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PATENTS ACT 1990
NOTICE OF ENTITLEMENT

We, **Lyfjathroun H.F.** AND **Statens Seruminstitut**, the applicants/Nominated Persons in respect of Application No. 61065/94 state the following:-

The Nominated Persons are entitled to the grant of the patent because the Nominated Persons derive title to the invention from the inventors by assignment.

The Nominated Persons are entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Persons made the application listed in the declaration under Article 8 of the PCT.

DATED this THIRTEENTH day of SEPTEMBER 1995

a member of the firm of
DAVIES COLLISON CAVE
for and on behalf of the
applicant(s)

(DCC ref: 1763300)

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A PHARMACEUTICAL COMPOSITION FOR TOPICAL ADMINISTRATION OF ANTIGENS AND/OR VACCINES TO MAMMALS VIA A MUCOSAL MEMBRANE

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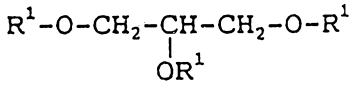
(57) Claim

1. A pharmaceutical composition for topical administration of antigens and/or vaccines to mammals via a mucosal membrane, CHARACTERIZED by comprising one or more antigens and/or vaccines and as an adjuvant/vehicle a caprylic/capric acid glyceride of the general formula



wherein each R^1 independently is H or a C_8-C_{10} acyl group containing 1-6% free glycerol, 45-50% monoglycerides, 30-40% diglycerides and 5-9% triglycerides.

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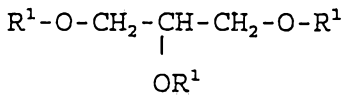
12. Use of caprylic/capric acid glycerides of the general formula



wherein each R^1 independently is H or a C_8-C_{10} acyl group containing 1-6% free glycerol, 45-50% monoglycerides, 30-40% diglycerides and 5-9% triglycerides as adjuvants/vehicles in pharmaceutical compositions for the topical administration of antigens and/or vaccines to mammals.

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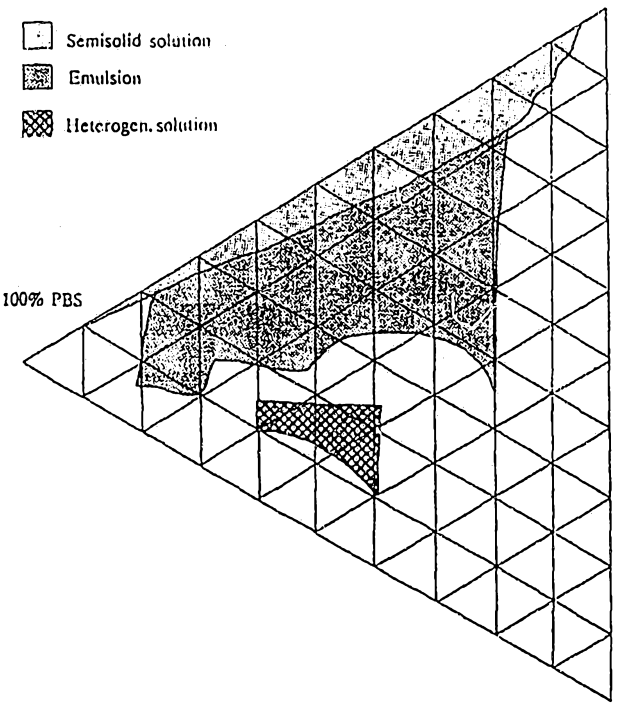
(54) Title: A PHARMACEUTICAL ~~PREPARATION~~ Composition FOR TOPICAL ADMINISTRATION OF ANTIGENS AND/OR VACCINES TO MAMMALS VIA A MUCOSAL MEMBRANE

(57) Abstract

A novel type of formulation for the topical administration of antigens and/or vaccines to mammals via mucosal membrane comprising one or more adjuvants/vehicles selected from (a) polyoxyethylene sorbitan monoesters, (b) polyoxyethylene castor oil, (c) caprylic/capric acid glycerides and (d) gangliosides in an amount of 0.01 to 15 % (v/v) calculated on the total volume of the preparation. This formulation enhances the immunological response in a mammal following mucosal administration, e.g. nasal, oral, rectal or vaginal application.



A pharmaceutical ~~preparation~~ composition for topical administration of antigens and/or vaccines to mammals via a mucosal membrane

The present invention relates to novel pharmaceutical ~~preparations~~ compositions for topical administration of antigens and/or vaccines to mammals, including humans, via a mucosal membrane. The invention also relates to the use of certain compounds (to be defined in more detail below) as adjuvants or vehicles in such ~~preparations~~ compositions.

The parenteral (intramuscular and subcutaneous) administration of antigens and/or vaccines is normally regarded as the most convenient way of administration. However, the administration by injection presents a range of disadvantages. Thus it requires the use of sterile syringes and may cause pains and irritations, particularly in the case of repeated injections, including the risk of infection. More significantly, in the case of intramuscular injections there is also a risk of the infection being poorly tolerated. There is likely to be an induration (hardening of tissue), haemorrhage (bleeding) and/or necrosis (local death of tissue) at the injection site. Besides, injections cannot be administered satisfactorily by untrained persons.

Administration of attenuated virus, bacteria or parasites has been attempted intranasally as well as through other mucosal surfaces. The elicitation of an immune response by such antigens through mucosal surfaces cannot be considered unexpected in such cases, because the modified live pathogens of the vaccine is following the natural route of infection of the wild-type pathogen creating immunity through a sub-clinical infection. The use of modified live pathogen to effect immunization entails a certain risk, however, because the more purified antigens are very poor

immunogens and thus require effective formulations and adjuvants to produce a clinically protective immune response.

Mucosal administration is currently receiving special interest, attempting to stimulate locally produced antibodies (secretory IgA antibodies) and also to avoid the inconveniences caused by the direct intervention into the organism in connection with parenteral administration. Additionally, this route of administration may conveniently be used as an alternative to parenteral injection, since it may well be performed by an untrained person. Furthermore, small children will avoid the psychological irritation during injection (vaccination).

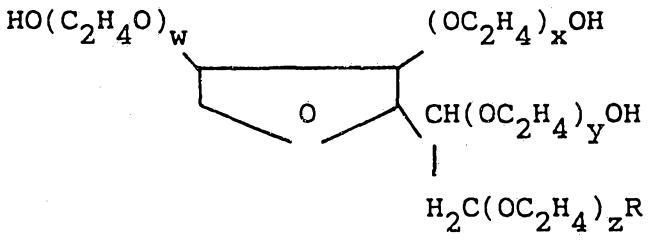
In order to be an attractive alternative to parenteral administration, the intranasal administration should be capable of stimulating humoral and cellular immune factors both systemically (mainly of the IgG isotype) and at mucosal surfaces where most pathogens enter the host by locally produced antibodies of the secretory IgA (IgA_s) isotype. Several oral vaccines have been shown to induce appropriate IgA_s responses in remote secretions including saliva, lachrymal fluid and fluids obtained from nasal and gastrointestinal washes. Such intranasally administered vaccines and/or antigens may not cause any considerable pain or irritation to the patient nor any irreversible damage or irritation to the mucosal surfaces.

In nasal administration, the antigen and/or vaccine must be applied to the mucosa in such a condition that it is able to penetrate or to be absorbed through the mucosa. In order to penetrate the mucus the vehicle must be biocompatible with the mucus and hence have a certain degree of hydrophilicity.

Vaccines and/or antigens are not able to be administered in pure form. It is necessary to blend them with other components to obtain a preparation which is ready for use. Dependent on the chemical properties of the antigen and/or vaccine it will be necessary to take various considerations into account before a pharmaceutical ~~preparation~~ composition for humans or animals can be produced.

It has now surprisingly been found that the topical administration of antigens and/or vaccines to mammals via mucosal membranes can be performed in a new and significantly improved manner by using a novel type of formulation, said ~~preparation~~ composition being characterized by comprising one or more adjuvants/vehicles selected from

(a) polyoxyethylene sorbitan monoesters of the general formula



wherein R is selected among laurate, palmitate, stearate and oleate, and wherein the sum of w, x, y and z is 4, 5 or 20;

(b) polyoxyethylene castor oil produced by reacting 1 mole of castor oil or hydrogenerated castor oil with 10-45 moles of ethylene oxide;

(c) caprylic/capric acid glycerides of the general formula

$$R^1\text{-}O\text{-}CH_2\text{-}CH(OR^1)\text{-}CH_2\text{-}O\text{-}R^1$$

wherein each R^1 independently is H or a C_8-C_{10} acyl group containing 1-6% free glycerol, 45-50% monoglycerides, 30-40% diglycerides and 5-9% triglycerides, and

(d) gangliosides of the general formula

$$R^2\text{-Gal-Glc-Cer}$$
$$|$$
$$R^3\text{-NeuAc}$$

wherein Gal is galactose, Glc is glucose, Cer is ceramide (N-fatty acyl sphingosine) and NeuAc is N-acetyl neuraminic acid (sialic acid), and wherein R^2 may be one or more substances selected among N-acetyl galactosamine, galactose, N-acetyl neuraminic acid or combinations thereof, and R^3 is H or N-acetyl neuraminic acid

in an amount of 0.01 to 15% (v/v) calculated on the total volume of the ~~preparation~~ composition.

The nasal epithelial membrane consists of practically a single layer of epithelial cells (pseudostratified epithelium) and it is therefore even more suited for antigen and/or vaccine administration than other mucosal surfaces having squamous epithelial layers, such as the mouth, vagina, etc. These surfaces, however, are also well suited for the application of antigens and/or vaccines with the delivery system according to the invention. The extensive network of blood capillaries under the nasal mucosa is - together with the high density of T and B cells - parti-

cularly suited to provide a rapid recognition of the antigen and/or the vaccine, which may also provide a quick immunological response.

For liquid compositions it is essential that the effective amount of the antigen and/or the vaccine can be administered in a volume of less than about 300 µl for human subjects. A larger volume can be disagreeable to the patient and will evidently drain out anteriorly through the nostrils or posteriorly toward the pharynx. The result is that a part of the antigen and/or the vaccine is lost from the absorption site.

The volume is preferably from about 20 µl to about 125 µl and preferably administered into both nostrils.

A variety of vehicle systems for the delivery of antigens and/or vaccines have been developed. The literature to date has suggested that uptake of antigens and/or vaccines from the nasal mucosa is frequently made possible by incorporation of a special vehicle system into the formulation, adding certain amount of absorption enhancing agents or a certain amount of adjuvants.

Much has been written regarding the potential use of various vehicles as drug delivery systems for intranasal administration. In such vehicle systems, the medicament is rapidly absorbed into the blood stream. One of the problems encountered in using such vehicle systems is that the antigen and/or the vaccine is absorbed and degraded without recognition and, therefore, without stimulating an immunological response. The system according to the invention describes a vaccine/antigen delivery system which provides a clear immunological response in spite of the short contact time inside the nasal cavity.

A possible enhancement of the immunological response after mucosal administration of polyoxyethyl-35-castor oil, caprylic/capric acid glycerides and/or gangliosides together with an antigen or a vaccine has not been suggested anywhere in the prior art.

US patent No. 4,610,868 describes a lipid matrix carrier for parenteral administration of drugs. This system requires a lipid matrix carrier comprising a hydrophobic compound, an amphipathic compound and a bioactive agent with a globular structure of a diameter between 500 and 100,000 nm. Here the hydrophobic compound may comprise a mixture of glycerides and the amphipathic compound may comprise a sphingolipid. Furthermore, this formulation may be administered into the nasal area. However, this system is not acceptable as a nasal formulation, due to the rapid clearance inside the nose and the large globular structure. Therefore, this system will be transferred into the stomach by the cilia before the bioactive agent is released.

US patent No. 4,985,242 describes an intranasally applicable powdery pharmaceutical composition comprising a polypeptide with physiological activity, a quaternary ammonium compound, and a lower alkyl ether of cellulose. Typical surfactants in this composition are polyoxyethylene sorbitan fatty acid esters. This powdery pharmaceutical composition is stated to have an excellent preservability and chemical stability of the polypeptides. Further, when the composition is administered to the nasal cavity in the form of a spray, the polypeptides are absorbed effectively through the nasal mucosa. However, the surfactant concentration is critical since, on the one hand, high concentrations lead to sticky preparations without powder characteristics. On the other hand, low concentrations will not enable the induction of an immunological response. If

the ~~purpose~~ purpose of US patent No. 4,985,242 had been to induce an immunological response, which is not the case, this would be regarded as a serious drawback when protein and peptide drugs were to be administered. These surfactants would therefore not be usable for the purpose of the present invention.

Several other references relating to the use of a polyoxyethylene derivative of a sorbitan ester in nasal preparations are known. However, no reference describes the substance according to the invention as an adjuvant or as an immunomodulator. This effect is indeed surprising and unexpected. A novel method of administering the natural female sex hormones 17β-oestradiol and progesterone as solutions, suspensions, gels and ointments, containing 1% to 2% Tween 80, is described in US Patent No. 4,315,925. From EP Patent No. 246,625 is known an aqueous steroid formulation for nasal administration of an anti-inflammatoric steroid preparation containing propylene glycol, polyethylene glycol 400 and 1% to 4% Tween 20. EP Patent No. 242,643 describes an intranasal administration of drugs, especially insulin, using e.g. 0.01% to 0.5 % Tween 80 to reduce the nasal irritation by other absorption promoters. Finally, in PCT/AT87/00015 a sprayable, Tween-containing formulation for e.g. benzodiazepines is described. However, this formulation requires the use of a propeller gas.

The present invention presents a new and significantly improved method for the administration of antigens/vaccines, using the above new type of formulation. The method provides protective immune response in recipients of the antigen and/or the vaccine, both systemically and locally, which are elicited after intranasal immunization.

The primary preferred object of the invention is to provide an intranasal composition, which is capable of producing a high systemic immune response (humoral and cellular, mainly of the IgG isotype) as well as locally produced antibodies of the secretory IgA isotype at mucosal surfaces without causing unacceptable damage to the nasal epithelial membrane.

It is another object of the invention to provide a controlled delivery system for intranasal application, which is biocompatible with the mucus and which is capable of dissolving required amounts of antigens and/or vaccines in small volumes.

According to an aspect of the invention the present delivery system is also usable for other mammalian surfaces such as the vagina, eye, mouth, lungs, ear, genital tract, gastrointestinal tract, rectum, skin etc.

As mentioned previously, the pharmaceutical ~~preparation~~ composition of the present invention is characterized by comprising one or more substances selected from

(a) polyoxyethylene sorbitan monoesters, (b) polyoxyethylene glycerol triesters, (c) caprylic/capric acid glycerides, and (d) gangliosides.

The preferred polyoxyethylene sorbitan monoester (a) is Polysorbate 20, which is a laurate ester of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

The polyoxyethylene glycol triester (b) is preferably Polyoxyl-35-castor oil. This compound is mainly the triricinoleate ester of ethoxylated (about 35 moles) gly-

cerol with smaller amounts of polyethylene glycol ricinoleate and the corresponding free glycols. Polyoxyl-35-castor oil is commonly known as Cremophor EL.

The caprylic/capric acid glycerides (c) are principally a mixture of mono-, di- and triglycerides in which the acid groups are only caprylic and capric acid groups. They are known commercially under the trade name Imwitor.

The gangliosides (d) of the above formula IV are principally a mixture of asialo-, monosialo-, disialo- and trisialogangliosides.

The composition according to the invention may comprise one or more additional pharmaceutical excipients, selected among surfactants and absorption promoters, such as polyoxyethylene alcohol ethers, bile salts and derivatives thereof, fusidic acid and derivatives thereof, oleic acid, lecithin, lysolecitines, Tween 21 to 85, etc, water absorbing polymers, such as glycofurol, polyethylene glycol 200 to 7500, polyvinylpyrrolidone, propylene glycol or polyacrylic acid, gelatine, cellulose and derivatives, etc.; substances which inhibit enzymatic degradation, such as aprotinin, etc.; alcohols, such as ethanol, glycerol, benzyl alcohol, etc.; organic solvents such as ethyl acetate, benzyl alcohol, etc.; hydrophobic agents, such as vegetable oil, soybean oil, peanut oil, coconut oil, maize oil, olive oil, sunflower oil, "Miglyols" or mixtures thereof, etc.; pH-controlling agents, such as nitric acid, phosphoric acid, acetic acid, citrates, etc.; preservatives and osmotic pressure controlling agents, such as glycerol, sodium chloride, methyl paraoxybenzoate, benzoic acid, etc.; liposome and/or emulsion formulations, such as lecitines, etc.; microencapsulated formulations; propellants, such as butane; water etc. The use of propellants is not compulsory in the ~~preparation~~ composition according to the

invention.

The pharmaceutical composition of the invention may comprise any antigens and/or vaccines. The vaccines may be selected among all the vaccines causing diseases in humans or animals. These include bacterial vaccines such as chlamydia, cholera, diphtheria, haemophilus influenzae, leprosy, meningococcal, pertussis, pneumococcal, shigella, tetanus, tuberculosis, etc.; virus vaccines such as hepatitis viruses, herpes viruses, human immunodeficiency viruses (HIV), influenza viruses, measles virus, mumps virus, parainfluenza virus, paramyxo viruses, polio virus, rabies viruses, respiratory syncytial viruses, rhinovirus types, rotavirus, rubella virus, etc., and parasite vaccines such as vaccines for leishamaniasis, schistosomiasis and trypanosomiasis, which may be used to produce local and/or systemic antibodies.

Preferred pharmaceutical compositions of the invention based on 100 mℓ of the composition contain:

from 0.01 to 90 ml active vaccine/antigen component,
from 0.01 to 75 ml caprylic/capric acid glycerides,
from 0.1 to 95 ml polyoxyethylene sorbitan monoesters,
and optionally one or more adjuvants or excipients;

from 0.01 to 90 ml active vaccine/antigen component,
from 0.01 to 90 ml gangliosides,
from 0.1 to 95 ml polyoxyethylene castor oil or polyoxyethylene sorbitan monoesters,
from 0.1 to 75 ml caprylic/capric acid glycerides,
and optionally one or more adjuvants or excipients; or

from 0.01 to 90 ml active vaccine/antigen component,
from 0.01 to 90 ml gangliosides and/or polyoxyethylene castor oil,
from 0.01 to 95 ml polyoxyethylene sorbitan monoesters,
from 0.1 to 75 ml caprylic/capric acid glycerides,
from 0.01 to 99 ml PBS/saline,
from 0.01 to 90 ml distilled water,
and optionally one or more adjuvants or excipients.

The invention is described in further detail in the following examples.

EXAMPLE I

A tetanus vaccine formulation consists of (a) tetanus toxoid (22.5 µl), gangliosides (10.0 µl) and Tween-20 (7.5 µl); (b) tetanus toxoid (22.5 µl) and a solution of an Imwitor/cremophor mixture (1:1) (17.5 µl); (c) tetanus toxoid (22.5 µl) and isotonic saline (17.5 µl). Formulations a, b and c are administered intranasally to mice (2.5 µl/nostril) under i.p. nembutal anaesthesia. Each mouse received 1.5 Lf tetanus toxoid. Three weeks later the mice are boosted with the same formulations and one week after, they are sacrificed and serum and nasal wash antibodies are measured. The excess serum samples are furthermore measured in living animals receiving live tetanus toxoid in the neutralisation test. The following

results were obtained:

Formulation	Blood IgG	Nasal IgA	Neutralisation
Control (s.c.)[a)]	1.09	105	0.5
Formulation a	2.45	625	0.5
Formulation b	1.54	1132	0.8
Formulation C	0.0007	30	0.000

a) Commercially available product, single administration.

EXAMPLE II

A diphtheria vaccine formulation consists of (a) diphtheria toxoid (7.5 µl), gangliosides (12.5 µl) and Tween-20 (20.0 µl); (b) diphtheria toxoid (7.5 µl), PBS-saline (12.5 µl) and a solution of an Imwitor/cremophor mixture (1:1) (20.0 µl); (c) diphtheria toxoid (7.5 µl) and isotonic saline (32.5 µl). Formulations a, b and c are administered intranasally to mice (2.5 µl / nostril) under i.p. nembutal anaesthesia. Each mouse received 1.5 Lf diphtheria toxoid. Three weeks later the mice are boosted with the same formulations and one week after they are sacrificed and serum and nasal wash antibodies are measured. The excess serum samples are furthermore measured in the neutralisation test. The following results were obtained:

Formulation	Blood IgG	Nasal IgA	Neutralisation
Control (s.c.)[a)]	0.354	34	0.012
Formulation a	0.004	36	0.025
Formulation b	2.22	352	0.020
Formulation c	0.0004	30	0.000

a) Commercially available product, single administration.

EXAMPLE III

An influenza vaccine formulation consists of (a) influenza virus vaccine (5.0 µl), gangliosides (10.0 µl), a solution of an Imwitor/cremophor mixture (1:1) (6.0 µl), distilled water (16.5 µl) and a PBS solution (2.5 µl); (b) influenza virus vaccine (5.0 µl) and isotonic saline (35.0 µl). The formulation was administered intranasally to mice (2.5 µl / nostril) under i.p. nembutal anaesthesia. Each mouse received 0.2 µg influenza HA. Four weeks later the mice were sacrificed and the serum HI titer measured. The following results were obtained:

Formulation	HI test
Control (s.c.)[a)]	1/80
Formulation a	1/160
Formulation b	1/20

a) Commercially available product.

EXAMPLE IV

A tetanus and diphtheria vaccine formulation consists of (a) tetanus toxoid (510 µl), diphtheria toxoid (169 µl), gangliosides (75 µl) and Tween-20 (750 µl); (b) tetanus toxoid (510 µl), diphtheria toxoid (169 µl) and a solution of an Imwitor/cremophor mixture (1:1) (220 µl). Six rabbits were divided into 3 groups of 2 rabbits each (4 nostrils in each group). Formulations a and b were administered intranasally (50 µl into each nostril) under unanaesthesized condition. Each rabbit received 18 Lf tetanus toxoid and 18 Lf diphtheria toxoid. The last group served as control and received only a single intranasal dose of isotonic saline. The rabbits were sacrificed by intravenous injection of pentobarbital 3½ h after dosing. Each nasal cavity was opened and individually evaluated macroscopically. The evaluator was blind as to the dosing scheme. The data show that the lesions observed were distributed almost evenly over the control and the test groups. Small focal nature and anterior location of some lesions were obtained, corresponding to the abrasion from the tip of the applicatior pipette. No macroscopic difference was observed between isotonic saline and the formulations a and b.

EXAMPLE V

Three solvents, phosphate buffered saline (PBS), caprylic/capric acid glycerides (CCG) and polyoxyethylene sorbitan monoesters (PS), were mixed together in various concentrations in order to see their interrelationship (phase diagram). The figure shows that within certain concentration rages an emulsion or a semisolid solution is achieved. CCG and PBS show a heteogeneous solution upon mixing when little or no PS is present in the system.

Viscosity, bioadhesiveness, sprayability and homogenicity (in the case of an emulsion delivery system) may be controlled, dependent on the concentration of each substance.

EXAMPLE VI

A tetanus vaccine formulation consists of (a) tetanus toxoid (510 µl), gangliosides (75 µl), polyoxyethylene sorbitan monoesters (750 µl) and saline (169 µl); (b) commercially available tetanus/diphteria vaccine, adsorbed to aluminum hydroxide. Formulation a was administered intranasally to rabbits (50 µl/nostril) using no anaesthesia nor sedation, and formulation b was administered subcutaneously. Each rabbit received 18 Lf tetanus toxoid and 18 Lf diphtheria toxoid. Three weeks later the rabbits received a booster of the same formulations. Weekly serum samples were collected from the marginal ear vein, and the samples were measured using the ToBi technique. The following results were obtained (IU/ml):

Formulation	2 weeks	3 weeks	4 weeks
a	0.034	1.012	0.847
b	0.477	1.572	1.456

EXAMPLE VII

The synergistic effect between caprylic/capric acid glycerides (CCG) and polyoxyethylene sorbitan monoesters (PS) was determined as follows:

Six diphtheria (1.5 Lf) vaccine formulations were made: (a) in phosphate buffered saline (PBS); (b) commercially available $Al(OH)_3$ adsorbed vaccine for subcutaneous injection; (c) in PBS solution containing 40% polysorbate

20; (d) in PBS solution containing 40% polysorbate 20 and 25% polyoxyethylene castor oil; (e) in 40% polysorbate 20 and 10% caprylic/capric acid glyceride (*mono*- and *di*-glycerides); and (f) in 40% polysorbate 20, 25% polyoxyethylene castor oil and 10% caprylic/capric acid glyceride (*mono*- and *di*-glycerides). The formulations were administered intranasally to mice (2.5 µl/nostril) under i.p. nembutal anaesthesia. Three weeks later the mice received a booster containing the same formulations, and a further week later they were sacrified and serum antibodies were measured. The following results were obtained:

Formulations	Diphth. IgG
a	0.0004
b	0.354
c	0.448
d	0.127
e	7.3
f	0.115

It appears that neither PS nor CCG alone can provide a satisfactory effect. This is only the case with combinations of PS and CCG.

EXAMPLE VIII

In this example the synergistic effect between caprylic/capric acid glycerides (CCG) and polyoxyethylene sorbitan monoesters (PS) was investigated further.

Seven influenza A vaccine formulations were made: (a) in phosphate buffered saline (PBS); (b) in PBS solution containing 25% polyoxyethylene castor oil; (c) in PBS solution containing 25% polyoxyethylene castor oil and 10%

caprylic/capric acid glyceride (*mono*- and *di*-glycerides); (d) in PBS solution containing 40% polysorbate 20; (e) in PBS solution containing 40% polysorbate 20 and 25% polyoxyethylene castor oil; (f) in 40% polysorbate 20 and 10% caprylic/capric acid glyceride (*mono*- and *di*-glycerides); and (g) in 40% polysorbate 20, 25% polyoxyethylene castor oil and 10% caprylic/capric glyceride (*mono*- and *di*-glycerides). The formulations were administered intranasally to mice (2.5 µl/nostril) under i.p. nembutal anaesthesia. Three weeks later the mice received a booster, containing the same formulations, and a further week later they were sacrificed and the serum antibodies were measured. The following results were obtained:

Formulation	IgG
a	0.072
b	0.053
c	0.073
d	0.114
e	0.038
f	0.354
g	0.037

Caprylic/capric acid glycerides were not tested alone, since they are insoluble in water.

EXAMPLE IX

This example illustrates the selection of the optimal CCG and PS concentration.

Seven diphtheria vaccine formulations were made: (a) in phosphate buffered saline (PBS); (b) in PBS solution containing 35% polysorbate 20; (c) in PBS solution containing

57.5% polysorbate 20; (d) in PBS solution containing 35% polysorbate 20 and 10% caprylic/capric acid glyceride (*mono*- and *di*-glycerides); (e) in PBS solution containing 57.5% polysorbate 20 and 10% caprylic/capric acid glyceride (*mono*- and *di*-glycerides); (f) in PBS solution containing 35% polysorbate 20 and 24% caprylic/capric acid glyceride (*mono*- and *di*-glycerides); and (g) in 57.5% polysorbate 20 and 24% caprylic/capric acid glyceride (*mono*- and *di*-glycerides). The formulations were administered intranasally to mice (2.5 µl/nostril) under i.p. nembutal anaesthesia. Three weeks later the mice received a booster, containing the same formulations, and one further week later they were sacrificed and the serum antibodies were measured. The following results were obtained:

Formulation	IgG
a	0.365
b	1.22
c	0.092
d	9.65
e	2.33
f	1.31
g	26.6

Caprylic/capric acid glycerides were not tested alone, since they are insoluble in water.

EXAMPLE X

The selection of the optimal CCG and PS concentration is further illustrated in this example.

Seven diphtheria and tetanus vaccine formulations were made by using fixed caprylic/capric acid glyceride (*mono*- and *di*-glycerides) concentration (10%) but variable polysorbate 20 (*mono*-ester) concentration, ranging from 28% (a) with 2% increments up to 40% (g). The formulations were administered intranasally to mice (2.5 µl/nostril) under i.p. nembutal anaesthesia. Three weeks later the mice received a booster, containing the same formulations, and one additional week later they were sacrificed and the serum antibodies were measured. The following results were obtained:

Formulation	Diphth. IgG	Tetan. IgG
a	0.07	0.04
b	0.17	0.04
c	0.10	0.02
d	0.16	0.03
e	1.60	0.01
f	1.25	0.06
g	0.27	0.004

EXAMPLE XI

This example concerns the selection of polyoxyethylene fatty acid esters. Such polyoxyethylene fatty acid esters are found as mono- and tri-esters. Diphtheria toxoids were formulated in the following different compositions: (a) in isotonic phosphate buffered saline (PBS); (b) in PBS solution containing 47% polysorbate 80 (*tri*-ester); and (c) in PBS solution containing 47% polysorbate 20 (*mono*-ester). The formulations were administered intranasally to mice (2.5 µl/nostril) under i.p. nembutal anaesthesia. Four weeks later the mice were sacrificed and the serum antibodies were measured. The following results were obtained:

Formulation	IgG
a	0.001
b	0.002
c	0.006

EXAMPLE XII

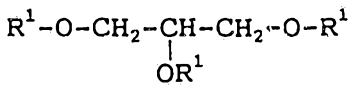
The selection of glyceride esters was performed as follows: Six tetanus (1.5 Lf) and diphtheria (1.5 Lf) vaccine formulations were made. The formulations were administered intranasally to mice (2.5 µl/nostril) under i.p. nembutal anaesthesia. Four weeks later the mice were sacrificed and serum and nasal wash antibodies were measured. The following results were obtained:

Formulation	Diphth. IgG	Tetan. IgG
Negative control	0.0013	0.0078
$C_{8 \text{ and } 10}$ diglyceride ester (Miglyol 829)(3.5%)	0.0003	0.0030
$C_{8 \text{ and } 10}$ mono-diglyceride ester (Imwitor 742)(7%)	0.0027	0.2580
C_{16} triglyceride ester (Dynasan 116)(2.5%)	0.0014	0.0057

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

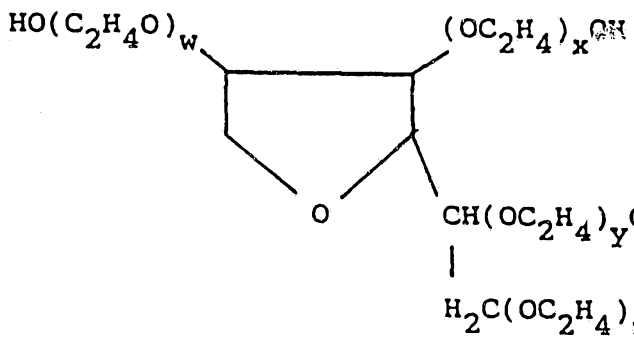
1. A pharmaceutical composition for topical administration of antigens and/or vaccines to mammal via a mucosal membrane, CHARACTERIZED by comprising one or more antigens and/or vaccines and as an adjuvant/vehicle a caprylic/capric acid glyceride of the general formula



wherein each R^1 independently is H or a C_8-C_{10} acyl group containing 1-6% free glycerol, 45-[illegible]% monoglycerides, 30-40% diglycerides and 5-9% triglycerides.

2. A pharmaceutical composition according to claim 1, CHARACTERIZED by further comprising one or more adjuvants selected from

(a) polyoxyethylene sorbitan monoesters of the general formula

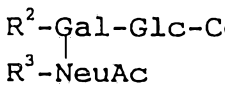


(I)

wherein R is selected among laurate, palmitate, stearate and oleate, and wherein the sum of w, x, y and z is 4, 5 or 20;

(b) polyoxyethylene castor oil produced by reacting 1 mole of castor oil or hydrogenated castor oil with 10-45 moles of ethylene oxide; and

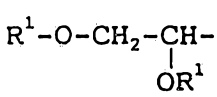
(c) gangliosides of the general formula



wherein Gal is galactose, Clc is glucose, Cer is ceramide (N-fatty acyl sphingosine) and NeuAc is N-acetyl neuraminic acid (sialic acid), and wherein R^2 may be one or more substances selected among N-acetyl galactosamine, galactose, N-acetyl neuraminic acid or combinations thereof, and R^3 is H or N-acetyl neuraminic acid.

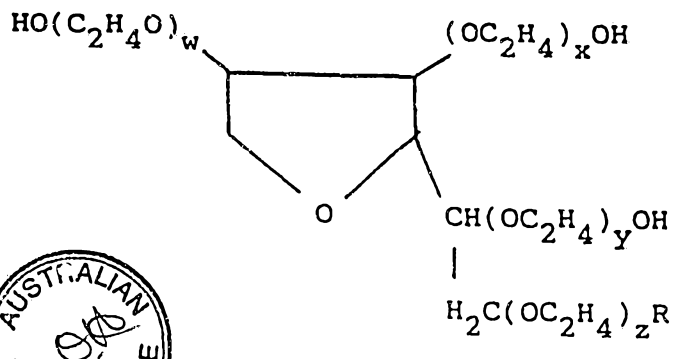
3. A pharmaceutical composition according to claim 2, CHARACTERIZED by comprising an adjuvant/vehicle mixture of

a caprylic/capric acid glyceride of the general formula



wherein each R^1 independently is H or a C_8-C_{10} acyl group containing 1-6% free glycerol, 45-50% monoglycerides, 30-40% diglycerides and 5-9% triglycerides, and

a polyoxyethylene sorbitan monoester of the general formula



wherein R is selected among laurate, palmitate, stearate and oleate, and wherein the sum of w, x, y and z is 4, 5 or 20.

4. A pharmaceutical composition according to any one of the claims 1 to 3, CHARACTERIZED in that the antigens and/or vaccines are selected among bacterial vaccines, virus vaccines and parasite vaccines, which may be used to produce local or systemic antibodies, or mixtures thereof.

5. A pharmaceutical composition according to claim 4, CHARACTERIZED in that the antigens and/or vaccines are bacterial vaccines selected among chlamydia, cholera, diphteria, haemophilus influenzae, leprosy, meningococcal, pertussis, pneumococcal, shigella, tetanus and tuberculosis, or mixtures thereof.

6. A pharmaceutical composition according to claim 4, CHARACTERIZED in that the antigens and/or vaccines are virus vaccines selected among hepatitis viruses, herpes viruses, human immunodeficiency viruses (HIV), influenza viruses, measles virus, mumps virus, parainfluenza virus, paramyxo viruses, polio virus, rabies viruses, respiratory syncytial viruses, rhinovirus types, rotavirus and rubella virus, or mixtures thereof.

7. A pharmaceutical composition according to claim 4, CHARACTERIZED in that the antigens and/or vaccines are parasite vaccines selected among vaccines for leishamaniasis, schistosomiasis and trypanosomiasis, or mixtures thereof.

8. A pharmaceutical composition according to any one of the claims 1 to 7, CHARACTERIZED by further comprising at least one compound selected from the group consisting of surfactants and/or absorption promoters, water absorbing

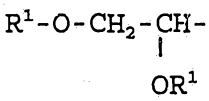
polymers, oils, emulsions, liposomes, substances inhibiting enzymatic degradation, alcohols, organic solvents, water, hydrophobic agents, pH-controlling agents, preservatives and osmotic pressure controlling agents, cyclodextrines and propellants or mixtures thereof.

9. A pharmaceutical composition according to any one of the claims 1 to 8 CHARACTERIZED in that 100 ml of the compositions contains:
from 0.01 to 90 ml active vaccine/antigen component,
from 0.01 to 75 ml caprylic/capric acid glycerides,
from 0.1 to 95 ml polyoxyethylene sorbitan monoesters,
and optionally one or more adjuvants or excipients.

10. A pharmaceutical composition according to any one of the claims 1 to 8, CHARACTERIZED in that 100 ml of the composition contains:
from 0.01 to 90 ml active vaccine/antigen component,
from 0.01 to 90 ml gangliosides,
from 0.1 to 95 ml polyoxyethylene castor oil or polyoxyethylene sorbitan monoesters,
from 0.1 to 75 ml caprylic/capric acid glycerides,
and optionally one or more adjuvants or excipients.

11. A pharmaceutical composition according to any one of the claims 1 to 8, CHARACTERIZED in that 100 ml of the composition contains:
from 0.01 to 90 ml active vaccine/antigen component,
from 0.01 to 90 ml gangliosides and/or polyoxyethylene castor oil,
from 0.01 to 95 ml polyoxyethylene sorbitan monoesters,
from 0.1 to 75 ml caprylic/capric acid glycerides,
from 0.01 to 99 ml PBS/saline,
from 0.01 to 90 ml distilled water,
and optionally one or more adjuvants or excipients.

12. Use of caprylic/capric acid glycerides of the general formula



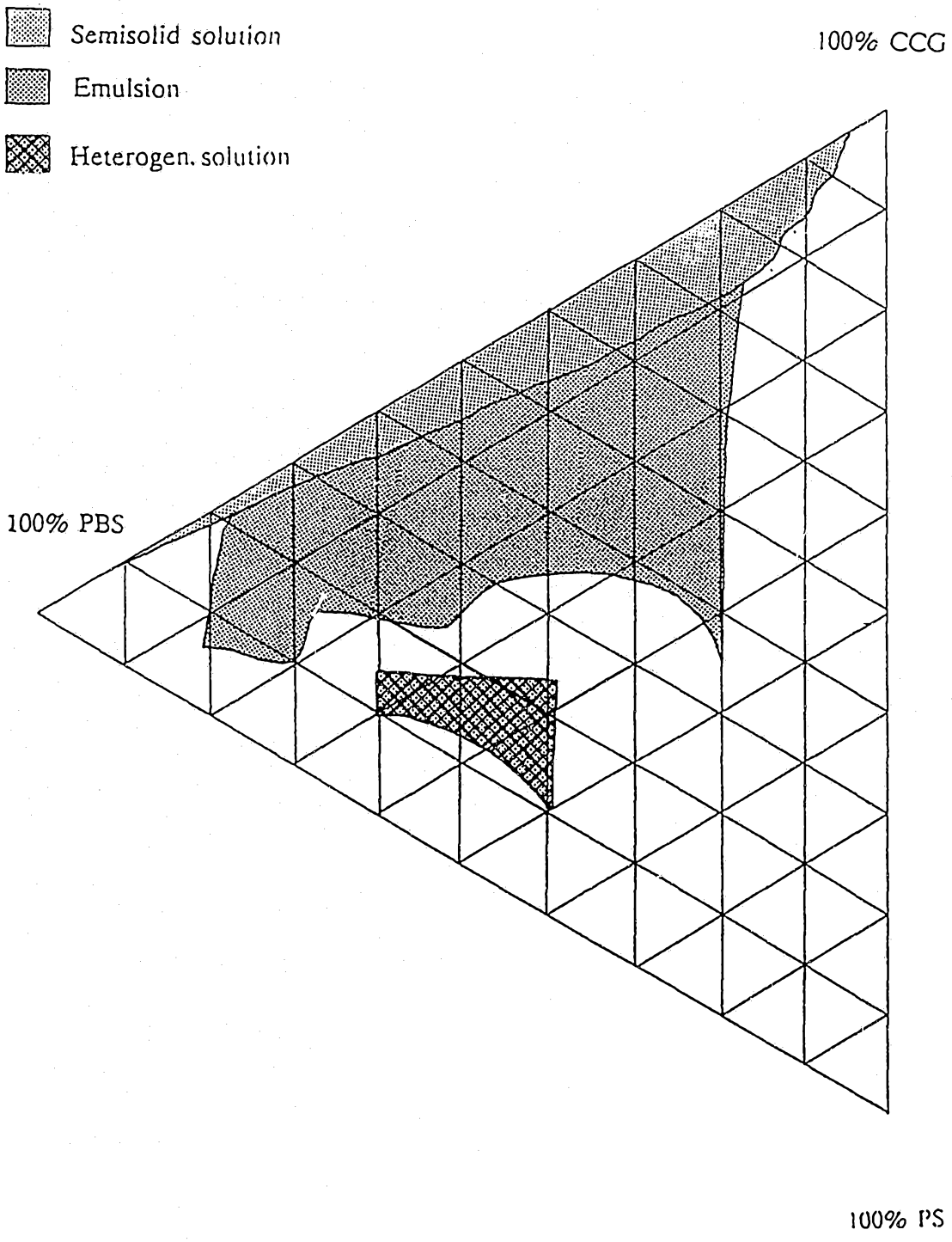
wherein each R^1 independently is H or a C_8-C_{10} acyl group containing 1-6% free glycerol, 45-50% monoglycerides, 30-40% diglycerides and 5-9% triglycerides as adjuvants/vehicles in pharmaceutical compositions for the topical administration of antigens and/or vaccines to mammals.

13. Use of a pharmaceutical composition according to any one of the claims 1 to 11 for topical administration of antigens and/or vaccines to mammals via a mucosal membrane, whereby the application of the preparation is directed to the mucosa of the nose, mouth, eye, ear, vagina or rectum.

14. Use according to claim 13, whereby the application of the composition is directed to the mucosa of the nose.

15. Pharmaceutical compositions or uses thereof, substantially as hereinbefore described with reference to the Examples and/or drawing.

DATED this 22nd day of February, 1996
Lyfjathroun H.F. and Statens Seruminstitut
By Its Patent Attorneys
DAVIES COLLISON CAVE


Semisolid solution
Emulsion
Heterogen. solution
100% CCG
100% PBS
100% PS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00062

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 39/39, A61K 9/06
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)

IPC5: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A1, 9203162 (THE WELLCOME FOUNDATION LIMITED), 5 March 1992 (05.03.92), page 9, line 1 - line 23 --	1-5,9
Y	DE, A1, 3911442 (NATIONAL INSTITUTE OF HEALTH), 2 November 1989 (02.11.89) --	1-5,9
A	US, A, 4985242 (KUNIO SEKINE ET AL), 15 January 1991 (15.01.91), column 5, line 62, claim 3 --	1-5,9
A	EP, A1, 0440289 (DUPHAR INTERNATIONAL RESEARCH B.V), 7 August 1991 (07.08.91) --	1-5,9

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 May 1994	18 -07- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl Olof Gustafsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00062

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DE, B2, 1960714 (INSTITUT MERIEUX), 28 May 1975 (28.05.75) --	1-5,9
Y	DE, A1, 3446515 (BEHRINGWERKE AG), 26 June 1986 (26.06.86), see claims --	1-5,9
P,X	EP, A2, 0544612 (THE NISSHIN OIL MILLS, LTD.), 2 June 1993 (02.06.93), see examples 10-12 and page 4 --	1-5,9
Y	GB, A, 1171125 (GLAXO LABORATORIES LIMITED), 19 November 1969 (19.11.69), page 3 - page 7 -- ---------	1-5,9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00062

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See attached sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5 and 9 (partially)

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

1. Pharmaceutical preparation for topical administration of antigens or vaccines to mammals, comprising polylxyethylene sorbitan monoesters of lauric, palmitic, stearic of oleic acid, or use of these adjuvants in pharmaceutical preparations for topical administration of antigens or vaccines according to claims 1-5 and 9 (partially).

2. Pharmaceutical preparation for ropical administration of antigens or vaccines to mammals, comprising polyoxyethylene castor oil, or use of this adjuvant in pharmaceutical preparations for topical administration of antigens or vaccines according to claims 1-5 and 9 (partially).

3. Pharmaceutical preparation for topical administration of antigens or vaccines to mammals, comprising caprylic/capric acid glycerides, or use of this adjuvant in pharmaceutical preparations for topical administration of antigens or vaccines according to claims 1-5 and 9 (partially).

4. Pharmaceutical preparation for topical administration of antigens or vaccines to mammals, comprising gangliosides of the general formula according to (d) of claim 1, or use of this adjuvant in pharmaceutical preparations for topical administration of antigens or vaccines according to claims 1-5 and 9 (partially).

5. Pharmaceutical preparation for topical administration of antigens or vaccines to mammals, comprising a mixture of caprylic/capric acid glycerides and polyoxyethylene sorbitane monoesters and vaccines or antigen formulations containing these components and optionally further components (e.g. gangliosides), or use of these adjuvants in pharmaceutical preparations for topical administration of antigens or vaccines according to claims 1-9 (partially).

6-? Different pharmaceutical preparations for topical administration of antigens or vaccines to mammals, comprising mixtures of adjuvants/vehicles (a) - (d) of claim 1, not covered by alternative 5 above, or use of these adjuvants in pharmaceutical preparations for topical administration of antigens or vaccines according to claims 1-5 and 9 (partially). Each of these mixtures is considered to represent a separate invention in view of the fact that mixtures of adjuvants are commonly used in the art.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/05/94

International application No.
PCT/DK 94/00062

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9203162	05/03/92	EP-A- 0546036	16/06/93
DE-A1- 3911442	02/11/89	FR-A,B- 2629717	13/10/89
		GB-A,B- 2217600	01/11/89
		JP-A- 2243633	27/09/90
		US-A- 5182109	26/01/93
US-A- 4985242	15/01/91	EP-A,B- 0193372	03/09/86
EP-A1- 0440289	07/08/91	AU-B- 647070	17/03/94
		AU-A- 6999091	01/08/91
		JP-A- 4210925	03/08/92
		NL-A- 9000207	16/08/91
DE-B2- 1960714	28/05/75	BE-A- 742585	03/06/70
		FR-A,B- 2025217	04/09/70
		LU-A- 57464	04/06/70
		NL-A- 6918176	08/06/70
DE-A1- 3446515	26/06/86	EP-A- 0187286	16/07/86
EP-A2- 0544612	02/06/93	AU-A- 2960892	17/06/93
GB-A- 1171125	19/11/69	BE-A- 699678	08/12/67
		DE-A- 1617502	10/02/72
		NL-A- 6707702	11/12/67