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(54) FORMULATION OF LIPOSOMAL VESICLES IN AQUEOUS SOLUTIONS WITH TEAR FILM CHARACTERISTICS

(57) Formulation of liposomal vesicles in aqueous vehicles with tear film characteristics. The present invention addresses the preparation of a pharmaceutical liposomal system in an aqueous solution that incorporates a substance or polymer with mucomimetic and/or mucoadhesive properties and that, owing to its components and characteristics, can replace the precorneal film. This invention is applicable to the areas of pharmacy and medicine.

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Description

OBJECT OF THE INVENTION

[0001] The present invention relates to the formulation of liposomal vesicles in aqueous solutions with tear film characteristics. The present invention describes a formulation of liposomes in aqueous vehicles which contain mucin or substances similar to mucin, mucomimetic substances or polymers with mucoadhesive properties which, at the temperature of the corneal surface, have characteristics similar to the precorneal film of the human eye. Said preparation can be used to replace the natural film and as medicinal preparation in some ocular pathologies such as the case of dry eye syndrome.

[0002] This invention is applicable to the areas of pharmacy and medicine.

STATE OF THE ART

[0003] The ocular surface is known to be formed by the conjunctival epithelium, the accessory lacrimal glands and the meibomian glands. Said surface is coated by a continuous film, with a thickness of approximately 10 μm, called precorneal film or tear film. Until a few years ago, the theoretical structure, generally accepted, included three types of components (lipid, serum-aqueous, mucinous) distributed in three layers: lipid, aqueous and mucinous (Ibrahim H, Buri P, Gurny R. Pharm Acta Helv 1988, 63: 146-53).

[0004] Recent studies consider that the precorneal film is a structure formed by the aqueous-protein and mucinous components combined to form a hydrated gel. In turn, this gel would be protected by a film of lipid character, whose components would be mainly produced by the meibomian glands and whose function would be to prevent the evaporation of the tear and improve the stability of the tear film (Pflugfelder SC, Solomon A, Stern ME. Cornea 2000; 19 (5): 644-649. McCulley JP, Shine W. Tr Am Ophth Soc 1997; 95: 79-93).

[0005] In accordance with the proposed model, the precorneal film would consist of two phases:

- Hydrophilic polar phase, in contact with the aqueous-mucinous layer which is composed of phospholipids, sphingomyelin, ceramides and cerebrosides.
- Hydrophobic non-polar phase in contact with the atmosphere and composed of non-polar lipids such as wax esters, cholesterol esters, triglycerides, free fatty acids and hydrocarbons.

[0006] The fraction of phospholipids represents approximately between 1-5% of the total of lipid secretion, the greatest concentration being phosphatidylcholine (PC) with a percentage close to 40% of the total phospholipids. Other phospholipids, such as phosphatidylethanolamine appear in a percentage of 18%, the remainder (a total of 10) being found in a range between 3 and 9%. Probably, this fraction produces a reduction in the surface tension of the aqueous phase, facilitating the extensibility of the precorneal film during the blinking movement.

[0007] The usual treatment of dry eye consists of relieving the symptoms by applying tear replacements topically. The typical composition of these preparations includes polymeric solutions such as that included in US patent 4,973,580 (Babiole) in which the ophthalmic formulation includes hyaluronic acid using hydrogen peroxide as preservative. Formulations are also disclosed wherein components similar to tear film are provided such as hypotonic lecithin solutions including viscosity agents derived from cellulose as appears in the US patent no. 4,421,748 (Trager). The use of phospholipids for the treatment of dry eye appear in various patents. Emulsion-type systems including positively-charged phospholipids such as those described in the following are disclosed: US patent 4,914,088 (1990) (Korb:); 5,278,151 (1994) (Korb:); 5,371,108 (1994) (Korb:); 5,294,607 (1994) (Korb:). Likewise, positively-charged liposomes are disclosed (US patent no. 4,804,539 (Guo) (1989) and US patent no. 4,818,537 (Guo) wherein positively-charged liposomes are used which are suspended in aqueous solutions containing high-viscosity polymers such as hydroxyethyl cellulose, methylcellulose, hydroxypropyl cellulose and vinyl derivatives such as polyvinylpyrrolidone, polyvinyl alcohol and their mixtures. They also include emulsions containing phospholipids, non-polar oils and emulsifiers such as US patent no. 6,656,460 (Benita).

[0008] In none of these patents does there appear the use of neutral or negatively-charged liposomes that are destabilized at the temperature of the precorneal film nor are they associated with mucin or with mucoadhesive substances or similar to mucin or mucomimetic as is the case of the invention described below.

DESCRIPTION OF THE INVENTION

[0009] The method object of the invention described herein relates to the preparation of a pharmaceutical form which acts as replacement of the precorneal film. The formulation incorporates liposomal vesicles of phospholipids as hydrophilic polar phase and non-polar lipids, both vehiculized in aqueous solutions which contain mucin or substances with mucomimetic substances or mucoadhesive polymers. The most relevant advantages of this invention consist of the use of phosphatidylcholine whose transition temperature is lower than the temperature of the corneal surface and also incorporates mucoadhesive and/or mucomimetic polymers or substances (mucin or polymers such as hyaluronic acid, cellulose derivatives, chondroitin sulphate, chitosan, colominic acid, thiolic derivatives or other similar components).

[0010] The components of the formulation and specifically the phospholipids which compose the liposomes are going to permit the formation, on the corneal surface, after the destabilization of the liposomal vesicles, of a water soluble monomolecular film which acts by prevent-

ing the evaporation of the aqueous phase and, furthermore, the surface tension of the latter will decrease which favours its rapid extensibility. The liposomes are prepared with phosphatidylcholine obtained from soy lecithin as majority component, cholesterol and α-tocopherol. Phosphatidylcholine contains acyl residues of fatty acids having a transition temperature lower than the temperature of the corneal surface, which guarantees the rapid formation of the film on the aqueous phase, once applied to the corneal surface. Cholesterol, for its part, stabilizes this film on reducing the fluidity of the matrix formed by the polyunsaturated residues of phosphatidylcholine. Finally, α-tocopherol ensures the chemical stability of the double bonds avoiding possible peroxidation.

[0011] The liposomes are vehiculized in aqueous solution containing an isotonizing agent (trehalose, sodium chloride, glucose...) to achieve the suitable osmolarity according to its clinical use. The solutions can be isotonic, or hypotonic. Once formed, the liposomes are incorporated in aqueous solutions which contain one or several substances or polymers with mucoadhesive or mucomimetic characteristics with the purpose of producing an increase in the permanence time of the formulation and of the components of the destabilizing components on the ocular surface. Thus, the maintenance of the new film is favoured once formed and the aqueous evaporation of the corneal surface is avoided. The concentrations of this last component will depend on the desired final viscosity in the formulation, of its interaction with the mucin, of its surface tension and of the expected rheological composition after its administration. Proteins are also included in the formulation in order to favour the stability of the film formed and improve its lubricating properties. These proteins are found in the natural tears and can be α-macroglobulin, lysozyme, lipocalin and lactoferrin.

[0012] To this formulation it is possible to add different components, in their majority components of natural tear film, which improve the characteristics of the formation and permanence of the precorneal film and/or which act as re-ephithelizers, anti-inflammatory agents and antioxidants of the ocular surface, and/or favouring corneal and conjunctival epithelial differentiation. Within these substances we have:

- · Mucoadhesive polymers such as hyaluronic acid, cellulose derivatives, chondroitin sulphate, chitosan, colominic acid, thiolic derivatives (or another similar component).
- · Neutral lipids and low polarity lipids such as waxes, cholesterol esters, triglycerides, free fatty acids and hydrocarbons.
- · Vitamin A.
- · Sodium, potassium, calcium, chloride and bicarbonate ions.
- · Albumin or pre-albumin.
- · Immunoglobin A (IGA).
- · Epithelial growth factor (EGF).
- · Beta transforming growth factor (THF-β).
- · Acidic fibroblast growth factor (aFGF).
- · Basic fibroblast growth factor (bFGF).
- · Antiproteases such as macroglobulin.
- · Neural factors such as substance P and insulinlike growth factor.
- · Antibacterial agents such as Ig G, lysozyme and complement.
- · Long-chain fatty acids such as gadoleic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, linolenic, eicosenoic, lignoceric, lactic and myristic acid.
- · Hydrophilic lipids such as phospholipids, sphingomyelin, ceramides and cerebrosides.

EMBODIMENT OF THE INVENTION

[0013] The present invention, which relates to the formulation of liposomal vesicles in aqueous solutions with characteristics of tear film, is additionally illustrated by the following examples, which are not limitative of their scope, which is defined by the attached note of claims.

[0014] The liposomal vesicles object of the invention were performed according to the classic Bangham method. To do this phosphatidylcholine, cholesterol and α-tocopherol (in different proportions) were dissolved in chloroform producing a final end concentration of phosphatidylcholine of 8 mg/ml. Once this solution is saturated with nitrogen it was introduced in the volumetric flask of the rotovapor at a temperature of 30-35ºC with moderate vacuum. After evaporating the solvent, a fine lipid film was formed on the walls which was then hydrated. The hydration phase was carried out with an aqueous solution saturated with nitrogen which contained the isotonizing agent at a temperature of 37ºC, using glass pearls which due to shear produced the formation of multilamellar vesicles. The final concentration of phosphatidylcholine was adjusted in accordance with the volume of the isotonizing vehicle.

[0015] After two hours of rest and in the absence of light, sonication of the dispersion was performed maintaining the product temperature between 5 and 10ºC with crushed ice. The preparation finalized performing 5 runs of the dispersion through 0.8 μm filters.

[0016] The mucin or mucoadhesive and/or mucomimetic substance was adding on diluting the liposomes to the desired final concentration. The final concentrations of the liposomes in the polymeric solution may range from 1 mg/ml to 40 mg/ml.

[0017] The other possible components are added, in accordance with their physicochemical characteristics, with the isotonizing agent or with the mucomimetic substances.

[0018] The *base* liposomes were prepared from phosphatidylcholine from soy, and cholesterol (8:1) and they were reconstituted with water and hypotonic solutions of sodium chloride. The influence of the sonication process on the end size of the vesicles was studied comparing the use of an ultrasound probe during 2.5 min and an ultrasound bath during 15 min (Fig. 1). The yield of the

preparation process of the lipid vesicles, in both cases, was over 90%.

[0019] The dispersions of the liposomes in water at a PC concentration of 20 mg/ml had pH values between 6.9 and 7.2. The average particle diameters for the different batches prepared with ultrasound bath varied from 392 to 478 nm. The percentage of particles over 1 μm was, in all cases, under 2%.

[0020] Measurements of surface tension was carried out with solutions of different liposome concentrations, obtaining the date in Figure 2.

[0021] Cell viability tests were carried out with aqueous hypotonic solutions of *base* liposomes and *base* liposomes with vitamin E in cell cultures of macrophages. To study the cytotoxicity, the reduction technique was used, on a mitochondrial level, of the bromide salt of 3 (4,5- dimethylthiazol- 2- yl)- 2,5- diphenyltetrazolium (MTT) to a coloured product (formazan) (Mossman T.J. Immum Methods 1983, 65:55-63). Peritoneal macrophages obtained from male Swiss mice were used. The cells were exposed to formulations which contained aqueous hypotonic solutions of *base* liposomes. As negative control, culture medium was used and as positive control 0.005% benzalkonium chloride. The solutions were incubated at 37ºC for 1 and 4 hours. The results obtained demonstrated an optimum tolerance for the *base* liposomes with and without vitamin E (Figures 3 and 4).

BRIEF DESCRIPTION OF THE FIGURES

[0022]

Figure 1: Influence of the sonication process on the final size of the vesicles comparing the use of an ultrasound probe during 2.5 minutes (-♦-) and an ultrasound bath during 15 minutes (-■-). The frequency of each class is represented, expressed in percentage, compared with the average size thereof in μm.

Figure 2: Surface tension of the aqueous dispersion of liposomes (mN/m) in accordance with its concentration. The concentration of the liposomes in the solution is expressed in phosphatidylcholine concentration (mM).

Figure 3: Cell viability (%) with aqueous hypotonic solutions of *base* liposomes with (■) and without (□) vitamin E incubated at 37ºC for 1 hour. Two concentrations of liposomes are studied (20 and 40 mg/ml) and two controls, one positive (0.005% benzalkonium chloride) and another negative (culture medium).

Figure 4: Cell viability (%) with aqueous hypotonic solutions of *base* liposomes with (■) and without (□) vitamin E incubated at 37ºC for 4 hours. Two concentrations of liposomes are studied (20 and 40 mg/ml) and two controls, one positive (0.005% benzalkonium chloride) and another negative (culture medium).

Claims

1. Ophthalmic composition intended to act as replacement of the precorneal film, **characterized in that** it contains liposomal vesicles of neutral or negatively-charged phospholipids as hydrophilic polar phase and non-polar lipids, both vehiculized in aqueous solutions which contain mucin or substances with properties similar to mucin or mucoadhesive polymers.

2. The ophthalmic composition of claim 1 which contains mucoadhesive polymers such as hyaluronic acid, cellulose derivatives, chondroitin sulphate, chitosan, colominic acid, thiolic derivatives (or another similar component).

3. The ophthalmic composition of claim 1 which contains a substance with mucomimetic properties.

4. The ophthalmic composition of claim 1 which contains neutral lipids and low-polarity lipids such as waxes, cholesterol esters, triglycerides, free fatty acids and hydrocarbons.

5. The ophthalmic composition of claim 1 which contains lipocalins.

6. The ophthalmic composition of claim 1 which contains vitamin A.

7. The ophthalmic composition of claim 1 which contains sodium, potassium, calcium, chloride and bicarbonate ions

8. The ophthalmic composition of claim 1 which contains vitamin C.

9. The ophthalmic composition of claim 1 which contains lactoferrin.

10. The ophthalmic composition of claim 1 which contains albumin or pre-albumin.

11. The ophthalmic composition of claim 1 which contains immunoglobulin A (IGA).

12. The ophthalmic composition of claim 1 which contains epithelial growth factor (EGF).

13. The ophthalmic composition of claim 1 which contains beta transforming growth factor (TGF-β).

14. The ophthalmic composition of claim 1 which contains acidic fibroblast growth factor (aFGF).

15. The ophthalmic composition of claim 1 which contains basic fibroblast growth factor (bFGF).

16. The ophthalmic composition of claim 1 which contains antiproteases such as macroglobulin.

17. The ophthalmic composition of claim 1 which contains neural factors such as substance P and insulin like growth factor.

18. The ophthalmic composition of claim 1 which contains antibacterial agents such as Ig G, lysozyme and complement.

19. The ophthalmic composition of claim 1 which contains long-chain fatty acids such as gadoleic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, linolenic, eicosenoic, lignoceric, lactic and myristic acid.

20. The ophthalmic composition of claim 1 which contains hydrophilic lipids such as phospholipids, sphingomyelin, ceramides and cerebrosides.

21. Use, in accordance with the preceding claims, of these medicinal preparations in determined pathologies such as the case of dry eye syndrome.

FIG. 1

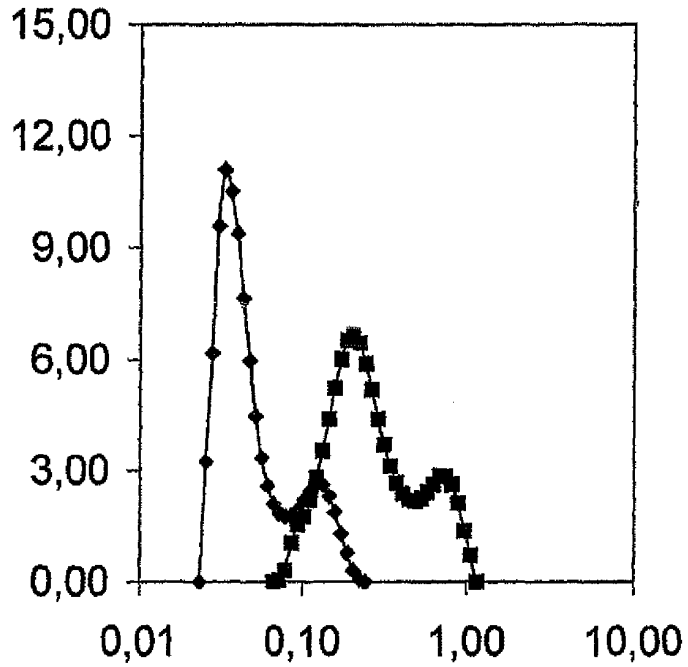


FIG. 2

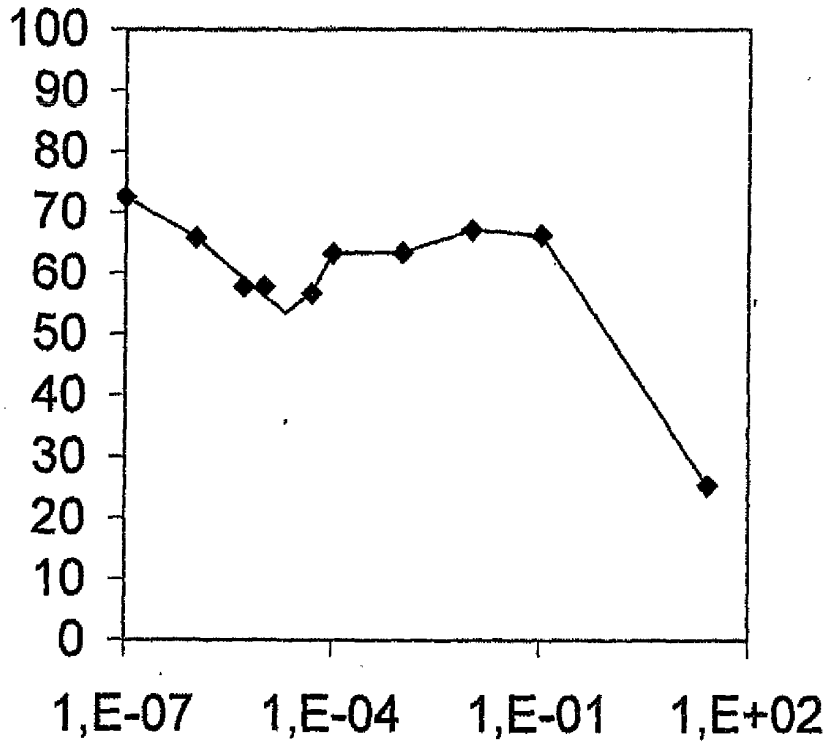


FIG. 3

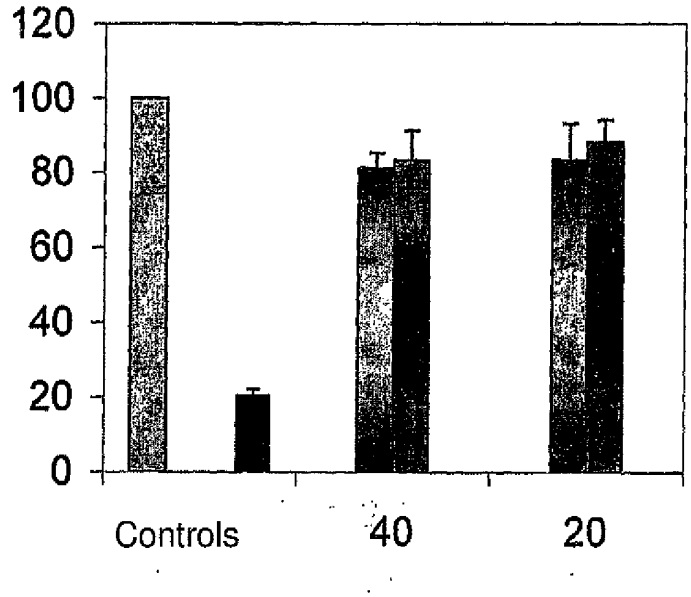
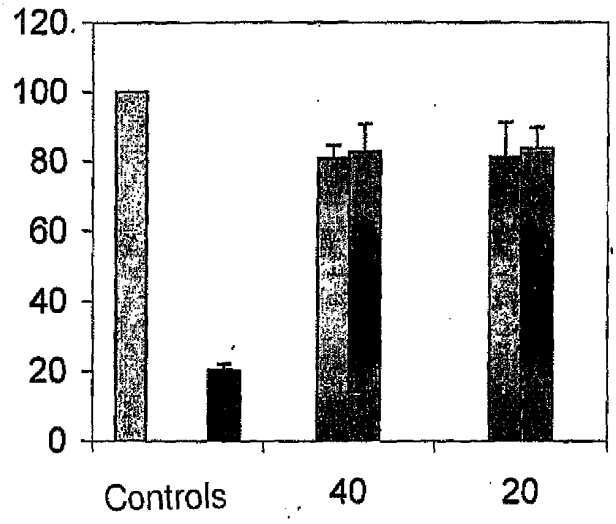


FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/ ES 2006/000208

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/127 (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

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

CIBEPAT,EPODOC, BIOSIS, EMBASE, MEDLINE, NPL, XPESP.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9011781 A1 (ALCON LABORATORIES, INC) 18.10.1990, pages 4-7, examples, claims 1-10.	1-21.
X	WO 9843616 A1 (UNIVERSITY OF IOWA RESEARCH FOUNDATION) 08.10.1998, pages 6-10, claims.	1-21.
A	WO 0051619 A1 (VISTA SCIENTIFIC LLC) 08.09.2000, page 5, lines 22- 27, pages 12-13.	1-21.
A	US 20050202097 A1 (MASKIN) 15.09.2005, page 3, paragraphs 28, 31, claim 25.	1-21.

☒ Further documents are listed in the continuation of Box C. ☒ 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" earlier 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 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
23 November 2006 (23.11.2006)	(27-12-2006)
Name and mailing address of the ISA/ O.E.P.M. Paseo de la Castellana, 75 28071 Madrid, España. Facsimile No. 34 91 3495304	Authorized officer H. Aylagas Cancio Telephone No. +34 91 349 8563

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/ES 2006/000208

C (continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005084635 A2 (INSTITUTE OF OPHTALMOLOGY) 15.09.2005, page 7-11.	1-21.

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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Form PCT/ISA/210 (patent family annex) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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. [X] Claims Nos.: **Claim 21**
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 21 relates to a method for treatment of the human or animal body by therapy. The search was carried out on the basis of the possible effects of the compositions.

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 No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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 additional fees, this Authority did not invite payment of additional fees.

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.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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