

LIPOSOME ENCAPSULATED APOMORPHINE**1. FIELD OF THE INVENTION**

The present invention relates to Aposomes (liposome encapsulated apomorphine), methods of making Aposomes and to the use of such in the treatment of Parkinson's disease.

2. BACKGROUND TO THE INVENTION

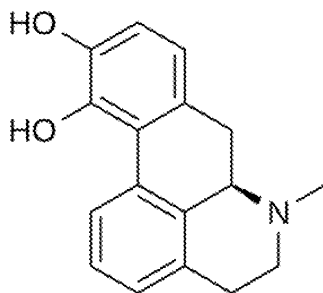
Neurodegenerative diseases are becoming more prevalent as populations age. Parkinson's disease (PD) is one of the most severe progressive neurodegenerative disorders, having a mortifying effect on the health of millions of people around the globe.

In PD, the neural cells producing dopamine in the substantia nigra of the brain die out. Dopamine is a chemical neurotransmitter that is utilised by brain cells to transmit impulses that control or modulate peripheral muscle movement. The degeneration of dopamine-containing neurons reduces the amount of dopamine in the brain. This process is thought to disturb nerve cell functions such that impulses are not transmitted properly, resulting in a loss of muscle control and function. This leads to symptoms like hypokinesia, rigidity, bradykinesia, and rest tremor.

Currently, there is no cure for PD. Treatments are typically aimed at alleviating PD symptoms, primarily by replacing the dopamine with either (levo)-3,4-dihydroxyphenylalanine (L-DOPA), which is metabolised to dopamine, or by administering chemical agents that stimulate the dopamine receptors. These receptors fall into two broad classes, D1-type and D2-type receptors. The former is divided into D1 and D5 receptors, while the D2 receptor family consists of D2, D3 and D4 receptors.

Delivering drugs to the brain for treating Parkinson's disease is very challenging. The blood-brain barrier acts as a highly selective semi-permeable barrier, preventing most drugs from reaching the brain. Conventional drug delivery systems used for PD do not readily cross the blood-brain barrier, leading to severe side-effects.

Apomorphine is (6aR)-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline-10,11-diol. It has the chemical formula:



Apomorphine is an extremely potent dopamine agonist which acts on both D1 and D2 receptors. It is highly lipophilic and so readily crosses the blood-brain barrier. Unfortunately, it has poor oral absorption and a short half-life. Current formulations for clinical use include subcutaneous injection, continuous subcutaneous infusion, sublingual
5 films and pulmonary inhalation as a dry powder. Intranasal administration of a dry powder has been trialled but discontinued due to toxicity and efficacy issues. In all of these delivery forms, apomorphine has a rapid onset of up to 15 minutes but a short elimination half-life of 30 minutes, providing a clinical effect duration of only about 30-60 minutes. The exception is continuous subcutaneous infusion, which is highly invasive.

- 10 Animal studies have indicated that transdermal delivery or delivery via implants may provide useful modes of administration of apomorphine. However, when the delivery of apomorphine from implants was studied in monkeys it was found that in most cases, the animals had to be treated with the immunosuppressant dexamethasone to prevent local irritation and other complications following implantation surgery. Transdermal delivery of
15 apomorphine has also been associated with local skin irritation and colouration.

Problems with apomorphine delivery have caused some to consider whether a liposomal delivery vehicle might be advantageous. Liposomes are self-assembled (phospho)lipid-based vesicles that form a bilayer or concentric series of multiple bilayers enclosing a central aqueous intraliposomal compartment. They range in size from 30 nm to the
20 micrometer scale, with the phospholipid bilayer being about 4-5 nm thick.

Liposomes can be outstanding drug delivery vehicles. The encapsulated drug is protected from physiological degradation, extending its half-life. The rate and location of drug release can be controlled and liposomes have excellent biocompatibility and safety.

However, there are many challenges to overcome, before one achieves a clinically viable
25 liposome formulation that can be administered to a patient. These challenges include:

- (a) achieving a sufficient level of drug loading into the liposome;
- (b) maintaining the drug in the liposomes when exposed to the blood plasma or patient tissues;
- (c) releasing the drug at the target site at a rate and level that is sufficient to
30 result in the desired therapeutic efficacy; and
- (d) achieving a pharmaceutically acceptable product in terms of shelf-life stability.

For *in vivo* and clinical applications, liposomes should ideally contain a maximal amount of therapeutic agent per volume of liposome, while maintaining minimal drug leakage.

(Wen, C-J., et al., 2012) describe theranostic liposomes co-loaded with quantum dots and apomorphine for brain targeting and bioimaging. The liposomes were prepared by Wen et al using passive encapsulation of apomorphine and co-dispersed quantum dots via the thin-film hydration method using phosphatidylcholine (2% w/v), cholesterol (0.8% w/v), stearylamine (0.4% w/v) and DSPE-PEG2000 (0.8% w/v). The liposomes were rehydrated with apomorphine solution at a concentration of 0.04% (w/v) resulting in >80% encapsulation efficiency. This equates to a drug to lipid ratio (apomorphine to total lipid constituents) of approximately 0.001:1 (w/w). Using these methods, apomorphine was completely soluble with no drug crystals observable, which is consistent with the low drug to lipid ratios obtained.

Similarly, (Hsu, S-H., et al., 2014) describe similar passively-loaded liposome encapsulated apomorphine compositions for brain targeting, manufactured using the thin-film hydration method. In this case, the liposome composition comprises soy phosphatidylcholine (3% w/v), cholesterol (1% w/v), a cationic surfactant, for example, stearylamine (0.2%), and a non-ionic surfactant, for example Brij 78® (0.3% w/v). The liposomes were rehydrated with apomorphine solution at a concentration of 0.1% (w/v) which resulted in an encapsulation efficiency of 99% when Brij 78® was used as the non-ionic surfactant. However, the high encapsulation efficiency for apomorphine in this composition still only equates to a drug to lipid ratio (apomorphine to total lipid constituents) of approximately 0.02:1 (w/w). The liposomes of Hsu et al. also have a high rate of passive leakage, with an average of approximately 25% total encapsulated apomorphine remaining after 2 hours at 37 °C, and an average of approximately 2% total encapsulated apomorphine remaining after 6 hours. In these compositions the apomorphine was completely soluble with no drug crystals observable, which is consistent with the low drug to lipid ratios obtained and high leakage rates observed.

There is therefore still a need to overcome the problems associated with the liposomal delivery of apomorphine, and/or to provide liposome encapsulated apomorphine having a favourable drug to lipid ratio and/or that has reduced passive leakage of the apomorphine. It is an object of the invention to overcome at least one of these problems and/or to at least provide the public with a useful choice.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

3. SUMMARY OF THE INVENTION

The invention relates to a high performance liposome encapsulated apomorphine which has significant advantages over currently used apomorphine formulations.

5 In one aspect the invention provides a liposome comprising lipids and apomorphine or a salt thereof, the lipids forming a lipid membrane enclosing an aqueous intraliposomal compartment containing the apomorphine or salt thereof. Such a liposome is described herein as an Aposome.

10 In one aspect the invention provides an Aposome in which the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (mol/mol).

In one aspect the invention provides an Aposome in which the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (wt/wt).

15 In one aspect the invention provides a composition comprising Aposomes of the invention and a carrier. In one embodiment the composition comprises a suspension of Aposomes in buffer.

In one embodiment the composition is a pharmaceutical composition and the carrier comprises one or more pharmaceutically acceptable excipients.

In another aspect the invention provides a process for making Aposomes comprising:

- 20 (a) preparing an aqueous suspension comprising liposomes of 50-400 nm size wherein the liposomes comprise a lipid membrane enclosing an aqueous intraliposomal compartment containing an ammonium salt that is (i) at a concentration of about 150 mM to about 550 mM and (ii) that is greater than the concentration of ammonium salt in the aqueous suspension;
- 25 (b) mixing the aqueous suspension with a solution of apomorphine salt wherein the apomorphine enters the intraliposomal compartment and forms a salt with anions of the ammonium salt, wherein the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (mol/mol) and/or about 0.05:1 to about 5:1 (wt/wt);
- 30 (c) optionally removing unencapsulated apomorphine from the aqueous suspension.

In one embodiment the intraliposomal compartment comprises solid or semi-solid apomorphine salt, preferably apomorphine sulfate.

In another aspect the invention provides a method of treating a condition in which an increase in dopaminergic receptor activation would be beneficial in a subject in need

thereof, the method comprising administering the pharmaceutical composition of the invention to the subject.

- In one embodiment the condition is a neurological condition. In one embodiment the condition is Parkinson's disease. In one embodiment the condition is Restless Leg Syndrome. In one embodiment the condition is Erectile Dysfunction.

4. BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described by way of example only and with reference to the drawings in which:

- 10 **Figure 1** shows a schematic drawing depicting the loading equilibrium between intraliposomal ammonium and ammonia, and external apomorphine and apomorphine- H^+ .
- Figure 2** shows a graph of fluorescence intensity vs initial intraliposomal ammonium salt, with and without MTAB.
- 15 **Figure 3** is a graph showing the change in fluorescence intensity with time in suspensions of Aposomes which were loaded under different conditions as described in Example 3.
- Figure 4A** shows the equilibrium between protonated and unprotonated apomorphine upon changing pH. **Figure 4B** is a graph showing the change in fluorescence intensity with time in compositions comprising Aposomes that were prepared at different pHs. **Figure 4C** shows the change in fluorescence intensity with time in comparable unencapsulated apomorphine compositions, as described in Example 3.
- 25 **Figure 5A** is a graph showing the relationship between phosphatidylcholine and ammonium ferrothiocyanate, used to quantify the phosphatidylcholine concentration of the Aposomes as set out in Example 4. **Figures 5B-C** are graphs showing the total phospholipid concentration and the total apomorphine concentration encapsulated (i.e. the fully-diluted total concentration of apomorphine encapsulated following liposome lysis) against the initial ammonium sulfate concentration used to prepare the liposomes of step (a) of the process of the invention – see Example 4.
- 30 **Figure 6** shows the impact of internal ammonium sulfate gradient on entrapment efficiency of apomorphine when liposomes are incubated with 4 mM apomorphine hydrochloride solution at pH 6.3. Encapsulation efficiency is shown as the molar ratio of encapsulated apomorphine (measured by apomorphine fluorescence intensity upon lysis of liposomes with MTAB) and total phospholipid (measured by Stewart Assay/UV-Vis

spectroscopy to confirm the initial amount of phospholipid used in the preparation). The total mass of apomorphine and total mass of phospholipid was calculated from the concentration determined and volume used. Data shows ammonium sulfate concentrations of 300-1000 mM produce the highest encapsulation efficiency/drug:lipid ratios on a mol:mol basis.

Figure 7 shows the impact of the internal ammonium sulfate gradient on the entrapment efficiency of apomorphine when liposomes are incubated with 4 mM apomorphine hydrochloride solution at pH 6.3. Encapsulation efficiency is shown as the ratio between the total mass of apomorphine (measured by apomorphine fluorescence intensity upon lysis of liposomes with MTAB and total phospholipid concentration (measured by Stewart Assay/UV-Vis spectroscopy to confirm the initial amount of phospholipid used in the preparation). The total mass of apomorphine and total mass of phospholipid was calculated from the concentration determined and volume used. Data shows ammonium sulfate concentrations of 300-1000 mM produce the highest encapsulation efficiency/drug:lipid ratios on a wt:wt basis.

Figure 8 is a set of cryo-transmission electron micrographs of liposomal apomorphine compositions of the invention, as discussed in Example 5.

Figure 9 is a cryo-transmission electron micrograph of Aposomes encapsulating apomorphine sulfate (Fig 9A). Figure 9B shows passive release of apomorphine from Aposomes at physiological temperature over 12 hours. Note: "Half-life" denotes theoretical time to 100% passive drug release (leakage rate) from liposomes *in vitro* at 37°C. It is not an *in vivo* drug half-life.

Figure 10 is a graph showing the hydrodynamic diameter of ammonium sulfate liposomes and subsequent Aposomes prepared by active loading of apomorphine (4 mM) against said ammonium sulfate liposomes, as described in Example 2. Ammonium sulfate concentration ranges include 300 mM, 500 mM, and 2M, following on from the encapsulation efficiency experiment. Data shows a significant change in hydrodynamic diameter for Aposomes prepared from 500 mM or 2 M ammonium sulfate liposomes. An increase in hydrodynamic diameter infers liposomal instability, suggesting that Aposomes prepared from 300 mM ammonium sulfate liposomes (or thereabouts) produce the most stable suspensions.

Figure 11 shows the polydispersity index of ammonium sulfate liposomes and subsequent Aposomes prepared by active loading of apomorphine (4 mM) against said ammonium sulfate liposomes. Ammonium sulfate concentration ranges include 300 mM, 500 mM, and 2M, following on from the encapsulation efficiency and hydrodynamic

diameter experiments. Data shows a significant increase in polydispersity for Aposomes prepared from 500 mM or 2M ammonium sulfate liposomes. Increase in polydispersity infers liposomal instability and heterogeneity, suggesting that Aposomes prepared from 300 mM ammonium sulfate liposomes (or thereabouts) produce the most stable suspensions. A polydispersity index of equal to or less than 0.1 is generally considered to be a stable, homogenous liposome suspension and is pharmaceutically acceptable.

Figure 12 is a set of cryo-transmission electron micrographs of Aposomal compositions as previously described in Examples 1-5 using 300 mM ammonium citrate (Fig 12A), ammonium trimesate (Fig 12B), carboxyfluorescein ammonium salt (Fig 12C) or 250 mM ammonium oxalate (Fig 12 D) as the intraliposomal ammonium salt. Negative-stain TEM demonstrated the presence of solid intraliposomal apomorphine salt particles in all cases demonstrating that ammonium salts with a broad range of divalent or trivalent counterions may be used in the preparation of stable Aposomes.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 Definitions and abbreviations

As used herein the term "comprising" means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

The term "about" as used herein means a reasonable amount of deviation of the modified term such that the end result is not significantly changed. For example, when applied to a value, the term should be construed as including a deviation of +/- 5% of the value.

The term "liposome" refers to a small artificial vesicle, spherical in shape, having at least one lipid bilayer. The "liposomal membrane" is the outer lipid bilayer of a liposome. The term "intraliposomal compartment" refers to the volume sequestered inside the liposomal membrane. The lipid bilayer is an arrangement of amphiphilic lipid molecules characterised by a hydrophilic moiety and a hydrophobic moiety, arranged in two-dimensional sheets in the which the hydrophobic moieties are oriented inwards while the hydrophilic moieties are oriented outward. Liposomes can be unilamellar (with one lipid bilayer) or multilamellar (with more than one lipid bilayer).

Liposomes generally have a spherical shape but reference to a liposomal diameter should not be taken to imply that the liposomes are completely spherical. References to liposomes as having a particular size should also not be taken to mean that every

liposome with a population of liposomes has that size. In general, a liposome's size refers to the average size of a population of liposomes with a bell-curve distribution of sizes.

As used herein the term "Aposome" means a "liposome comprising lipids and
5 apomorphine or a salt thereof, the lipids forming a lipid membrane enclosing an aqueous intraliposomal compartment containing the apomorphine or salt thereof" and can be replaced with the full description in any context in this specification.

The term "hydrodynamic diameter" means the diameter that a liposome would have if it were a perfect solid sphere exhibiting the same hydrodynamic friction as the liposome in
10 question when moving in a liquid. The hydrodynamic diameter takes account of the chemical chains extending from a liposome's exterior lipid membrane. The hydrodynamic diameter of a liposome may be larger than the liposome's core diameter since associated molecules and ions, for example solvation spheres, extending from the core may increase drag on the liposome, giving it the same diffusion properties of a hard
15 sphere of larger diameter. Unless otherwise specified, the "size" of a liposome refers to its average hydrodynamic diameter.

The average hydrodynamic diameter of a liposome may be measured by dynamic light scattering using, for example, a Malvern Zetasizer Nano, or other such Zetasizer or
20 particle sizing instrument known to those of ordinary skill in the art. Liposome size can also be measured using Transmission Electron Microscopy (TEM) which in most cases, will provide a good correlation with the hydrodynamic diameter measured by dynamic light scattering.

The term "therapeutically effective amount" (or "effective amount") refers to an amount sufficient to effect beneficial or desired results, including clinical results, but not limited
25 thereto. A therapeutically effective amount of the composition of the invention can be administered in one or more administrations of each agent separately or together. The therapeutically effective amount of the agents to be administered to a subject depends on, for example, the purpose for which the agents are administered, mode of administration, nature and dosage of any co-administered compounds, and
30 characteristics of the subject, such as general health, other diseases, age, sex, genotype, body weight and tolerance to drugs. A person skilled in the art will be able to determine appropriate dosages having regard to these any other relevant factors.

The term "subject" refers to a human or a non-human animal, preferably a vertebrate that is a mammal. Non-human mammals include, but are not limited to; livestock, such
35 as, cattle, sheep, swine, deer, and goats; sport and companion animals, such as, dogs,

cats, and horses; and research animals, such as, mice, rats, rabbits, and guinea pigs. Preferably, the subject is a human.

The term "treating" and grammatical variations thereof as used herein refers to both therapeutic and prophylactic or preventative measures, wherein the object is to prevent or slow down the targeted conditions.

It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7) and, therefore, all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

Whenever a range is given in the specification, for example, a temperature range, a time range, or a composition range, all intermediate ranges and subranges, as well as all individual values included in the ranges given are intended to be included in the disclosure. In the disclosure and the claims, "and/or" means additionally or alternatively. Moreover, any use of a term in the singular also encompasses plural forms.

5.2 The Aposomes of the invention and process of manufacture

The present invention provides Aposomes with surprisingly high drug to lipid ratios - between about 0.05:1 to about 5:1 (mol/mol) and/or about 0.05:1 to about 5:1 (wt/wt) apomorphine:total lipid constituents. This is much higher than the drug to lipid ratios found in known liposomal apomorphine formulations.

This superior product profile allows for a 30 to 60 fold reduction in the volume of a pharmaceutically acceptable product administered to a patient. For example, administering 3 mg of apomorphine to a patient using a formulation produced by the methods described by Wen et al. would require a 7.5 mL volume of liposomal composition to be administered, either orally, subcutaneously, intravenously, by inhalation, or intranasally. In contrast, the volume required to administer the same 3 mg dose of apomorphine to a patient using the methods and compositions of the present invention would be 0.25 to 0.125 mL.

In one aspect the invention provides an Aposome in which the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (mol/mol) and/or about 0.05:1 to about 5:1 (wt/wt).

In one embodiment the Aposomes are multilamellar or unilamellar, preferably unilamellar.

The inventor found that multivalent salts of apomorphine are insoluble and form a nanoscale precipitate in the intraliposomal compartment of the Aposomes when present at a sufficiently high concentration. In one embodiment the intraliposomal compartment of the Aposomes comprises solid or semi-solid apomorphine salt, preferably apomorphine sulfate. In one embodiment the solid or semi-solid apomorphine salt takes up about 10 to about 60% of the volume of the intraliposomal compartment. In another embodiment, the solid or semi-solid apomorphine salt takes up about 20 to about 50% of the volume of the intraliposomal compartment. In yet another embodiment, the solid or semi-solid apomorphine salt takes up about 30 to about 40% of the volume of the intraliposomal compartment.

In one embodiment the Aposomes are about 50 to 400 nm size, preferably about 50 to 330 nm, more preferably about 50 to 250 nm, and most preferably, about 50 to 150 nm size.

The lipid membrane of the liposomes/Aposomes comprises at least one liposome-forming lipid. Liposome-forming lipids include but are not limited to glycerophospholipids and sphingomyelins. Glycerophospholipids are lipids with a glycerol backbone where at least one, preferably two, of the hydroxyl groups of the head group are substituted by one or two of an acyl, alkyl or alkenyl chain, a phosphate group or combination of any of these and/or derivatives of the same. Glycerophospholipids may contain a chemically reactive group such as an amine, acid, ester, aldehyde or alcohol at the head group, thereby providing the lipid with a polar moiety. Examples of glycerophospholipids include but are not limited phosphatidylcholine (lecithin), phosphatidylglycerol, phosphatidylcholine, phosphatidyl ethanolamine, phosphatidylserine, phosphatidylinositol and hydrogenated glycerophospholipids such as hydrogenated soybean phosphatidyl choline (HSPC).

The sphingomyelins consist of a ceramide unit with a phosphorylcholine moiety attached at position 1 and therefore in fact are N-acyl sphingosines. The phosphorylcholine moiety is hydrophilic. The term "sphingomyelin" as used herein, includes dihydrosphingomyelin (DHSM).

DHSM for use in the methods of the invention can be obtained from natural or synthetic sources. Naturally sourced DHSM includes but is not limited to egg yolk or brain derived DHSM. Synthetic DHSM can be obtained by hydrogenating natural sphingomyelin, such as chicken egg or brain derived sphingomyelin. Alternatively, totally synthetic methods of preparing dihydrosphingomyelin can obtain 98% or more of the compound having a

long-chain alkyl group having 16 carbon atoms and a long-chain alkyl group having 18 carbon atoms.

In the liposome-forming lipids the acyl, alkyl or alkenyl chain is typically between 14 to about 24 carbon atoms in length, and has varying degrees of saturation being fully,
5 partially or non-hydrogenated naturally occurring lipids, semi-synthetic or fully synthetic lipids. The level of saturation may affect the rigidity of the liposome thus formed. Typically lipids with saturated chains are more rigid than lipids of same chain length in which there are un-saturated chains, especially having cis double bonds.

In one embodiment, the lipid membrane comprises a single type or a combination of
10 liposome-forming lipids. In one embodiment, the liposome-forming lipid is a phospholipid such as a glycerophospholipid or sphingosine. When the liposome-forming lipid is a phospholipid, the amount thereof in the liposome/Aposome can be determined as organic phosphorous by the modified Bartlett method (Shmeeda, Even-Chen, Honen, Cohen, & Weintraub, 2003).

15 In one embodiment, the liposome-forming lipid is a choline-type phospholipid such as diacylglycero-phosphocholine (the acyl, alkyl or alkenyl chain being as defined above).

In one embodiment the liposome-forming lipid is di-lauroyl-sn-glycero-2-phosphocholine (DLPC). In one embodiment the liposome-forming lipid is 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC). In one embodiment the liposome-forming lipid is 1,2-
20 dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). In one embodiment the liposome-forming lipid is 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). In one embodiment the liposome-forming lipid is 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine. In one embodiment the liposome-forming lipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). In one embodiment the liposome-forming lipid is 1,2-dinonadecanoyl-sn-
25 glycero-3-phosphocholine. In one embodiment the liposome-forming lipid is 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DBPC). In one embodiment the liposome-forming lipid is 1,2-diheneiarachidoyl-sn-glycero-3-phosphocholine. In one embodiment the liposome-forming lipid is 1,2-dibehenoyl-sn-glycero-3-phosphocholine 1,2-ditricosanoyl-sn-glycero-3-phosphocholine. In one embodiment the liposome-forming
30 lipid is 1,2-dilignoceroyl-sn-glycero-3-phosphocholine. In one embodiment the liposome-forming lipid is 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine. In one embodiment the liposome-forming lipid is 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (PSPC). In one embodiment the liposome-forming lipid is 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (SPPC). In one embodiment the liposome-forming lipid is 1,2-
35 di-oleoyl-sn-glycero-3-phosphocholine (DOPC). In one embodiment the liposome-forming lipid comprises at least hydrogenated soy phosphatidylcholine (HSPC). In

addition to the at least one liposome-forming lipid, the lipid membrane typically includes other lipophilic components.

In one embodiment the lipid membrane also comprises sphingomyelin. In one embodiment the lipid membrane comprises DHSM. In one embodiment the DHSM has
5 two long chain alkyl groups having 16 carbon atoms or a long-chain alkyl group having 16 carbon atoms and a long chain alkyl group having 18 carbon atoms or a long chain alkyl group having 16 carbon atoms and a long chain alkyl group having 20 to 24 carbon atoms. Preferably, the DHSM contains a long-chain alkyl group having 16 carbon atoms and a long chain alkyl group having 18 carbon atoms.

10 In one embodiment the lipid membrane also comprises a sterol, such as cholesterol.

In one embodiment the lipid membrane also comprises a lipopolymer. Lipopolymers comprise lipids modified at their head group with a polymer moiety, such as polyethylene glycol (PEG), having a molecular weight equal or above 750 Da. The head
15 group may be polar or apolar, to which a large (>750 Da) flexible hydrophilic polymer is attached. The attachment of the hydrophilic polymer head group to the lipid region may be a covalent or non-covalent attachment, however, it is preferably via the formation of a covalent bond (optionally via a linker).

The lipids modified into lipopolymers may be neutral, negatively charged, as well positively charged, i.e. there is no restriction to a specific (or no) charge. For example
20 the neutral distearoyl glycerol and the negatively charged distearoyl phosphatidylethanolamine can both be covalently attached to methoxy poly(ethylene glycol) (mPEG or PEG) of Mw 750, 2000, 5000, or 12000 (Priev, A., et al., 2002) (O, 2005) (Lasic, 1992).

The most commonly used and commercially available lipids derivatized into lipopolymers
25 are those based on phosphatidyl ethanolamine (PE), usually, distearylphosphatidylethanolamine (DSPE). A specific family of lipopolymers employed by the invention includes methoxy PEG-DSPE (with different lengths of PEG chains) in which the PEG polymer is linked to the DSPE primary amino group via a carbamate linkage. The PEG moiety preferably has a molecular weight of the head group from about
30 750 Da to about 20,000 Da. More preferably, the molecular weight of the head group is from about 750 Da to about 12,000 Da and most preferably between about 1,000 Da to about 5,000 Da. One specific methoxy PEG-DSPE employed herein is that wherein PEG has a molecular weight of 2000 Da, designated herein 2000PEG-DSPE, DSPE-PEG2000 or 2kPEG-DSPE (Lasic, 1992).

In one embodiment the lipid membrane comprises about 1 to about 10 mol% lipopolymer. In one embodiment the lipid membrane comprises at least about 1, 2, 3, 4, 5, 6, 7, 8 or 9 to about 10 mol% lipopolymer.

5 In one embodiment the lipid membrane comprises the liposome-forming lipid (which may be one or a combination of such lipids), one or more sterols and one or more lipopolymers. The mole ratio between these three components may vary. In one embodiment the lipid membrane comprises hydrogenated soybean phosphatidylcholine (HSPC), a lipopolymer of 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (2kPEG-DSPE) and cholesterol.

10 In one embodiment the lipid membrane comprises one or more of HSPC, cholesterol, DSPC, sphingomyelin, DHSM, cholesterol and methoxy DSPE-PEG (preferably DSPE-PEG2000).

15 In one embodiment the lipid membrane comprises about 20-60 mol% HSPC or DSPC and/or about 5-60 mol% sphingomyelin or DHSM and/or about 20-60 mol% cholesterol and/or about 1-15 mol% DSPE-PEG.

In one embodiment the lipid membrane comprises 20-60 mol% HSPC or DSPC, about 20-60 mol% cholesterol, about 5-60 mol% sphingomyelin or DHSM and about 1-15 mol% DSPE-PEG.

In one embodiment, the DSPE-PEG is selected from DSPE-PEG2000 or DSPE-PEG5000.

20 In one embodiment, the lipid membrane comprises HSPC or DHSM, cholesterol, and DSPE-PEG2000 in an about 56:39:5 mol% ratio.

In one embodiment, the lipid membrane comprises HSPC or DHSM, cholesterol, and DSPE-PEG2000 in an about 57:38:5 mol% ratio.

25 In one embodiment the lipid membrane comprises DSPC, sphingomyelin, cholesterol, and DSPE-PEG2000 in an about 100:5:5:4 mol ratio.

Liposomes/Aposomes are characterised by the amount (in moles or weight) of each component relative to the amount of lipid present in the lipid bilayer(s). For the avoidance of doubt, the total lipid present in the lipid bilayer includes sterols and lipopolymers as well as glycerophospholipids, sphingomyelins and other phospholipids.

30 The mass of total lipid present in the liposomes can be calculated based on the amount of lipid used to prepare the liposomes. Where the lipid membrane comprises mostly phospholipids, the total lipid concentration can also be estimated using the Stewart assay (Stewart, 1980), which measures the phospholipid concentration. Where the lipid membrane comprises a significant proportion non-phospholipid compounds, such as

sterols (e.g. cholesterol), the total lipid concentration in solution can be estimated by determining the phospholipid concentration using the Stewart Assay and dividing the determined concentration of phospholipid by the initial fraction of phospholipid:total lipid used in the preparation. Alternatively, the total lipid concentration can be determined by

5 high performance liquid chromatography, quantitative mass spectrometry, nuclear magnetic resonance spectroscopy, or other such methods of quantification known to those skilled in the art.

Once known, the total lipid concentration can be converted to mass and moles, using standard calculations.

10 In one embodiment the ratio of apomorphine to lipid in the Aposomes is between about 0.05:1 and about 5:1 (mol/mol), about 0.1:1 to about 4:1, about 0.2:1 to about 3:1 or about 0.4:1 to about 2:1 (mol/mol).

In one embodiment the ratio of apomorphine to lipid is greater than 0.5:1 (mol/mol).

In one embodiment the ratio of apomorphine to lipid in the Aposomes is about 0.05:1 to

15 about 5:1 (wt/wt), about 0.1:1 to about 3:1, about 0.2:1 to about 2:1 or about 0.5:1 to about 1:1 (wt/wt).

In one embodiment the ratio of apomorphine to lipid is greater than 0.2:1 wt/wt.

In one aspect the invention provides a composition comprising Aposomes and a carrier.

In one embodiment the carrier is a buffer.

20 The Aposomes within the composition are stable. Stability can be defined by rate of release of apomorphine from the liposomes when the liposomal composition is kept under storage at 4°C.

In one embodiment the Aposomes release less than about 20 wt%, preferably less than 10 wt% apomorphine into the carrier when stored at 4°C for one month. In one

25 embodiment the Aposomes release less than about 20 wt%, preferably less than about 10 wt% apomorphine, into the carrier when stored at 4°C for two, three, four, five, six months, nine, 12 or even 24 months.

The Aposomes of the invention are prepared by active loading of an apomorphine salt across the liposomal membrane using an ion gradient.

30 In another aspect the invention provides a process for making Aposomes comprising:

- (a) preparing an aqueous suspension comprising liposomes of 50-400 nm size wherein the liposomes comprise a lipid membrane enclosing an aqueous intraliposomal compartment containing an ammonium salt that is (i) at a

concentration of about 150 mM to about 550 mM and (ii) that is greater than the concentration of ammonium salt in the aqueous suspension;

- (b) mixing the aqueous suspension with a solution of apomorphine salt wherein the apomorphine enters the intraliposomal compartment and forms a salt with anions of the ammonium salt, wherein the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (mol/mol) and/or about 0.05:1 to about 5:1 (wt/wt);

(c) optionally removing unencapsulated apomorphine from the aqueous suspension.

The process of the invention utilises an ion gradient across the liposomal membrane to load a apomorphine into the liposomes.

Active liposomal loading methods are described in US Patents 5,316,771 and 11,413,244, incorporated by reference herein. However, the inventors have surprisingly identified that apomorphine, unlike other dopamine agonists used in the treatment of Parkinson's disease such as ropinirole and pramipexole, forms stable liposome suspensions with very high drug to lipid ratios, making them suitable for clinical use.

In step (a) of the method an aqueous suspension of liposomes is prepared. The liposomes are about 50-400 nm size and comprise a lipid membrane enclosing an aqueous intraliposomal compartment, which in turn comprises ammonium salt.

In one embodiment the concentration of ammonium salt inside the intraliposomal compartment is about 200 mM to about 500 mM. It is assumed that the concentration of ammonium salt inside the intraliposomal compartment is about the same as the concentration of ammonium salt used in the preparation of the liposomes.

In one embodiment the concentration of ammonium salt inside the intraliposomal compartment is about 200 mM to about 500 mM. In one embodiment the concentration of ammonium salt inside the intraliposomal compartment is about 250 mM to about 400, preferably about 150 to about 350 mM, more preferably about 250 mM to about 350 mM, most preferably about 300 mM.

In one embodiment the ammonium salt comprises ammonium and a multivalent counterion. In one embodiment the ammonium salt is selected from the group comprising ammonium sulfate, ammonium citrate, ammonium oxalate, ammonium phosphate, ammonium trimesate, sucrose ammonium octasulfate and carboxyfluorescein ammonium salt.

The concentration of ammonium salt inside the intraliposomal compartment is also greater than the concentration of ammonium salt in the aqueous suspension. Methods

of preparing liposomes of high ammonium salt concentration are known in the art, including in US Patents 5,316,771 and 11,413,244. An exemplary method is described in Example 1.

5 In one embodiment the aqueous suspension contains substantially no, or trace amounts of ammonium ions.

In one embodiment the aqueous suspension comprises an aqueous buffer. The aqueous buffer may include ethylenediaminetetraacetic acid or a pharmaceutically acceptable salt thereof, and/or citric acid or a pharmaceutically acceptable salt thereof; and buffering agents and buffer formulations, for example, but not limited to, BES (N,N-bis[2-
10 hydroxyethyl]-2-aminoethanesulfonic acid) Buffered Saline, Bicine (2-(Bis(2-hydroxyethyl)amino)acetic acid), Carbonate-Bicarbonate, CHES (N-Cyclohexyl-2-aminoethanesulfonic acid), Diethanolamine, EBBS (Earle's Balanced Salt Solution), Glycine-Sodium Hydroxide Buffer, HEPES ((4-(2-hydroxyethyl)-1-
15 piperazineethanesulfonic acid), HBSS (Hank's Balanced Salt Solution), HEPPSO (4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid) hydrate), HHBS (Hank's Buffer with HEPES), Imidazole-HCl, Maleic Acid, MES (2-(N-morpholino) ethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PBS (Phosphate Buffered Saline), Sodium Borate Buffer, TAE Buffer (Tris Base, Acetic Acid, EDTA), TBS (Tris Buffered Saline), TE Buffer (Tris EDTA), Tricine (N-(2-Hydroxy-1,1-
20 bis(hydroxymethyl)ethyl)glycine), TRIS (tris(hydroxymethyl)aminomethane) and/or Trizma (2-Amino-2-(hydroxymethyl)-1,3-propanediol).

The aqueous suspension may also include one or more of sodium chloride, sucrose, antioxidants and preserving agents.

25 In step (b) the aqueous suspension is mixed with a solution of apomorphine salt wherein the apomorphine is taken into the liposomes and forms a salt with anions of the ammonium salt.

Apomorphine salts are soluble in aqueous solution and dissociate to provide positively charged apomorphine ions at low pH. Where the pH is higher, apomorphine is largely deprotonated. Apomorphine has a pKa of 7.2.

30 Without being bound by theory, it is assumed that loading occurs as a complex equilibrium process between the dissolved ammonium salt and ammonia within the liposome, and protonated and unprotonated apomorphine external to the liposome, where the uncharged species can freely cross the liposome membrane resulting in the intraliposomal formation of insoluble apomorphine salt (for example – apomorphine
35 sulfate) (Figure 1).

In one embodiment the apomorphine salt is selected from hydrochloride salt and methanesulfonate salt. Hydrochloride salt is preferred.

In one embodiment the solution comprises apomorphine salt in buffer. The buffers described above for the aqueous liposome suspension are also suitable for use in the
5 solution of apomorphine salt. In one embodiment the solution comprises apomorphine salt in PBS buffer.

The concentration of apomorphine in the loading solution should be as high as practicable, taking into account the solubility of the apomorphine salt. In one embodiment the loading solution comprises about 4 mM apomorphine hydrochloride.

10 In one embodiment the concentration of lipid in the aqueous liposome suspension is about 1 mg/mL to about 100 mg/mL, about 5 mg/mL to about 50 mg/mL, about 10 mg/mL to about 30 mg/mL. In another embodiment the concentration of lipid in the aqueous liposome suspension is about 10 mg/mL.

In one embodiment the aqueous suspension of liposomes is mixed with the solution of
15 apomorphine salt in a ratio of about 1:100 to about 100:1 v/v, preferably about 1:20 to about 20:1. In one embodiment the apomorphine salt solution is about 4 mM concentration. Different ratios of liposomes to apomorphine salt give different effects. Assuming a liposome concentration of about 10 mg/mL, if the liposomal suspension is in excess, the system is driven to load all of the apomorphine into the liposomes. This
20 leaves less apomorphine to be removed from the suspension but results in less apomorphine sulfate per liposome (and so a lower drug to lipid ratio). If the apomorphine is in excess, the system is driven to exhaust the ammonium sulfate gradient resulting in a higher concentration of apomorphine sulfate in the intraliposomal compartment (about equivalent to the concentration of ammonium sulfate in the
25 liposomes). This results in a higher drug to lipid ratio but leaves excess apomorphine to be removed from the aqueous suspension.

In one embodiment the liposomal suspension is mixed with the solution of apomorphine salt in a ratio of about 1:5 v/v.

The process of the invention utilises an ion gradient across the liposomal membrane to
30 move apomorphine into the intraliposomal compartment, achieving a concentration of about 200 mM to about 500 mM of apomorphine salt in the intraliposomal compartment. As the concentration of ammonium salt increases, osmotic stress across the liposome membrane becomes a barrier to efficient drug loading and the preparation of a stable product. Consequently, it is preferred that the ammonium salt concentration in the
35 liposomes not exceed about 550 mM.

This increased osmotic pressure exhibited across the liposome membrane can be balanced by the inclusion of appropriate salts, for example sodium chloride, or sugars, for example sucrose, in the loading solution containing the apomorphine salt or the aqueous suspension.

- 5 In one embodiment the solution of apomorphine salt comprises one or more of sodium chloride and sucrose. In one embodiment the concentration of sodium chloride or sucrose is approximately osmotically equivalent to the concentration of ammonium salt in the liposomes.

- 10 The pH of the mixture is important because it determines the ratio of protonated to deprotonated apomorphine (see Figure 4). As shown in Example 3, apomorphine loading increases as the pH increases to 7.

- In one embodiment the pH of the mixture of the aqueous liposomal suspension and apomorphine salt solution is between about pH 5 and about pH 8. In another embodiment, the pH of the mixture is between about pH 6 and about pH 7. Where one
15 of the two components is in excess, the pH of the mixture can be approximated by the pH of the excess component.

- In one embodiment the aqueous suspension of liposomes comprising about 10 mg/L lipid, is mixed with the solution of apomorphine salt (preferably about 4 mM) in a ratio of about 1:100 to about 1:5 v/v and the pH of the solution of apomorphine salt is between
20 about pH 5 and about pH 8. In another embodiment, the pH of the solution is between about pH 6 and about pH 7.

- A person skilled in the art would know how long and under what conditions to mix the aqueous suspension of liposomes with the solution of apomorphine salt. For example, the opacity of the mixture can be monitored. As apomorphine salt precipitates in the
25 intraliposomal compartment, the mixture becomes more opaque. The process is complete when the opacity stops changing.

- In one embodiment the aqueous suspension of liposomes is mixed with the solution of apomorphine salt overnight at room temperature. This process results in Aposomes in which the ratio of apomorphine in the intraliposomal compartment to the total lipid in the
30 lipid membrane of the Aposome is about 0.05:1 to about 5:1 (mol/mol) and/or about 0.05:1 to about 5:1 (wt/wt).

In one embodiment ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane of the Aposome is about 0.1:1 to about 4:1, preferably about 0.2:1 to about 3:1 and more preferably, about 0.4:1 to about 2:1 (mol/mol).

In one embodiment the ratio of apomorphine to lipid in the Aposomes is about 0.1:1 to about 3:1, preferably about 0.2:1 to about 2:1 and more preferably, about 0.5:1 to about 1:1 (wt/wt).

5 Optionally in step (c) the unencapsulated apomorphine salt is removed from the aqueous suspension.

In one embodiment the unencapsulated apomorphine salt is removed dialysis or size exclusion chromatography.

In another aspect the invention provides a process for making Aposomes comprising:

10 (a) mixing an aqueous suspension of liposomes of 50-400 nm size with a solution of apomorphine salt wherein:

(i) the liposomes comprise a lipid membrane enclosing an aqueous intraliposomal compartment containing an ammonium salt that is (i) at a concentration of about 150 mM to about 550 mM and (ii) that is greater than the concentration of ammonium salt in the aqueous suspension; and

15 (ii) the apomorphine enters the intraliposomal compartment and forms a salt with anions of the ammonium salt, wherein the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (mol/mol) and/or about 0.05:1 to about 5:1 (wt/wt);

(b) optionally removing unencapsulated apomorphine from the aqueous suspension.

20 In one aspect the invention provides a composition comprising Aposomes of the invention and a carrier. In one embodiment the carrier is a buffer. In one embodiment the composition is a pharmaceutical composition and the carrier comprises pharmaceutically acceptable excipients.

25 In one embodiment the composition comprises a concentration of apomorphine that is sufficient to provide a therapeutic effect upon administration to a subject in need thereof.

30 Pharmaceutically acceptable excipients are carriers that are safe, non-toxic and not biologically undesirable. In one embodiment the pharmaceutically acceptable excipient is a aqueous or non-aqueous, isotonic sterile injection or infusion solution, which may contain anti-oxidants, buffering agents, and solutes including but not limited to saline, buffer, sugars, thickening agents, stabilisers and preservatives.

The concentration of apomorphine in the pharmaceutical composition of the invention depends on its intended use but is sufficient to achieve a therapeutic effect upon administration to a subject in need thereof, wherein the amount of pharmaceutical

composition administered is below the dose which would cause unacceptable side effects such as nausea, emesis, tachycardia and bladder pain. The amount of apomorphine required to achieve a therapeutic effect without causing unacceptable side effects depends on various parameters known to those skilled in the art, including the type and severity of disease, the mode of administration, and the gender, age, weight and other determinants of the subject to be treated. Achieving a therapeutic effect encompasses ameliorating undesired disease symptoms, preventing the manifestation of such symptoms before they occur, slowing down progression of the disease and/or symptoms, enhancing the onset of remission of the disease, slowing down damage caused by progressive chronic stages of the disease, delaying onset of progressive stages, lessening severity of the disease, improving the survival rate and/or curing or preventing the disease from occurring.

In one embodiment the pharmaceutical composition is formulated as a dosage form. In one embodiment the dosage form is an oral, parenteral, topical, etc dosage form. In one embodiment the dosage form comprises a total amount of apomorphine that is sufficient to provide a therapeutic effect upon administration to a subject in need thereof.

In another aspect the invention provides a method of treating a condition in which an increase in dopaminergic receptor activation would be beneficial in a subject in need thereof, the method comprising administration of a pharmaceutical composition of the invention to the subject.

In one embodiment the condition is a neurological condition. In one embodiment the condition is Parkinson's disease. In one embodiment the condition is Restless Leg Syndrome. In one embodiment the condition is Erectile Dysfunction. In one embodiment the condition is Female Sexual Dysfunction. In one embodiment the condition is Alzheimer's disease and/or dementia.

6. EXAMPLES

General Experimental Methods

30 *Apomorphine fluorescence standard curve*

Apomorphine is a weak amphipathic base with physiochemical properties suited for active loading against an ammonium salt gradient. Apomorphine is fluorescently active, with excitation and emission wavelengths of approximately 320 and 435 nm respectively.

10 μM and 100 μM apomorphine solutions were prepared containing phosphate buffered saline and 2 mg mL^{-1} ascorbic acid, and serially diluted to give final apomorphine concentrations between 0 and 9 μM , and 10 and 90 μM respectively. Fluorescence intensity from 100 μM aliquots of the solution were subsequently recorded on a

5 CLARIOstar® fluorescence plate reader with an excitation and emission wavelength of 320 nm and 435 nm respectively using Nunc™ 96-well microtitre fluorescence plates.

Additionally, the effect of liposomal encapsulation and lysing surfactant myristyltrimethylammonium bromide (MTAB) were investigated through the addition of 10 μM of a 10 mM liposome suspension (as described in Example 2), or the addition of

10 10 μM of a liposome suspension with an additional 10 μM of 25 mM MTAB. Since the fluorescence of a compound can change in the presence of surfactant micelles, this information allowed the total concentration of apomorphine in the Aposome suspension to be accounted for when measuring after complete lysis of the liposome suspension by surfactant addition.

15

General procedure for TEM and Cryo-TEM experiments

Negative stain TEM was performed using a Philips CM100 BioTWIN transmission electron microscope (Philips/FEI Corporation, Eindhoven, Neatherlands) combined with a LaB 6 emitter, fitted with a MegaView III Olympus digital camera. Samples were prepared by

20 depositing 10 μM of a diluted liposome suspension onto a 3 mm plasma-ionised carbon-coated copper specimen grid and removing it by capillary wicking using Whatman® filter paper after 60 seconds. Subsequently 10 μL of 1% phosphotungsted acid solution was deposited onto the grid and immediately removed by capillary wicking, and the grid was dried under heat lamp. Cryo-TEM was performed using a JEOL 2200FS field emission

25 scanning electron microscope (STEM) with an omega energy filter (JEOL Ltd., Tokyo Japan), fitted with a TVIPS F416 CMOS camera (TVIPS GmbH, Germany) and Direct Electron DE-20 detector (Direct Electron LP, California, USA). Specimen holders used included a standard JEOL holder with a single tilt axis (JEOL Ltd., Tokyo, Japan) and a Gatan model 914 high tilt cryo holder (Gatan Inc., California, USA). Images were

30 acquired using SerieIEM software (University of Colorado, Colorado, Boulder, USA) in conjunction with TVIPS EM-Menu and DE Imaging Manager. Image analysis was performed using the IMOD software package (Boulder Laboratory for 3D Microscopy, Colorado, USA).

Example 1: Preparation of liposomes comprising high ammonium salt concentration

35

Liposomes were prepared using a variation of the thin-film rehydration method and adapted for active drug loading. Liposomes were generally prepared using a lipid composition comprising hydrogenated soy phosphatidylcholine, cholesterol and DSPE-PEG2000 in a 57:38:5 mol% ratio, or DSPC, sphingomyelin, cholesterol, DSPE-PEG2000 in a 100:5:5:4 mol ratio by combining the appropriate volumes of the membrane components dissolved in chlorinated solvent (e.g. chloroform, dichloromethane) (see Table 1) and subsequently removing the solvent *in vacuo* to form a lipid film.

Table 1: Lipid concentrations and volumes generally used in preparation of liposomes

Lipid	Conc (mg mL ⁻¹)	Vol (μL)
HSPC	16	987
Cholesterol	2	192
DSPE-PEG2000	10	224
DSPC	16	987
Sphingomyelin	4	200

The dry lipid film was rehydrated with a solution containing the desired internal ammonium salt gradient, (for example: ammonium sulfate, ammonium citrate, ammonium oxalate, ammonium trimesate, carboxyfluorescein ammonium salt), at the appropriate concentration (50 to 2000 mM), without any additional buffering agents. To ensure dispersion of the lipid film, the suspension was heated to approximately the phase transition temperature of the lipid bilayer (approximately 50 °C) and vortexed or sonicated for five minutes, then extruded 15 times through polycarbonate membranes (1000 nm, 400 nm, 200 nm, and/or 100 nm in diameter) at 60°C to give a set of liposomes varying in size, ammonium salt concentration and ammonium salt anion (Olson, F., et al., 1979). The resulting liposomal suspension contained the same concentration of ammonium salt within and outside of the liposome. The external ammonium salt was removed via dialysis against phosphate buffered saline at pH 7.4. Dialysis was generally performed with 3 x 500 mL buffer changes at room temperature over 12 hours or until a sufficient ammonium salt gradient was established. The suspension of ammonium-containing liposomes in PBS was then stored at 4°C for later loading with apomorphine. The physical size characteristics of some resulting liposomes are described in Table 2.

Table 2: Physical characterization of some ammonium sulfate liposome preparations

Liposome Batch Description	Hydrodynamic Diameter (Z-Ave, nm)	Error	Polydispersity Index	Error
300 mM Ammonium Sulfate	150	2	0.11	0.01
500 mM Ammonium sulfate	162	3	0.09	0.003
2000 mM Ammonium sulfate	168	5	0.09	0.01

Example 2: Active loading of apomorphine into liposomes

- 5 The active loading process was initially investigated with 200 nm liposomes containing 300 mM ammonium sulfate (prepared as set out in Example 1). A 4 mM solution of apomorphine hydrochloride was prepared containing phosphate buffered saline (PBS), 2 mg mL⁻¹ ascorbic acid as an antioxidant at pH 6.3 and sodium chloride or sucrose in an approximately osmotically equivalent amount to the ammonium sulfate concentration in
- 10 the liposomes. Apomorphine has a pK_a of 7.2, so loading was performed at pH 6.3 to ensure an adequate proportion of neutral apomorphine.

- A 1 in 5 dilution of the liposome suspension (10 mg/mL lipid) was made using the apomorphine solution (400 µL liposome suspension to 1.6 mL apomorphine solution in a 2 mL glass HPLC tube). Apomorphine loading was performed at room temperature over
- 15 12 hours in a sealed tube with stirring. Any residual unencapsulated apomorphine was subsequently removed by dialysis against PBS at pH 7.4 containing ascorbic acid (2 mg mL⁻¹).

- After the removal of any residual unencapsulated apomorphine by dialysis the baseline fluorescence of the Aposome was measured using excitation and emission wavelengths
- 20 of 320 and 435 nm respectively with and without the inclusion of myristylmethylammonium bromide (MTAB) to ascertain the approximate amount of apomorphine encapsulated. Over the 12 hour period, the Aposome suspension was observed to turn noticeably opaque.

- The baseline fluorescence of the Aposome suspension indicated a relatively consistent
- 25 baseline of approximately 14000 ±1000 fluorescence units regardless of the initial concentration of an ammonium salt used (see Figure 2). However, a dramatic increase in the fluorescence intensity was observed upon lysis of the Aposomes with MTAB, demonstrating a sufficient degree of encapsulation when an internal ammonium salt

concentration of 300 mM or greater is used, with maximal fluorescence intensity achieved using a concentration of 500 mM (Figure 2). Interestingly, the total apomorphine fluorescence of the lysed suspension decreased when the internal concentration of an ammonium salt was increased above 500 mM, possibly due to the high osmotic stress across the liposomal membrane as a result of the high internal salt concentration leading to liposome rupture or partial loss of the internal gradient. (Levin & Idiart, 2004)

Example 3: Effect of osmotic stress and pH on apomorphine liposome loading

Osmotic stress can influence the behaviour and stability of liposomal drugs. In this example, the inventors studied the influence of osmotic stress on the apomorphine loading process, and optimisation of the conditions of manufacture to maximise encapsulation.

The effect of osmotic stress on liposome loading was investigated using Aposomes loaded using a 2000 mM ammonium sulfate gradient, with and without an equivalent concentration of sodium chloride in the apomorphine solution. The Aposomes were produced in accordance with Example 2. Their fluorescence intensity was measured, as described above. The results are shown in Figure 3.

Little change in fluorescence intensity was observed for a solution of apomorphine, or apomorphine with control liposomes containing no ammonium salt in the presence of 2000 mM sodium chloride over a period of 120 minutes. The addition of liposomes containing 2000 mM ammonium salt to the apomorphine solution with and without the inclusion of sodium chloride resulted in a significant decrease in fluorescence intensity, indicating the active accumulation of apomorphine within the Aposome interior.

However, a significant increase in the degree of loading was observed with addition of 2000 mM sodium chloride (Figure 3), demonstrating that the inclusion of an equivalent concentration of sodium chloride to the intraliposomal ammonium salt concentration within the apomorphine solution leads to a far superior loading outcome, where the ammonium salt concentration is high. The large degree of error in the fluorescence intensity recorded during the loading of apomorphine without the inclusion of sodium chloride is likely the result of osmotic stress induced liposome disruption.

Because the pH of the environment controls the ratio of protonated to deprotonated apomorphine (Figure 4A), and because deprotonated apomorphine is the predominant species able to freely cross the liposome membrane, the inventors postulated that the rate of active loading could be influenced by the external pH of the solution of apomorphine salt.

Using liposomes containing 300 mM ammonium salt, as these were demonstrated previously to have the highest encapsulation efficiency (see Example 4), the effect of the loading pH on the active accumulation of apomorphine was investigated. Ammonium salt liposomes prepared in accordance with Example 1 were mixed with a 4 mM apomorphine solution in a ratio of 1:5 v/v. The apomorphine solution contained a concentration of sodium chloride that was osmotically approximately equivalent to the concentration of ammonium salt. The mixture was buffered between pH 5.5 and 7.5 at intervals of 0.5. The fluorescence intensity was monitored over a period of 120 minutes (Figure 4B). In comparison to an apomorphine solution without the addition of liposomes (Figure 4C), a decrease in the fluorescence intensity was observed at all pHs indicating that active liposome loading of apomorphine occurs under all the pH ranges evaluated. However, a significant increase in the rate of change in the fluorescence intensity is observed with increasing pH. This demonstrates that as the equilibrium between protonated and deprotonated apomorphine is shifted to increase the proportion of the uncharged species, the rate of liposome loading can be dramatically increased.

In summary, both pH and osmotic stress are factors critical to the manufacture of Aposomes. Optimal drug loading is achieved when osmolality, or the salt concentration of the internal liposome volume and external medium, are approximately equal. Furthermore, the rate of drug loading is primarily controlled by the pH of the medium, with higher pH resulting in a greater rate of drug loading and encapsulation. However, the solubility of the apomorphine salt must be taken into account such that a pH of less than 7 is preferred.

Example 4: Effect of an ammonium salt gradient magnitude on loading capacity

The apomorphine loading capacity of liposomes prepared from increasing internal ammonium salt gradients was investigated. The liposomes were prepared in accordance with Example 2.

The apomorphine loading capacity was defined as the ratio of apomorphine to total lipid concentration. This was determined via analysis of the total phospholipid concentration and the total apomorphine concentration upon complete lysis of the liposomes. Although the lipid membrane comprises lipids that are not phospholipids, for most Aposomes, the phospholipid concentration approximates the total lipid concentration. In situations where the total lipid composition comprises a substantial proportion of non-phospholipid constituents, the total lipid concentration can be estimated by determining the phospholipid concentration in solution and dividing by the fraction of phospholipid to total lipid.

The total lipid concentration can also be calculated from the amount of lipids used to produce the dry lipid film when the liposomes comprising ammonium salt are first prepared.

- 5 The phospholipid concentration within the liposome suspensions was estimated using the Stewart assay, a colorimetric method for determining lipid concentration based on the reaction between phospholipids and ammonium ferrothiocyanate. (Stewart, 1980).

A linear relationship was confirmed between phosphatidylcholine and ammonium ferrothiocyanate (Figure 5A), which was used to quantify the phospholipid concentrations in the liposome suspensions post-dialysis after apomorphine loading using the formula
10 $y=10.866x+0.0033$, wherein y is the UV-Vis absorbance value, and x is the phospholipid concentration in μM . Briefly, 10 μL of Aposome suspension was combined with 10 μL of Stewart Assay ammonium ferrothiocyanate solution and 0.98 mL chloroform and shaken well for 15 minutes. The chloroform solution was analyzed colorimetrically to determine the diluted phospholipid concentration in chloroform. The concentration of phospholipid
15 in the Aposome preparation was then estimated by dividing the chloroform concentration by the dilution factor (0.02). In all cases, the phospholipid concentration detected in the Aposome preparation was below the initial preparation concentration of 2 mM. However, a decrease in detectable phospholipid was observed with increasing initial ammonium salt gradient (Figure 5B). Without wishing to be bound by theory, the inventors believe
20 this may result from osmotically-induced destruction of the liposomes with higher internal ammonium concentrations during the loading process, and subsequent removal of a portion of the phospholipid content via dialysis.

The apomorphine concentration in the Aposome suspension was determined by fluorescence spectroscopy as described in previous examples using the linear
25 relationship determined between apomorphine concentration and fluorescence intensity in the General Methods, using the formula $y=1514x+14159$, wherein y is the fluorescence intensity and x is the apomorphine concentration in μM . Briefly, 100 μL of Aposome suspension was combined with 100 μL MTAB (0.1%) and 1.8 mL phosphate buffered saline (pH 7.4) in a 2 mL quartz reduced volume cuvette and mixed well.
30 Fluorescence intensity was then measured with an excitation and emission wavelength of 320 nm and 435 nm respectively to give the apomorphine concentration in the diluted solution. The apomorphine concentration in the Aposome suspension was then estimated by dividing the diluted apomorphine concentration determined by fluorescence spectroscopy by the dilution factor (0.05).

- 35 The drug to lipid ratios of the Aposomes ranged from approximately 0.5 to 1.7 on a mol:mol basis, or approximately 0.2 – 0.6 on a mass:mass (mg:mg) basis. Stable

Aposomes with the highest drug to lipid ratios were achieved with 300, 500, and 1000 mM initial ammonium sulfate gradients, resulting in high drug:lipid ratios of between 1.5 – 1.7 on a mol:mol basis. Aposomes prepared from 300 mM and 500 mM ammonium sulfate resulted in the highest drug to lipid ratios of 1.5 to 1.7 on a mol:mol basis or 0.5 – 0.6 on a mass:mass basis.

These results demonstrate the surprisingly high loading efficiency of apomorphine with ~30-60x more apomorphine per lipid carrier on a mass:mass basis than the apomorphine liposomes reported by Wen and Hsu (Table 3; Figures 6 - 7), and 5x higher than the drug to lipid ratio of commercially available DOXIL[®] (liposomal doxorubicin) is 0.125 (wt/wt). (Nordstrom, R., et al., 2021)

Table 3: Drug to lipid ratios obtained from 200 nm liposomes encapsulating apomorphine prepared by active loading against a 100 – 2000 mM ammonium sulfate gradient.

Ammonium Sulfate Gradient	Drug to lipid ratio (mol:mol)	Error	Drug to lipid ratio (mg:mg)	Error
100 mM	0.55	0.05	0.19	0.02
300 mM	1.49	0.06	0.50	0.02
500 mM	1.73	0.2	0.58	0.07
1000 mM	1.65	0.4	0.56	0.1
2000 mM	0.82	0.2	0.28	0.05

As with liposomes loaded in the absence of external sodium chloride (Figure 5C), a similar trend in the total concentration of apomorphine encapsulated within the liposome suspension was observed, with an increasing encapsulated concentration observed with increasing initial ammonium salt gradient up to 500 mM, and subsequently decreasing for greater initial ammonium salt concentrations (Figure 5C). However, when normalising for the decreasing phospholipid concentration by considering the total apomorphine to phospholipid concentration ratio, no difference in the loading efficiency for 300 mM, 500 mM and 1000 mM ammonium salt liposomes was apparent (Figure 5C). The physical characteristics of some apomorphine liposome preparations are displayed in Table 4.

Table 4: Physical characteristics of liposomal apomorphine

Liposome Batch Description	Hydrodynamic Diameter (Z-Ave, nm)	Error	Polydispersity Index	Error
Aposomes from 300 mM Ammonium Sulfate liposomes	152	2	0.07	0.01
Aposomes from 500 mM Ammonium sulfate liposomes	206	10	0.2	0.04
Aposomes from 2000 mM Ammonium sulfate liposomes	211	6	0.16	0.004

In summary, the initial concentration of an ammonium salt encapsulated within the liposome determines the magnitude of apomorphine encapsulation after the loading process. Higher initial gradients generally lead to higher apomorphine encapsulations, with optimal loading occurring with an initial ammonium salt concentration of about 300 mM to about 500 mM. Initial gradient concentrations beyond these parameters are seen to diminish the magnitude of apomorphine encapsulation.

Example 5: Liposome-encapsulated nanoparticulate apomorphine sulfate

The morphology of the Aposomes was investigated to demonstrate that apomorphine is stably confined within the aqueous compartment of the liposome. Negative stain transmission electron microscopy (TEM) was performed on Aposomes loaded using a 300 mM ammonium salt gradient at pH 5.5 to 7.0 (as per Example 2) in order to investigate any pH-dependent change in liposome morphology. In all cases, solid apomorphine was evident within the liposome structures (Figure 8, A-D). A smaller proportion of liposomes comprising nanoscale precipitates of apomorphine salt were evident in the micrographs of liposomes loaded at pH 5.5, which is consistent with the lesser degree of loading observed by fluorescence spectroscopy. Interestingly however, similar morphologies were observed between Aposomes loaded at all pHs. Aposomes tended to contain one spherical precipitate per particle which appeared to form at one side of the liposome as opposed to centrally within the aqueous core.

Cryo-TEM studies of Aposomes confirm the formation of an apomorphine sulfate nanoparticle that generally occupies approximately 20-30% of the liposome volume

(Figure 9A). Passive apomorphine released from 300 mM Aposomes was assessed by incubating the liposome suspension at physiological temperature (37 °C) for 12 hours. A passive release rate of approximately 0.6% per hour was measured over the period at physiological temperature (Figure 9B) suggesting a stable liposome suspension.

- 5 Furthermore, dynamic light scattering (DLS) of the apomorphine liposomes in comparison to the initial 300 mM ammonium salt liposomes indicates no change in the size distribution and a small decrease in polydispersity index (Figures 10 and 11).

- In comparison, an increase in both average liposome size and polydispersity index was observed for Aposomes prepared from liposomes containing an ammonium ion
10 concentration greater than 300 mM, suggesting that Aposomes prepared from liposomes encapsulating about 300 mM ammonium sulfate form the most stable suspensions.

- Additionally, Aposomes were prepared as previously described in Examples 1-5 using 300 mM ammonium citrate, ammonium trimesate, carboxyfluorescein ammonium salt or 250 mM ammonium oxalate as the intraliposomal ammonium salt. Negative-stain TEM
15 demonstrated the presence of solid intraliposomal apomorphine salt particles in all cases demonstrating that ammonium salts with a broad range of divalent or trivalent counterions may be used in the preparation of stable Aposomes (Figure 12).

- In summary, methods for producing and characterizing liposomal structures encapsulating nanoscale precipitates of apomorphine salt have been demonstrated. The
20 nanoparticulate drug can be produced with size and shape control by actively or remotely loading the drug against a concentration gradient (such as an ammonium salt, for example ammonium sulfate, ammonium citrate, ammonium oxalate, ammonium trimesate). Aposomes prepared from liposomes encapsulating about 300 mM of an ammonium salt led to apomorphine liposomes with the greatest stability and the highest
25 drug:lipid ratios.

Example 6: Liposomal drug compositions encapsulating alternative D1 and D2 specific agonists

- Liposomes were prepared containing either ammonium citrate (300 mM), ammonium sulfate (300 mM), or ammonium trimesate (300 mM) to form the initial ammonium
30 gradient and incorporate a range of structurally diverse counterions. The active loading process described in Example 2 was carried out on alternative dopamine D1- and D2-agonists ropinirole and pramipexole. Both of these compounds are organic amines of similar molecular weight to apomorphine, which are also used clinically for the treatment of Parkinson's disease.
- 35 Surprisingly, unlike apomorphine, active loading of both ropinirole and pramipexole was unsuccessful in response to all ammonium ion gradients, with no intraliposomal drug

nanoparticle visible by TEM, demonstrating the serendipity of stably encapsulating apomorphine salts within a liposome preparation.

Example 7: Further prophetic examples of Aposomes

Aposomes comprising high drug to lipid ratios can be further produced using liposomes comprising alternative liposome-forming lipids and alternative methods of production.

As further non-limiting examples of Aposomes of the invention, the liposome may comprise a hydrophilic polymer-modified diacylphosphatidylethanolamine (e.g. DSPE-PEG-2000), a sphingomyelin or DHSM, and cholesterol. Example compositions and molar ratios of liposome components are described in Table 4.

Table 4: Molar ratio of components of the liposome membrane of prophetic Aposomes

Example Composition	PEG Lipid	Cholesterol	Sphingomyelin	Dihydrosphingomyelin
1	5%	37%	58%	0%
2	5%	42%	53%	0%
3	5%	42%	54%	0%
4	5%	47%	48%	0%
5	5%	38%	57%	0%
6	5%	39%	56%	0%
7	5%	38%	0%	57%
8	5%	36%	0%	59%
9	5%	40%	0%	55%
10	4%	36%	0%	60%
11	5%	43%	0%	52%
12	10%	40%	0%	50%
13	5%	40%	0%	55%
14	0%	43%	0%	57%
15	0%	45%	55%	0%

The sphingomyelin for use in the Aposomes of Table 4 may be obtained from synthetic or natural sources, including sphingomyelin manufactured synthetically or isolated and purified from brain or egg sources, such as chicken egg sphingomyelin and DHSM obtained by hydrogenating natural sphingomyelin or by synthetic manufacture.

By way of example, Aposomes comprising sphingomyelin or DHSM may be manufactured by thin-film rehydration and extrusion methods as previously described. In this case, the appropriate amounts of each lipid constituent described in Table 4 are weighed and

dissolved in organic solvent, such as ethanol or chloroform. The organic solvent is then removed by evaporation under vacuum or elevated temperature to obtain a dry film, which is subsequently rehydrated and dissolved in aqueous ammonium sulfate solution (150-350 mM) at 50-65 °C. The suspended lipid mixture is then extruded through
5 membranes with a controlled pore size, for example polycarbonate membranes with pore sizes ranging from 50 to 1000 nm, and preferably with pore sizes ranging from 50 – 200 nm, to produce ammonium sulfate liposomes with a diameter of 50 – 200 nm. The unencapsulated ammonium sulfate is then removed by dialysis against, for example
10 3.15% by mass NaCl solution or other liquid phase dialysis solution devoid of any ammonium ions. Apomorphine is then encapsulated in the 50-200 nm liposome particles by active (remote) loading, whereby apomorphine is dissolved in aqueous solution and pH adjusted with HCl to 3.5-6.5 and then combined with the liposome solution in a 1:5 to 5:1 volume ratio and heated to 50-60 °C for 60-120 minutes. Liposomal apomorphine is then purified, removing excess (unencapsulated) apomorphine by liquid dialysis
15 against, for example a phosphate buffered saline solution or a sucrose/histadine buffer containing approximately 9.4% sucrose and 10 mM histadine at room temperature.

By way of further example, Aposomes may be manufactured by emulsification and extrusion by:

- 20 1. Preparing an Oil Phase by dissolving the lipid constituents, for example those described in Table 4 or those described in any of Examples 1 – 6, in a volume of ethanol at 65 °C. For example dissolving 11.5 g of sphingomyelin or dihydrosphingomyelin, 4.32 g of cholesterol, and 4.32 g of PEG phospholipid in 381 mL of ethanol at 65 °C.
- 25 2. Preparing a first water phase, Water Phase 1, by dissolving an appropriate amount of ammonium sulfate into a large volume (e.g. 1000 mL) of water to achieve an ammonium sulfate concentration of 150 – 350 mM. For example dissolving 25.2 g of ammonium sulfate in 1000 mL of water to achieve an ammonium sulfate concentration of 170 mM.
- 30 3. Preparing a second water phase, Water Phase 2, by dissolving an appropriate amount of ammonium sulfate into a smaller volume (e.g. 200 mL) of water to achieve an ammonium sulfate concentration of 150 – 350 mM. For example dissolving 5.04 g of ammonium sulfate in 233.7 mL of water to achieve an ammonium sulfate concentration of 170 mM.
- 35 4. Producing a liposome suspension by emulsification, by heating Water Phase 1 to 65 °C and adding to it the whole Oil Phase, with mixing using a precision emulsification disperser at a peripheral speed of e.g. 25-26 m/s for 60 minutes. Subsequently the Water Phase 2 is added at room temperature with continuous

stirring at a reduced peripheral speed of e.g. 0.1 m/s while heating to 65 °C to evaporate the organic solvent and water until a reduced total aqueous volume of e.g. 600 mL is obtained.

- 5 5. Removing the excess unencapsulated ammonium sulfate from the outer aqueous phase by liquid dialysis. For example, an aqueous solution of saline (e.g. 3.15 w% NaCl) can be used as a dialysis liquid. Using this liquid, the liposome suspension prepared in (4) is subjected to cross-flow filtration at room temperature to remove ammonium sulfate present in the outer water phase to obtain purified ammonium sulfate liposomes with a low external concentration of ammonium ions.
- 10 6. Encapsulating apomorphine by active loading. Apomorphine hydrochloride is dissolved in water to a concentration of, for example, 5 mg/mL. Further, while stirring the apomorphine solution well, the pH is adjusted to pH 3 – 6.5 with hydrochloric acid. Liposomes are added to the resulting apomorphine solution at a volume ratio of between 1:5 and 5:1, preferably 1:1, and heated at 60 °C for 60 – 120 minutes.
- 15 7. Purification of liposomal apomorphine by dialysis. Excess, unencapsulated apomorphine is then removed from the liposomal apomorphine suspension by liquid dialysis using, for example, a phosphate buffered saline solution (pH ~7.4) or a sucrose/histidine buffer solution (e.g. 9.4 w% sucrose and 10 mM histidine). Using the dialysis liquid, the liposomal apomorphine suspension is subjected to cross-flow filtration at room temperature with the dialysis liquid to remove any unencapsulated apomorphine in the outer water phase (i.e. the outer water phase is replaced with the dialysis liquid to produce an Aposome composition of the invention.
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WHAT WE CLAIM:

1. A liposome comprising lipids and apomorphine or a salt thereof, the lipids forming a lipid membrane enclosing an aqueous intraliposomal compartment containing the apomorphine or salt thereof (Aposome), wherein the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (mol/mol), preferably about 0.05:1 and about 5:1 (mol/mol), more preferably about 0.1:1 to about 4:1, even more preferably about 0.2:1 to about 3:1 and most preferably, about 0.4:1 to about 2:1 (mol/mol).
2. An Aposome of claim 1 in which the intraliposomal compartment of the Aposome comprises a solid or semi-solid apomorphine salt, wherein preferably the apomorphine counter anion is a multivalent anion.
3. An Aposome of claim 2 wherein the apomorphine salt is selected from the group comprising apomorphine sulfate, apomorphine citrate, apomorphine oxalate, apomorphine sucrose octasulfate and apomorphine phosphate.
4. An Aposome of claim 2 or claim 3 in which the solid or semi-solid apomorphine salt takes up about 10 to about 60% of the volume of the intraliposomal compartment.
5. An Aposome of any preceding claim in which the lipid membrane comprises lipids selected from the group comprising phospholipids, sphingolipids, glycerolipids, polymer-conjugated lipids, and sterols.
6. An Aposome of any preceding claim which the lipid membrane comprises lipids selected from the group comprising HSPC, DHSM, DSPC, DPPC, DOPC, Soy PC, cholesterol, DSPE-PEG2000, sphingomyelin.
7. An Aposome of any preceding claim in which the lipid membrane comprises HSPC or DHSM, cholesterol and DSPE-PEG2000 in about a 57:38:5 mol% ratio or DSPC, sphingomyelin, cholesterol, and DSPE-PEG2000 in about a 100:5:5:4 mol ratio.
8. A composition comprising an Aposome of any one of claims 1 to 7 and buffer wherein the Aposomes release less than about 20 wt%, preferably less than 10 wt% apomorphine into the carrier when stored at 4°C for one, three, 6, 9, 12 or 24 months.
9. A process for making Aposomes comprising:

- 5 (a) preparing an aqueous suspension comprising liposomes of 50-400 nm size wherein the liposomes comprise a lipid membrane enclosing an aqueous intraliposomal compartment containing an ammonium salt that is (i) at a concentration of about 150 mM to about 550 mM and (ii) that is greater than the concentration of ammonium salt in the aqueous suspension;
- 10 (b) mixing the aqueous suspension with a solution of apomorphine salt wherein the apomorphine enters the intraliposomal compartment and forms a salt with anions of the ammonium salt, wherein the ratio of apomorphine in the intraliposomal compartment to the total lipid in the lipid membrane is about 0.05:1 to about 5:1 (mol/mol) and/or about 0.05:1 to about 5:1 (wt/wt); and
- (c) optionally removing unencapsulated apomorphine from the aqueous suspension.
10. A process of claim 9 wherein the concentration of ammonium salt inside the intraliposomal compartment is about 200 mM to about 500 mM, preferably about 250 mM to about 350 mM, more preferably about 300 mM.
- 15 11. A process of claim 9 or 10 wherein the ammonium salt is selected from the group comprising ammonium sulfate, ammonium citrate, ammonium oxalate, ammonium phosphate, ammonium trimesate, sucrose ammonium octasulfate and carboxyfluorescein ammonium salt.
- 20 12. A process of any one of claims 9 to 11 wherein the solution of apomorphine salt is about 4 mM concentration.
13. A process of any one of claims 9 to 12 wherein the concentration of lipid in the aqueous suspension is about 10 mg/mL.
- 25 14. A process of any one of claims 9 to 13 wherein the aqueous suspension of liposomes is mixed with the solution of apomorphine salt in a ratio of about 1:100 to about 100:1 v/v, preferably about 1:20 to about 20:1, more preferably about 1:5 v/v.
15. A process of any one of claims 9 to 14 wherein the pH of the mixture of the aqueous liposomal suspension and apomorphine salt solution is between about pH 5 and about pH 8, preferably about pH 6 and about pH 7.
- 30 16. A process of any one of claims 9 to 15 wherein optional step (c) is carried out.
17. A process of any one of claims 9 to 16 that provides an Aposome of any one of claims 1 to 7.
18. A pharmaceutical composition comprising an Aposome of any one of claims 1 to 7 and one or more pharmaceutically acceptable excipients.

19. A method of treating a condition in which an increase in dopaminergic receptor activation would be beneficial in a subject in need thereof, the method comprising administration of a pharmaceutical composition of claim 18.
20. A method of claim 19 wherein the condition is Parkinson's disease.