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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Emulsions suitable for use as Adjuvants for Immunological Agents

We, MERCK & CO., INC., a corporation duly organized and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is concerned with a novel adjuvant composition which when employed as a vehicle for the administration of injectable immunological substances produces higher antibody titers than are obtainable by the use of an equivalent aqueous dose thereof. In addition to potentiating the antibody titer, the adjuvant compositions of this invention also maintain these high titers over a much longer period of time than is achieved by an equivalent aqueous dose of the immunological substances.

The invention also is concerned with the use of the novel adjuvant composition in the preparation of vaccines for use in the immunization of man or animal and for use in animals to produce antisera for diagnostic and therapeutic purposes.

The need for an adjuvant in the administration of immunological substances has long been recognized and considerable work has been done to discover substances which when added to an antigen or other immunological substance would potentiate its antigenic activity and thereby its antibody stimulating capacity. To date, many such adjuvants have been discovered such as the use of alum precipitation of antigens, combining certain specific antigens some of which would potentiate the activity of the others in the mixture, the use of calcium phosphate particularly to potentiate influenza antibody production, the similar use of staphylococcus toxin which appeared to improve the antibody response to certain antigens. Several other adjuvant substances also have been considered such as tapioca, calcium, or magnesium salts, tannin, and the like which when added to certain specific antigens would increase the antibody titer over that obtainable when the antigen alone was administered. While most of these antigens gave satisfactory responses when administered along with one or at most a few antigens, none of these agents could be used successfully as an adjuvant with all immunological substances in order to potentiate the antibody response.

While the prior discoveries appeared to provide a satisfactory solution to the problem because of the relatively few antigens which were available for use to immunize man and animals, today an ever increasing number of antigens are being made available for immunization and prophylaxis and it becomes of increasingly more importance that the antigens be combined for administration in a single dosage form. To accomplish this it is necessary to use smaller quantities of the antigen in order to have them contained in a sufficiently small volume for for administration in a single dosage form. The need therefore exists for an adjuvant which is relatively nontoxic to the host and which will potentiate the antibody response to all antigens and additionally will maintain the titer over a long period of time thus endowing the host with a long period of immunity.

In an attempt to satisfy the current needs, it had been proposed to use a mineral oil emulsion in which the antigen was incorporated in the aqueous phase. While this seemed to present some promise of providing an adjuvant-type composition, it was found that it was not in fact a suitable solution because the mineral oil was not metabolized by the animal host and therefore could be a carcinogen. These workers then tried to overcome the toxic properties of the mineral oil by replacing it with peanut oil. They found,

[*Price* 4*s*. 6*d*.]

however, that the vegetable oil did not potentiate the antigenic properties of the immunological agents and was not a useful substitute for the mineral oil (Annual Review Microbiology, 1947, page 297).

It has now been discovered as a feature of this invention that an adjuvant composition in the form of a stable emulsion comprising a vegetable oil, water (or aqueous phase), mannide monooleate and aluminum monostearate, can be used as a vehicle for all immunological substances and when combined with an antigen or multiple antigens will produce abnormally high antibody titers and an abnormally long duration of immunity and additionally will be non-toxic to the host. While the vegetable oil and aqueous phase of the adjuvant composition can be varied within the limits described hereinbelow, it is essential that the other ingredients be present in the emulsion in order to obtain an adjuvant effect when an antigen or antigens are incorporated therein.

The adjuvant composition of this invention comprises from 20—75% by volume of a vegetable oil, 1.25—25% by volume of mannide monooleate, 0.5—5% by volume of aluminum monostearate, and sufficient quantity of water or aqueous solution to bring the volume to 100%.

The novel adjuvant compositions are prepared by admixing the mannide monooleate in the vegetable oil. The bivalent mixture then is combined with the aluminum monostearate. The complete trivalent oil vehicle is heated slowly, with simultaneous mixing, to a temperature sufficient to disperse the aluminum monostearate and impart some degree of thixotropy. The solution then is cooled to room temperature before addition of the aqueous phase. The aqueous phase then is added portionwise preferably with stirring, to the mixture and is emulsified by homogenizing by any number of known mechanical means, for example by the use of a colloid mill, Waring or other blendor, homogenizers, vibrators, pulsators, or other variable speed mechanical stirring devices.

While the emulsion thus prepared forms a stable adjuvant composition which can safely be used as a vehicle for the administration of immunological agents to man and animals, other materials such as secondary emulsifiers, as "Tween 80" (Registered Trade Mark), polysorbate 80 (Atlas Powder Co.) can be incorporated in the emulsion.

The emulsion prepared by this procedure is of a water in oil variety as determined by the drop test and does not conduct electricity. Its breaking point when placed in gradually heated water is greater than 80° C. and it can withstand 56° C. for at least one week, and 37° C. for at least two weeks without separation of the aqueous phase. The composition additionally is stable for at least 4 months at 37° C. and at 4° C. without evidence of breaking. The emulsion also does not break into oil and water phases after centrifugation at speeds up to 2500 r.p.m. for an hour and does not produce more than 20% excess oil phase after such centrifugation. The particle size of the internal phase, that is the water phase, is less than 100 microns and the viscosity of the whole emulsion is between 2000 to 8500 c.p.s. when tested in a Brookfield viscometer utilizing spindle No. 2 at 5 r.p.m. These specifications, however, do not have to be found in all emulsions of this invention as variations therein can be made without destroying the useful character of the adjuvant vehicle.

When the adjuvant composition of this invention is employed as a vehicle for an immunological substance, the antigen (or antigens) is incorporated in the aqueous phase prior to the addition of the aqueous phase to the vegetable oil dispersion in the process described above. The immunological agent advantageously is used in purified or concentrated form although it can as well be added in any other desired aqueous medium such as in the form of a vaccine in which it is normally administered or dissolved in a balanced salt solution, physiological saline solution, phosphate buffered saline solution, or tissue culture fluids as Eagle Basal Media [Science (1959) Volume 130, page 432, Eagle, H]; Medium 199 [Proc. Soc. Exptl. Biol. and Med., Vol. 73, page 1 (1959) Morgan, J. F. et al] or other media in which the organism may have been grown as embryonated egg amniotic, allantoic, or yolk sac material. The aqueous phase also may contain preservatives and/or substances conventionally incorporated in vaccine preparations.

The antigen itself may be in the form of purified or partially purified antigen derived from bacteria, viruses, rickettsia or their products, or extracts of bacteria, viruses, or rickettsia, or the antigen may be an allergen such as pollens, dusts, danders, or extracts of the same or the antigen may be in the form of a poison or a venom derived from poisonous insects or reptiles. In all cases the antigens will be in the form in which their toxic or virulent properties have been reduced or destroyed and which when introduced into a suitable host will either induce active immunity by the production therein of antibodies against the specific micro-organisms, extract or products of micro-organisms used in the preparation of the antigen or, in the case of allergens, they will aid in alleviating the symptoms of the allergy due to the specific allergen. The antigens can be used either singly or in combination, for example, multiple bacterial antigens can be employed, multiple viral antigens, multiple rickettsial antigens, multiple bacterial or viral toxoids, multiple allergens or com-

binations of any of the foregoing products can be combined in the aqueous phase of the adjuvant composition of this invention. Antigens of particular importance are derived from bacteria such as *H. pertussis, Leptospira pomona* and *icterohaemorrhagiae, S. typhosa, S. paratyphi A & B, C. diphtheriae, C. tetani, C. botulinum, C. perfringens, C. feseri* and other gas gangrene bacteria, *B. anthracis, P. pestis, P. multocida,* and *V. cholerae* from viruses as poliovirus (multiple types), adeno virus (multiple types), parainfluenza virus (multiple types), measles, mumps, respiratory syncytial virus, influenza (various types), Shipping fever virus (SF4), Western and Eastern equine encephalomyelitis, Jananese B. encephalomyelitis, Russian Spring Summer encephalomyelitis, Hog cholera virus, Newcastle disease virus, Fowl pox, Rabies, Feline and Canine distemper viruses, from rickettsiae as epidemic and endemic typhus or other members of the spotted fever group, from various spider and snake venoms or any of the known allergens for example from ragweed, house dust, pollen extracts, or grass pollens.

Any vegetable oil known to be suitable for use in injectable preparations and which satisfy the specifications of U.S. Pharmacopeia or National Formulary can be employed in the adjuvant compositions of this invention. Oils which have been used successfully in the preparation of these compositions are peanut oil, corn oil, olive oil, sesame oil, chaulmoogra oil, cotton-seed oil, oil of sweet almond, and persic oil. Other vegetable oils known to be useful in injectable preparations can, of course, be substituted for any of these vegetable oils.

When antigens are incorporated in the novel adjuvant composition of this invention prepared as described above, an antibody response which is adjuvant-like in nature will be elicited in the host upon administration.

The following examples describe various adjuvant compositions falling within the scope of this invention which are suitable for use in the preparation of vaccines in which any of the antigens mentioned above are incorporated either singly or in combination.

EXAMPLE 1

	By Volume
Peanut oil	30.0%
Mannide monooleate	5.0%
Aluminum monostearate	1.4%
Phosphate buffered saline	63.6%

The mannide monooleate is added to the peanut oil and thoroughly mixed with an overhead stirrer. Aluminum monostearate is added slowly with stirring to the peanut oil-mannide monooleate bivalent oil vehicle and the homogeneous mixture then is heated slowly with stirring to about 120° C. to ensure thorough dispersion of the aluminum monostearate in the bivalent oil vehicle. The homogeneous mixture is permitted to cool to room temperature (20—30° C.) after which the aqueous phase is incorporated slowly in the mixture with stirring using a variable speed mechanical stirring device to form the emulsion. The emulsion thus formed has the following characteristics, viz.:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	>95°C.
Electrical conductivity	Neg.
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	No Separation
Viscosity*	Excellent

* Viscosity data represents the rate of flow through a 27 gauge needle.

EXAMPLE 2

	By Volume
Peanut oil	35.0%
Mannide monooleate	5.0%
Aluminum monostearate	1.6%
Phosphate buffered saline	58.4%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplet in water at room temperature	Yes
Breaking point of emulsion	>95° C.
Electrical conductivity	Neg.
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	No Separation
Viscosity	Excellent

EXAMPLE 3

	By Volume
Peanut oil	65.0%
Mannide monooleate	5.0%
Aluminum monostearate	2.8%
Phosphate buffered saline	27.2%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	92° C.
Electrical conductivity	Neg.
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	<1% Separation
Viscosity	Excellent

EXAMPLE 4

	By Volume
Peanut oil	45.5%
Mannide monooleate	2.5%
Aluminum monostearate	2.0%
Phosphate buffered saline	50.0%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	>95° C.
Electrical conductivity	Neg.
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	No Separation
Viscosity	Excellent

EXAMPLE 5

	By Volume
Peanut oil	23.0%
Mannide monooleate	25.0%
Aluminum monostearate	2.0%
Phosphate buffered saline	50.0%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	>95° C.
Electrical conductivity	Neg.
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	<1% Separation
Viscosity	Excellent

EXAMPLE 6

	By Volume
Peanut oil	44.5%
Mannide monooleate	5.0%
Aluminum monostearate	0.5%
Phosphate buffered saline	50.0%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	92° C.
Electrical conductivity	Neg.
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	<1% Separation
Viscosity	Excellent

EXAMPLE 7

	By Volume
Peanut oil	60.0%
Mannide monooleate	5.0%
Aluminum monostearate	5.0%
Phosphate buffered saline	30.0%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	93° C.
Electrical conductivity	Neg.
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	No Separation
Viscosity	Excellent

EXAMPLE 8

	By Volume
Peanut oil	70.0%
Mannide monooleate	5.0%
Aluminum monostearate	3.0%
Phosphate buffered saline	22.0%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	92° C.
Electrical conductivity	Slight
Per cent oil phase produced at	
200 r.p.m. for 45 minutes	No Separation
2000 r.p.m. for 45 minutes	10% Separation
Viscosity	Fair

EXAMPLE 9

	By Volume
Peanut oil	43.0%
Mannide monooleate	5.0%
Aluminum monostearate	2.0%
Phosphate buffered saline containing Influenza Virus, polyvalent, types A and B containing a total of 500 C.C.A. units per cc (including 200 C.C.A. units type A—2, Asian strain and 100 C.C.A. units each of A/Ann Arbor, A/PR8 and B/Great Lakes per cc.)	50.0%

The above ingredients were admixed and converted into an emulsion by the method described in Example 1. The emulsion thus formed had the following stability characteristics:

Stable droplets in water at room temperature	Yes
Breaking point of emulsion	96° C.
Electrical conductivity	Neg.
Per cent oil phase produced at 200 r.p.m. for 45 minutes 2000 r.p.m. for 45 minutes	 No Separation 9.7% Separation
Stability at 4° C. for 36 days	Excellent
Stability at 25° C. for 36 days	Trace of creaming
Stability at 37° C. for 36 days	Trace of creaming
Stability at 56° C. for 36 days	40% creaming with trace of aqueous separation

EXAMPLE 10

Emulsions were prepared as described in Example 9 but replacing the peanut oil by sesame oil, corn oil, olive oil, cottonseed oil, sweet almond oil, persic oil and chaulmoogra oil, each at a concentration of 43% by volume and in each instance a stable water in oil emulsion is obtained which complies with the standards set forth hereinabove for a stable water-in-oil emulsion.

The adjuvant compositions described in the above Examples or modifications thereof can be employed as vehicles for the preparation of injectable antigen preparations. As any of the antigens described above either singly or in combination can be incorporated in the aqueous phase, only a few representative examples are given below of vaccines so prepared to illustrate their preparation and the adjuvant response obtained when administered to animals. Exhaustive examples are not included to describe every possible vaccine as to do so would only unduly lengthen the disclosure without materially adding to the knowledge concerning the use of the adjuvant preparations.

In the following Examples and discussion, the abbreviation "CCA" refers to Chick Cell Agglutinin which is determined by the method described in J. Immunology 45:273 (1942), Hirst and Pickles.

EXAMPLE 11

	By Volume
Peanut oil	53.75%
Mannide monooleate	1.25%
Aluminum monostearate	5.0%
Influenza virus vaccine, polyvalent, types A and B containing a total of 500 CCA units per cc (including 200 CCA units type A—2, Asian strain, 100 CCA units each of A/Ann Arbor, A/PR8 and B/Great Lakes per cc.)	40.0%

The above ingredients were admixed and formed into an emulsion by substantially the same method described in Example 1, and was found to comply with the standards set forth herein above for a stable water-in-oil emulsion.

EXAMPLE 12

	By Volume
Peanut oil	54.0%
Mannide monooleate	5.0%
Aluminum monostearate	1.0%
Influenza virus vaccine, polyvalent, types A and B containing a total of 500 CCA units per cc (including 200 CCA units type A—2, Asian strain, 100 CCA units each of A/Ann Arbor, A/PR8 and B/Great Lakes per cc)	40.0%

The above ingredients were admixed and formed into an emulsion by substantially the same method described in Example 1, and was found to comply with the standards set forth herein above for a stable water-in-oil emulsion.

EXAMPLE 13

	By Volume
Peanut oil	53.0%
Mannide monooleate	5.0%
Aluminum monostearate	2.0%
Influenza virus vaccine, polyvalent, types A and B containing a total of 500 CCA units per cc (including 200 CCA units type A—2, Asian strain, 100 CCA units each of A/Ann Arbor, A/PR8 and B/Great Lakes per cc.)	40.0%

The above ingredients were admixed and formed into an emulsion by substantially the same method described in Example 1, and was found to comply with the standards set forth herein above for a stable water-in-oil emulsion.

EXAMPLE 14

	By Volume
Peanut oil	52.0%
Mannide monooleate	5.0%
Aluminium monostearate	3.0%
Influenza virus vaccine, polyvalent, types A and B containing a total of 500 CCA units per cc (including 200 CCA units type A—2, Asian strain, 100 CCA units each of A/Ann Arbor, A/PR8 and B/Great Lakes per cc)	40.0%

The above ingredients were admixed and formed into an emulsion by substantially the same method described in Example 1, and was found to comply with the standards set forth herein above for a stable water-in-oil emulsion.

EXAMPLE 15

	By Volume
Peanut oil	43.0%
Mannide monooleate	5.0%
Aluminum monostearate	2.0%
Phosphate buffered saline solution containing 660 CCA units per ml polyvalent influenza virus vaccine (including 264 CCA units A/Asian, 132 CCA unit A/Ann Arbor, 132 CCA units A/PR8, 132 CCA units B/Great Lakes per ml.)	50.0%

The above ingredients were admixed and formed into an emulsion by substantially the same method described in Example 1, and was found to comply with the standards set forth herein above for a stable water-in-oil emulsion.

EXAMPLE 16

	By Volume
Peanut oil	43.0%
Mannide monooleate	5.0%
Aluminum monostearate	2.0%
Purified poliomyelitis virus vaccine, trivalent, types I, II and III containing a total of 5—6 gamma [including 4.0 gamma of type I (Parker), and 0.8 gamma each of types II (MEFI) and III (Saukett)] per cc.	50.0%

The above ingredients were admixed and formed into an emulsion by substantially the same method described in Example 1, and was found to comply with the standards set forth herein above for a stable water-in-oil emulsion.

The adjuvant effect of the vaccines described in the foregoing Examples was tested in animals by the following method.

Dual injections at zero time of 0.25 to 1.0 ml. were administered intramuscularly into each of 10 to 20 Hartley strain albino guinea pigs weighing between 450 and 550 grams each. In all experiments, several bleedings were taken at 4, 8 and 12 week intervals, and a measured aliquot of the individual sera for each bleeding was tested for antibody titer by the following technique:

For influenza antibody Standard-pattern hemagglutination-inhibition (HI) test by the procedure described in: (J.A.M.A., *172*, 1230—1238 (1960) Jensen, Woodhour and Bailey).

The results were compared to the antibody response of control guinea pigs simultaneously inoculated with 0.25 to 1.0 ml. of phosphate buffered saline containing the antigenic test dose.

The results observed are recorded in Tables I to III.

TABLE I

Guinea pig immune response to adjuvant vehicle containing influenza virus vaccine

Dose: 1.0 ml. I.M. containing 200 CCA units at day "O"

Material Inoculated	Geometric mean serum HI titer at indicated week														
	4					8					12				
	A Jap 305	A Ann Arbor	B Great Lakes 1739	A PR8	No. of Sera	A Jap 305	A Ann Arbor	B Great Lakes 1739	A PR8	No. of Sera	A Jap 305	A Ann Arbor	B Great Lakes 1739	A PR8	No. of Sera
Control Influenza vaccine (phosphate buffered saline)	5	8	18	60	10	.5	3	8	37	9	5	26	24	60	8
Adjuvant preparation of Example 11	147	181	169	446	10	128	147	128	631	7	79	181	128	388	6

TABLE II

Guinea pig immune response to adjuvant vehicle containing influenza virus vaccine

Dosage: 0.5 ml. I.M. containing 100 CCA units at day "O"

Material Inoculated	Geometric mean serum HI titer at indicated week									
	4					8				
	A Jap 305	A Ann Arbor	B Great Lakes 1739	A PR8	No. of Sera	A Jap 305	A Ann Arbor	B Great Lakes 1739	A PR8	No. of Sera
Control Influenza vaccine (Phosphate buffered saline)	3	4	9	28	19	2	7	12	69	15
Adjuvant preparation of example 12	45	69	39	294	18	37	45	23	416	17
Adjuvant preparation of example 13	52	73	56	338	19	26	39	26	274	16
Adjuvant preparation of example 14	79	119	79	338	17	45	49	34	338	15

TABLE III

Guinea pig immune response to adjuvant vehicle containing polyvalent influenza virus vaccine

Dose: 0.25 ml. I.M. containing 62.5 CCA virus units at day "O"

Vaccine	Geometric mean HI antibody titer at 4 weeks				
	A Jap 305	A Ann Arbor	B Great Lakes 1739	A PR8	No. of Sera
Control Influenza vaccine (phosphate buffered saline)	3	6	9	56	18
Adjuvant preparation of Example 9	97	158	97	548	23

WHAT WE CLAIM IS:—

1. A water-in-oil emulsion comprising, on a volume basis, from 20 to 75% of vegetable oil, from 1.25 to 25% of mannide monooleate, from 0.5 to 5% of aluminum monostearate, and sufficient aqueous phase to bring the volume to 100%.

2. A water-in-oil emulsion as claimed in claim 1, in which the vegetable oil is sesame oil, peanut oil, chaulmoogra oil, corn oil, olive oil, cottonseed oil, oil of sweet almond or persic oil.

3. An emulsion as claimed in claim 1 or 2, comprising an immunological agent incorporated in the aqueous phase.

4. An emulsion as claimed in claim 3 in which the immunological agent is influenza antigen.

5. An emulsion as claimed in claim 3 in which the immunological agent is poliomyelitis antigen.

6. A process for producing a water-in-oil emulsion, comprising dispersing aluminum monostearate in a preformed mixture of vegetable oil and mannide monooleate, adding an aqueous phase to the dispersion and emulsifying the resulting mixture.

7. A process as claimed in claim 6, in which the aluminum monostearate is dispersed in the preformed mixture of vegetable oil and mannide monooleate with agitation and simultaneous heating and the resulting dispersion is cooled before adding the aqueous phase.

8. A process as claimed in claim 7, in which the aqueous phase is added with stirring to form the emulsion.

9. A process as claimed in any one of claims 6—8 in which the vegetable oil is sesame oil,

TABLE III

Guinea pig immune response to adjuvant vehicle containing polyvalent influenza virus vaccine

Dose: 0.25 ml. I.M. containing 62.5 CCA virus units at day "O"

Vaccine	Geometric mean HI antibody titer at 4 weeks				
	A Jap 305	A Ann Arbor	B Great Lakes 1739	A PR8	No. of Sera
Control Influenza vaccine (phosphate buffered saline)	3	6	9	56	18
Adjuvant preparation of Example 9	97	158	97	548	23

WHAT WE CLAIM IS:—

1. A water-in-oil emulsion comprising, on a volume basis, from 20 to 75% of vegetable oil, from 1.25 to 25% of mannide monooleate, from 0.5 to 5% of aluminum monostearate, and sufficient aqueous phase to bring the volume to 100%.

2. A water-in-oil emulsion as claimed in claim 1, in which the vegetable oil is sesame oil, peanut oil, chaulmoogra oil, corn oil, olive oil, cottonseed oil, oil of sweet almond or persic oil.

3. An emulsion as claimed in claim 1 or 2, comprising an immunological agent incorporated in the aqueous phase.

4. An emulsion as claimed in claim 3 in which the immunological agent is influenza antigen.

5. An emulsion as claimed in claim 3 in which the immunological agent is poliomyelitis antigen.

6. A process for producing a water-in-oil emulsion, comprising dispersing aluminum monostearate in a preformed mixture of vegetable oil and mannide monooleate, adding an aqueous phase to the dispersion and emulsifying the resulting mixture.

7. A process as claimed in claim 6, in which the aluminum monostearate is dispersed in the preformed mixture of vegetable oil and mannide monooleate with agitation and simultaneous heating and the resulting dispersion is cooled before adding the aqueous phase.

8. A process as claimed in claim 7, in which the aqueous phase is added with stirring to form the emulsion.

9. A process as claimed in any one of claims 6—8 in which the vegetable oil is sesame oil,

peanut oil, chaulmoogra oil, corn oil, olive oil, cottonseed oil, oil of sweet almond or persic oil.

10. A process as claimed in any one of claims 6—9 in which an immunological agent is incorporated in the aqueous phase before the aqueous phase is added to the dispersion.

11. A water-in-oil emulsion according to claim 1 substantially as hereinbefore described in any one of Examples 1—8.

12. A water-in-oil emulsion containing an immunological agent according to claim 3, substantially as hereinbefore described in any one of Examples 9—16.

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