(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 March 2019 (14.03.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/049145 A1

(51) International Patent Classification:
A61K 9/00 (2006.01) *A61K 47/24* (2006.01)
A61K 47/02 (2006.01) *A61P 23/00* (2006.01)
A61K 47/10 (2017.01)

(21) International Application Number:
PCT/IL2018/051004

(22) International Filing Date:
06 September 2018 (06.09.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/556,509 11 September 2017 (11.09.2017) US

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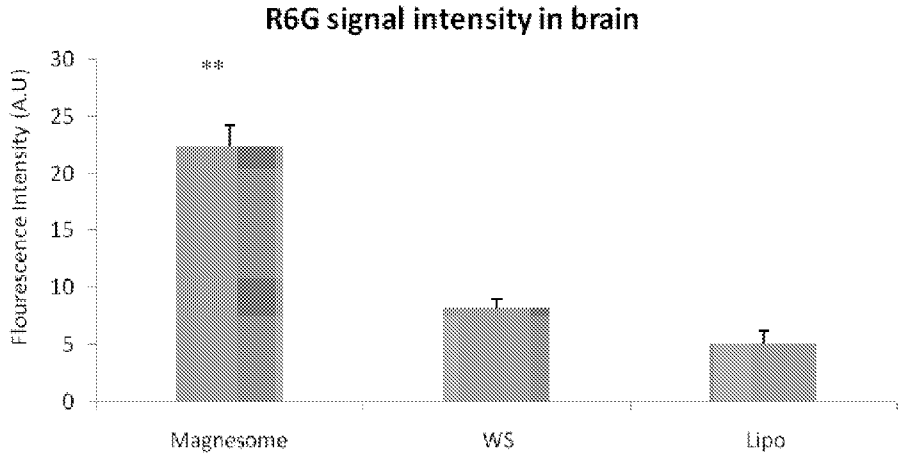
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) Title: COMPOSITIONS AND METHODS FOR NASAL ADMINISTRATION OF DRUGS TO BRAIN AND FOR SYSTEMIC EFFECT

Figure 2



(57) Abstract: The invention relates to a nasally administrable composition comprising at least one active substance in magnesium-containing vesicular carrier, said carrier comprising glycol, phospholipids, water and at least one magnesium source. Methods for nasal administration of the composition, for example, for pain relief, are also provided.

WO 2019/049145 A1

Compositions and methods for nasal administration of drugs to brain and for systemic effect

The present invention is directed to pharmaceutical compositions for nasal administration of biologically active agents.

Nasal delivery can provide drug absorption into the systemic circulation and it has also been suggested that this route of administration can offer a pathway to transport drugs the brain. There exists a need for an effective carrier to enable enhanced nasal delivery of pharmaceutically active agents.

In WO 01/06987 a carrier is disclosed, in which the major component is an ethanol/propylene glycol mixture. The carrier contains from 10 to 80% by volume of an aliphatic alcohol, 10 to 80% by volume of a glycol and 0.1 to 5% by weight of a bile salt or lecithin for administering anticonvulsive agent. The examples of WO 01/06987 illustrate a vehicle with water content of 10%.

In US 8,911,751, Touitou et al. describe the intranasal administration of active ingredients to the systemic circulation or brain with the aid of a vesicular carrier containing water : alcohol : glycol : phospholipids proportioned >30% : 12-30% : 1-30% : 0.2-10%, respectively, expressed by weight percentage.

Delivery to brain of many molecules is difficult to achieve and presents a challenge. We have surprisingly found that addition of magnesium ion to a carrier containing what we call soft phospholipid vesicles, enhances the delivery to brain and systemic action of the active molecule.

The composition of the invention, i.e., a soft phospholipid vesicle that contains magnesium, displaying enhanced nasal delivery properties to the brain and systemic action, is named herein "phospholipid magnesome" (magnesium-containing vesicular carrier).

The invention provides a nasally administrable composition comprising an active substance (in particular a physiologically/pharmaceutically active substance) in a magnesium-containing vesicular carrier, i.e., a carrier comprising glycol, phospholipids, at least one magnesium source and water. Hence, in its most general form, phospholipid magnesome is based on a vesicular carrier made of glycol: phospholipids : magnesium ion and water.

The invention also provides a method of administering a pharmaceutically active ingredient to a mammal in need thereof, for treating various conditions as described below, which method comprises the intranasal administration of a composition comprising a therapeutically effective amount of said active ingredient in a magnesium-containing vesicular carrier, i.e., a carrier comprising glycol, phospholipids, at least one magnesium source and water.

The experimental results reported below indicate that the magnesium-containing vesicular carrier of the invention enhances the delivery of drugs to the brain via the intranasal route. Accordingly, another aspect of the invention is a method of increasing the delivery of a physiologically active compound from a nasally administrable composition to the brain and /or bloodstream of mammals, the method comprises incorporating magnesium source into a vesicular carrier comprising glycol,

phospholipids, water and physiologically active compound, e.g., CNS-active drugs.

The concentration ranges of the constituents of phospholipid magnesome as set forth herein are by weight percentage, based on the total weight of the composition (unless indicated otherwise).

The concentration of the glycol in the composition (e.g., propylene glycol) is not less than 5% by weight, e.g., up to 50%, for example, 10-50 % by weight (e.g., 10-40%). The water content of phospholipid magnesome is not less than 20% by weight, e.g., not less than 30%, e.g., not less than 50%.

Phospholipids suitable for use in the preparation of the composition according to the present invention include phosphoglycerides, e.g., phosphatidylcholine (lecithin, such as soy and egg lecithin). Other phospholipids are hydrogenated phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol. Preferably, the phospholipids are present in the composition of the invention at a concentration of 0.1 to 15% by weight, e.g., 0.2 to 10% by weight. Suitable products are phosphatidylcholine commercially available from various sources, for example, from Lipoid under the brand names of Phospholipon® : the 85 G, 90G and 80 H, 90H grades; Lipoid®: Lipoid 100S PC, Lipoid S 100, Lipoid S 75, and others.

Suitable magnesium sources are compounds which dissociate in the liquid carrier to release magnesium ion (Mg^{2+}), e.g., pharmaceutically acceptable water-soluble magnesium salts such as magnesium sulfate, magnesium chloride, magnesium L-aspartate, magnesium stearate and magnesium alginate, including their hydrated forms. The concentration of the magnesium salt

is not less than 0.002%, e.g., from 0.005 to 5.0% by weight, preferably not less than 0.01% by weight, e.g., from 0.01 to 1.0% by weight or from 0.01 to 5% by weight. But the magnesium source could also be added at higher concentrations, e.g., up to 20% by weight.

Mucoadhesive agents can be added to improve adhesion of phospholipid magnesome to the nasal mucous membrane, for example, hydroxypropyl cellulose, carbomer and alginates, e.g., gel-forming agents. The desired concentration of the mucoadhesive agent depends on the properties of the agent selected and the desired mucoadhesivity. In general, the concentration of this additive varies from 0.1 to 10 % by weight.

Alcohol, such as ethanol and isopropanol, is not a mandatory component of phospholipid magnesome, but can be part of the composition, e.g., up to 25% by weight, for example, from 1 to 25% by weight. However, as shown below, the compositions are preferably free of aliphatic monohydroxy alcohols (e.g., devoid of C2-C5 alcohols, such as ethanol and isopropanol).

Antioxidants can be added to maintain the stability of the product. Some examples of antioxidants include tocopherols (vitamin E), butyl hydroxytoluene, sodium metabisulfite, potassium metabisulfite, ascorbyl palmitate and the like. These antioxidants may be present in the formulations in a concentration of from about 0.05 to 1.0 %w/w.

One method to prepare phospholipid magnesome is by dissolving phospholipids in the glycol component, or in a mixture of glycol and alcohol, adding the physiologically active substance and the other ingredients, including of course the magnesium source, and finally combining the glycolic/alcoholic component

and the aqueous component. The magnesium source is generally added in the form of a separately prepared aqueous solution, but of course, it could also be employed in a solid form to be dissolved directly in the carrier.

In some cases, the total amount of water needed for the composition is divided such that a portion, say, up to 20-30% of the total water amount, is added in conjunction with the physiologically active substance or other ingredients. Some physiologically active substances require basification or acidification of the medium to facilitate their dissolution. These physiologically active substances are first separately dissolved in an alkaline aqueous solution (or acidic aqueous solution, as required) followed by respective pH adjustment, and the clear aqueous solution with the physiologically active substance is mixed with the magnesium salt solution and the remaining amount of water and the resultant aqueous phase is combined with the phospholipids solution to form the composition. The preparation of the composition is carried out by mixing under various methods, homogenization or stirring, typically at room temperature or at an elevated temperature. To prepare forms of phospholipid magnesome capable of being adhered to nasal mucous membrane, as mentioned above, a gel component (e.g., alginate, carbomer) is prepared separately, the aqueous solution of the magnesium compound is added to the gel which is ultimately combined with the phospholipids solution under stirring.

The experimental results reported below show that phospholipid magnesome achieves more efficient delivery via the nasal route into mice brain than controls. That is, a more efficient transport of an optical probe (rhodamine 6G; abbreviated R6G) via the nasal route into mice brain, that is, deeper delivery of the optical probe was measured in comparison with either the

corresponding, magnesium-free vesicular carrier or a conventional liposome carrier. Enhanced delivery was also noted following nasal delivery of insulin-labeled with fluorescein isothiocyanate (FITC) and near-infrared (NIR) fluorescently-labeled recombinant human epidermal growth factor (EGF).

Phospholipid magnesome has also been shown to have no negative effect of the nasal cavity in a rat model, e.g., mucosal epithelium remains intact with no indication of development of inflammation following application of phospholipid magnesome.

Additional experimental work supporting this invention includes a comparison of the analgesic activity of a painkiller delivered either from phospholipid magnesome or from a conventional liposome (analgesic activity of the test formulation/compounds is indicated by decrease in the frequency of writhes in the animal model). Very good writhing inhibition was achieved with the aid of phospholipid magnesome. Pharmacokinetic study (with ketoprofen as a drug model) demonstrates rapid absorption of the drug from phospholipid magnesome via the nasal route, as compared to significantly slower absorption from the oral administration of an equal dose of drug.

Lastly, the transition temperature (Tm) values reported below suggests that phospholipid magnesome consists of soft vesicles, i.e., vesicles exhibiting higher phospholipid fluidity as compared to corresponding conventional liposome.

In addition to the components already listed above, phospholipid magnesome may further include auxiliary agents, such as surfactants, preservatives, thickening agents, buffers, viscosity and absorption enhancing agents and agents capable of adjusting the pH and osmolarity of the formulation.

Phospholipid magnesome may also include agents such as tolerance enhancers to reduce or prevent drying of the mucus membrane and to prevent irritation thereof.

Suitable preservatives that can be used with phospholipid magnesome include preservatives acceptable for nasal use, for example, benzyl benzalkonium salts.

Regarding buffers, phospholipid magnesome may include a buffer for maintaining the formulation at a pH of about 7.0. The particular buffer, of course, can vary depending upon the particular nasal delivery system used, as well as the specific active molecule selected. Buffers that are suitable for use in the present invention include, for example, acetate, citrate, prolamine, carbonate and phosphate buffers and combinations thereof. The pharmaceutical formulations of the present invention may include a pH adjusting agent.

Regarding thickening agents, the viscosity of phospholipid magnesome can be maintained at a desired level using a pharmaceutically acceptable thickening agent. Thickening agents that can be added to the compositions of the present invention include for example, methyl cellulose, xanthan gum, tragacanth, adhesives, guar gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, polyvinyl alcohol, alginates, acacia, chitosans, mucoadhesive polymer-systems like poly(acrylates), cellulose derivatives, hyaluronic acid, hyaluronic acid derivatives, chitin, collagen, pectin, starch, poly(ethylene glycol), sulfated polysaccharides, carrageenan, Na-alginate, gelatine, pectin and combinations thereof. The desired concentration of the thickening agent will depend on the agent selected and the viscosity desired.

The invention also provides a nasally administrable composition comprising an active substance (in particular a physiologically/pharmaceutically active substance) in a carrier comprising glycol, phospholipids, water, at least one magnesium source as described above and at least one oil, e.g., vegetable oil such as hemp seed oil, sesame oil or olive oil, for example, up to 5% by weight.

The compositions of the invention can be prepared as liquid, viscous liquid or gel. It can also be incorporated into different dosage forms acceptable for the nasal route of administration, e.g., they may be incorporated into various nasal creams, nasal ointments, nasal suspensions and nasal gels in addition of course to nasal liquids.

As used herein, nasally administering or nasal administration includes administering the compositions into naristilles of the nose to the mucous membranes of the nasal passage or nasal cavity of the mammal. For example, the compositions of the invention can be delivered to the nasal cavity as drops; liquid delivered to the nasal cavity as non-aerosol spray (packaged in a bottle with an atomizer attachment, such as a pump-sprayer) or as an aerosol spray packed in a container under pressure to emit pressurized suspension, as described in detail in Remington's Pharmaceutical Sciences (16th edition, Chapters 83 and 92). Suitable devices [nasal sprays, metered-dose sprays, squeeze bottles, liquid droppers, disposable one-dose droppers, nebulizers, cartridge systems with unit-dose ampoules, single-dose pumps, bi-dose pumps, multiple-dose pumps] are of course commercially available from various sources. Regarding spray devices, it should be noted that both single (unit) dose or multiple dose systems may be used. Typically, a spray device comprises a bottle and a pump. Typically, the volume of liquid that is dispensed in a single spray actuation is in the range

of from 5 to 250 microlitters/each nostril/single administration and the concentration of the active ingredient in the formulation may be readily adjusted such that one or more spray into the nostrils will comply with the dosage regimen. Administration of compositions of the present invention may also take place using a nasal tampon or nasal sponge containing the compositions.

The nasal administration may be used for systemic delivery of active compounds through the circulation or for CNS delivery for treating CNS-originating diseases or conditions.

A wide range of physiologically active substances can be administered via the nasal route with the aid of phospholipid magnesome to treat one or more of the following diseases and conditions: neurological disorder, muscular disturbances, ticks, brain, CNS, insomnia, pain, anxiety, migraine, glioma, epilepsy, blastoglioma, cancer, acne, IBD, Chron's disease, loss of appetite, fear, distress, panic, tremor, multiple sclerosis, autism, Alzheimer, menopause, Parkinson, awakens, good mood, *post-traumatic*, alcoholic and nonalcoholic fatty liver, hysteria, seizure and types of encephalopathy, including hepatic- encephalopathy.

Phospholipid magnesome is, under a preferred aspect of the invention, suitable for enhancing the transport of hydrophilic compounds of any molecular size, inter alia, peptides, proteins and hydrophilic small molecule compounds (in the form of pharmaceutically acceptable salts), and nucleic acid based molecules such as antisense, iRNA, siRNA, micro RNA and anti-microRNA to the brain or systemically, as indicated by the results reported below. That is, peptides, amino acids, proteins and steroid hormones (e.g. insulin, insulin derivatives, insulin detemir, insulin monomeric, oxytocin,

LHRH, LHRH analogues, adreno-corticotropic hormone, somatropin, leuprolide, calcitonin, parathyroid hormone, estrogens, testosterone, adrenal corticosteroids, megestrol, progesterone, sex hormones, growth hormones, growth factors, etc.)

But other active compounds can benefit from being delivered from phospholipid magnesome, for example cannabinoids and in particular derivatives of cannabinoids that have been rendered hydrophilic. The cannabinoid compound, either natural or synthetic, may be utilized in a solid form or in the form of an extraction concentrate, solvent extract, oil extract and oil solution, possibly surfactant-containing extracts and solutions. A non-limiting list of cannabinoids is given below:

Δ^9-THC, available under the name dronabinol; and Δ^8-THC.

CBD (chemical named 2-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenedi-ol). The synthesis of CBD was described, for example, by *Gaoni Y, Mechoulam R [Tetrahedron Letters. 26 (8): 1083-1086 (1985)]; and by Petil*ka et al. [Helv. Chim. Acta, 52:1102 (1969); and in J. Am. Chem. Soc., 87:3273 (1965)].

CBN (chemically named 6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol). The synthesis of CBN was described by Novak et al., Tetrahedron Letters, 23:253 (1982); and by Jesse A. Teske and Alexander Deiters *Org. Lett.*, 2008,*10* (11), pp 2195-2198.

Nabilone (chemically named: 3-(1,1-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-9-H-dibenzo[b,d]pyran-9-one). The preparation of this synthetic cannabinoid is described, for example, in US 3,968,125.

Levonantradol (chemically named: (-)-(6S,6aR,9R,10aR)-5,6,6a,7,8,9,10,10a-octahydro-6-methyl-3-[(R)-1-meth- yl-4-phenylbutoxy]-1,9-phenanthridinediol 1-acetate. The preparation of this synthetic cannabinoid is described, for example, in US 4,206,225, US 4,232,018, US 4,260,764, US 4,235,913, US 4,243,674, US 4,263,438, US 4,270,005, and US4,283,569.

(-)-HU-210 (chemically named: (-)-(3S,4S)-7-hydroxy-Δ^6-tetrahydrocannabinol-1,1-dimethylhept- yl).The preparation of this synthetic cannabinoid can is found in US 4,876,276 and US 5,521,215.

(+)-HU-210 (chemically named: (+)-(3S,4S)-7-hydroxy-Δ^6-tetrahydrocannabinol-1,1-dimethylhept-yl). The preparation of this synthetic cannabinoid is described in US 4,876,276 and US 5,521,215.

11-hydroxy-Δ^9-THC, which can be prepared via the synthetic route describedby Siegel et al., J. Org. Chem., 54:5428 (1989).

Δ^8-tetrahydrocannabinol-11-oic acid, which is naturally occurring derivative and can be produced synthetically employing methods described in US 6,162,829.

CP 55,940 (chemically named: 4-(1,1-dimethylheptyl)-2,3' dihydroxy-6'alpha-(3-hydroxypropyl)-1',2',3',4',5',6'-hexahydrobiphenyl), which is commercially available from Tocris Cookson, Inc., Its preparation has been described; see for example US 4,371,720 and US 4,663,474.

R(+)-WIN 55,212-2 (chemically named: (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-de]-1- ,4-benzoxazin-6-yl]-1-naphthalenyl-methanone) is commercially available in the form of its mesylate salt from various

manufacturers.

Crude herbal cannabis - in countries and jurisdictions where it is, or will become, legally allowed - can also be delivered using the composition of this invention.

It should be noted that the compositions of the invention is not limited to the delivery of a single active ingredient, and it may be used to provide combination therapy, that is, a second active ingredient could be added to the composition.

The following active ingredients (and of course, pharmaceutically acceptable salts thereof) can also be incorporated into phospholipid magnesome:

-Antimalarial agents (e.g. artemisinin derivatives, dihydroartemisinin, artemotil, chloroquine, primaquine, doxycillin, quinine, aminoquinolines, cinchona alkaloids, antifolates, quinidine, melfoquine, halofantrine, lumefantrine, amodiaquine, pyronaridine, tafenoquine, artesunates, artemether, artemotil, biguanides, proguanil, chloproguanil, diaminopyrimidines, pyremethamine, trimethoprim, dapsone, sulfonamides, atovaquone, sulfadoxine-pyrimethamine, N-acetyl cysteine, piperaquine, DHA-piperaquine, lumefantrine, dermaseptins, bisphosphonates, quercitin etc. The drugs could be used alone or in combinations.

-OTC drugs (e.g. antipyretics, anesthetics, cough suppressants, etc.)

-Antiinfective agents

-Antibiotics (e.g. penicillins, cephalosporins, macrolids, tetracyclines, aminoglycosides, anti-tuberculosis agents, doxycycline, ciprofloxacine, moxifloxacine, gatifloxacine, carbapenems, azithromycine, clarithromycine, erythromycine, ketolides, penems, tobramyicin, filgrastim, pentamidine, microcidin, clerocidin; amikacine, etc.)

-Genetic molecules (e.g. Anti-sense oligonucleotides, nucleic acids, oligonucleotides, DNA, RNA, iRNA, siRNA, micro RNA and anti-microRNA)

-Anti-cancer agents (e.g. anti-proliferative agents, anti-vascularization agents, taxol, etopside, cisplatin, etc.)

-Anti-protozoal agents

-Antivirals (e.g. acyclovir, gancyclovir, ribavirin, anti-HIV agents, anti-hepatitis agents, famciclovir, valaciclovir, didanosine, saquinavir, ritonavir, lamivudine, stavudine, zidovudine, etc.)

-Anti-inflammatory drugs (e.g. NSAIDs, steroidal agents, cannabinoids, leukotriene-antagonists, tacrolimus, sirolimus, everolimus, etc.)

-Anti-allergic molecules (e.g. antihistamines, fexofenadine)

-Bronchodilators

-Vaccines and other immunogenic molecules (e.g. tetanus toxoid, reduced diphtheria toxoid, acellular pertussis vaccine, mums vaccine, smallpox vaccine, anti-HIV vaccines, hepatitis vaccines, pneumonia vaccines, influenza vaccines, TNF-alpha-antibodies etc.)

-Anesthetics, local anesthetics.

-Antipyretics (e.g. paracetamol, ibuprofen, diclofenac, aspirin, etc.)

-Agents for treatment of severe events such cardiovascular attacks, seizures, hypoglycemia, etc.

-Afrodisiacs from plants or synthetics

-Anti-nausea and anti-vomiting.

-Immunomodulators (immunoglobulins, etc.)

-Cardiovascular drugs (e.g. beta-blockers, alpha-blockers, calcium channel blockers, etc.)

-Peptide and steroid hormones (eg. insulin, insulin derivatives, insulin detemir, insulin monomeric, oxytocin, LHRH, LHRH analogues, adreno-corticotropic hormone, somatropin, leuprolide, calcitonin, parathyroid hormone, estrogens,

testosterone, adrenal corticosteroids, megestrol, progesterone, sex hormones, growth hormones, growth factors, etc.)

-Peptide and protein related drugs (e.g. amino acids, peptides, polypeptides, proteins)

-Vitamins (e.g. Vit A, Vitamins from B group, folic acid, Vit C, Vit D, Vit E, Vit K, niacin, derivatives of Vit D, etc.)

- Autonomic Nervous System Drugs

-Fertilizing agents

-Antidepressants (e.g. buspirone, venlafaxine, benzodiazepins, selective serotonin reuptake inhibitors (SSRIs), sertraline, citalopram, tricyclic antidepressants, paroxetine, trazodone, lithium, bupropion, sertraline, fluoxetine, etc.)

-Agents for smoking cessation (e.g. bupropion, nicotine, etc.)

-Agents for treating alcoholism and alcohol withdrawal

-Lipid-lowering agents (e.g. inhibitors of 3 hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, simvastatin, atrovastatin, etc.)

-Drugs for CNS or spinal cord (benzodiazepines, lorazepam, hydromorphone, midazolam, Acetaminophen, 4'-hydroxyacetanilide, barbiturates, anesthetics, etc.)

- Anti-epilepsic agents (e.g. valproic acid and its derivatives, carbamazepin, etc.)

-Angiotensin antagonists (e.g. valsartan, etc.)

-Anti-psychotic agents and anti-schizophrenic agents (e.g. quetiapine, risperidone)

-Agents for treatment of Parkinsonian syndrome (e.g. L-dopa and its derivatives, trihexyphenidyl, etc.)

-Anti-Alzheimer drugs (e.g. cholinesterase inhibitors, galantamine, rivastigmine, donepezil, tacrine, memantine, N-methyl D-aspartate (NMDA) antagonists).

-Agents for treatment of non-insulin dependent diabetes (e.g. metformine,

-Agents against erectile dysfunction (e.g. sildenafil, tadalafil, papaverine, vardenafil, PGE1, etc.)

-Prostaglandins

-Agents for bladder dysfunction (e.g. oxybutynin, propantheline bromide, trospium, solifenacin succinate etc.)

-Agents for treatment menopausal syndrome (e.g estrogens, non-estrogen compounds, etc.)

-Agents for treatment hot flashes in postmenopausal women

-Agents for treatment primary or secondary hypogonadism (e.g. testosterone, etc.)

-Cytokines (e.g. TNF, interferons, IFN-alpha, IFN-beta, interleukins etc.)

-CNS stimulants

-Muscle relaxants

-Anti paralytic gas agents

-Appetite stimulators/depressors (e.g. cannabinoids, etc.)

-Gastrointesinal absorption modifiers

-Narcotics and Antagonists (e.g. opiates, oxycodoneetc.)

-Painkillers (opiates, endorphins, tramadol HCl, codein, NSAIDs, gabapentine, fentanil and pharamaceutically acceptable salts thereof etc.)

-Hypnotics (Zolpidem, benzodiazepins, barbiturates, ramelteon, etc.)

-Histamines and Antihistamines

-Antimigraine Drugs (e.g. imipramine, propranolol, sumatriptan, eg.)

-Diagnostic agents (e.g. Phenolsulfonphthalein, Dye T-1824, Vital Dyes, Potassium Ferrocyanide, Secretin, Pentagastrin, Cerulein, etc.)

- anti-inflammatory drugs

-ADHD related medication (e.g. methylphenidate, dexmethylphenidate, dextroamphetamine, d- and l-amphetamin racemic mixture, pemoline, etc.)

-Diuretic agents

-Anti-osteoporotic agents (e.g. bisphosphonates, aledronate, pamidronate, tirphostins, etc.)

-Drugs for treatment of asthma

-drugs for post trauma, crisis , anxiety treatment

-Anti-Spasmotic agents (e.g. papaverine, etc.)

-Agents for treatment of multiple sclerosis and other neurodegenerative disorders (eg. mitoxantrone, glatiramer acetate, interferon beta-1a, interferon beta-1b, etc.)

-Plant derived agents from leave, root, flower, seed, stem or branches extracts.

-Anti anxiety drugs.

The experimental work reported below shows that the carrier of the invention can be used for delivery to the brain via the nasal route of pharmaceutically active agents spanning a wide range of molecular weight and physiochemical properties, e.g.:

large molecules (e.g., molecular weight >1000 g/mol) such as peptide hormones (insulin, oxytocin) and proteins.

small molecules, for example, with molecular weights up 500 g/mol including compounds bearing hydrophilic functional groups (carboxylic acid, hydroxyl) and salt-forming groups, e.g., compounds administered as acid addition salts, such as tramadol hydrochloride (analgesic), butorphanol tartrate (opioid); rizatriptan benzoate (antimigraine) safinamide mesylate (anti-Parkinson); and other small molecules such as mannitol, ketoprofen and brotizolam, and also cannabinoids (CBD, CBN, THC or mixtures thereof).

Accordingly, specific aspects of the invention include compositions comprising a pharmaceutically active agent selected from:

an analgesic, antimigraine and/or antipyretic agent (e.g., tramadol, ketoprofen, rizatriptan and pharmaceutically acceptable salts thereof);

an opioid (e.g., butorphanol and pharmaceutically acceptable salts thereof);

an anti-Parkinson drug (e.g., safinamide and pharmaceutically acceptable salts thereof);

a sedative and/or hypnotic drug (e.g., brotizolam or a pharmaceutically acceptable salt thereof);

a peptide or a polypeptide (e.g., peptide hormone selected from the group consisting of insulin and oxytocin, or bacitracin);

a cannabinoid or a mixture of cannabinoids (CBD, THC, CBN or a mixture thereof).

It should be noted that concentration of the active agent in the composition of the invention may vary broadly, from 0.01 to 20% by weight.

Additional aspect of the invention relates to a method of administering an active substance to a mammal in need thereof, comprising intranasal administration of a composition comprising a therapeutically effective amount of the active substance (e.g., an analgesic, antimigraine and/or antipyretic agents; an opioid; an anti-Parkinson drug; a sedative and/or hypnotic drug; peptide or a polypeptide; a cannabinoid) in a magnesium-containing vesicular carrier that contains glycol, phospholipids, water and at least one magnesium source.

Use of a vesicular carrier that contains glycol, phospholipids, water and at least one magnesium source as described herein (e.g., monohydroxy aliphatic alcohol-free carrier), for intranasal administration of pharmaceutically active agents for systemic delivery or CNS (central nervous system) delivery, is another aspect of the invention.

Owing to its ability to offer a rapid onset of an analgesic effect, the composition of the invention is especially useful in a method for pain relief, the method comprising the intranasal administration to a patient of a composition comprising a carrier and therapeutically effective amount of an active substance selected from the group consisting of an analgesic, an opioid, an antimigraine agent and an anti-inflammatory, wherein the carrier in a magnesium-containing vesicular carrier comprising glycol, phospholipids, water and at least one magnesium source.

The nasal vesicular composition without an added drug/pharmaceutically active compound, can also be used, e.g., for CNS effect. The carrier of the invention may contain high concentration of magnesium, useful for such purpose. Hence nasally administrable vesicular composition comprising glycol, phospholipids, water and at least one magnesium source, wherein the composition is devoid of an active substance, constitutes another aspect of the invention. Concentration ranges for the components are as previously indicated.

In the drawings:

Figure 1: Three-dimensional micrographs of the olfactory region for mice brain treated with R6G at a dose of 10mg/kg from phospholipids magnesome, water solution and Liposome. Height: 589µm, width: 589µm, depth: 606µm, lens x20 (A1-MP microscope NIKON- Japan).

Figure 2: a bar diagram showing fluorescent intensity (A.U.) in the olfactory region of mice brain at 10 min after treatment with R6G at a dose of 3mg/kg in phospholipids magnesome, water solution and Liposome; Mean±SD. Auto fluorescence was subtracted. **P value <0.01 considered very significant.

Figure 3: Three-dimensional micrographs of the olfactory region for mice brain treated with R6G at a dose of 10mg/kg from phospholipid magnesome vs. soft vesicles. Height: 979µm, width: 979µm, depth: 507µm, lens x20 (A1-MP microscope NIKON-Japan).

Figure 4: a bar diagram showing fluorescent intensity (A.U.) in the olfactory region of mice brain at 10 min after treatment with Insulin-FITC at a dose of 1mg/kg in phospholipids magnesome, WS and Lipo; Mean±SD. Auto fluorescence was subtracted. **P value <0.01 considered very significant.

Figure 5: NIR images for mice brains treated with EPG IRDye 800CW at a dose 1mg/Kg from phospholipid magnesome as compared to water solution and liposome.

Figure 6: a bar diagram showing fluorescent intensity (A.U.) in the olfactory region of mice brain at 10 min after

treatment with EGF-IRDye 800CW at a dose of 1mg/kg in phospholipids magnesome, as compared to water solution and liposome; Mean±SD. Auto fluorescence was subtracted. **P value <0.01 considered very significant.

Figure 7: a bar diagram showing the writhing counts for mice treated with Oxytocin at a dose of 0.4mg/kg phospholipid magnesome, WS, Lipo and Untreated control 5, 30 and 120 min prior to IP injection of acetic acid; Mean±SD.P<0.05 for phospholipid magnesome vs. untreated, WS and Lipo 5 min. P<0.01 for phospholipid magnesome vs. untreated, WS and Lipo at 30 and 120 min time points.

Figures 8A-8D: Representative micrographs of nasal cavities excised from rats that (A) received no treatment or treated with (B) phospholipid Magnesome, (C) NS and (D) SLS nasal solution.

Figures 9A-9B: DSC thermograms for (A) phospholipids magnesome, (B) liposome obtained by Mettler Toledo DSC-1 STAR system (China).

Figure 10: a graph showing the pharmacokinetic profile of Ketoprofen in plasma following nasal administration of Ketoprofen from the composition of the invention vs. Ketoprofen oral administration, each at a dose of 14mg/Kg. Results (mean±SD)** P<0.01, very significant at 10min, and 30min.

Examples

Abbreviations used in the examples:

PL- Phospholipid

PG- Propylene Glycol

WS- Water solution

DDW- Double distilled water

ETOH- Ethanol

HSO- Hemp Seed Oil

SO- Sesame oil

Lipo- Liposome

The following materials were used: Magnesium Sulfate, anhydrous from J.T.Baker, USA; Phospholipon 90 G - Lipoid, Phospholipid GmbH, Germany; Propylene Glycol - Tamar, Israel and Vitamin E Acetate- Tamar, Israel.

Example 1

Nasal delivery to brain

Nasal delivery of R6G in the composition of the invention to the olfactory region of mice brain visualized by a Multiphoton Microscope (A1-MP microscope NIKON- Japan).

Part I

Nine female C57Bl/6J mice (8-9 weeks), were divided equally into 3 groups of administration. The following formulations were prepared:

	Phospholipid magnesome (%w/w)	Water solution %w/w	Liposome % w/w
R6G	1	1	1
PL	3	-	3
PG	15	-	-
Sodium alginate	0.6	-	0.6
Magnesium sulfate	0.03	-	-
DDW	To 100	To 100	To 100

Preparation: PL was dissolved in PG, R6G was added and then 20% of the total DDW amount was added, through mixing. In a separate vessel, Sodium Alginate was dispersed in 10% of the total amount of DDW and mixed with Magnesium Sulfate aqueous solution (10 mg/ml). The PL solution was then added to the alginate gel through mixing with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany), then the remaining DDW was added.

R6G was administered nasally to mice from the three above formulations at a dose of 10 mg/kg animal (~0.2mg\ 20µl/ mouse). Ten minutes after treatments, the animals were sacrificed, the brains were removed, washed with normal saline and examined by the Multiphoton Microscope using the following conditions: excitation λ 850 nm, field of image of 589x589x606 nm (width x height x depth), lens x60 laser, intensity 5%, scanning 512, scan speed 0.5, line skipping 2, Luts1500 and zoom1. The fluorescence intensity of the probe (arbitrary units A.U.) in the olfactory region in brain was further assessed using Image Pro-Plus software.

The micrographs are presented in Figure 1 (left: phospholipid magnesome; middle: water solution; right: liposome). Figure 2 is a bar diagram showing the fluorescent intensity. The micrographs of Multiphoton Microscopic examination and the semi- quantification showed that higher fluorescent signals are found in the olfactory region in the groups treated with phospholipid magnesome containing R6G relative to different control nasal compositions Water Solution (WS) and Liposome (Lipo). It is worthy to notice that the fluorescence intensity in the WS group was apparently higher (yet not statistically significant) than that achieved in the animals receiving Lipo treatment.

These results point towards the efficiency of phospholipid magnesome to improve the hydrophilic probe delivery to the examined region in the brain.

Part II

In this part, the effect of phospholipid magnesome was evaluated in comparison with a composition without magnesium.

Female C57Bl/6J mice (8-9 weeks), were divided into 2 groups of administration. The following formulations were prepared:

	Phospholipid magnesome (%w/w)	No Mg %w/w
R6G	1	1
PL	3	3
PG	15	15
Magnesium sulfate	0.03	-
DDW	To 100	To 100

The administered dose and the experimental procedure was performed as described in Part I of this example.

Figure 3 shows the three-dimensional micrographs. The results of this part of the experiment show deeper delivery of R6G into the examined brain region. The semi quantification indicated a fluorescence of 13.2 A.U. to the phospholipid magnesome as compared to 8.0 A.U. for the soft vesicles, such results pointing towards the superiority of phospholipid magnesome as carrier for brain delivery of drugs.

Example 2

Nasal delivery of Insulin- FITC to brain

Nasal delivery of Insulin FITC to the olfactory region in mice brain from the composition of the invention and two control compositions, examined by a Multiphoton Microscope (A1-MP microscope NIKON- Japan).

Nine female C57Bl/6J mice (8-9 weeks) were divided equally into 3 groups of administration. The following formulations were prepared.

	Phospholipid magnesome (%w/w)	Water solution %w/w	Liposome % w/w
Insulin FITC	0.1	0.1	0.1
PL	3	-	3
PG	15	-	-
Sodium alginate	0.6	-	0.6
Magnesium sulfate	0.03	-	-
DDW	To 100	To 100	To 100

Preparation: PL was dissolved in PG, Insulin FITC was added then 20% of the total DDW amount was added, through mixing. In a separate vessel, Sodium Alginate was dispersed in 10% of the total amount of DDW and mixed with Magnesium Sulfate aqueous solution (10 mg/ml). The PL solution was then added to the alginate gel through mixing and then the remaining DDW was added.

Insulin FITC was administered nasally to mice from the three formulations at a dose of 1 mg/kg animal (~0.02mg\ 20µl/ mouse). Ten minutes after treatments, the animals were sacrificed, the brains were removed, washed with normal saline and examined by the Multiphoton Microscope using the following conditions: excitation λ of 860 nm, field 58.6x58.6x30.6 nm (width x height x depth), lens x20, laser intensity 11.1%,

scanning 512, scan speed 0.5, no line skipping, Luts: 1000 and zoom 9.9. The fluorescence intensity of the probe (arbitrary units A.U.) in the olfactory region in brain was further assessed using Image Pro-Plus software.

Multiphoton imaging of the olfactory region following Insulin-FITC administration in phospholipid magnesome indicates the presence of augmented fluorescent signal in this group relative to controls (micrographs are not shown). Semi-quantification of the fluorescence signals gave fluorescent intensities of ~24 A.U. in brain section of animals treated with phospholipid magnesome containing Insulin-FITC as compared to 3.4 and 7.0 A.U. for the controls WS and Lipo, respectively, as shown in the bar diagram of Figure 4.

Example 3

Nasal delivery of EGF-IRDye 800CW to brain

Nasal delivery of EGF-IRDye 800CW to brain of mice from phospholipid magnesome as compared with two controls, was examined by Odyssey® Infrared Imaging System (LI-COR, USA).

Twelve female C57Bl/6J mice (8-9 weeks) were divided equally into 3 groups of administration and Untreated Control group. The following formulations were prepared.

	Phospholipid magnesome (%w/w)	Water solution %w/w	Liposome % w/w
EGF-IRDye 800CW	0.1	0.1	0.1
PL	3	-	3
PG	15	-	-
Sodium alginate	0.6	-	0.6
Magnesium sulfate	0.03	-	-
DDW	To 100	To 100	To 100

Preparation: PL was dissolved in PG, EGF-IRDye 800CW was added and then 20% of the total DDW amount was added, through mixing. In a separate vessel, Sodium Alginate was dispersed in 10% of the total amount of DDW and mixed with Magnesium Sulfate aqueous solution (10 mg/ml). The PL solution was then added to the alginate gel through mixing, then the remaining DDW was added.

EGF-IRDye 800CW was administered nasally to mice from the three formulations at a dose of 1 mg/kg animal (~0.02mg\ 20µl/ mouse). Ten minutes after treatments, the animals were sacrificed; brains were removed, washed with normal saline and observed under the imaging system. The scanning was performed using offset 3, resolution 339.6µm, channel 800 nm and intensity 3.

The NIR images (Figure 5; from left to right: untreated group, phospholipid magnesome-treated group; water solution-treated group and liposome-treated group) and their semi-quantification (Figure 6, in the form of a bar diagram) indicate the presence of the labeled peptide signal in brain tissues following nasal administration from various systems as compared to Untreated Control. Figure 5 shows that following administration from phospholipid magnesome, EGF-IRDye 800CW accumulated in the cerebrum and in the olfactory bulb with remarkable accumulation in the cerebrum. The administration from water solution lead to peptide accumulation in the olfactory bulb (Figure 5). The fluorescent signals calculated to be 19.1 A.U. in phospholipid magnesome containing EGF-IRDye 800CW as compared to 10.0 and 6.7 A.U. in WS and Lipo, respectively (Figure 6).

Example 4

Analgesic effect of Oxytocin nasally delivered in phospholipid magnesome as compared to two control carriers.

The analgesic effect of intranasal administration of Oxytocin in phospholipid magnesome composition was evaluated in femaleC57Bl/6J mice. The acetic acid-induced pain mice model was used.

In this model, animals received analgesic treatment, then after predetermined time periods, pain was induced by 0.6% (v/v) acetic acid solution injected intraperitoneally at a dose of 10 ml/kg. The number of writhes in a 10 min period was counted, starting 5 min after the acetic acid injection. A writhe is characterized by a wave of contraction of the abdominal musculature followed by extension of at least one hind limb. Antinociception is expressed as percent inhibition of the number of writhes observed in treated animals in comparison to animals in the untreated group.

The Maximum Possible Effect (MPE %) of different treatments is expressed as the inhibition percent of the number of writhes in a drug-treated animal group, when compared to the mean number of writhes measured in a group of untreated control mice according to the following equation:

MPE %= [Mean of writhes in untreated control group - Mean of writhes in treated group] / [Mean of writhes in untreated control group] * 100

Twenty mice were divided into three equal treatment groups for testing three time points: 5, 30 or 120 min (n=5/group) and Untreated control group. The following formulations were prepared.

	Phospholipid magnesome (%w/w)	Water solution %w/w	Liposome % w/w
oxytocin	0.08	0.08	0.08
PL	3	-	3
PG	15	-	-
Sodium alginate	0.6	-	0.6
Magnesium sulfate	0.03	-	-
DDW	To 100	To 100	To 100

Preparation: PL was dissolved in PG, Oxytocin was added and then 20% of the total DDW amount was added, through mixing. In a separate vessel, Sodium Alginate was dispersed in 10% of the total amount of DDW and mixed with Magnesium Sulfate aqueous solution (10 mg/ml). The PL solution was then added to the alginate gel through mixing, then the remaining DDW was added.

Oxytocin was administered nasally to mice from the three formulations at a dose of 0.4 mg/kg animal (~0.008mg\ 10µl/ mouse).

Figure 7 and the table below give the writhes counts and MPE% values, respectively, following treatment of mice with 0.4mg/kg Oxytocin incorporated in various carriers. As shown in the bar diagram of Figure 7, a significant decrease in writhes number was observed following treatment with Oxytocin from phospholipid magnesome (the darkest bar). The effect of the peptide incorporated in various controls (WS and Lipo) was lower by 2 folds, underscoring the enhanced delivery of Oxytocin from phospholipid magnesome.

Below are tabulated calculated MPE% values following Oxytocin administration to mice at a dose of 0.4mg/kg from phospholipid magnesome and control nasal systems administrated at various time points before pain induction.

	Phospholipid magnesome	Water solution	Liposome
Time (min)	MPE%		
5	63.5	35.8	30.8
30	63.9	27.8	25.8
120	58.0	29.8	19.2

Example 5

Local safety

The effect of phospholipid magnesome on the nasal cavity was evaluated in rats. Female SD/H rats were divided into four groups:

Group 1- (phospholipid magnesome): Intranasal administration of phospholipid magensome composition as described in Example 1, Part II (15 µl/ rat)

Group 2 - (NS): Intranasal Normal saline (15 µl / rat).

Group 3 - (SLS): Intranasal Sodium lauryl sulfate Solution (1% w/w), (15 µl / rat).

Group 4- Untreated control animals.

The animals received the treatments twice a day for one week. At the end of the experiment, animals were sacrificed, nasal cavities were removed and fixed in 3.7% Formaldehyde PBS. Sections of the nasal cavity were cut serially at 7µm thickness and stained with Hematoxylin & Eosin. The sections were examined by professional histopathologist (Authority for Animal Facilities, Hebrew University of Jerusalem, Israel) by Olympus light microscope BX43 and Olympus digital camera DP21 with Olympus cellSens Entry 1.13 software (Olympus, Japan) using magnification x10. Local toxicity was assessed by evaluating the histopathological alterations in different regions of the nasal cavity (cartilage and turbinate bone, lamina propria and submucosa, mucosal epithelium and lumen).

No pathological findings were observed in the histopathological analysis of the nasal cavities excised from rats treated with phospholipid magnesome or NS. The micrographs for these groups were similar to untreated control group showing intact mucosal epithelium, empty lumen and no infiltration of inflammatory cells. Overall, there was no evidence of inflammation. Turbinate bone integrity was preserved. Epithelium was normal with no evidence of erosion or ulceration and ciliated epithelium was intact. On the other hand, minimal proteinaceous material in the lumen and focal aggregations of neutrophils were observed in the positive control group treated with SLS. Micrographs corresponding to the four groups are presented in Figure 8.

Example 6

Tramadol HCl-containing phospholipid magnesome

Ingredients	%w/w
Tramadol HCl	10
PL	3
PG	15
Sodium Alginate	0.6
Magnesium Sulfate	0.01
DDW	To 100

Preparation: PL was dissolved in PG, Tramadol and 20% of the total DDW amount were added, through mixing. In a separate vessel, Sodium Alginate was dispersed in 10% of the total amount of DDW and mixed with Magnesium Sulfate aqueous solution (10 mg/ml). The PL solution was then added to the alginate gel through mixing with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany), then the remaining DDW was added .

Example 7

Rizatriptan Benzoate-containing phospholipid magnesome

Ingredients	%w/w
Rizatriptan Benzoate	10
PL	3
PG	15
Magnesium Sulfate	0.03
DDW	To 100

Preparation: PL was dissolved in PG, then Rizatriptan was added, through mixing. To this mixture, Magnesium Sulfate aqueous solution (10 mg/ml) was added through mixing with a magnetic stirrer, then the remaining DDW was added.

Example 8

Mannitol-Containing phospholipid magnesome

Ingredients	%w/w
Mannitol	10
PL	3
PG	15
Magnesium Sulfate	0.07
DDW	To 100

Preparation: PL was dissolved in PG, then Mannitol was dispersed in the PL solution. To this mixture Magnesium Sulfate aqueous solution (10 mg/ml) was added through mixing with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany), then DDW was added.

Example 9

Cannabidiol-containing phospholipid magnesome

Ingredients	%w/w
CBD	1
PL	3
PG	25
Ethanol Absolute (ETOH)	15
Magnesium Sulfate	0.01
DDW	To 100

Preparation: PL was dissolved in ETOH and PG mixture, then CBD was dissolved in the PL solution. To this solution, Magnesium Sulfate aqueous solution (10 mg/ml) was added through mixing with a magnetic stirrer then DDW was added.

Example 10

Cannabidiol-containing phospholipid magnesome

Ingredients	%w/w
CBD	0.5
PL	5
PG	25
ETOH	17
Magnesium Sulfate	0.01
DDW	To 100

The composition is prepared with a high shear mixer. PL was dissolved in ETOH and PG mixture, then CBD was dissolved in the PL solution. To this solution, Magnesium Sulfate aqueous solution (10 mg/ml) was added through mixing with a magnetic stirrer then DDW was added.

Example 11

brotizolam-containing phospholipid magnesome

Ingredients	%w/w
Brotizolam	0.1
PL	3
PG	15
Carbopol 980	0.05
Ammonium hydroxide	0.05
Magnesium Sulfate	0.02
DDW	To 100

PL was dissolved in PG. Brotizolam was added to the solution. In a separate vessel, Carbopol 980 was suspended in DDW and ammonium hydroxide was added. Then Magnesium Sulfate aqueous solution (10 mg/ml) was added to this mixture followed by adding the PL solution through mixing with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany).

Example 12

Cannabidiol-containing phospholipid magnesome

Ingredients	%w/w
CBD	1
PL	10
PG	40
Vit E	0.4
Magnesium Sulfate	0.03
DDW	To 100

PL was dissolved in PG, then CBD and Vit E were dissolved in the PL solution. To this solution, Magnesium Sulfate aqueous solution (10 mg/ml) was added through mixing with a magnetic stirrer then DDW was added.

Example 13

Butorphanol tartrate (BUT) -containing phospholipid magnesome

Ingredients	%w/w
BUT	0.1
PL	2
PG	30
Sodium Hydroxide	0.5
Magnesium Sulfate	0.025
DDW	To 100

Preparation: PL was dissolved in PG, then BUT was dissolved in the PL solution. To this solution, DDW and magnesium sulfate solutions were added through mixing with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany). Finally, Sodium Hydroxide was added (for pH adjustment). Final pH- 5.5.

Example 14

Ketoprofen (KET) -containing phospholipid magnesome

Ingredients	%w/w
KET	20
PL	2
Magnesium Sulfate	0.025
PG	30
Sodium Hydroxide (10%)	40
Hydrochloric Acid (32%)	5.8
DDW	To 100

Preparation: PL was dissolved in PG. In a separate vessel, KET was suspended in 20% of DDW, then Sodium Hydroxide Solution was added (to dissolve KET) and followed by the addition of Hydrochloric Acid (for pH adjustment) and the rest amount of

DDW and magnesium sulfate solution wee added. Finally, the PL solution was added to this solution and mixed with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany).

Example 15

Ketoprofen (KET) -containing phospholipid magnesome

Ingredients	%w/w
KET	3
PL	2
PG	30
Sodium Hydroxide (10%)	10
Magnesium sulfate	0.02
HCl solution (32%)	2
DDW	To 100

Preparation: PL was dissolved in PG. In a separate vessel, KET was suspended in 20% of DDW, then Sodium Hydroxide Solution was added (to dissolve KET) and followed by the addition of Hydrochloric Acid (for pH adjustment). The Magnesium solution. and the rest amount of DDW were added. Finally, the PL solution was added to this solution and mixed with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany). Final pH-5.7

Example 16

Assessment of phospholipid magnesome softness (more fluid bilayers relative to liposome) by Transition Temperature (Tm) measurement by Differential Scanning Calorimetry (DSC)

Tm of the phospholipid was measured in the following compositions:

	phospholipid magnesome	Liposome
Ingredients	%w/w	%w/w
Magnesium sulfate	0.03	-
PL	5	5
PG	15	-
DDW	To 100	To 100

The measurements were carried out using a Mettler Toledo DSC-1 STAR system (Toledo, China). Samples of 20 mg were placed in aluminum metal dishes. Tomograms were generated recording Tm values at a heating rate of 10°C/min within the temperature range of -50°C to +50°C.

Results indicate that phospholipid magnesome systems had a Tm value of -7°C vs. +7°C for liposome. This lower Tm by 14°C could be the result of a fluidization of the PL lamellae in phospholipid magnesome vesicles in comparison with classic liposome. The thermograms are shown in Figure 9A and 9B.

Example 17

Assessment of Drug Concentration in Plasma and Pharmacokinetic Parameters

Plasma concentration of Ketoprofen was measured following *in vivo* nasal administration and compared to oral administration. The experiment was carried out using Male Sprague Dawley (SD/Hsd) rats (Harlan, Israel).

The method of Ketoprofen extraction from plasma was validated according to FDA regulations for bio-analytical method validation, assessing precision, recovery, selectivity and linearity. The precision was 8.7%, the recovery was 96.6±5.5%,

the limit of detection (LOD) was 0.84mcg/ml, and the limit of quantification (LOQ) was 2.53mcg/ml, Linear regression analysis of the plasma standard curve showed correlation with R^2=1.00 over the concentration range of 0.05-100mcg/ml Ketoprofen. The calibration curve equation: Y = 143453X, (Y=Area, X= Concentration).

Ketoprofen (14mg/kg) was administered nasally from Ketoprofen new nanovesicular carrier (KET-phospholipid Magnesome) and compared to oral administration (KET-PO). The compositions are tabulated below.

Compositions

	KET –phospholipid magnesome	KET-PO
Ingredients	%w/w	%w/w
Ketoprofen (KET)	20	2
PL	5	-
PG	30	-
Sodium Hydroxide (10%)	40	40
Hydrochloric Acid (32%)	5.8	5.8
Magnesium sulfate	0.02	-
Methyl Cellulose	-	2
DDW	To 100	To 100

Preparation: PL is dissolved in PG. In a separate vessel, KET is suspended in a part of water, then Sodium Hydroxide Solution is added (to dissolve KET) and followed by the addition of hydrochloric acid (for pH adjustment). The magnesium solution and the rest amount of DDW are added. Finally, the PL solution is added to this aqueous solution and mixed with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany) or Polytron homogenizer. Final pH-5.4

Blood samples were collected from rats' tails at 10, 30, 60, 120, 180, 240 and 300 min post drug administration. The blood samples were centrifuged at 3k rpm for 10 min at 25°C (HERMLE Z 160 M), and then 150 mcl of the plasma was taken. Plasma samples were frozen and kept at -20°C until analysis.

A volume of 300 mcl of ACN was added to the plasma and mixed by vortex for 3 minutes at level 10, followed by the addition of 300 mcl of acetate buffer 0.05M with pH 5, and mixed by vortex for additional 1 minute at level 10. Then, the samples were centrifuged for 5 min at 14k rpm at 25°C (HERMLE Z 160 M), and the supernatants were filtered through Bulk GHP Acrodisc® 13mm syringe filter with 0.45um GHP membrane (Pall Corporation, USA), and transferred into pre-labeled auto injector vials before being injected into HPLC-UV.

The relative bioavailability (*F* %) was calculated according to the following equation:

$$F\,\% = [(AUC_{NVC} * DOSE_{PO})] / [(AUC_{PO} * DOSE_{NCV})] * 100$$

The AUC_{NVC} and AUC_{PO} represent the means of individual AUC from nasal and oral experimental groups, respectively.

The pharmacokinetic study aimed to evaluate the influence of the new nanovesicular carrier on the absorption parameters of the drug model, Ketoprofen. For this purpose, Ketoprofen concentration was assessed in plasma of rats following drug administration from new nanovesicular carrier as compared to oral administration. Ketoprofen plasma concentration curves versus time are plotted in Figure 10 (rectangular: nasal

administration according to the invention; triangle: oral administration.

Ketoprofen was assayed in rat plasma starting from 10 min post nasal or oral administration. Results are tabulated below.

PK parameters	Nasal composition	Oral administration
$T_{1/2}$,min	138.2±21.1	328.7±83. 1
Tmax ,min	10.0±0.0	94.0±12.4
Cmax, mcg/ml	43.7±8.3	13.7±5.9
AUC_{0-5hr}	4130.4±730.6	2642.1±1153. 3
T_{last},min	300.00±0.00	300±0.00
Bioavailability (relative to oral, %)	156.3	

Results presented above show that very significant higher plasma concentrations ($P < 0.01$) were detected 10 and 30min after Ketoprofen administration in nasal vesicular nanocarrier. C_{max} plasma values calculated for new nanovesicular carrier and oral administration were 43.65±8.30 and 13.68±5.91 mcg/ml, respectively. The T_{max} values were 10 and 94 min for nasal vesicular carrier administration and oral administration, respectively.

These results above indicate that Ketoprofen was rapidly delivered from the nasal cavity to the systemic circulation following nasal administration showing that the phospholipid magnesome possesses enhanced delivery properties and enhanced effect for the first 90 minutes relative to the oral administration, with a very rapid onset of action and behaves similar to the oral administration for the next five tested hours.

Example 18

Cannabidiol-containing phospholipid magnesome

Ingredients	%w/w
CBD	5
PL	5
PG	30
Ethanol	15
Hemp Seed Oil (HSO)	4
Magnesium Sulfate	0.03
DDW	To 100

PL is dissolved in PG and Ethanol mixture, and then HSO is added. CBD is dissolved in this solution. Then, Magnesium Sulfate aqueous solution (10 mg/ml) is slowly added through vigorous mixing with a homogenizer. Finally, DDW is slowly added with mixing.

Example 19

Cannabidiol-containing phospholipid magnesome

Ingredients	%w/w
THC	0.5
PL	5
Vit E	0.5
PG	30
Olive Oil	3
Magnesium Sulfate	0.03
DDW	To 100

PL is dissolved in PG, then Vit E, Oil and THC are added to the PL solution. To this solution, Magnesium Sulfate aqueous solution (10 mg/ml) is added slowly through mixing with an overhead stirrer (Heidolph digital 200 RZR-2000, Germany).

Examples 20-21

Insulin-containing phospholipid Magnesome

	Example 20 %w/w	Example 21 %w/w
Insulin	0.1	0.1
PL	3.0	3.0
PG	15.0	15.0
Vitamin E	0.3	0.3
Magnesium sulfate	1.0	5.0
DDW	80.6	76.6

Preparation: PL was dissolved in PG, then Vitamin E was added. In a separate vessel, Magnesium Sulfate was dissolved in DDW. The Magnesium solution was added gradually to the PL solution and mixed well. Finally, Insulin (from Bovine Pancreas- Sigma Aldrich, USA) was added and mixed well. The mixing through the entire preparation process was performed using an overhead Heidolph® stirrer (Heidolph Digital 200 RZR-2000, Germany).

Examples 22-23

Bacitracin-containing phospholipid Magnesome

	Example 22 %w/w	Example 23 %w/w
Bacitracin	0.1	0.1
PL	3.0	3.0
PG	15.0	15.0
Vitamin E	0.3	0.3
Magnesium sulfate	10	20
DDW	71.6	61.6

PL was dissolved in PG, then Vitamin E was added. In a separate vessel, Magnesium Sulfate was dissolved in DDW. The Magnesium solution was added gradually to the PL solution and mixed well. Finally, Bacitracin (Sigma Aldrich, USA) was added and mixed well. The mixing through the entire

preparation process was performed using an overhead Heidolph® stirrer (Heidolph Digital 200 RZR-2000, Germany).

Example 24

Tramadol HCl - containing phospholipid Magnesome

	Example 24 %w/w
Tramadol HCl	1.0
PL	3.0
PG	15.0
Vitamin E	0.3
Magnesium sulfate	5.0
DDW	75.7

PL was dissolved in PG, then Vitamin E was added. In a separate vessel, Tramadol HCl (Chemagis, Israel) was dissolved in DDW and followed by dissolving the Magnesium Sulfate. The aqueous solution of Tramadol HCl and Magnesium was added gradually to the PL solution and mixed well. The mixing through the entire preparation process was performed using an overhead Heidolph® stirrer (Heidolph Digital 200 RZR-2000, Germany).

Example 25

Safinamide Mesylate - containing phospholipid Magnesome

	Example 25 %w/w
Safinamide mesylate	20
PL	5.0
PG	15.0
Vitamin E	0.5
Magnesium sulfate	0.5
DDW	to 100

PL is dissolved in PG, then Vitamin E is added. In a separate vessel, Safinamide mesylate is dissolved in DDW and is added

gradually to the PL solution and mixed well using an overhead Heidolph® stirrer (Heidolph Digital 200 RZR-2000, Germany). Finally, the Magnesium Sulfate is added to the composition and mixed.

Examples 26-34

phospholipid Magnesome with varying Mg content

	Ex. 26 %w/w	Ex. 27 %w/w	Ex. 28 %w/w	Ex. 29 %w/w	Ex. 30	Ex. 31	Ex. 32	Ex.33	Ex. 34
PL	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
PG	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin E	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3
Magnesium sulfate	0.01	0.1	0.3	0.5	1.0	2.0	5.0	10.0	20.0
DDW	81.69	81.6	81.4	81.2	80.7	79.7	76.7	71.7	61.7

PL was dissolved in PG, then Vitamin E was added. In a separate vessel, Magnesium Sulfate was dissolved in DDW. The Magnesium solution was added gradually to the PL solution and mixed well using an overhead Heidolph® stirrer (Heidolph Digital 200 RZR-2000, Germany).

Claims

1) A nasally administrable composition comprising at least one active substance in magnesium-containing vesicular carrier, said carrier comprising glycol, phospholipids, water and at least one magnesium source.

2) The composition of claim 1, comprising from 5 to 50% by weight propylene glycol, from 0.2 to 10% by weight phospholipids, not less than 20% by weight water and not less than 0.01% by weight magnesium source.

3) The composition of claim 2, comprising from 0.01 to 20.0% by weight magnesium salt.

4) The composition of claim 3, comprising from 0.01 to 5.0% by weight magnesium salt.

5) The composition of any one of claims 1 to 4, further comprising an antioxidant, a mucoadhesive agent or both.

6) The composition according to any one of the preceding claims, which is free of aliphatic monohydroxy alcohol.

7) The composition according to any one of claims 5 and 6, wherein the magnesium source is magnesium sulfate and the mucoadhesive agent is an alginate salt.

8) The composition according to any one of the preceding claims, further comprising a vegetable oil.

9) The composition of any one of claims 1 to 8, wherein the active substance is an analgesic, antimigraine and/or antipyretic agent.

10) The composition of claim 9, wherein the analgesic, antimigraine and antipyretic agent is selected from the group consisting of tramadol, ketoprofen, rizatriptan and pharmaceutically acceptable salts thereof.

11) The composition of any one of claims 1 to 8, wherein the active substance is an opioid.

12) The composition of claim 11, wherein the opioid is butorphanol or a pharmaceutically acceptable salt thereof.

13) The composition of any one of claims 1 to 8, wherein the active substance is an anti-Parkinson drug.

14) The composition of claim 13, wherein the anti-Parkinson drug is safinamide or a pharmaceutically acceptable salt thereof.

15) The composition of any one of claims 1 to 8, wherein the active substance is a sedative and/or hypnotic drug.

16) The composition of claim 15, wherein the sedative and/or hypnotic drug is brotizolam or a pharmaceutically acceptable salt thereof.

17) The composition of any one of claims 1 to 8, wherein the active substance is a peptide or a polypeptide.

18) The composition according to claim 15 comprising peptide hormone selected from the group consisting of insulin and oxytocin, or bacitracin.

19) The composition according to any one of claims 1 to 6, wherein the active substance is mannitol.

20) A method of administering an active substance to a mammal in need thereof, comprising intranasal administration of a composition comprising a therapeutically effective amount of the active substance in a magnesium-containing vesicular carrier that contains glycol, phospholipids, water and at least one magnesium source.

21) A method according to claim 20, wherein the composition further comprises an antioxidant, a mucoadhesive agent or both.

22) A method according to claim 20 or 21, wherein the composition is free of aliphatic monohydroxy alcohol.

23) A method according to any one of claims 20 to 22, wherein the active substance is selected from the group consisting of an analgesic, antimigraine and/or antipyretic agents; opioids; an anti-Parkinson drug; a sedative and/or hypnotic drug.

24) A method according to any one of claims 20 to 22, wherein the active substance is selected from the group consisting of peptide or polypeptides.

25) A method of increasing the delivery of a physiologically active compound from a nasally administrable composition to the bloodstream/brain of mammals, the method comprises incorporating magnesium source into a vesicular carrier which

contains glycol, phospholipids, water and said physiologically active compound, for enhanced delivery.

26) A method according to claim 25, wherein the active compound is selected from the group consisting of an analgesic, antimigraine and/or antipyretic agents; an opioid; an anti-Parkinson drug; a sedative and/or hypnotic drug; and a hormone peptide.

27) A method for pain relief, comprising the intranasal administration to a patient of a composition comprising a carrier and therapeutically effective amount of an active substance selected from the group consisting of an analgesic, an opioid, an antimigraine agent, and anti-inflammatory agent, wherein the carrier in a magnesium-containing vesicular carrier comprising glycol, phospholipids, water and at least one magnesium source.

28) A nasally administrable vesicular composition comprising glycol, phospholipids, water and at least one magnesium source, wherein the composition is devoid of an active substance.

Figure 1

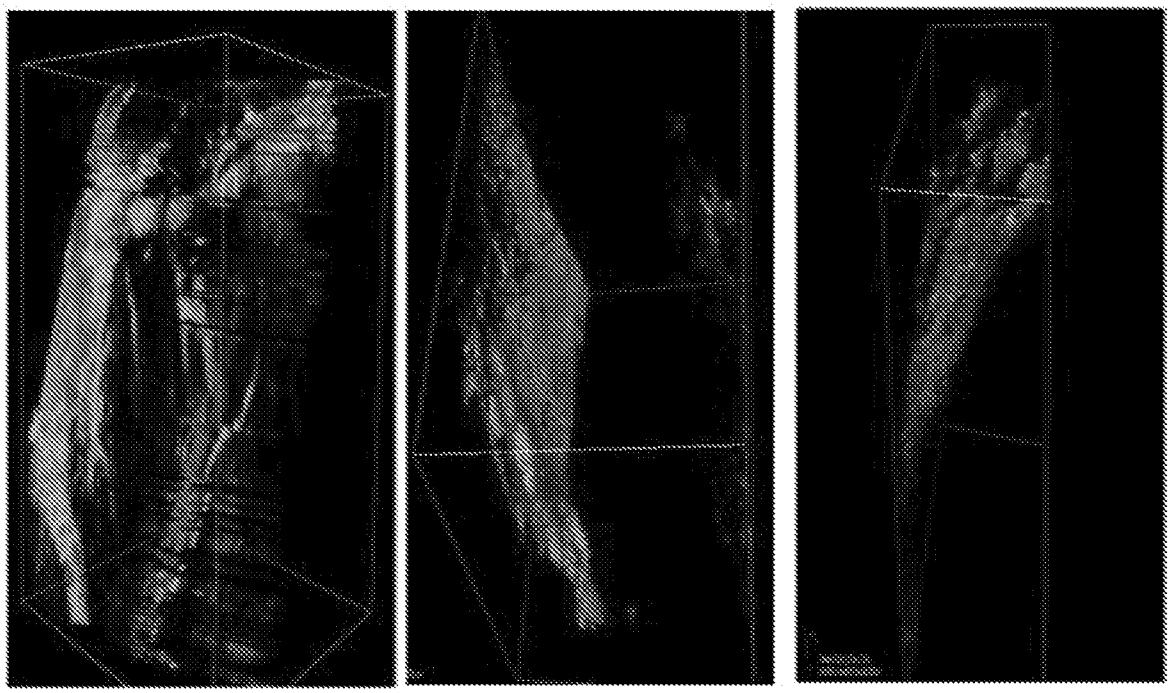


Figure 2

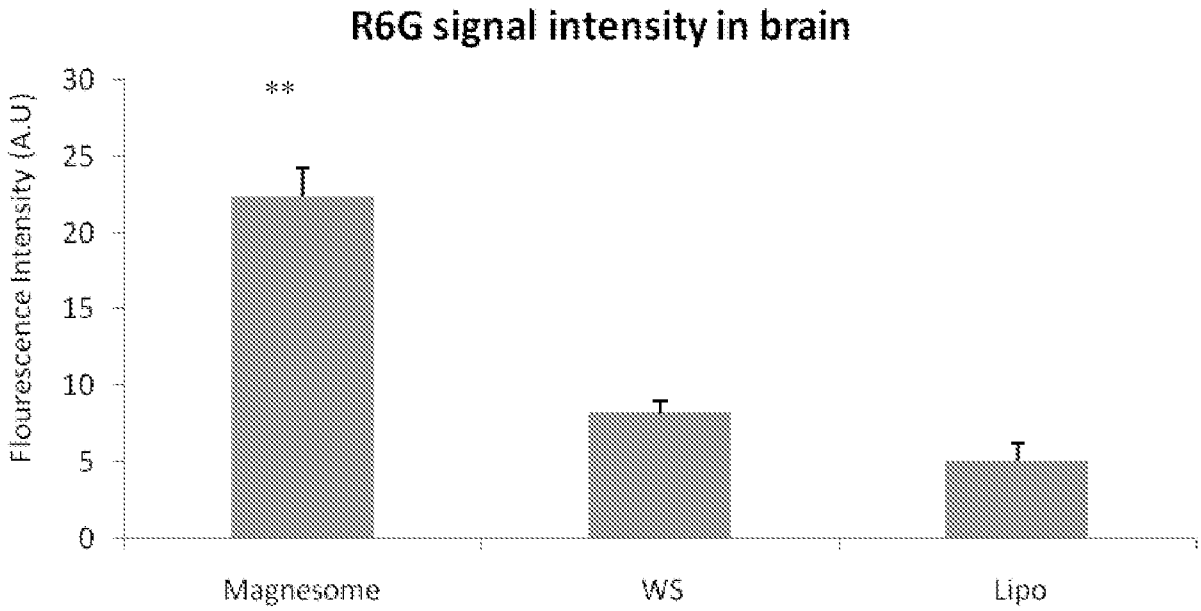


Figure 3

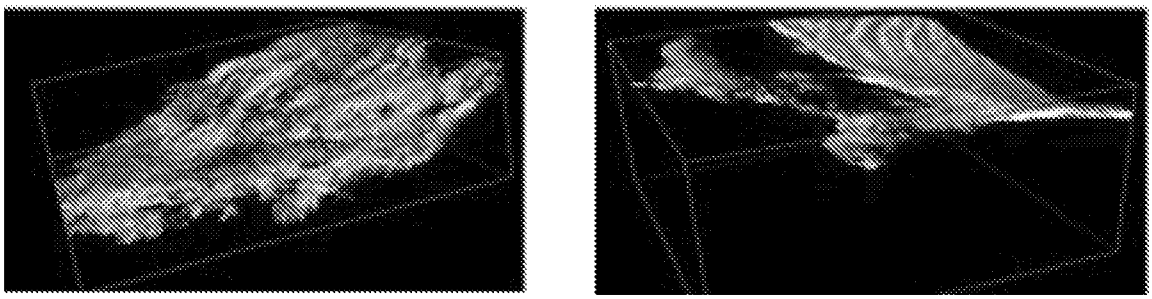


Figure 4

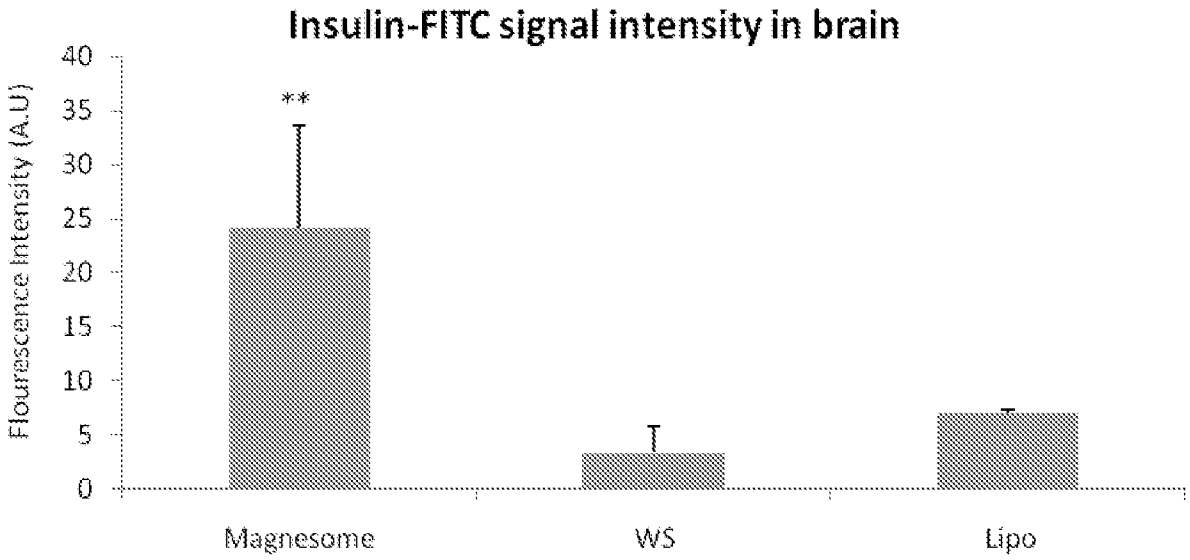


Figure 5

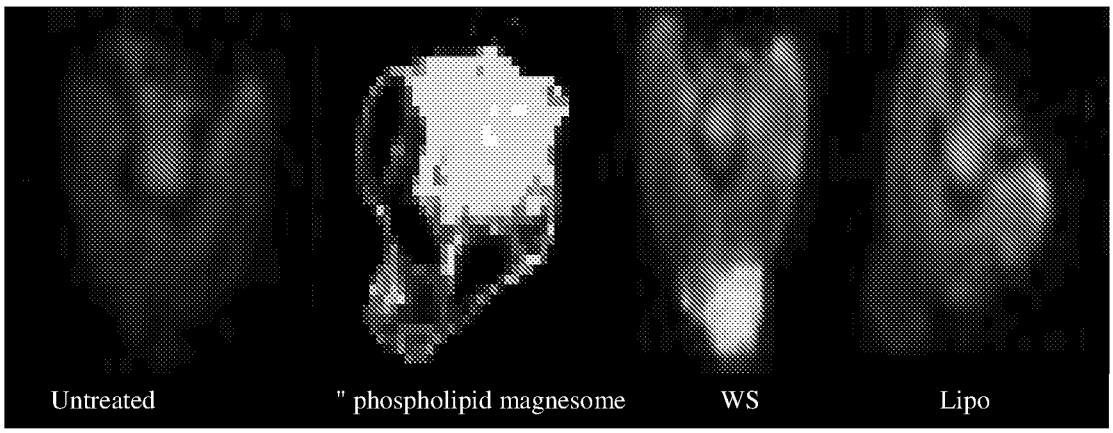


Figure 6

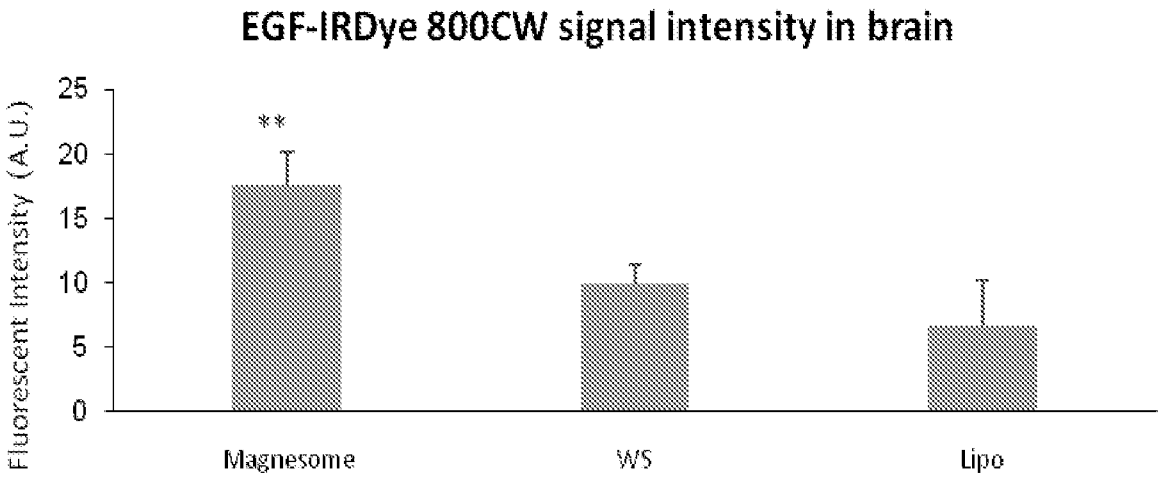


Figure 7

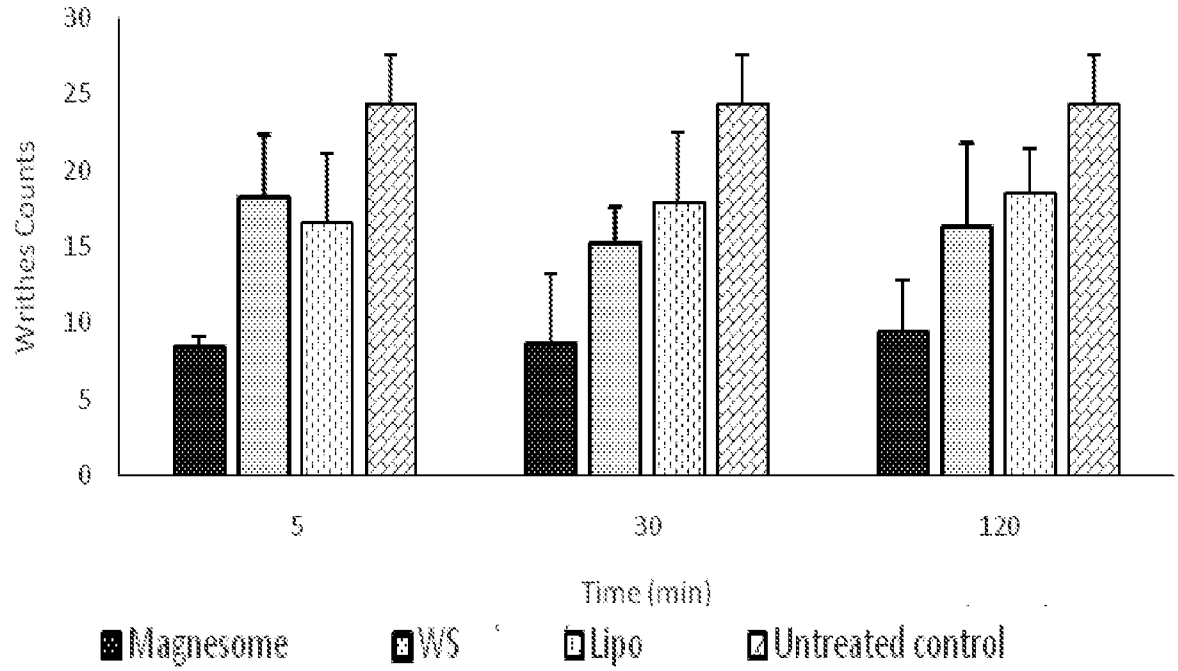


Figure 8a

Figure 8b

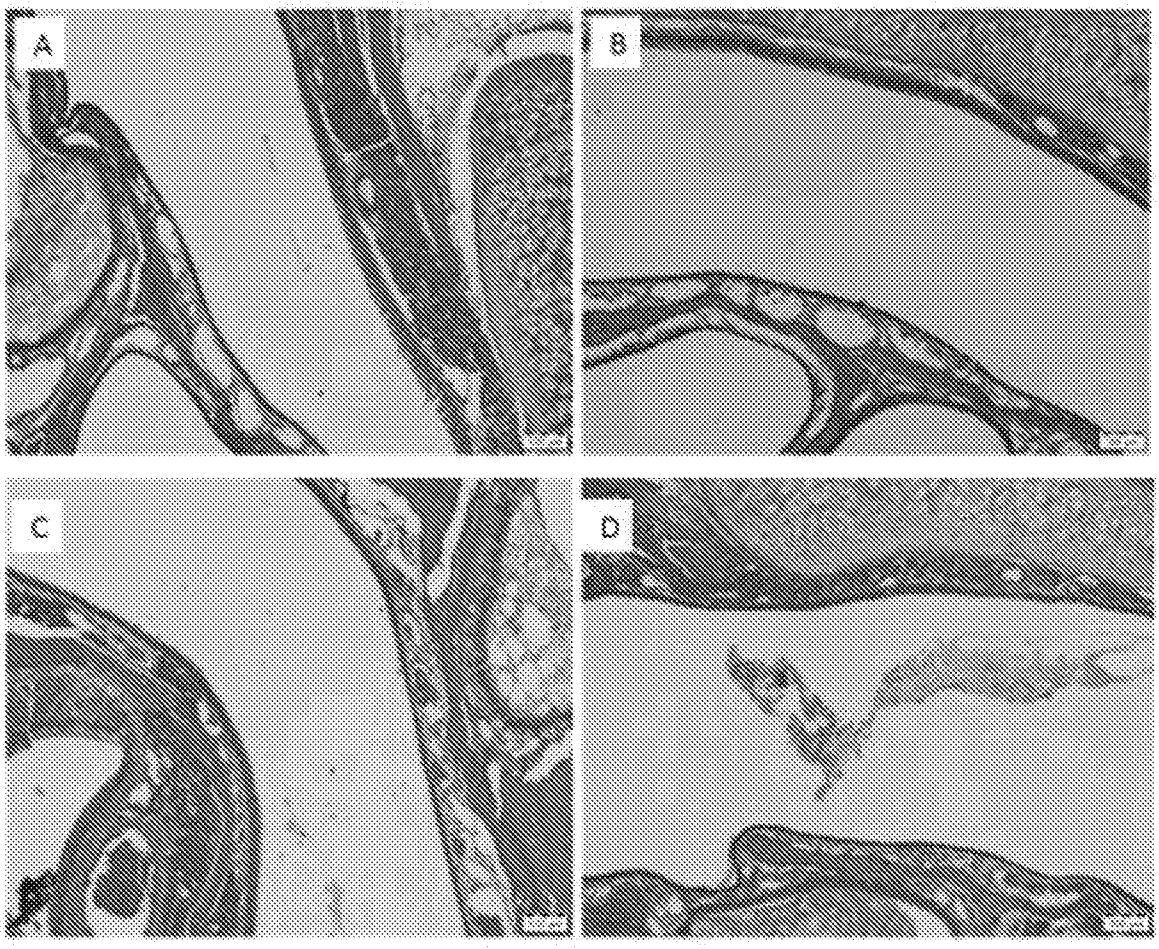
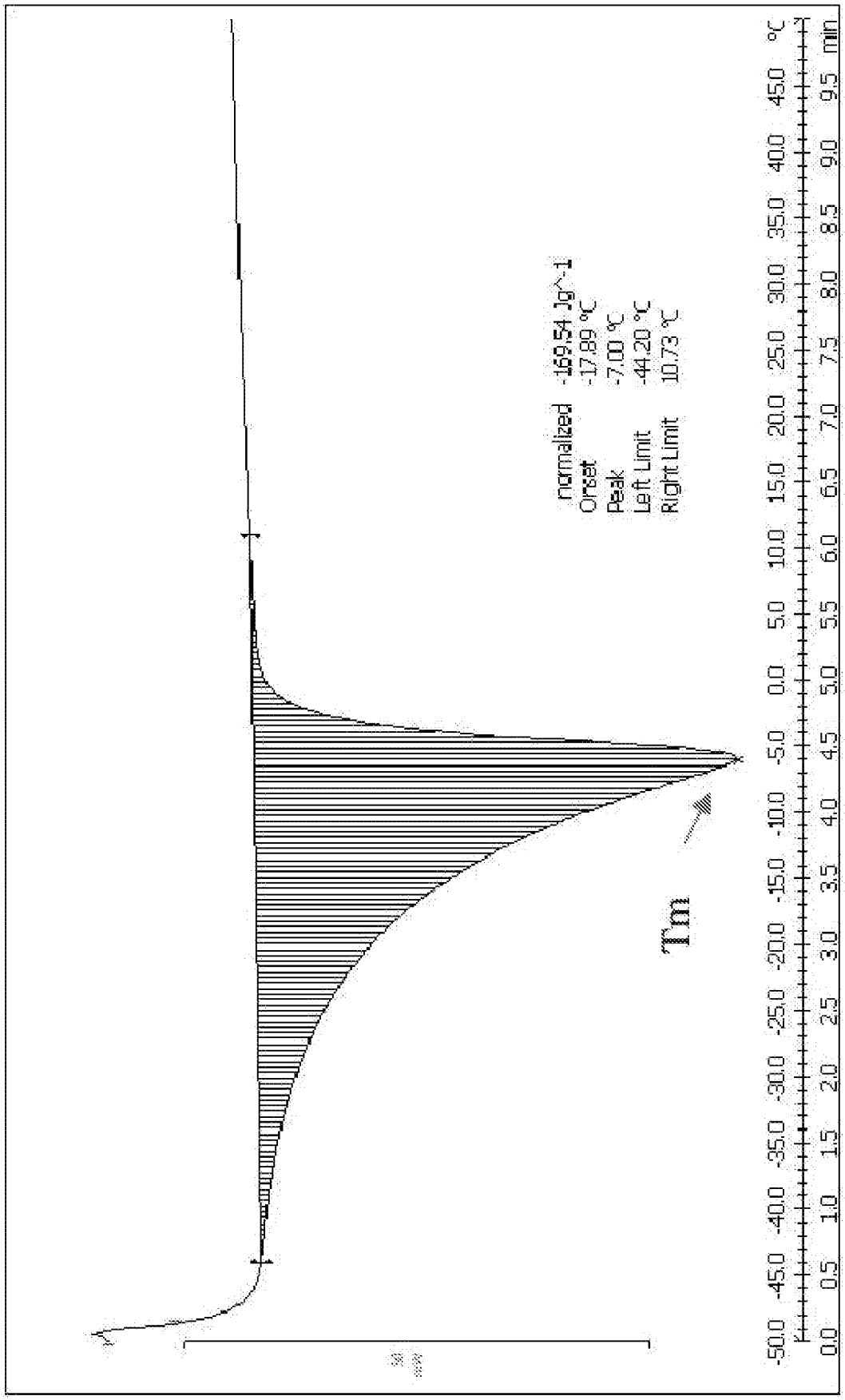


Figure 8c

Figure 8d

Figure 9A


normalized -169.54 Jg^-1
Onset -17.89 °C
Peak -7.00 °C
Left Limit -44.20 °C
Right Limit 10.73 °C
Tm
-50.0 -45.0 -40.0 -35.0 -30.0 -25.0 -20.0 -15.0 -10.0 -5.0 0.0 5.0 10.0 15.0 20.0 25.0 30.0 35.0 40.0 45.0 °C
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 min

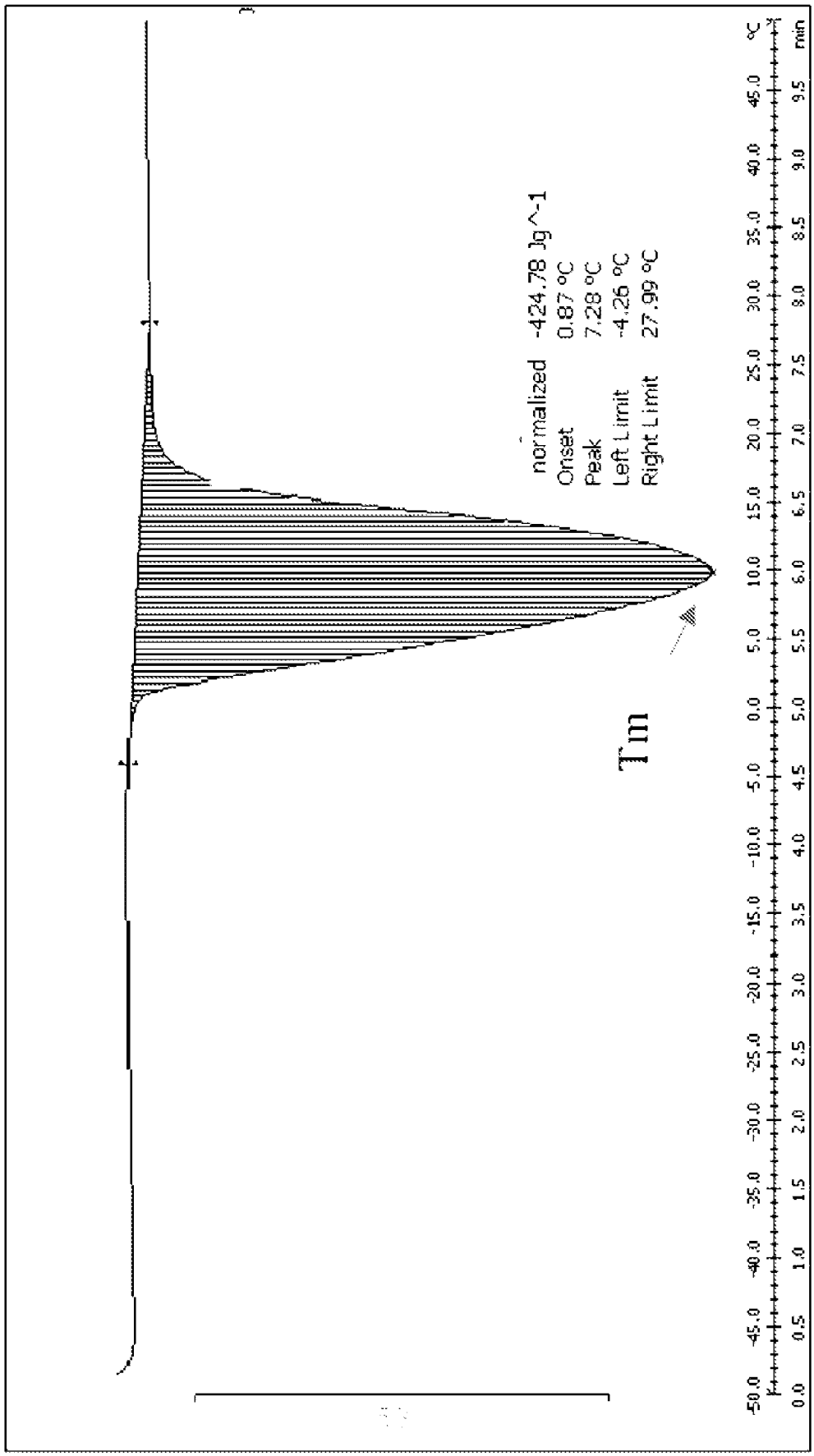
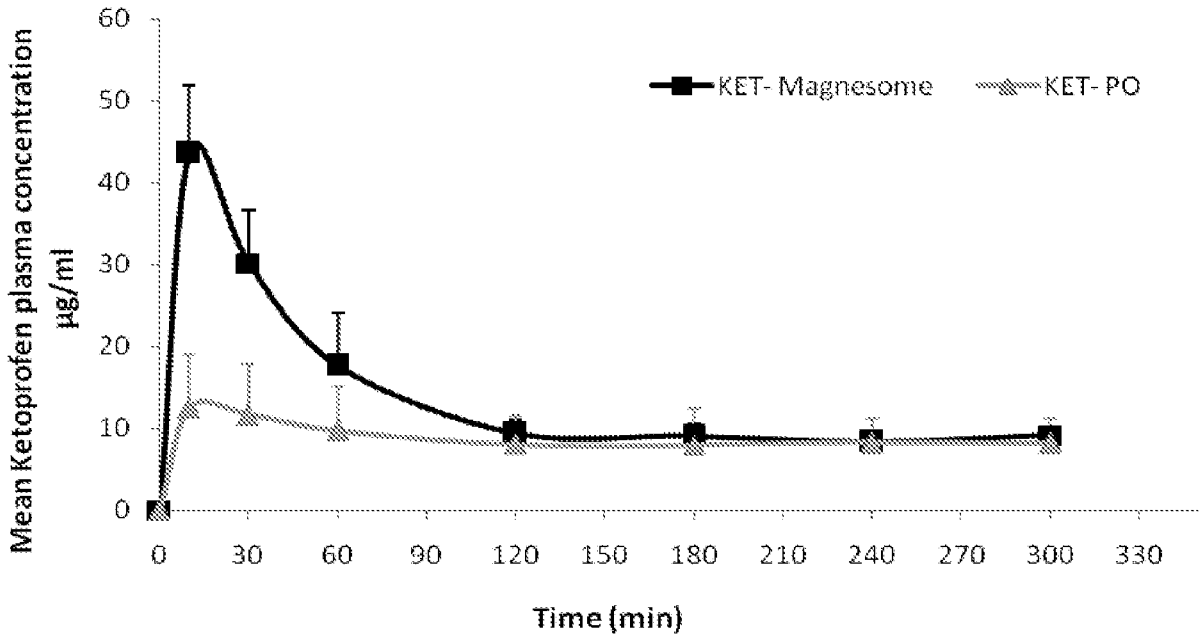

normalized -424.78 Jg^-1
Onset 0.87 °C
Peak 7.28 °C
Left Limit -4.26 °C
Right Limit 27.99 °C
Tm
°C
min

Figure 9B

Figure 10


Mean Ketoprofen plasma concentration µg/ml
60
50
40
30
20
10
0
KET- Magnesome
KET- PO
0
30
60
90
120
150
180
210
240
270
300
330
Time (min)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2018/051004

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/00 A61K47/02 A61K47/10 A61K47/24 A61P23/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/043057 A2 (YISSUM RES DEV CO [IL]; TOUITOU ELKA [IL]; GODIN BIANA [IL]; DUCHI SHA) 19 April 2007 (2007-04-19) claims 1-45 examples 1-25 -----	1-28
A	FIGEN ESEN ET AL: "Effect of magnesium sulfate administration on blood-brain barrier in a rat model of intraperitoneal sepsis: a randomized controlled experimental study", CRITICAL CARE, vol. 9, no. 1, 23 November 2004 (2004-11-23), page R18, XP055535299, GB ISSN: 1364-8535, DOI: 10.1186/cc3004 abstract ----- -/--	1-28

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2018	07/01/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Hörtner, Michael

1

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2018/051004

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	NATSHEH HIBA ET AL: "Phospholipid Magnesome-a nasal vesicular carrier for delivery of drugs to brain", DRUG DELIVERY AND TRANSLATIONAL RESEARCH, SPRINGER, GERMANY, vol. 8, no. 3, 9 March 2018 (2018-03-09), pages 806-819, XP036495855, ISSN: 2190-393X, DOI: 10.1007/S13346-018-0503-Y [retrieved on 2018-03-09] the whole document -----	1-28

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IL2018/051004

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