Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2996580 C 2020/06/30

(11)(21) 2 996 580

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 2011/02/11

(41) Mise à la disp. pub./Open to Public Insp.: 2011/08/18

(45) Date de délivrance/Issue Date: 2020/06/30

(62) Demande originale/Original Application: 2 827 049

(30) Priorité/Priority: 2010/02/10 (DE102010007562.0)

(51) Cl.Int./Int.Cl. *A61K 47/02* (2006.01),
A61K 31/711 (2006.01), *A61K 9/113* (2006.01)

(72) Inventeurs/Inventors:
SCHMIDTS, THOMAS, DE;
GARN, HOLGER, DE;
RUNKEL, FRANK, DE

(73) Propriétaire/Owner:
STERNA BIOLOGICALS GMBH & CO. KG, DE

(74) Agent: MBM INTELLECTUAL PROPERTY LAW LLP

(54) Titre : COMPOSITION PHARMACEUTIQUE DERMATOLOGIQUE ADAPTEE AUX OLIGONUCLEOTIDES
(54) Title: DERMATOLOGICAL PHARMACEUTICAL COMPOSITION SUITABLE FOR OLIGONUCLEOTIDES

(57) Abrégé/Abstract:
The invention relates to a cosmetic and/or dermatological and/or pharmaceutical composition for the topical use and application of oligonucleotides, in particular antisense-oligonucleotides such as DNAzyme, siRNAs, as DNAs or ribozymes for use as an agent against inflammatory diseases by means of emulsions having a dispersed, internal, discontinuous aqueous phase.

ABSTRACT

The invention relates to a cosmetic and/or dermatological and/or pharmaceutical composition for the topical use and application of oligonucleotides, in particular antisense-oligonucleotides such as DNAzyme, siRNAs, as DNAs or ribozymes for use as an agent against inflammatory diseases by means of emulsions having a dispersed, internal, discontinuous aqueous phase.

Dermatological Pharmaceutical Composition Suitable for Oligonucleotides

The present invention relates to a cosmetic and/or dermatological and/or pharmaceutical preparation for topical use and administration of oligonucleotides as agents against diseases. The invention relates in particular to emulsions having a dispersed, discontinuous, internal aqueous phase with which oligonucleotides are formulated and administered as agents for topical application against inflammatory diseases.

State of the Art

Oligonucleotides are substances comprised of a few nucleotides (DNA or RNA building blocks) whose nucleotide sequence consists in general of approx. 10-100 nucleotide units. Oligonucleotides are known as primers, for example, which are used in the polymerase chain reaction (PCR). Antisense oligonucleotides are oligonucleotides which have a base sequence that is complimentary to a cellular, viral or synthetic RNA or DNA and which can bind them by Watson-Crick base pairing. Such molecules are often directed against functional mRNAs. Due to the specific binding to the mRNA, ultimately the formation of the respective protein which is encoded by this mRNA is prevented by blockade of translation.

Antisense molecules may belong to various molecular classes to which antisense DNA (asDNA), "small inhibitory" RNA (siRNA), ribozymes and DNAzymes also belong. The last two groups of molecules are characterized by an inherent catalytic activity, which may lead to direct cleavage of the bound target RNA among other things.

As an example, but not exclusively, the DNAzymes of DE 103 46 487.5 are disclosed as examples of DNAzymes for producing an agent against inflammatory diseases.

It is known in general that oligonucleotides are very sensitive to naturally occurring or recombinant nucleases. Nucleases are a group of ubiquitous enzymes which usually act as hydrolases at ester linkages and catalyze the degradation of oligonucleotides. Those skilled in the art are familiar with nucleases as DNAses or RNAses.

The sensitivity of oligonucleotides to nucleases is problematical in medical use of oligonucleotides in particular. In comparison with traditional drugs, oligonucleotides can be degraded rapidly by DNAses and RNAses, which leads to a short half-life and thus to a lower bioavailability in the target cells.

In order for oligonucleotides to also be usable medicinally (in particular therapeutically), they must be protected effectively from the nucleases. To do so, great efforts are being made throughout the world with various technical approaches to modify the structure of oligonucleotides by chemical modifications, for example, in order to increase their stability or to develop drug carrier systems, i.e., so-called drug delivery systems. These drug delivery systems include, for example, liposomes, nanoparticles, viral shell capsids and protamine oligonucleotide particles.

Many of these drug delivery systems have disadvantages; for example, they do not provide adequate protection from degradation by nucleases, they have poor uptake into the target cells and tissue or they retain toxic or problematical constituents, which have a negative effect on the efficacy of the oligonucleotides. For topical application in particular, there is not currently a suitable preparation with which oligonucleotides such as DNAzymes can be formulated and administered as agents against inflammatory diseases because the formulations are not adequately protected against bacteria, fungi and nucleases. Successful use of oligonucleotides in dermatology has so far been prevented by this lack of options for protection.

Object

The object of the present invention is to eliminate the disadvantages of the prior art and to provide a preparation for topical application of oligonucleotides.

Solution

This problem is solved by an emulsion having a dispersed, discontinuous, internal aqueous phase.

A stable effective formulation in the form of an emulsion with a dispersed, discontinuous, internal aqueous phase holding oligonucleotides in a stable emulsion, effectively protecting the oligonucleotides from enzymatic degradation by nuclease while permitting good uptake into the target cells and tissue has surprisingly been found.

1. Characterization of the formulation

The formulation according to the invention in the form of an emulsion with a dispersed, discontinuous, internal aqueous phase is formed by using the galenical form of the water-in-oil-

in-water emulsion, the so-called WOW emulsion, and/or the water-in-oil emulsion, the so-called WO emulsion, with the addition of magnesium or sodium ions. It has surprisingly been found that adding magnesium or sodium ions to the formulation is very advantageous for the effect of the oligonucleotides, improving the stability of the oligonucleotides by protecting them from enzymatic degradation and thus permitting good absorption into the target cells, e.g., skin cells, lung and intestinal epithelial cells, mucosa, nasal epithelial cells and cells of the pharyngeal space.

Substances of the formulation according to the invention

The formulation according to the invention comprises at least one lipophilic emulsifier, at least one consistency agent, at least one occlusive component, at least one organic and/or inorganic additive, water and at least one oligonucleotide.

Alternatively, the formulation according to the invention additionally comprises at least one hydrophilic emulsifier and/or at least one amphiphilic component.

Alternatively, the formulation according to the invention additionally comprises at least one humectant factor.

Alternatively, the formulation according to the invention comprises at least one preservative.

Alternatively, the formulation according to the invention comprises at least one natural or synthetic oil or wax.

The at least one lipophilic emulsifier is selected from the group comprised of sorbitan fatty acid esters, glycerol derivatives (e.g., Span™, also glycerol stearate, glycerol dioleate, glycerol monooleate).

The at least one consistency agent is selected from the group comprised of fatty acid esters (e.g., cetyl palmitate, myristyl myristate), polyethylene glycols, cera alba, microcrystalline wax, lanolin and alcohols thereof, hydrogenated castor oil, carbomers (e.g., crosslinked acrylic acid polymers) or cellulose and its derivative.

The at least one occlusive component is selected from the group comprised of saturated hydrocarbons, e.g., paraffin or polysiloxanes such as silicone oils.

The at least one organic and/or inorganic additive is a salt or an ionic liquid whose cation component comprises Na, Mg, K, Li, Ca, Fe, Cu, Ag or a combination of these elements (e.g., a mixture of NaCl, $MgSO_4$) or a combination of these elements with organic cations (e.g., a mixture of $Mg(N(SO_2CF_3)_2)_2$, $Mg(OSO_2CF_3)_2$ in an ionic liquid, e.g., 1-ethyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium sulfate, 1-ethyl-3-methylimdazolium trifluoromethane sulfonate, 1-ethyl-3-methylimidazolium dicyanamide or 1-ethyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)amide).

The at least one hydrophilic emulsifier is selected from the group comprised of polysorbates or ethoxylated polyethylene glycols (e.g., Tween™, steareths, laureths, ceteareths) or ethoxylated ether and/or esters.

The at least one amphiphilic component is selected from the group of phospholipids, preferably lecithin or one of its derivatives.

The at least one humectant factor is selected from the group of glycerol, polyols, osmolytes.

The at least one oil is selected from the group of esterified fatty acids (e.g., decyl oleate), waxes (e.g., jojoba wax) or partially esterified glycerides (e.g., medium-chain length triglycerides) or natural oils such as soy oil, peanut oil, avocado oil, olive oil, castor oil and nut oils or seed oils.

The at least one preservative is paraben, for example.

The at least one oligonucleotide is selected from the group comprised of primers, aptamers, antisense oligonucleotides, e.g., DNAzymes, siRNAs, asDNAs or ribozyme.

As an example but not exclusively, the DNAzymes of DE 103 46 487.5 are cited as the DNAzymes; they are directed against the mRNA of the proteins GATA-3 and T-bet and are disclosed for producing an agent against inflammatory diseases. For example, the following DNAzymes are used (each individually or in combination with the others):

Name of the DNAzyme against GATA-3 mRNA sequence:

hgd1	5'-TCGGTCAGAggctagctacaacgaTGCGTTGCT-3'
hgd2	5'-GGCGTACGAggctagctacaacgaCTGCTCGGT-3'
hgd3	5'-GGCGGCGTAggctagctacaacgaGACCTGCTC-3'

hgd4	5'-CTCGGGTCAggctagctacaacgaCTGGGTAGC-3'
hgd5	5'-TCCTCTGCAggctagctacaacgaCGGGGTCCT-3'
hgd6	5'-ACTCTGCAAggctagctacaacgaTCTGCGAGC-3'
hgd7	5'-GGGCGACGAggctagctacaacgaTCTGCAATT-3'
hgd8	5'-AAGGGGCGAggctagctacaacgaGACTCTGCA-3'
hgd9	5'-AAAACGGGAggctagctacaacgaCAGGTTGTA-3'
hgd10	5'-AGAATAAAAggctagctacaacgaGGGACCAGG-3'
hgd11	5'-ATGGCAGAAggctagctacaacgaAAAACGGGA-3'
hgd12	5'-AACTGGGTAggctagctacaacgaGGCAGAATA-3'
hgd13	5'-ATCCAAAAAggctagctacaacgaTGGGTATGG-3'
hgd14	5'-AGGGGAAGAggctagctacaacgaAAAAATCCA-3'
hgd15	5'-TTTTAAAAAggctagctacaacgaTATCTTGGA-3'
hgd16	5'-GTGGGGGGAggctagctacaacgaGGGAAGGCT-3'
hgd17	5'-GTTGAATGAggctagctacaacgaTTGCTTTCG-3'
hgd18	5'-GTCGTTGAAggctagctacaacgaGATTTGCTT-3'
hgd19	5'-GGCCCGGAAggctagctacaacgaCCGCGCGCG-3'
hgd20	5'-TCACCTCCAggctagctacaacgaGGCCTCGGC-3'
hgd21	5'-CCGCCGTCAggctagctacaacgaCTCCATGGC-3'
hgd22	5'-GGTGGCTCAggctagctacaacgaCCAGCGCGG-3'
hgd23	5'-CGTTGAGCAggctagctacaacgaGGCGGGGTG-3'
hgd24	5'-CCGCGTCCAggctagctacaacgaGTAGGAGTG-3'
hgd25	5'-CAGCGGGTAggctagctacaacgaTGCGCCGCG-3'
hgd26	5'-GCACATCCAggctagctacaacgaCTCCTCCGG-3'
hgd27	5'-AAAAGCACAggctagctacaacgaCCACCTCCT-3'
hgd28	5'-TAAAAAGCAggctagctacaacgaATCCACCTC-3'

hgd29	5'-GACCGTCGAggctagctacaacgaGTTAAAAAG-3'
hgd30	5'-TTGCCTTGAggctagctacaacgaCGTCGATGT-3'
hgd31	5'-AGGGCGGGAggctagctacaacgaGTGGTTGCC-3'
hgd32	5'-TGGCCCTGAggctagctacaacgaCGAGTTTCC-3'
hgd33	5'-ACCTCTGCAggctagctacaacgaCGTGGCCCT-3'
hgd34	5'-CGGAGGGTAggctagctacaacgaCTCTGCACC-3'
hgd35	5'-GGCGGCACAggctagctacaacgaCTGGCTCCC-3'
hgd36	5'-CGGGCGGCAggctagctacaacgaACCTGGCTC-3'
hgd37	5'-AGGGATCCAggctagctacaacgaGAAGCAGAG-3'
hgd38	5'-GGGTAGGGAggctagctacaacgaCCATGAAGC-3'
hgd39	5'-GGGCTGAGAggctagctacaacgaTCCAGGGGG-3'
hgd40	5'-GTGGATGGAggctagctacaacgaGTCTTGGAG-3'
hgd41	5'-CGTGGTGGAggctagctacaacgaGGACGTCTT-3'
hgd42	5'-GGGGGTAGAggctagctacaacgaGGAGAGGGG-3'
hgd43	5'-GGAGGAGGAggctagctacaacgaGAGGCCGGG-3'
hgd44	5'-GCCCCCCGAggctagctacaacgaAAGGAGGAG-3'
hgd45	5'-CCGGGGAGAggctagctacaacgaGTCCTTCGG-3'
hgd46	5'-GGACAGCGAggctagctacaacgaGGGTCCGGG-3'
hgd47	5'-TGGGGTGGAggctagctacaacgaAGCGATGGG-3'
hgd48	5'-CTTGAGGCAggctagctacaacgaTCTTTCTCG-3'
hgd49	5'-CACCTGGTAggctagctacaacgaTTGAGGCAC-3'
hgd50	5'-GCAGGGGCAggctagctacaacgaCTGGTACTT-3'
hgd51	5'-CCAGCTTCAggctagctacaacgaGCTGTCGGG-3'
hgd52	5'-GTGGGACGAggctagctacaacgaTCCAGCTTC-3'
hgd53	5'-GGAGTGGGAggctagctacaacgaGACTCCAGC-3'

hgd54 5'-ATGCTGCCAggctagctacaacgaGGGAGTGGG-3'

hgd55 5'-GGGCGGTCAggctagctacaacgaGCTGCCACG-3'

hgd56 5'-GAGGCTCCAggctagctacaacgaCCAGGGCGG-3'

hgd57 5'-GTGGGTCGAggctagctacaacgaGAGGAGGCT-3'

hgd58 5'-AGGTGGTGAggctagctacaacgaGGGGTGGTG-3'

hgd59 5'-ACTCGGGCAggctagctacaacgaGTAGGGCGG-3'

hgd60 5'-GGAGCTGTAggctagctacaacgaTCGGGCACG-3'

hgd61 5'-GGACTTGCAggctagctacaacgaCCGAAGCCG-3'

hgd62 5'-GGGCCTGGAggctagctacaacgaTTGCATCCG-3'

hgd63 5'-TGTGCTGGAggctagctacaacgaCGGGCCTTG-3'

hgd64 5'-GTTCACACAggctagctacaacgaTCCCTGCCT-3'

hgd65 5'-CAGTTCACAggctagctacaacgaACTCCCTGC-3'

hgd66 5'-CACAGTTCAggctagctacaacgaACACTCCCT-3'

hgd67 5'-GTTGCCCCAggctagctacaacgaAGTTCACAC-3'

hgd68 5'-TCGCCGCCAggctagctacaacgaAGTGGGGTC-3'

hgd69 5'-CCCGTGCCAggctagctacaacgaCTCGCCGCC-3'

hgd70 5'-GGCGTTGCAggctagctacaacgaAGGTAGTGT-3'

Name of the DNAzymes against T-bet mRNA sequence:

td1 5'-TGGCTTCTAggctagctacaacgaGCCCTCGTC-3'

td2 5'-GGGCTCTGAggctagctacaacgaGCCTGGCTT-3'

td3 5'-GGGACCCCAggctagctacaacgaCGGAGCCCG-3'

td4 5'-GGTGGGGGAggctagctacaacgaCCCACCGGA-3'

td5 5'-GGCGGGGGAggctagctacaacgaCCGAGGGCC-3'

td6 5'-GGGCTGGGAggctagctacaacgaGGGCAGGGA-3'

td7 5'-CGTCGAGGAggctagctacaacgaCCGCCCCTC-3'

td8	5'-GGGCTGGCAggctagctacaacgaCTTCCCGTA-3'
td9	5'-CGATGCCCAggctagctacaacgaCCGGGGCGG-3'
td10	5'-GCTCCACGAggctagctacaacgaGCCCATCCG-3'
td11	5'-CCGGCTCCAggctagctacaacgaGATGCCCAT-3'
td12	5'-TCTCCGCAAggctagctacaacgaCCGGCTCCA-3'
td13	5'-CCGTCAGCAggctagctacaacgaGTCTCCGCA-3'
td14	5'-TCCCCGGCAggctagctacaacgaCGGCTCGGT-3'
td15	5'-CCCCCGCGAggctagctacaacgaGCTCGTCCG-3'
td16	5'-GTAGGGAGAggctagctacaacgaCCCAGGCTG-3'
td17	5'-GGGCGGGCAggctagctacaacgaCAAGGCGCC-3'
td18	5'-CGGGAAGGAggctagctacaacgaTCGCCCGCG-3'
td19	5'-TAGTCCTCAggctagctacaacgaGCGGCCCCG-3'
td20	5'-TCCCCGACAggctagctacaacgaCTCCAGTCC-3'
td21	5'-TTTCCCCGAggctagctacaacgaACCTCCAGT-3'
td22	5'-TGAGCGCGAggctagctacaacgaCCTCAGTTT-3'
td23	5'-GGACCACAAggctagctacaacgaAGGTGGTTG-3'
td24	5'-CTTGGACCAggctagctacaacgaAACAGGTGG-3'
td25	5'-AAACTTGGAggctagctacaacgaCACAACAGG-3'
td26	5'-CTGATTAAAggctagctacaacgaTTGGACCAC-3'
td27	5'-TGGTGCTGAggctagctacaacgaTAAACTTGG-3'
td28	5'-TGATGATCAggctagctacaacgaCTCTGTCTG-3'
td29	5'-TGGTGATGAggctagctacaacgaCATCTCTGT-3'
td30	5'-GCTTGGTGAggctagctacaacgaGATCATCTC-3'
td31	5'-ATGGGAACAggctagctacaacgaCCGCCGTCC-3'
td32	5'-GAATGGGAAggctagctacaacgaATCCGCCGT-3'

td33 5'-TGACAGGAAggctagctacaacgaGGGAACATC-3'

td34 5'-AGTAAATGAggctagctacaacgaAGGAATGGG-3'

td35 5'-CACAGTAAAggctagctacaacgaGACAGGAAT-3'

td36 5'-GCCCGGCCAggctagctacaacgaAGTAAATGA-3'

td37 5'-CCACAAACAggctagctacaacgaCCTGTAGTG-3'

td38 5'-GTCCACAAAggctagctacaacgaATCCTGTAG-3'

td39 5'-CCACGTCCAggctagctacaacgaAAACATCCT-3'

td40 5'-CCAAGACCAggctagctacaacgaGTCCACAAA-3'

td41 5'-CCACCAAGAggctagctacaacgaCACGTCCAC-3'

td42 5'-GCTGGTCCAggctagctacaacgaCAAGACCAC-3'

td43 5'-GCTCTGGTAggctagctacaacgaCGCCAGTGG-3'

td44 5'-CTGCACCCAggctagctacaacgaTTGCCGCTC-3'

td45 5'-CACACTGCAggctagctacaacgaCCACTTGCC-3'

td46 5'-CTTTCCACAggctagctacaacgaTGCACCCAC-3'

td47 5'-GCCTTTCCAggctagctacaacgaACTGCACCC-3'

td48 5'-TTCCTGGCAggctagctacaacgaGCTGCCCTC-3'

td49 5'-GTGGACGTAggctagctacaacgaAGGCGGTTT-3'

td50 5'-CCGGGTGGAggctagctacaacgaGTACAGGCG-3'

td51 5'-CCTGGCGCAggctagctacaacgaCCAGTGCGC-3'

td52 5'-CAAATGAAAggctagctacaacgaTTCCTGGCG-3'

td53 5'-TTTCCCAAAggctagctacaacgaGAAACTTCC-3'

td54 5'-ATTGTTGGAggctagctacaacgaGCCCCCTTG-3'

td55 5'-TGGGTCACAggctagctacaacgaTGTTGGACG-3'

td56 5'-TCTGGGTCAggctagctacaacgaATTGTTGGA-3'

td57 5'-GCACAATCAggctagctacaacgaCTGGGTCAC-3'

td58 5'-GGAGCACAAggctagctacaacgaCATCTGGGT-3'

td59 5'-ACTGGAGCAggctagctacaacgaAATCATCTG-3'

td60 5'-ATGGAGGGAggctagctacaacgaTGGAGCACA-3'

td61 5'-TGGTACTTAggctagctacaacgaGGAGGGACT-3'

td62 5'-GGGCTGGTAggctagctacaacgaTTATGGAGG-3'

td63 5'-TCAACGATAggctagctacaacgaGCAGCCGGG-3'

td64 5'-CCTCAACGAggctagctacaacgaATGCAGCCG-3'

td65 5'-TCACCTCAAggctagctacaacgaGATATGCAG-3'

td66 5'-CGTCGTTCAggctagctacaacgaCTCAACGAT-3'

td67 5'-GTAAAGATAggctagctacaacgaGCGTGTTGG-3'

td68 5'-AAGTAAAGAggctagctacaacgaATGCGTGTT-3'

td69 5'-GGCAATGAAggctagctacaacgaTGGGTTTCT-3'

td70 5'-TCACGGCAAggctagctacaacgaGAACTGGGT-3'

td71 5'-AGGCAGTCAggctagctacaacgaGGCAATGAA-3'

td72 5'-ATCTCGGCAggctagctacaacgaTCTGGTAGG-3'

td73 5'-GCTGAGTAAggctagctacaacgaCTCGGCATT-3'

td74 5'-TATTATCAAggctagctacaacgaTTTCAGCTG-3'

td75 5'-GGGTTATTAggctagctacaacgaCAATTTTCA-3'

td76 5'-AAGGGGTTAggctagctacaacgaTATCAATTT-3'

td77 5'-CTCCCGGAAggctagctacaacgaCCTTTGGCA-3'

td78 5'-GTACATGGAggctagctacaacgaTCAAAGTTC-3'

Components of the WOW emulsion:

The WOW emulsion comprises

at least one lipophilic emulsifier, at least one amphiphilic component, at least one consistency agent, at least one occlusive component, at least one organic or inorganic additive, at least one hydrophilic emulsifier.

In particular the WOW emulsion comprises

at least one lipophilic emulsifier, where the lipophilic emulsifier is selected from the group comprised of sorbitan, fatty acid esters, glycerol derivatives (e.g., Spans™).

At least one amphiphilic component, where this amphiphilic component is selected from the group comprised of phospholipids, preferably lecithin or one of its derivatives.

At least one consistency agent, where the consistency agent is selected from the group comprised of fatty acid esters (e.g., cetyl palmitate, myristyl myristate), polyethylene glycols, cera alba, microcrystalline wax, lanolin, hydrogenated castor oil, protegin™ W, protegin™ WX, carbomers (e.g., crosslinked acrylic acid polymers) or cellulose and its derivatives.

At least one occlusive component, where the occlusive component is a saturated hydrocarbon selected from the group comprised of paraffin or polysiloxanes such as silicone oils

Alternative an oil selected from the group comprised of esterified fatty acids (e.g., decyl oleate), waxes (e.g., jojoba wax) or partially esterified glycerides (e.g., medium chain length triglycerides) or natural oils such as soy oil, peanut oil, avocado oil, olive oil, castor oil and nut or seed oils.

At least one organic and/or inorganic additive preferably a salt or an ionic liquid whose cation component comprises Na, Mg, K, Li, Ca, Fe, Cu, Ag or a combination of these elements.

At least one hydrophilic emulsifier where the hydrophilic emulsifier is selected from the group comprised of polysorbates, ethoxylated polyethylene glycols (e.g., Tweens™, steareths, laureths, ceteareths), ethoxylated ether, ethoxylated esters

At least one oligonucleotide selected from the group of antisense oligonucleotides, e.g., DNAzymes, siRNAs, asDNAs or ribozymes or primers or aptamers.

Alternatively, the WOW emulsion additionally comprises at least one humectant factor where the humectant factor is selected from the group comprised of glycerol, polyols, osmolytes.

Alternatively, the WOW emulsion additionally comprises a least one preservative, e.g., paraben.

Ingredients of the WO emulsion:

The WO emulsion comprises at least one lipophilic emulsifier, at least one occlusive component, at least one consistency agent, at least one organic and/or inorganic additive, at least one hydrophilic emulsifier.

In particular the WO emulsion comprises at least one lipophilic emulsifier, where the lipophilic emulsifier is selected from the group comprised of sorbitan, fatty acid esters, glycerol derivatives (e.g., glycerol stearate, glycerol dioleates, glycerol monooleates).

At least one occlusive component wherein the occlusive component is a saturated hydrocarbon selected from the group comprised of paraffin or polysiloxanes such as silicone oils

Alternatively, an oil selected from the group comprised of esterified fatty acids (e.g., decyl oleate), waxes (e.g., jojoba wax) or partially esterified glycerides (e.g., medium chain-length triglycerides) or natural oils such as soy oil, peanut oil, avocado oil, olive oil, castor oil as well as nut or seed oils

At least one consistency agent, where the consistency agent is selected from the group comprised of fatty acid esters (e.g., cetyl palmitate, myristyl myristate), polyethylene glycols, cera alba, microcrystalline wax, lanolin or alcohols thereof, hydrogenated castor oil, protegin™ W, protegin™ WX, carbomers (e.g., crosslinked acrylic acid polymers) or cellulose and derivatives thereof

At least one organic and/or inorganic additive, preferably a salt or an ionic liquid whose cationic component comprises Na, Mg, K, Li, Ca, Fe, Cu, Ag or a combination of these elements

Alternatively, the WO emulsion additionally contains at least one humectant factor wherein the humectant factor is selected from the group comprised of glycerol, polyols, osmolytes

At least one oligonucleotide selected from the group of comprised of antisense oligonucleotides, e.g., DNAzymes, siRNAs, asDNAs or ribozymes or primers or aptamers.

Alternatively, the WO emulsion additionally comprises a least one preservative, e.g., paraben.

Preparation of the emulsions

The pharmaceutical composition according to the invention for topical application, comprising at least one lipophilic emulsifier, at least one consistency agent, at least one occlusive component, at least one organic and/or inorganic additive and at least oligonucleotide is added by methods with which those skilled in the art are familiar.

The pharmaceutical composition according to the invention for topical application, comprising at least one lipophilic emulsifier, at least one consistency agent, at least one occlusive component, at least one organic and/or inorganic additive and at least one oligonucleotide is more or less fluid, is a shampoo, a solution, a lotion, a cream, ointment, milk, paste or foam. Alternatively, it is an aerosol and is administered via the lungs.

The pharmaceutical composition according to the invention for topical application, comprising at least one lipophilic emulsifier, at least one consistency agent, at least one occlusive component, at least one organic and/or inorganic additive and at least one oligonucleotide is more or less fluid, is used in particular in infants especially in human infants.

2. Use of an agent for treatment and prevention of diseases

The formulation according to the invention in the form of an emulsion having a dispersed internal continuous aqueous phase is suitable in particular for protecting the oligonucleotides through the addition of magnesium or sodium ions because it stabilizes the oligonucleotides and protects them from enzymatic degradation enabling good absorption into the target cells. Therefore the formulation according to the invention is suitable as a cosmetic and/or dermatologic and/or pharmaceutical preparation for topical application and administration. Due to the use of oligonucleotides, which are known to be effective against inflammatory diseases, for example, the DNAzymes of DE 103 46 487.5, the formulation according to the invention is therefore suitable for production of an agent for treatment and prevention of inflammatory diseases which is used for topical application.

The formulation according to the invention has good absorption of the oligonucleotides, e.g., the DNAzyme, in particular the DNAzyme of DE 103 46 487.5 into target cells in topical

application, e.g., skin cells, lung cells and intestinal epithelial cells, mucosa, nasal epithelial cells and cells of the pharyngeal area.

3. Exemplary embodiments

3.1 Exemplary embodiment of WOW emulsion

The DNAzyme-containing water-in-oil-in-water emulsions (WOW emulsions) according to the invention preferably include the following ingredients:

WOW	(%) (range)
Lipophilic emulsifier preferably sorbitan fatty acid ester, glycerol derivatives (e.g., Spans™)	0.5-20
Amphiphilic component phospholipids preferably lecithin or one of its derivatives,	0.05-5
Consistency agents, such as fatty acid esters (e.g., cetyl palmitate, myristyl myristate), polyethylene glycols, cera alba, microcrystalline wax, lanolin, hydrogenated castor oil, carbomers (e.g., crosslinked acrylic acid polymers), protegin™ W, protegin™ WX or cellulose and its derivatives	0.1-5
Occlusive component based on saturated hydrocarbons such as paraffin or polysiloxanes such as silicone oils	1-25
Alternatively, an oil selected from the group of esterified fatty acids (e.g., decyl oleate), waxes (e.g., jojoba wax) or partially esterified glycerides (e.g., medium chain length triglycerides) or natural oils such as soy oil, peanut oil, avocado oil, olive oil, castor oil and nut or seed oils	1-25
Organic and/or inorganic additives, e.g., a salt or ionic liquid whose	0.01-2

cationic component comprises Na, Mg, K, Li, Ca, Fe, Cu, Ag or a combination of these elements	
Oligonucleotide, e.g., one of the DNAzymes of DE 103 46 487.5	0.01-5
Water	as needed
Hydrophilic emulsifier such as polysorbates or ethoxylated polyethylene glycols (e.g., Tweens™, steareths, laureths, ceteareths) or ethoxylated ethers and/or esters	1-8
Humectant factors (e.g., glycerol, polyols, osmolytes)	0.1-10
Preservatives such as parabens	as needed

3.1.1. Selected exemplary embodiments (WOW 167)

WOW 167	(% w/w)
Sorbitan monooleate	4
Lecithin	0.2
Paraffin	15.8
NaCl	0.074
DNAzyme hdg 40	0.4
Water	to a total of 100
Steareth 20	1
Water + paraben	59

3.1.2. Selected exemplary embodiments (WOW 146)

WOW 146	(% w/w)
Sorbitan monooleate	4
Lecithin	0.2
Paraffin	15.8

$MgSO_4 \times 7H_2O$	0.308
DNAzyme hdg 40	0.4
Water	to a total of 100
Water + paraben, preserved	59
Water (external phase)	

3.2 Exemplary embodiment of a WO emulsion

The water-in-oils emulsions according to the invention containing DNAzyme (WO emulsions) comprise the following ingredients:

WOW	(%) Range
A lipophilic emulsifier or a mixture of same such as sorbitan fatty acid esters or glycerol derivatives (glycerol stearates, glycerol dioleates, glycerol monooleates)	1-15
Occlusive component based on saturated hydrocarbons such as paraffin or polysiloxanes such as silicone oils	10-70
Alternatively, an oil selected from the group of esterified fatty acids (e.g., decyl oleate), waxes (e.g., jojoba wax) or partially esterified glycerides (e.g., medium chain length triglycerides) or natural oils such as soy oil, peanut oil, avocado oil, olive oil, castor oil as well as nut or seed oils	10-70
Consistency agents such as fatty acid esters (e.g., cetyl palmitate, myristyl myristate), polyethylene glycols, cera alba, microcrystalline wax, lanolin, hydrogenated castor oil, protegin™ W, protegin™ WX, carbomers (e.g., crosslinked acrylic acid polymers), or cellulose and its derivatives	0.5-10

Lanolin or its alcohols	1-8
Preservatives (e.g., parabens)	as needed
Organic and/or inorganic additives, e.g., a salt or ionic liquid whose cation component comprises Na, Mg, K, Li, Ca, Fe, Cu, Ag or a combination of these elements	0.01-2
Oligonucleotide, e.g., one of the DNAzymes of DE 103 46 487.5	0.01-5
Humectant factors (e.g., glycerol, polyols, osmolytes)	0.1-10

3.2.1. Selected exemplary embodiments (WO 126)

WO 126	(% w/w)
Glycerol stearate	1
Glycerol monooleate (Imwitor 946)	2
Glycerol dioleate (Crossential GDO)	2
Paraffin	38
Cetyl palmitate	2
Lanolin	3
Hydrogenated castor oil	2
Preserved water + parabens	to a total of 100
NaCl	0.5
DNAzyme hdg 40	0.4
Glycerol	3

3.2.2. Selected exemplary embodiments (WO 162)

WO 162	(% w/w)
Glycerol stearate	1
Glycerol monooleate (Imwitor 946)	2

Glycerol dioleate (Crossential GDO)	2
Paraffin	38
Cetyl palmitate	2
Lanolin	3
Water + paraben, preserved	to a total of 100
Water	
$MgSO_4 \times 7H_2O$	1
DNAzyme hdg 40	0.4
Glycerol	3

The oligonucleotide used is preferably any of the DNAzymes hgd 1-70 and td 1-78 disclosed in DE 103 46 487.5, preferably hgd 40 as a DNAzyme directed against GATA-3 at td 69 and/or td 70 as DNAzymes directed against T-bet.

4. Stability measurements on the formulations

The formulations WOW 167 and WOW 146 containing DNAzyme according to the invention differ in the added electrolytes in the internal aqueous phase, which is represented graphically in Figure 1. Solutions of 0.13M are preferably used here to prepare WOW 167 and WOW 146, because formulations with higher concentrations of electrolytes reduce the stability of the formulations. Stability is measured by comparison and over a period of 150 days.

Immediately after preparing the formulation, the viscosity of the WOW 167 (formulation with NaCl) is somewhat higher at 1.4 Pa·s than the viscosity of WOW 146 (formulation with $MgSO_4$) at 1 Pa·s. The viscosity of the WOW 146 formulation (formulation with $MgSO_4$) drops slightly to 0.75 Pa·s within the first 5 days, then remains largely unchanged at 0.75 Pa·s. The viscosity of

the WOW 167 (formulation with NaCl) drops to 0.6 Pa·s by day 50 and then drops further to 0.4 Pa·s.

WOW 146 (formulation with $MgSO_4$) is thus much more stable than the WOW 167 (formulation with NaCl). It should be pointed out that the slight decline in viscosities does not have a negative effect on the stability or the protective action, and the emulsions do not exhibit any phase inversion or separation and are stable.

The measured droplet size is in the range of 10-20 µm.

The DNAzyme-containing formulations WO 162 and WO 126 according to the invention also differ in the electrolytes added to the internal water phase. The stability is measured by comparison and over a period of 150 days, where the viscosity remains unchanged for 150 days at approx. 4 Pa·s in the case of WO 126 and 4.2 for WO 162.

The droplet size here is in the range of 0.5-2.5 µm.

5. Stability with respect to degradation by nucleases such as DNAses

The DNAzyme-containing WOW 167 and WOW 146 formulations according to the invention contain the DNAzymes of DE 103 46 487.5, namely DNAzymes hgd 1 to hgd 70 against GATA-3 and DNAzymes td 1 to td 78 against T-bet and protect them from degradation due to nucleases, in particular DNAses. It is important to protect the DNAzymes from degradation due to the DNAse, so that they can manifest their therapeutic efficacy.

A commercially available DNAse I with an activity of 105 U is used experimentally to measure the stability of the DNAzyme-containing formulations according to the invention with respect to DNAse and this was added to the WOW 167 and WOW 146 formulations. In addition, the

degradation by a dermal lysate is used as a positive control. The degradation of the DNAzymes is determined by HPLC.

The measurement is performed using the following method:

Degradation of DNAzymes by DNAse from the skin

1) Method

Skin: approx. 50 mg

Solution: 1 mL DNAzyme standard (0.1625 mg/mL) + 3 mL RO water (water from reverse osmosis)

Method: Skin pulverized using a scalpel and mixed with solution and shaken lightly, then filtered and degraded by HPLC after 2 min, 52 min, 102 min and 152 min.

Figures 2a and 2b show that the DNAses naturally occurring in skin begin to degrade the DNAzyme after only 2 minutes; after 152 minutes almost the entire amount of DNAzyme is degraded.

6. Protecting the DNAzymes from degradation by DNAse using the formulations WOW 146, WOW 167, WO 126, WO 162 according to the invention

Galenical formulation 20 mg

Solution: DNAse I with an activity of 105 U in Tris buffer + 10 mM $MgSO_4$

Method: 20 mg of the respective formulation is mixed with 1 mL of a DNAse I solution. After an incubation time of 1 min, the mixture is agitated lightly at a temperature of 99°C for 10 minutes in a thermomixer to stop the activity of the DNAse. To break the emulsion, the batch is incubated in an ultrasonic bath at 50°C for 10 minutes. Then the batch is filtered through a 0.45 µm syringe filter and HPLC analysis is performed.

Figure 3 shows that the WOW 146 formulation offers approx. 61% protection of the DNAzymes from DNAse degradation, whereas 51% protection is achieved with the WOW 167 formulation. A standard solution containing DNAzyme (0.4% DNAzyme hgd 40 in PBS buffer (PBS = phosphate buffered saline solution – 137 mM NaCl, 2.7 mM KCl, 12 mM Na_2HPO_4 and KH_2PO_4, pH = 7.4)) was used as the control, but no active ingredient could be recovered here. The WOW 167 formulation thus provides better protection on the whole. A comparable OW galenical formulation (microemulsion: preserved water, oil components, glycerol, hydrophilic emulsifier, magnesium sulfate) with the same amount of DNAzyme also failed to show any protection and 100% of the DNAzymes was degraded.

7. Stability in comparison with oil-in-water formulations (OW formulations)

For detecting the stability of the active ingredient in the galenical formulations, the recovery of 0.4% of the DNAzyme hgd 40 active ingredient in WO 162, WOW 146 was analyzed by HPLC after one month, using as the reference a comparable OW galenical formulation (microemulsion: preserved water, oil component, glycerol, hydrophilic emulsifier, magnesium sulfate).

The recovery of DNAzyme hgd 40 in comparison with the starting value of 100% after one month was 35.98 ± 0.16% in the OW comparative galenical formulation. The recovery after one month in the WO 162 formulation was 95.66 ± 2.77%. The recovery after one month in the WOW formulation was 103.15 ± 2.29%.

8. Detecting the efficacy of GATA-3 DNAzyme formulations in the animal model

The efficacy of the pharmaceutical composition according to the invention for topical application comprising at least one lipophilic emulsifier, at least consistency agent, at least one occlusive component, at least one organic and/or inorganic additive and at least one oligonucleotide as the

agent for treatment of inflammatory skin diseases is illustrated on the example of GATA-3 DNAzyme-containing formulations in an animal model.

To do so, a mouse model is used (see Figure 4) in which a specific inflammation reaction in the skin of the mouse is induced by allergic sensitization using a model allergen such as ovalbumin UOVA) and subsequent repeated cutaneous administration by skin patches. In addition to the typical histological changes in the skin, the expected increased expression of the target gene, e.g., GATA-3 and the Th2 cytokines IL-4, IL-5 and IL-13 in the inflamed area of skin is triggered and detected. By using the DNAzyme-containing formulations according to the invention as the agents for treatment of inflamed skin diseases, a therapeutic efficacy becomes apparent. Thus a significantly reduced inflammation score in mice was achieved after treating them with DNAzyme formulations. Furthermore, it was demonstrated histologically that after this treatment, pure inflammation cells in particular CD4+ T-lymphocytes could be found in the inflamed areas of skin.

For example, the following were used:
Sensitization: 10 µg OVA + 10 µg $Al(OH)_3$ in 100 µL PBS, administered intraperitoneally
Patches: 100 µg OVA + 10 µg $Al(OH)_3$ administered cutaneously by the patch test method
Treatment: 200 µg DNAzyme in 50 µL PBS applied topically intradermally and/or epicutaneously in the formulations WOW 146, WO 162 and WOW 167

The analyses have shown that the formulations containing DNAzyme are effective against experimentally-induced inflammatory skin diseases.

[Legend to figures and list of reference numerals]

Figure 1 shows the viscosity (Pa·s) of the DNAzyme-containing formulations. Figure 1a shows WOW 146 (formulation with $MgSO_4$) and Figure 1b shows 167 (formulation with NaCl) in comparison over a period of 150 days. The stability of the WOW 146 (formulation with $MgSO_4$) is higher than the stability of the WOW 167 (formulation with NaCl).

Figure 2 shows the degradation of DNAzyme by DNAse from the skin, measured with HPLC.
Figure 2a shows the HPLC measurement of DNAzyme on the skin after 2 minutes.
Figure 2b shows the HPLC measurement of DNAzyme on the skin after 152 minutes.
Figures 2a and 2b show that unprotected degradation of DNAzymes on the skin begins already after 2 minutes due to the DNAses present there and after 152 minutes the DNAzyme is almost completely degraded. This reaction takes place equally all oligonucleotides and is not limited to DNAzymes.

Figure 3 shows the protection of the formulations containing DNAzyme with respect to degradation by DNAse. This shows the WOW 146 formulation containing DNAzyme, which exhibit 90% protection of the DNAzymes, whereas the WOW 167 formulation containing DNAzyme exhibits only 60% protection. A standard solution (0.4% DNAzyme in PBS buffer) containing DNAzyme is used as the control.

Figure 4 shows the experimental setup of an animal model in which an inflammatory skin reaction is induced experimentally and the efficacy of agents for treatment of these diseases is tested.

In this model, the inflammatory reaction typical of inflammatory skin diseases is induced in the skin of mice (strain Balb) by allergic sensitization using a model allergen, e.g., ovalbumin (OVA) and subsequent repeated epicutaneous application over a skin patch.

The sensitization is accomplished by intraperitoneal administration (i.p.), while the treatment with DNAzyme is achieved by intradermal (i.d.) administration or epicutaneous (e.c.) administration of various formulations.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A pharmaceutical composition for topical application in the form of either (i) a water-in-oil-in-water emulsion or (ii) a water-in-oil emulsion, each of said emulsions comprising a dispersed, discontinuous, internal aqueous phase, the composition comprising:

(a) at least one lipophilic emulsifier selected from the group consisting of a sorbitan fatty acid ester and a glycerol derivative;
(b) at least one consistency agent;
(c) at least one synthetic oil, natural oil, wax, or saturated hydrocarbon selected from the group consisting of paraffin and a polysiloxane;
(d) at least one additive comprising a salt or an ionic liquid, wherein the cation component of the salt or of the ionic liquid comprises an element selected from the group consisting of Na, Mg, K, Li, Ca, Fe, Cu, Ag and a combination of these elements; and,
(e) at least one antisense oligonucleotide held by the internal aqueous phase, wherein the at least one antisense oligonucleotide is a DNAzyme directed against the mRNA coding for T-bet protein, the DNAzyme selected from a group consisting of DNAzymes according to SEQ ID NO 71 to SEQ ID NO 148.

2. The pharmaceutical composition according to claim 1, further comprising at least one hydrophilic emulsifier selected from the group consisting of polysorbates, ethoxylated polyethylene glycols, ethoxylated ethers, and ethoxylated esters.

3. The pharmaceutical composition according to claim 1, further comprising at least one amphiphilic component.

4. The pharmaceutical composition according to claim 3, wherein the amphiphilic component is a phospholipid.

5. The pharmaceutical composition according to claim 1, further comprising at least one humectant factor.

6. The pharmaceutical composition according to claim 5, wherein the humectant factor is selected from the group consisting of glycerol, polyols, and osmolytes.

7. The pharmaceutical composition according to claim 1, further comprising at least one preservative.

8. The pharmaceutical composition according to claim 1, wherein the lipophilic emulsifier is a sorbitan monooleate.

9. The pharmaceutical composition according to claim 1, wherein the lipophilic emulsifier is a glycerol derivative selected from the group consisting of Spans™, glycerol stearate, glycerol dioleate, and glycerol monooleate.

10. The pharmaceutical composition according to claim 1, wherein the consistency agent is selected from the group consisting of a fatty acid ester, a polyethylene glycol, cera alba, a microcrystalline wax, lanolin, hydrogenated castor oil, protegin™ W, protegin™ WX, carbomers, and cellulose.

11. The pharmaceutical composition according to claim 1, wherein the synthetic oil is an esterified fatty acid or a partially esterified glyceride.

12. The pharmaceutical composition according to claim 1, wherein the natural oil is selected from the group consisting of a seed oil, and a nut oil.

13. The pharmaceutical composition according to claim 1, wherein the natural oil is selected from the group consisting of soy oil, peanut oil, avocado oil, olive oil and castor oil.

14. The pharmaceutical composition according to claim 1, wherein the wax is jojoba wax.

15. The pharmaceutical composition according to claim 7, wherein the preservative is paraben.

16. The pharmaceutical composition according to claim 1, wherein the cation is selected from the group consisting of Na, Mg, and a combination thereof.

17. The pharmaceutical composition according to claim 1, wherein the salt is selected from the group consisting of NaCl, $MgSO_4$, $Mg(N(SO_2CF_3)_2)_2$, and $Mg(OSO_2CF_3)_2$.

18. The pharmaceutical composition according to claim 1, wherein the ionic liquid is selected from the group consisting of 1-ethyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium sulfate, 1-ethyl-3-methylimdazolium trifluoromethane sulfonate, 1-ethyl-3-methylimidazolium dicyanamide, and 1-ethyl-1-methylpyrrolidiniumbis(trifluoromethylsulfonyl)amide).

(Appended drawings)

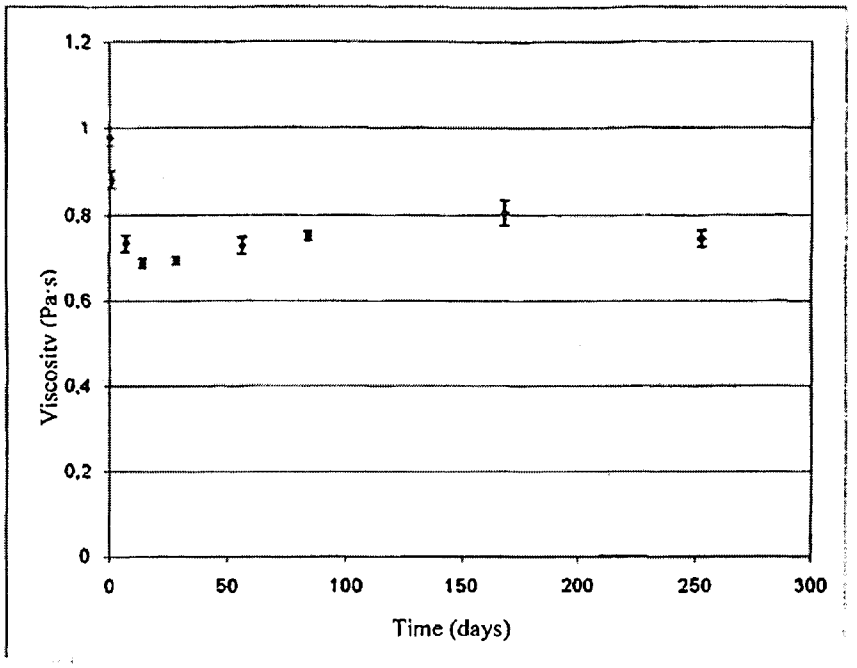


Figure 1a

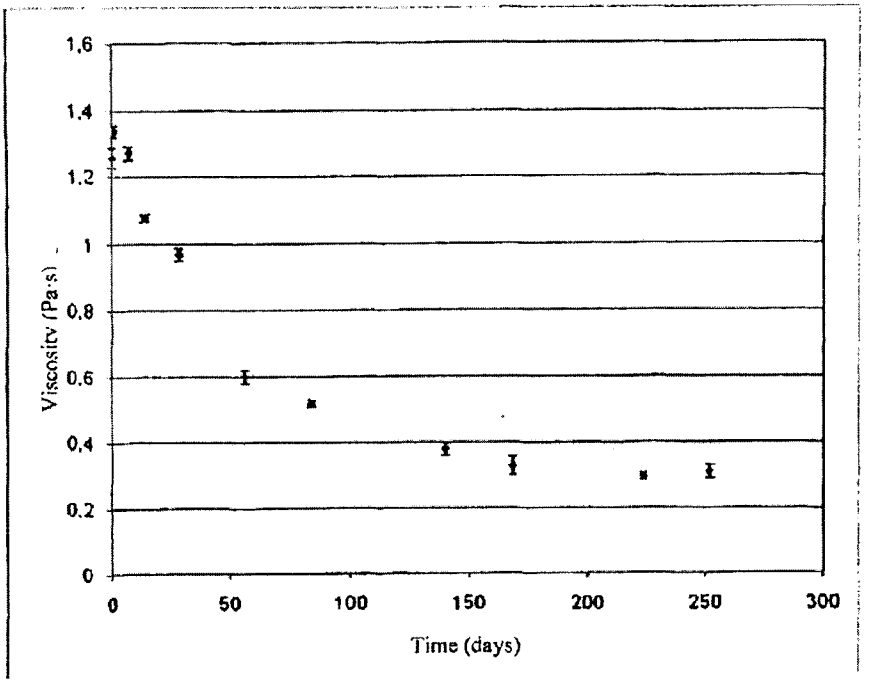

1,6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Viscosity (Pa·s)
0
50
100
150
200
250
300
Time (days)

Figure 1b

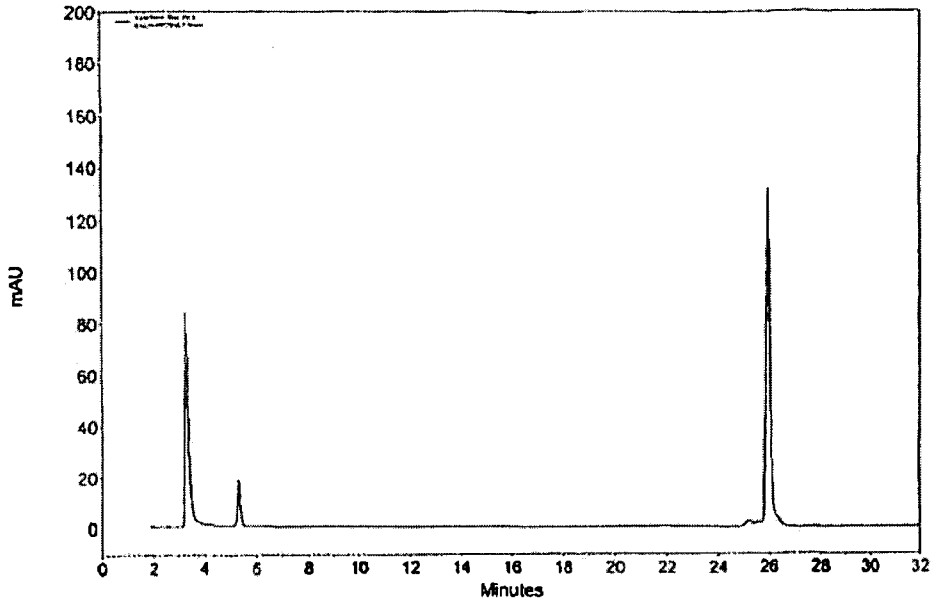

mAU
200
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Minutes

Figure 2a

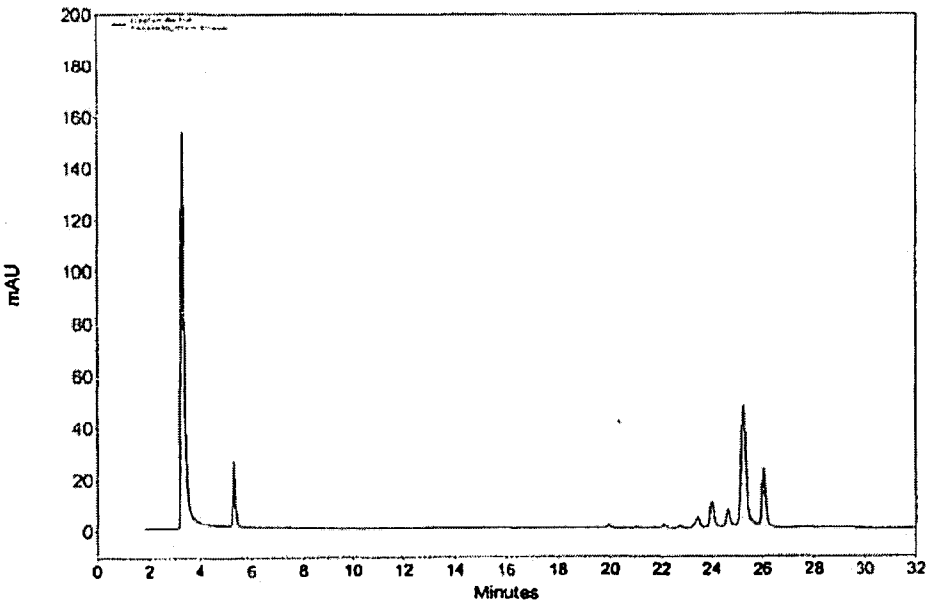

200
180
160
140
120
100
80
60
40
20
0
mAU
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Minutes

Figure 2b

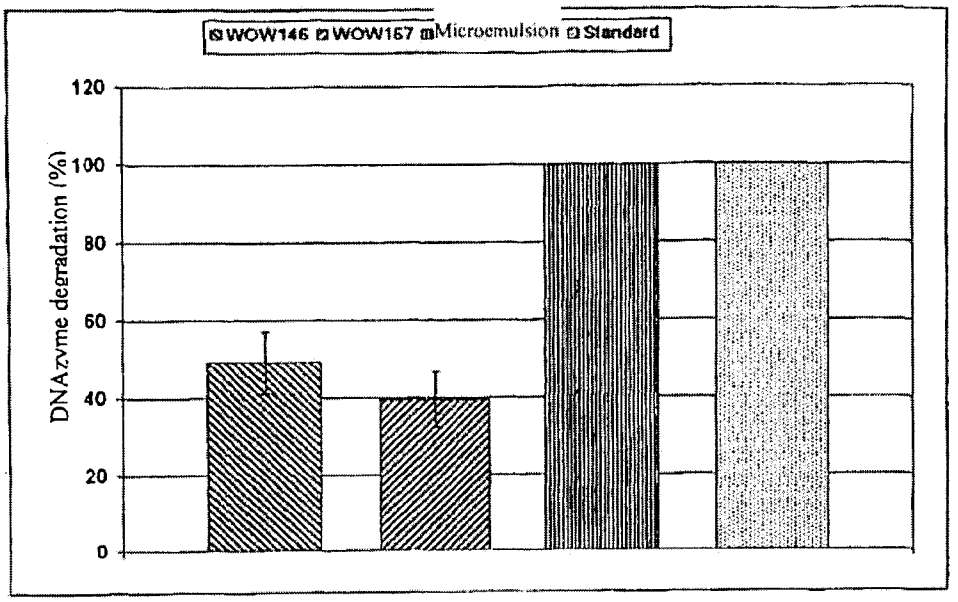
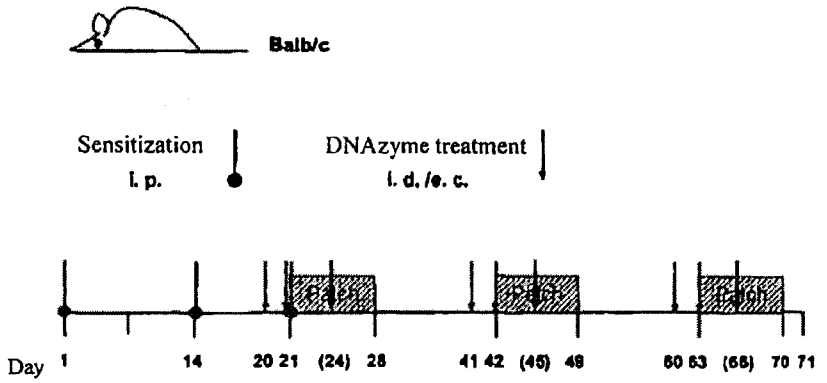

WOW146 WOW157 Microemulsion Standard
DNAzyme degradation (%)
120
100
80
60
40
20
0

Figure 3



Sensitization: 10 µg OVA + 10 µg $Al(OH)_3$ in 100 µL PBS administered intraperitoneally

Patches: 100 µg OVA + 10 µg $Al(OH)_3$ applied epicutaneously by the patch test method

Treatment: 200 µg DNAzyme in 50 µL PBS topically intradermally and/or epicutaneous application of three different formulations

Figure 4