferon-alpha, interferon-beta and interferon-gamma, blood coagulation factors such as Factor VIII, erythropoietin, cytokines such as Granulocyte Colony Stimulating Factor (G-CSF), Tumor Necrosis Factor (TNF), e.g. TNF-alpha, interleukins such as interleukin 2, agonists and antagonists of interleukins and interleukin receptors such as anakinra, agonists and antagonists of members of the TNF family and TNF family receptors, growth factors such as Vascular Endothelial Growth Factor, Insulin Like Growth Factor, Transforming Growth Factor (TGF), or Bone Morphogenetic Protein, as well as recombinant fusion proteins, e.g. immunoglobulin fusion proteins, enzymes such as lipases, cellulases, adeno-associated viruses or oncolytic herpes viruses, virus-like particles of any kind. The biomolecules can further be PEGylated or glycosylated, conjugated with another active agent or they can be modified in a different way.

All biomolecules as described above are well-known and can be produced by standard methods, for example by chemical synthesis or in biological systems, e.g. cellular systems such as *E. coli*, yeast, mammalian cells such as Chinese Hamster Ovary cells or Baby Hamster Kidney cells with subsequent purification.

In a preferred embodiment of the present invention, the biomolecules are selected from antibodies including complete antibodies of different classes, e.g. IgG, IgM, IgA, IgD and IgE, modified antibodies such as single chain antibodies, antibody fragments and conjugates of such antibodies, e.g. conjugates with reporter groups, pharmaceutically active groups such as cytotoxins or radioactive groups. For example, the antibody is an IgG antibody such as trastuzumab, rituximab or omalizumab.

In another preferred embodiment of the present invention, the biomolecules are selected from immunoglobulin fusion proteins, e.g. fusion proteins of cytokines or growth factors with constant immunoglobulin domains and conjugates of such immunoglobulin fusion proteins, e.g. conjugates with
5 reporter groups, pharmaceutically active groups such as cytotoxins or radioactive groups.

In another preferred embodiment of the present invention, the biomolecules are selected from cytokines, interleukins and interferons including interferon-
10 alpha, interferon-beta and interferon-gamma and conjugates thereof. For example, the cytokine is G-CSF and the interferon is interferon-alpha.

In another preferred embodiment of the present invention, the biomolecules are selected from agonists and antagonists of interleukins and interleukin
15 receptors, agonists and antagonists of members of the TNF family and TNF family receptors and conjugates thereof. For example, the biomolecule is an interleukin receptor antagonist such as anakinra.

The term "*active agent*" as used herein includes biomolecules as described
20 above and other types of pharmaceutically active agents such as proton pump inhibitors, e.g. omeprazole or pantoprazole, or antibiotics such as β -lactams, macrolides, aminoglycosides, quinolones/fluoroquinolones, glycopeptides (vancomycin), tetracyclines.

25 The excipients of the present invention from the group of diamides are advantageous in comparison to known excipients. They are, for example, effective at relatively low concentrations. They are chemically clearly defined substances. They reduce aggregation of biomolecules and other types of active agents after different forms of stress. Particularly preferred is the use
30 of the excipients in case of mechanic stress such as shaking stress, stirring stress, pumping stress, atomizing stress, nebulizing stress, dripping stress or dropping stress of a solution which can lead to cavitation.

The term "*diamide*" as used herein relates to a diamide of a dicarboxylic acid wherein both carboxy groups are present as carboxamide groups and wherein said diamide comprises at least one N-H amido group, i.e. a C(=O)-
5 N-H group, at least one unsubstituted or substituted N-hydroxyethyl amido group, and/or at least one unsubstituted or substituted N-hydroxymethyl amido group.

Substituents of N-hydroxyethyl amido groups N-hydroxymethyl amido groups
10 include C₁-C₁₀ hydrocarbon residues, particularly C₁-C₆ hydrocarbon residues and more particularly C₁-C₂ hydrocarbon residues optionally comprising at least one heteroatom, e.g. selected from halo, i.e. F, Cl, Br, or I; N, O, S and/or P, particularly selected from O. The hydrocarbon residues may be selected from substituted or unsubstituted alkyl residues, wherein the
15 term "*alkyl*" particularly includes methyl, ethyl, i-propyl, n-propyl, t-butyl, i-butyl or n-butyl. Hydrocarbon, e.g. alkyl residues may be unsubstituted or substituted by halo, e.g. F, OH, OCH₃, and/or =O.

In certain embodiments, the diamide has a solubility in water of at least about
20 0.02 % (w/v), of at least about 0.05 % (w/v), of at least about 0.1 % (w/v), of at least about 0.5 % (w/v) or of at least about 1 % (w/v) at 20°C, e.g. as determined by the column elution method according to the OECD Guidelines, Test No. 105. Preferably, the diamide is considered as soluble, well soluble or freely soluble according to the classifications of the European
25 Pharmacopoeia, which is herein incorporated by reference.

In certain embodiments, the diamide has a molecular weight in the range of about 120 Da to about 600 Da, e.g. about 150 Da to about 350 Da.

30 In certain embodiments, the diamide has both a solubility in water and a molecular weight in the ranges as indicated above.

In certain embodiments, the diamide is a compound of Formula (I) as described above.

In Formula (I) R^1 is selected from H and C_1 - C_{10} hydrocarbon residues, particularly C_1 - C_6 hydrocarbon residues and more particularly C_1 - C_2 hydrocarbon residues optionally comprising at least one heteroatom, e.g. selected from halo, i.e. F, Cl, Br, or I; N, O, S and/or P, particularly selected from O. The hydrocarbon residues may be selected from substituted or unsubstituted alkyl residues, wherein the term "*alkyl*" particularly includes methyl, ethyl, i-propyl, n-propyl, t-butyl, i-butyl or n-butyl. Hydrocarbon, e.g. alkyl residues may be unsubstituted or substituted by halo, particularly F, OH, OCH_3 , and/or $=O$.

In case, two residues R^1 form a ring, this ring is typically a carbocyclic or heterocyclic 3-6 membered ring.

In specific embodiments, R^1 does not contain any group, which carries a charge, i.e. a positive and/or negative charge, in an aqueous solution in the pH-range of 4-9 such as a carboxylic acid group or an amino group.

20

In certain embodiments of Formula (I), 2, 3 or 4 of R^1 are selected from H, unsubstituted or substituted hydroxyethyl amido groups of Formula (II) and/or unsubstituted or substituted hydroxymethyl amido groups of Formula (III). In certain embodiments, 2, 3 or 4 of R^1 are selected from H. In certain embodiments, 2, 3 or 4 of R^1 are selected from groups of Formula (II). In certain embodiments, 2, 3 or 4 of R^1 are selected from groups of Formula (III).

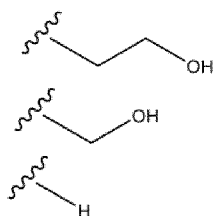
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In certain embodiments of Formula (I), all 4 of R^1 are selected from H, unsubstituted or substituted hydroxyethyl amido groups of Formula (II) and/or unsubstituted or substituted hydroxymethyl amido groups of Formula (III).

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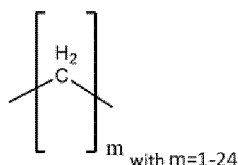
In the groups of Formula (II) and or Formula (III) each R^2 may be independently selected from H and C_1 - C_2 hydrocarbon residues, e.g. ethyl or methyl residues, wherein said hydrocarbon residues optionally comprise at least one heteroatom, e.g. selected from halo, i.e. F, Cl, Br, or I; N, O, S, and/or P, particularly selected from O. In specific embodiments, in the groups of Formula (II) and/or Formula (III) each or at least one R^2 is H, -CH₃, -OH and/or =O.

In certain embodiments R^1 is selected from



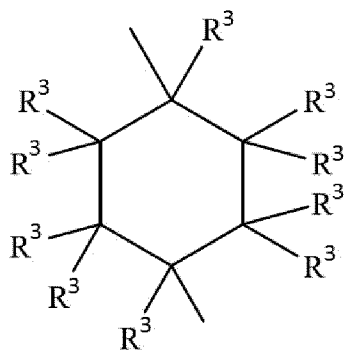
In certain embodiments, A is selected from linear or branched C_1 - C_6 hydrocarbon residues or cyclic C_3 - C_6 hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom, e.g. selected from halo, i.e. F, Cl, Br, or I; N, O, P and/or S, particularly selected from O.

In specific embodiments R is selected from



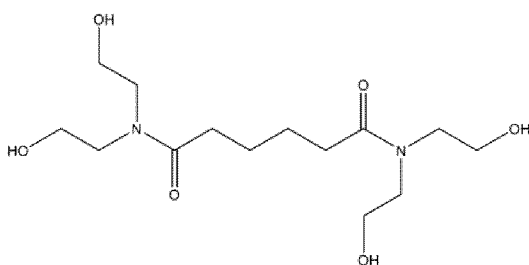
Particularly, $m = 1-6$, e.g. 1, 2, 3, 4, 5 or 6, more particularly $m = 3, 4$ or 5. In case $m = 4$, the compound is an adipinic acid diamide.

In further specific embodiments R is selected from



wherein each R^3 is independently selected from H, OH and C_{1-2} hydrocarbon residues, particularly from H and C_{1-2} hydrocarbon residues wherein said hydrocarbon residues optionally comprise at least one heteroatom which may
 5 be selected from N, O, P and/or S. Particularly, a heteroatom, if present, is O. In certain embodiments, at least 8, e.g. 8, 9 or 10 residues R^3 are H.

In a particular embodiment, the diamide is *N,N,N',N'*-tetrakis(2-hydroxyethyl) adipinic acid amide (*N*¹, *N*¹, *N*⁶, *N*⁶-tetrakis(2-hydroxyethyl)- hexanediamide -
 10 CAS# 6334-25-4), which is well soluble according to the classification of the European Pharmacopoeia:



15 The synthesis of *N,N,N',N'*-tetrakis-(2-hydroxyethyl) adipinic acid amide and related compounds is well-known (see, for example, US 6 235 933 B1 and WO 2011/110624 the contents of which are herein incorporated by reference).

20 Other specific examples of diamides include the following compounds:

*N*¹, *N*¹, *N*⁵, *N*⁵-tetrakis(2-hydroxyethyl)-pentanediamide (CAS# 114690-06-1);

*N*¹,*N*⁶-bis(2-hydroxyethyl)-*N*¹,*N*⁶-dimethyl- hexanediamide (CAS# 57843-54-6);

*N*¹,*N*⁶-bis(2-hydroxyethyl)-*N*¹,*N*⁶-bis(2-hydroxypropyl)- hexanediamide (CAS# 1918193-23-3);

5 *N*¹,*N*¹,*N*⁷,*N*⁷-tetrakis(2-hydroxyethyl)-4,4-dimethyl-heptanediamide (CAS# 331862-59-0); and

*N*¹,*N*¹,*N*⁶,*N*⁶-tetrakis(2-hydroxypropyl)- hexanediamide (CAS# 57843-53-5).

10 In certain embodiments, the diamides can be used as stabilizers of an active agent in a preparation. The preparation can be in any physical form, for example, a liquid preparation, e.g. a solution, emulsion, suspension, or aerosol, or a solid or semisolid preparation. The diamides and preparations containing them may also be used as film coating or other kind of surface coating.

15

The diamide is usually added to the preparation by dissolving in an aqueous medium, but also adding it in the form of a suspension. These examples are not final or restrictive, since there are also other possible ways to combine the diamide and active agent to be stabilized.

20

In a specific embodiment, the preparation is a liquid preparation, particularly an aqueous preparation and more particularly an aqueous solution. In a further specific embodiment, the preparation is a liquid preparation, which has been dried, for example, by freeze-drying, air-drying, spray drying, 25 freeze-spray drying, or foam drying. Such a dried preparation may be reconstituted by a suitable liquid, e.g. an aqueous liquid and eventually used for its intended purpose after reconstitution.

The preparation may have any suitable pH. Typically, the pH is from about 30 pH 4 to about pH 9 or from about pH 6 to about pH 8, e.g. about pH 7.

The preparation may further contain additional excipients such as preservatives, detergents, buffer substances or isotonicity agents.

In certain embodiments, the preparation comprises a diamide as stabilizer
5 and no further stabilizer. In certain embodiments, the preparation comprises a diamide as stabilizer in combination with at least one further stabilizer (i.e. a stabilizer different from a diamide). The at least one further stabilizer may be selected from a sugar such as glucose, sucrose or trehalose, a sugar alcohol such as mannitol or sorbitol, a salt such as NaCl, an amino acid such
10 as histidine, methionine or arginine, and/or an anti-oxidation agent, e.g. a thiol group-containing agent such as methionine.

In a specific embodiment of the present invention, the preparation does not contain a surfactant. Particularly the preparation does not contain a
15 polysorbate, a poloxamer, solutol HS15 and/or an ionic surfactant such as SDS.

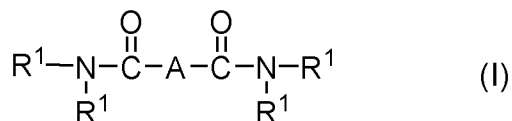
The suitable amount of diamide used in the present invention can easily be determined by the average skilled person. Typically, the concentration of the
20 diamide in a liquid preparation may be in the range of about 1 $\mu\text{mol/l}$ to about 1 mol/l, of about 100 mmol/l to about 500 mmol/l, of about 1 mmol/l to about 250 mmol/l or of about 10 mmol/l to about 100 mmol/l.

The suitable amount of active agent used in the present invention can easily
25 be determined by the average skilled person. Typically, the concentration of the active agent in a liquid preparation may be in the range of about 0.01 mg/ml to about 300 mg/ml, of about 0.1 mg/ml to about 200 mg/ml or of about 1 mg/ml to about 150 mg/ml of active agent. In a particularly preferred embodiment, the concentration of the active agent is between 10 mg/ml and
30 100 mg/ml. In higher concentrations of active agent, stabilization against aggregation is very important and can also be provided by the new excipients.

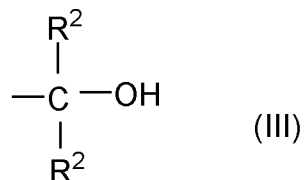
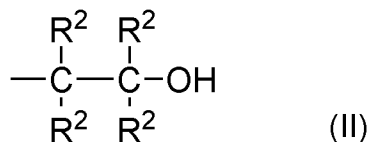
The following exemplary embodiments are part of the specification:

1. Use of a diamide of a dicarboxylic acid wherein said diamide comprises at least one N-H amido group, at least one unsubstituted or substituted N-hydroxyethyl amido group and/or at least one unsubstituted or substituted N-hydroxymethyl amido group as a stabilizer of an active agent, wherein the active agent is selected from peptides, polypeptides, nucleic acids, viruses or virus-like particles, proton pump inhibitors and antibiotics.

2. The use of item 1 wherein the diamide is a compound of Formula (I):



- wherein each R^1 is independently selected from H and $\text{C}_1\text{-C}_{10}$ hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom, and wherein two R^1 together may form a ring, with the proviso that at least one R^1 is H, a group of Formula (II) or a group of Formula (III):



wherein each R^2 is independently selected from H and C_{1-8} hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom,
and

5 wherein A is selected from linear, branched or cyclic C_1-C_{24} hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom.

10 3. The use of item 1 or 2 wherein the active agent is stabilized in a liquid preparation, particularly in an aqueous solution.

4. The use of item 3 wherein the concentration of the active agent in the preparation is in the range from about 0.01 mg/ml to about 300 mg/ml, of about 0.1 mg/ml to about 200 mg/ml or of about 1 mg/ml
15 to about 150 mg/ml.

5. The use of any one of items 3-4,
wherein the concentration of the diamide in the preparation is in the range of about 1 $\mu\text{mol/l}$ to about 1 mol/l, of about 100 mmol/l to
20 about 500 mmol/l, of about 1 mmol/l to about 250 mmol/l or of about 10 mmol/l to about 100 mmol/l.

6. The use of any one of items 1-5,
wherein the active agent is selected from peptides and polypeptides.
25

7. The use of item 6,
wherein the active agent is selected from antibodies such as IgG antibodies, immunoglobulin fusion proteins, interferons such as interferon-alpha, interleukins, interleukin receptors, agonists and
30 antagonists of interleukins and interleukin receptors, agonists and antagonists of members of the TNF family and TNF family receptors, cytokines, and enzymes.

8. The use of item 7,
wherein the active agent is selected from trastuzumab, rituximab,
omalizumab, interferon-alpha, G-CSF and anakinra.
- 5
9. The use of any one of items 1-8 as a stabilizer in the field of
medicine, cosmetics, diagnostics, and/or analytics.
10. The use of any one of items 2-9,
wherein 2, 3 or 4 of R^1 are H, groups of Formula (II) and/or groups of
Formula (III).
- 10
11. The use of any one of items 2-10,
wherein 2, 3 or 4 of R^1 are groups of Formula (II).
- 15
12. The use of any one of items 2-11,
wherein in the groups of Formula (II) and/or Formula (III) each R^2 is
independently selected from H and C_{1-2} hydrocarbon residues, said
hydrocarbon residues optionally comprising at least one heteroatom.
- 20
13. The use of item 12,
wherein in the groups of Formula (II) and/or Formula (III) each R^2 is
H.
- 25
14. The use of any one of items 2-13,
wherein in the compound of Formula (I) A is selected from linear or
branched C_1-C_6 hydrocarbon residues or cyclic C_3-C_6 hydrocarbon
residues, said hydrocarbon residues optionally comprising at least
one heteroatom.
- 30
15. The use of any one of items 1-14,

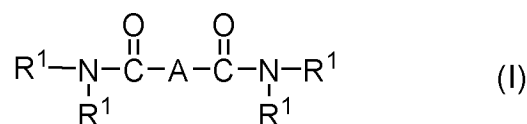
wherein the diamide has a solubility in water of at least about 0.02 % (w/v), of at least about 0.05 % (w/v), of at least about 0.1 % (w/v), of at least about 0.5 % (w/v) or of at least about 1 % (w/v) at 20°C.

5 16. The use of any one of items 1-15,
wherein the hydroxyalkylamide is N,N,N',N'-tetrakis-(2-hydroxyethyl)
adipinic acid amide.

10 17. The use of any one of items 1-16 as a stabilizer against
aggregation and/or particle formation.

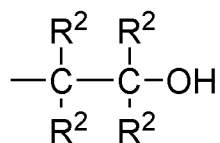
18. The use of any one of items 1-17 as a stabilizer against
aggregation and/or particle formation from freezing/thawing stress,
shaking stress and/or stirring stress.

15 19. A preparation comprising an active agent and a compound of
Formula (I):

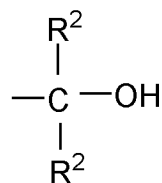


20 wherein each R¹ is independently selected from H and C₁-C₁₀
hydrocarbon residues, said hydrocarbon residues optionally
comprising at least one heteroatom,
and wherein two R¹ together may form a ring,
with the proviso that at least one R¹ is H, a group of Formula (II) or a
group of Formula (III):

25



(II)



(III)

wherein each R^2 is independently selected from H and C_{1-8} hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom,

5 wherein A is selected from linear, branched or cyclic $\text{C}_1\text{-C}_{24}$ hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom, and

wherein the active agent is selected from peptides, polypeptides, nucleic acids, viruses or virus-like particles, proton pump inhibitors
10 and antibiotics.

20. The preparation of item 19, which is a liquid preparation, particularly an aqueous solution.

15 21. The preparation of item 19 or 20, wherein the compound of Formula (I) is defined according to any one of items 10-15.

22. The preparation of any one of items 19-21,
20 wherein the compound of Formula (I) is N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide.

23. The preparation of any one of items 19-22,
wherein the active agent is defined according to any one of items 6-
25 8.

24. The preparation of any one of items 19-23, which has a pH from about pH 4 to about pH 9 or from about pH 6 to about pH 8.

25. The preparation of any one of items 19-23, which comprises a diamide as stabilizer and no further stabilizer.
- 5 26. The preparation of any one of items 19-23, which comprises a diamide as stabilizer in combination with at least one further stabilizer, which may be selected from a sugar such as glucose, sucrose or trehalose, a sugar alcohol such as mannitol or sorbitol, a salt such as NaCl, an amino acid such as histidine, methionine or arginine, and/or an anti-oxidation agent, e.g. a thiol group-
- 10 containing agent such as methionine.
27. The preparation of any one of items 19-26, which does not contain a surfactant, particularly a surfactant selected from polysorbates, poloxamers, solutol HS15 or SDS.
- 15 28. The preparation of any one of items 19-27, which has been dried and optionally has been reconstituted.
29. The preparation of claim 28, which has been dried by:
- 20 (a) freeze-drying,
(b) air-drying,
(c) spray drying,
(d) freeze-spray drying, or
- 25 (e) foam drying.
30. The preparation of any one of items 19-29 for use in the field of medicine, cosmetics, diagnostics, and/or analytics.
- 30 31. The preparation of item 30 for use in medicine.

The present invention shall be explained in more detail by the following Examples and Figures.

Figure Legends

5

Figure 1: Amount of particles after 3 freezing/thawing cycles. 50 mM phosphate buffer, pH 7.0; 5 mg/ml antibody IgG trastuzumab.

10 **Figure 2:** Amount of particles after stirring stress. 50 mM phosphate buffer, pH 7.0; 5 mg/ml antibody IgG trastuzumab.

Figure 3: Amount of particles after 3 freezing/thawing cycles. 50 mM phosphate buffer, pH 7.0; 1 mg/ml interferon-alpha-2a.

15 Examples

Example 1: Materials and Methods

20 Production of Test Solutions: The buffer solution consisted of 50 mM sodium phosphate at pH 7.0. Protein stock solutions were liberated from other excipients by IEX chromatography to remove potential impurities and surfactants and by dialysis over 24 h in buffer solution 100-200 times of their volumes. The buffer solution was renewed after 3 hours and after 14 hours. Stock solutions of test excipients were produced by dissolving 500 mM of the
25 excipient in 90% of the required amount of buffer solution. After that, the pH was adjusted and the remaining volume of buffer solution was added. Then, the solution was filtered with a 0.22 µm filter. The corresponding amount of protein stock solution was added. Sufficient homogenization was provided.

30 Freeze/Thaw-Cycle: The protein-containing solutions were filled into cleaned 2R vials and crimped. The samples were frozen from 20°C to -50°C in 3 cycles at a rate of 2K/min in a Christ 2D-6 freeze dryer and then thawed at

room temperature until the entire sample had reached the liquid state, before the cycle was started again.

5 Stirring Stress: The protein-containing solutions were filled into cleaned 2R vials and crimped. Afterwards, the samples were stirred for 2 h at 200 rpm with a magnetic stirrer (Variomag Poly 15, Thermofisher, 3 mm polytetrafluoroethylene-coated stirring bars).

10 Flow Imaging Microscopy: 165 µl of the sample solution were measured at 10x magnification using a flow imaging microscope (FlowCam, Fluid Imaging Technologies, Inc., Scarborough, ME, USA).

15 Size Exclusion Chromatography (Antibodies): The samples were analyzed by a Dionex Summit Chromatography system. As solid phase, a Superdex 200 Increase 10/300 GL Column (GE Healthcare) was used. The mobile phase was a 50 mM phosphate solution with 200 mM NaCl at pH 7.0. The monomer elution was detected by means of absorption of the UV signal at a wavelength of 280 nm. Recovery was defined as the proportion of the areas under the absorption curve of the monomer peak before and after stress.

20

Size Exclusion Chromatography (Interferon-alpha-2a): The samples were analyzed by a Dionex Summit Chromatography system. As solid phase, a Superose 12 10/300 GL Column (GE Healthcare) was used. The mobile phase was a 50 mM phosphate solution with 200 mM NaCl at pH 7.0. The protein concentration was detected by means of UV at a wavelength of 280 nm.

25

Example 2: Stabilization of an IgG antibody (trastuzumab) with N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide

30

N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (Ark Pharm Inc.), L-arginine (J. T. Baker) or D(+) trehalose (Sigma-Aldrich), respectively, was

added as a test excipient to an aqueous solution containing the recombinant IgG antibody trastuzumab so that the resulting solution had an IgG concentration of 5 mg/ml and contained 50 mM of excipient. The resulting solutions were subjected to freezing/thawing stress and stirring stress.

5

It was shown that stressed formulations comprising N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (compound A) contain less particles than formulations comprising standard excipients like, glycerol, NaCl, or D(+) trehalose or formulations without any excipients, and similar to L-arginine.

10 Polysorbate 20 leads to even less particles after freeze/thaw experiments, but this class of excipients is not preferred (Figures 1 and 2).

Example 3: Stabilization of interferon-alpha-2a by N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide

15

N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide was added to an aqueous solution containing interferon-alpha-2a so that the resulting solution had a protein concentration of 1 mg/ml and contained 50 mM of excipient. The resulting solutions were subjected to freeze/thawing stress. It was shown
20 that stressed formulations comprising N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (compound A) contain less particles than formulations without any excipient and formulations with arginine or polysorbate 20 and similar amounts as trehalose (Figure 3).

25 **Example 4: Stabilization of the IgG antibodies omalizumab and rituximab with N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide**

Production of Test Solutions: The buffer solution consisted of 20 mM histidine buffer pH 6.0. Protein stock solutions were liberated from other excipients by
30 dialysis over 24 h in buffer solution 100-200 times of their volumes. The buffer solution was renewed after 6 hours and after 20 hours. Stock solutions of test excipients were produced by dissolving 500 mM of the excipient in

90% of the required amount of buffer solution. After that, the pH was adjusted and the remaining volume of buffer solution was added. Then, the solution was filtered with a 0.22 µm filter. The corresponding amount of protein stock solution was added. Sufficient homogenization was provided.

5

Freeze/Thaw-Cycle: The protein-containing solutions were filled into cleaned 6R vials and crimped. The samples were frozen from 20°C to -70°C in 5 cycles in a freezer and then thawed at room temperature until the entire sample had reached the liquid state, before the cycle was started again.

10

Stirring Stress: The protein-containing solutions were filled into cleaned 6R vials and crimped. Afterwards, the samples were stirred for 2 h at 200 rpm with a magnetic stirrer (Variomag Poly 15, Thermofisher, 5 mm polytetrafluoroethylene-coated stirring bars).

15

Visual inspection: 3 Vials of each formulation were visually inspected according to the method described in the European Pharmacopoeia. Particular emphasis was given to the detection of turbidity.

20 N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (Ark Pharm Inc.), D(+) trehalose (Sigma-Aldrich), sucrose and mixtures of trehalose or sucrose with methionine (Sigma-Aldrich) respectively, were added as a test excipient to an aqueous solution containing a recombinant IgG antibody, either rituximab or omalizumab. The resulting solutions had an IgG concentration
25 of 10 mg/ml and contained 75 mM of excipient. In the case of sucrose or trehalose mixtures with methionine, the concentration of the sugar was 50 mM, and the concentration of methionine was 20 mM. The resulting solutions were subjected to freezing/thawing stress and stirring stress.

30 It was found that stressed formulations comprising N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (compound A) contained less particles and were less turbid than formulations comprising standard excipients like, D(+)

trehalose or sucrose, or formulations with mixtures of either trehalose with methionine or sucrose with methionine.

Example 5: Stabilization of the IgG antibodies rituximab and omalizumab with mixtures of N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide and further excipients

The production of test solutions, the freeze/thaw-cycle, the measurement of stirring stress and the visual inspection were performed as described in Example 4.

Mixtures of N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (Ark Pharm Inc.) at a concentration of 30 mM with either D(+) trehalose (Sigma-Aldrich) at a concentration of 30 mM, or sucrose at a concentration of 30 mM respectively, were prepared as aqueous test excipient solutions and a recombinant IgG antibody either rituximab or omalizumab in 20 mM histidine buffer pH 6.0 was dialyzed into these solutions. As a comparison, solutions containing either trehalose or sucrose, both either in a concentration of 30 mM or 60 mM were prepared and the antibody in a 20 mM histidine buffer pH 6.0 was dialyzed into these solutions. The resulting solutions had an IgG1 concentration of 10 mg/ml and contained 60 mM of excipients and were subjected to freezing/thawing stress and stirring stress.

It was found that stressed formulations comprising N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (compound A) in mixture with trehalose or sucrose contained less particles and were less turbid than formulations comprising standard excipients like, D(+) trehalose or sucrose alone.

Example 6: Stabilization of G-CSF by N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide

The production of test solutions, the freeze/thaw-cycle, the measurement of stirring stress and the visual inspection were performed substantially as described in Example 4.

The buffer solution consisted of 10 mM acetate buffer pH 4.5 and was renewed after 4 hours and after 16 hours. For the freeze/thaw cycle and the measurements of stirring stress, 2R vials were used.

N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (Ark Pharm Inc.), D(+) trehalose (Sigma-Aldrich), sucrose, and mixtures of trehalose or sucrose with methionine (Sigma-Aldrich) respectively, was added as a test excipient to an aqueous solution containing recombinant G-CSF. The resulting solutions had a G-CSF concentration of 1 mg/ml and contained 50 mM of excipients. In the case of sucrose or trehalose mixtures with methionine, the concentration of the sugar was 50 mM, and the concentration of methionine was 20 mM. The resulting solutions were subjected to freezing/thawing stress and stirring stress.

It was found that stressed formulations comprising N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (compound A) contained less particles and were less turbid than formulations comprising standard excipients like, D(+) trehalose, or sucrose, or formulations with mixtures of either trehalose with methionine or sucrose with methionine.

Example 7: Stabilization of anakinra by N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide

The production of test solutions, the freeze/thaw-cycle, the measurement of stirring stress and the visual inspection were performed substantially as described in Example 4.

The buffer solution consisted of 20 mM citrate buffer pH 6.5. The samples were stirred for 3 h.

10

N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (Ark Pharm Inc.), D(+) or trehalose (Sigma-Aldrich), or polysorbate 80, was added as a test excipient to an aqueous solution containing recombinant anakinra. The resulting solution had an anakinra concentration of 50 mg/ml and contained 15 50 mM of excipient. In the case of polysorbate 80 its concentration was 0,05%. The resulting solutions were subjected to freezing/thawing stress and stirring stress.

20

It was found that stressed formulations comprising N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide (compound A) contained less particles and were less turbid than formulations comprising trehalose, and contained about equally low amounts of particles and were about equally low in turbidity than formulations containing polysorbate 80.

25

30

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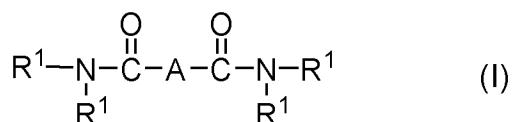
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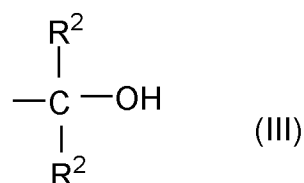
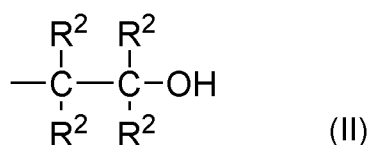
Claims

1. Use of a diamide of a dicarboxylic acid wherein said diamide comprises at least one N-H amido group, at least one unsubstituted or substituted N-hydroxyethyl amido group and/or at least one unsubstituted or substituted N-hydroxymethyl amido group as a stabilizer of an active agent, wherein the active agent is selected from peptides, polypeptides, nucleic acids, viruses or virus-like particles, proton pump inhibitors and antibiotics.

2. The use of claim 1 wherein the diamide is a compound of Formula (I):



wherein each R^1 is independently selected from H and $\text{C}_1\text{-C}_{10}$ hydrocarbon residues, said hydrocarbon residues optionally comprising at least one heteroatom, and wherein two R_1 together may form a ring, with the proviso that at least one R^1 , e.g. 1, 2, 3 or 4 of R^1 is H, a group of Formula (II) or a group of Formula (III):



wherein each R^2 is independently selected from H and $\text{C}_1\text{-C}_8$ hydrocarbon residues, particularly from $\text{C}_1\text{-C}_2$ hydrocarbon residues,

said hydrocarbon residues optionally comprising at least one heteroatom,

and

wherein A is selected from linear, branched or cyclic C₁-C₂₄ hydrocarbon residues, particularly from linear or branched C₁-C₆ hydrocarbon residues or cyclic C₃-C₆ residues, said hydrocarbon residues optionally comprising at least one heteroatom.

3. The use of claim 1 or 2 wherein the active agent is stabilized in a liquid preparation, particularly in an aqueous solution, particularly wherein (i) the concentration of the active agent in the preparation is in the range from about 0.01 mg/ml to about 300 mg/ml, of about 0.1 mg/ml to about 200 mg/ml or of about 1 mg/ml to about 150 mg/ml, and/or wherein (ii) the concentration of the diamide in the preparation is in the range of about 1 µmol/l to about 1 mol/l, of about 100 mmol/l to about 500 mmol/l, of about 1 mmol/l to about 250 mmol/l or of about 10 mmol/l to about 100 mmol/l.

4. The use of any one of claims 1-3, wherein the active agent is selected from antibodies such as IgG antibodies, antibody derivatives, immunoglobulin fusion proteins, interferons, interleukins, interleukin receptors, agonists and antagonists of interleukins and interleukin receptors, agonists and antagonists of members of the TNF family and TNF family receptors, cytokines, and enzymes.

5. The use of claim 4, wherein the active agent is selected from trastuzumab, rituximab, omalizumab, interferon-alpha, G-CSF and anakinra.

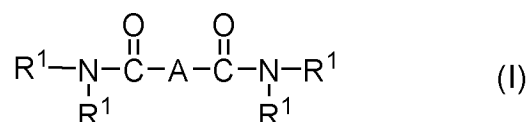
6. The use of any one of claims 1-5 as a stabilizer in the field of medicine, cosmetics, diagnostics, and/or analytics.

7. The use of any one of claims 1-6,
 wherein the diamide has a solubility in water of at least about 0.02 %
 (w/v), of at least about 0.05 % (w/v), of at least about 0.1 % (w/v), of
 at least about 0.5 % (w/v) or of at least about 1 % (w/v) at 20°C.

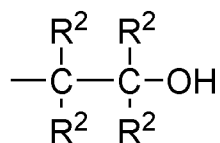
8. The use of any one of claims 1-7,
 wherein the diamide is N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic
 acid amide.

9. The use of any one of claims 1-8 as a stabilizer against
 aggregation and/or particle formation, particularly as a stabilizer
 against aggregation and/or particle formation from freezing/thawing
 stress, shaking stress and/or stirring stress.

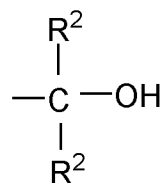
10. A preparation comprising aa active agent and a compound of
 Formula (I):



wherein each R¹ is independently selected from H and C₁-C₁₀
 hydrocarbon residues, said hydrocarbon residues optionally
 comprising at least one heteroatom,
 and wherein two R₁ together may form a ring,
 with the proviso that at least one R¹ e.g. 1, 2, 3 or 4 of R¹ is H, a
 group of Formula (II) or a group of Formula (III):



(II)



(III)

wherein each R^2 is independently selected from H and C_{1-8} hydrocarbon residues, e.g. 1, 2, 3 or 4 of R^1 is H, said hydrocarbon residues optionally comprising at least one heteroatom,

5 wherein A is selected from linear, branched or cyclic $\text{C}_1\text{-C}_{24}$ hydrocarbon residues, particularly from linear or branched $\text{C}_1\text{-C}_6$ hydrocarbon residues or cyclic $\text{C}_3\text{-C}_6$ residues, said hydrocarbon residues optionally comprising at least one heteroatom, and
 10 wherein the active agent is selected from peptides, polypeptides, nucleic acids, viruses or virus-like particles, proton pump inhibitors and antibiotics.

11. The preparation of claim 10, which is a liquid preparation, particularly an aqueous solution.

15

12. The preparation of claim 10 or 11,
 wherein the compound of Formula (I) is N,N,N',N'-tetrakis-(2-hydroxyethyl) adipinic acid amide.

20

13. The preparation of any one of claims 10-12,
 wherein the active agent is defined according to any one of claims 4 or 5.

25

14. The preparation of any one of claims 10-13,
 which does not contain a surfactant, particularly a surfactant selected from polysorbates, poloxamers, solutol HS15 or SDS.

15. The preparation of any one of claims 10-14 for use in medicine.

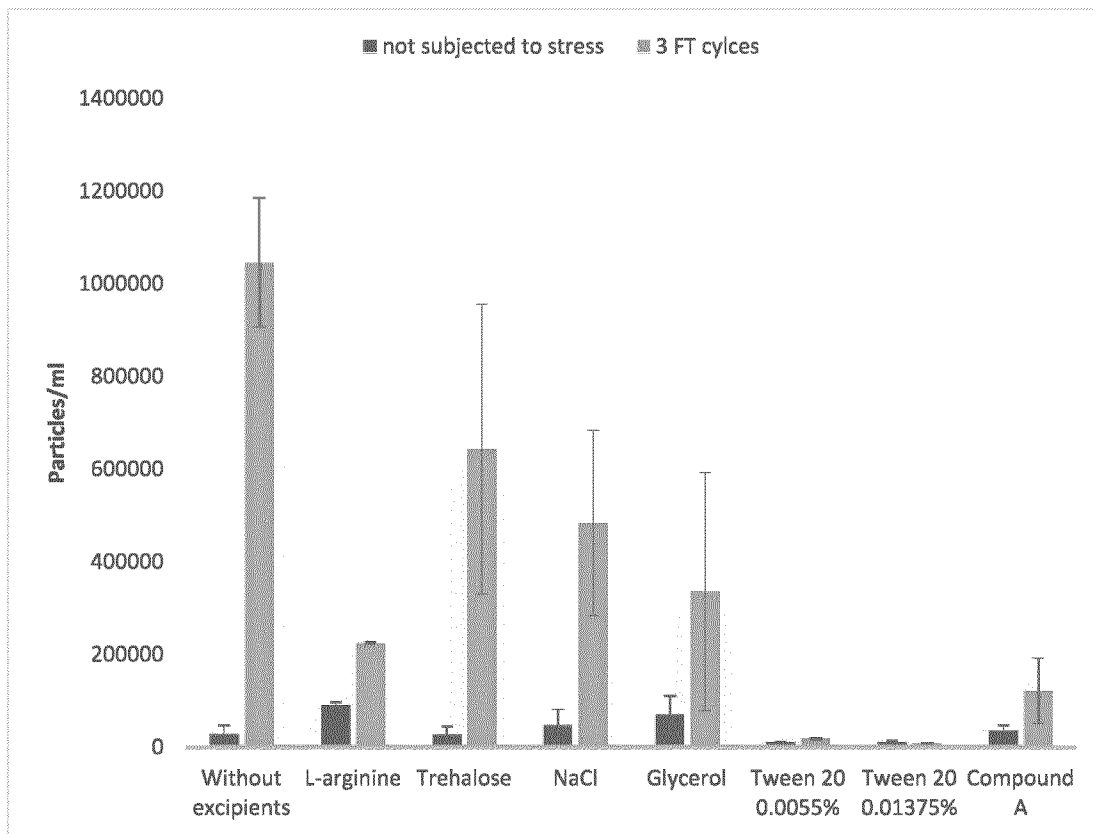
Figure 1

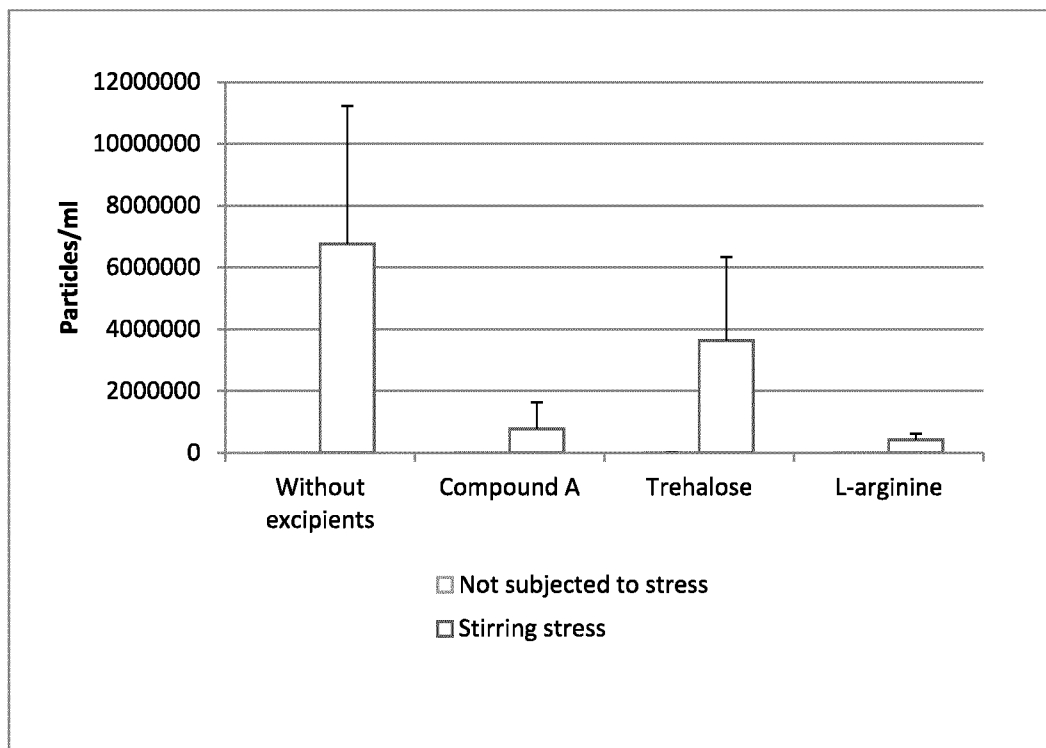
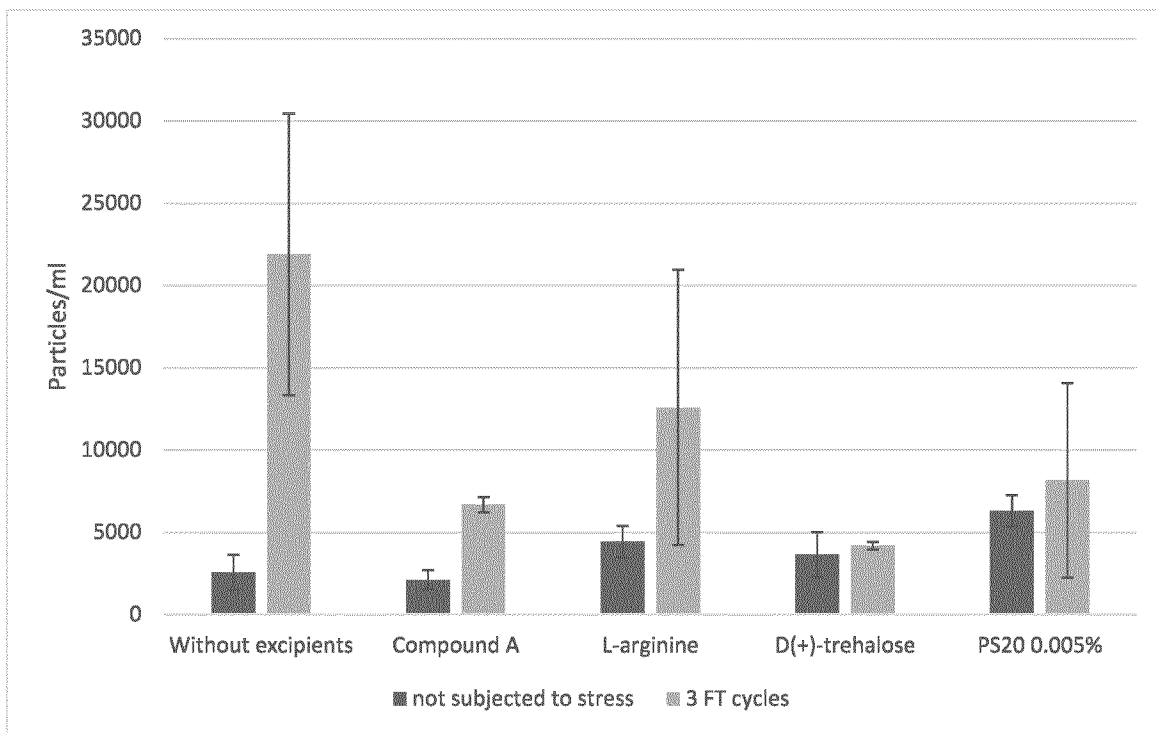
Figure 2

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/069494

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K9/08 A61K38/21 A61K39/395 A61K47/18 A61K9/00 C07K16/32 ADD. According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K C07K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, CHEM ABS Data, WPI Data														
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>US 2004/266921 A1 (RODRIGUES KLEIN A [US] ET AL) 30 December 2004 (2004-12-30) paragraph [0001] paragraph [0022] - paragraph [0027] paragraph [0029] paragraph [0041] -----</td> <td>1-15</td> </tr> <tr> <td>A</td> <td>EP 1 280 010 A2 (HEIDELBERGER DRUCKMASCH AG [DE]) 29 January 2003 (2003-01-29) paragraph [0011] examples 1-9 -----</td> <td>1-15</td> </tr> <tr> <td>A</td> <td>US 2006/251896 A1 (FERENCZ JOSEPH M [US] ET AL) 9 November 2006 (2006-11-09) example 10 ----- -/-</td> <td>1-15</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	A	US 2004/266921 A1 (RODRIGUES KLEIN A [US] ET AL) 30 December 2004 (2004-12-30) paragraph [0001] paragraph [0022] - paragraph [0027] paragraph [0029] paragraph [0041] -----	1-15	A	EP 1 280 010 A2 (HEIDELBERGER DRUCKMASCH AG [DE]) 29 January 2003 (2003-01-29) paragraph [0011] examples 1-9 -----	1-15	A	US 2006/251896 A1 (FERENCZ JOSEPH M [US] ET AL) 9 November 2006 (2006-11-09) example 10 ----- -/-	1-15
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.														
* Special categories of cited documents : <table border="0"> <tr> <td style="vertical-align: top;"> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td style="vertical-align: top;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family </td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family										
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Date of the actual completion of the international search 4 September 2020		Date of mailing of the international search report 16/09/2020												
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Ovens, Annabel												

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/069494

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Information on patent family members

International application No

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