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The invention provides methods for correcting an imbalance of one or more entities in a mammal, as well as therapeutic liposomes and compositions for use with such methods.
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Title: THERAPEUTIC LIPOSOMES

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THERAPEUTIC LIPOSOMES

Priority of Invention

The present application claims priority from United States Provisional Application Number 60/501,816 and from United States Provisional Application Number 60/501,818, both of which were filed on 09 September 2003. The entire contents of each of these provisional applications is hereby incorporated herein by reference.

Field of the Invention

The present invention relates to liposomes that are capable of correcting an imbalance of one or more entities in an animal.

Background of the Invention

Numerous diseases and conditions are caused by or lead to an imbalance of one or more entities in an animal. For example, hypercalcemia is a common clinical metabolic problem. It can occur as a manifestation of many disorders including: hyperparathyroidism, Paget's disease, vitamin A and vitamin D toxicity, tuberculosis, sarcoidosis, malignancies e.g. breast cancer, small cell lung cancer, squamous cell carcinoma, multiple myeloma, and others.

Hypercalcemia occurs in 10%-20% of people with cancer, making it one of the most common medical management problems facing physicians. Moreover, hypercalcemia is the most common life-threatening metabolic disorder associated with malignancy. The most effective therapy for treating acute hypercalcemia associated with malignancy is to remove or treat the underlying cancer. Until this is achieved, or if this cannot be achieved, the use of antihypercalcemic agents may be necessary. Therapy to treat acute hypercalcemia should be selected carefully with specific patient goals of preventing the consequences of acute hypercalcemia by decreasing serum calcium concentrations and preventing adverse experiences from antihypercalcemic medications. Bisphosphonates, calcitonin, furosemide, gallium
nitrates, glucocorticoids, normal saline and plicamycin have been used with some degree of success in the management of malignancy and offer clinicians a range of therapeutic options in the treatment of acute hypercalcemia. These options all, however, suffer from limitations.

One method of treating hypercalcemia includes hydration with intravenous normal saline (NaCl 0.9%) as the first step in the acute management of hypercalcemia. Since most patients suffering from acute hypercalcemia are volume contracted, the administration of NaCl 0.9% is important because it expands intracellular volume in addition to increasing renal calcium clearance.

The optimal administration rate of NaCl 0.9% is determined by the severity of hypercalcemia, the degree of volume contraction, the ability of the patient to tolerate fluid, and the overall clinical status of the patient. The usual rate of infusion for NaCl 0.9% to facilitate hydration and diuresis is 200 to 300 mL/hr.

Furosemide, a high-ceiling diuretic, may be used in the treatment of hypercalcemia to increase calcium excretion. Furosemide is important in the treatment of acute hypercalcemia because it inhibits calcium reabsorption by the kidneys and protects against volume overload from the administration of saline. The furosemide therapy, however has limitations. In the absence of adequate volume expansion, furosemide treatment may cause a reduction in glomerular filtration rate as well as calcium clearance. Therefore, the treatment should be administered only after adequate hydration. Intravenous doses up to 100 mg every 1-4 hours may be administered.

Regimens of NaCl 0.9% and furosemide result in a modest decrease in serum calcium concentrations. Clinical trials suggest that the combination of saline and furosemide typically decreases calcium levels by only 0.20-0.30 mmol within the first 48 hours. Therefore, the effectiveness of hydration and diuresis accomplished by the administration of NaCl 0.9% and furosemide only may be sufficient for the management of mild to moderate hypercalcemia, and is usually inadequate for severe hypercalcemia. In patients with severe hypercalcemia, the use of other antihypercalcemic agents may be warranted.

Salmon calcitonin primarily inhibits osteoclastic bone resorption and increases renal excretion of calcium. Rapid onset of action and analgesic properties contribute to the success of calcitonin in the treatment of acute
hypercalcemia. Typically, serum calcium concentrations decline by 1-3 mg/dL within a few hours. Maximal efficiency is usually reached within 24 hours after administering calcitonin.

Many clinical trials have demonstrated tachyphylaxis associated with calcitonin. Clinical studies have also demonstrated that calcitonin has a brief duration of action that lasts for only 24 hours. Despite reports of tachyphylaxis and a brief duration of action, calcitonin is a prominent agent in the early management of severe hypercalcemia. The recommended subcutaneous or intramuscular starting dose of calcitonin for treating acute hypercalcemia is 4 IU/kg administered every 12 hours. Important side effects of calcitonin are flushing, gastrointestinal upset and rash which make calcitonin unpleasant for animals and causes poor patient compliance.

Bisphosphonates are toxic to osteoclasts and inhibit osteoclast precursors, thereby decreasing osteoclast function. Currently, etidronate is commercially available in both an oral and intravenous dosage forms, and pamidronate is available in the intravenous dosage form.

Etidronate is administered intravenously (IV) in cases of acute hypercalcemia. The recommended dose is 7.5 mg/kg IV daily for three to seven days; each dose should be infused over two to four hours. Typically, serum calcium concentrations begin to decline within two days. Maximal efficacy occurs within seven days after initiation of treatment. Clinical trials have documented a return to normal serum calcium concentrations in approximately 40%-92% of patients after they have received etidronate. Adverse effects associated with etidronate include elevated serum phosphorus concentrations and renal insufficiency. Other adverse effects include transient pyrexia, rash, nausea, and nephrotoxicity. The potential of nephrotoxicity is reduced in patients who have serum creatinine levels less than 2.5 mg/dL.

The recommended dose for pamidronate treatment of acute hypercalcemia is 60-90 mg intravenously, infused over 2-24 hours. Although a single pamidronate dose may be effective in the treatment of acute hypercalcemia, subsequent doses may be given seven days after administering the last dose. Serum calcium concentration may decline within three days and maximal effects are not noted until seven days after administration. Clinical
trials demonstrate that pamidronate typically normalizes serum calcium concentrations in approximately 60%-100% of patients. Some adverse events associated with pamidronate include transient pyrexia, nausea, rash, nephrotoxicity, hypocalcemia and thrombophlebitis.

Similar to etidronate, the use of pamidronate is not recommended in patients with renal dysfunction or patients who have elevated serum creatinine levels greater than 2.5 mg/dL.

Zolendranate is more potent and requires less IV infusion time. The typical treatment time for metastatic bone disease is one 15 minute IV infusion every 3-4 weeks. However, zolendranate is not an adequate substitute as it is contraindicated in renal insufficiency. Although there are claims that biphosphonates reduce bone pain in metastatic disease, there is no evidence for this in acute therapy.

Glucocorticoids have been used in combination with other antihypercalcemic agents such as calcitonin to lower serum calcium concentrations. Glucocorticoids combat hypercalcemia by increasing urinary calcium excretion and decreasing intestinal calcium absorption when doses equivalent to hydrocortisone 200-300 mg IV are administered daily for three to five days. Typically, hypercalcemia due to lymphomas, multiple myeloma and granulomatous tumors responds most favorably to glucocorticoid therapy. Common adverse effects possibly associated with glucocorticosteroids include hyperglycemia and electrolyte disturbances such as hypokalemia. Less frequent affects include GI bleeding, diabetes and altered mentation.

Gallium nitrate inhibits bone resorption by inhibiting PTH-rp induced calcium resorption from the bone. The recommended dosage of gallium nitrate is 100-200 mg/m² daily for five consecutive days or until the desired calcium concentration is obtained. Gallium nitrate should be infused as a 24-hour continuous infusion. Serum calcium concentration declines within 48 hours. Maximal effects are achieved within 10 days after the first gallium nitrate dose.

Nephrotoxicity has been documented in 8%-15% of patients using gallium nitrate. To reduce the risk of renal complications, it is recommended that all patients are adequately hydrated before receiving this agent. In addition, gallium nitrate should be avoided in patients with renal dysfunction or patients with a
serum creatinine measurement greater than 2.5 mg/dL. Furthermore, concurrent use of nephrotoxic agents should be avoided in patients receiving this agent. Other adverse effects associated with gallium nitrate include hypophosphatemia, gastrointestinal upset, visual and hearing impairment, and tachycardia.

Plicamycin decreases serum calcium concentrations by inhibiting RNA synthesis in osteoclasts. Plicamycin is administered intravenously, either as a bolus injection or slowly as an in fusion. The occurrence of local irritation and other adverse effects may be reduced when plicamycin is administered by slow infusion. The recommended dose is 25 mcg/kg over a period of four to six hours. The serum calcium concentration declines within 12 hours after initiation of plicamycin. Maximal efficacy usually occurs within 96 hours. Clinical trials have demonstrated a median decrease in serum calcium concentration by 1.4 mg/dL within 48 hours after administration. When necessary, subsequent doses of plicamycin may be given after waiting at least 24 hours. Major side effects of plicamycin include hepatic toxicity, nephrotoxicity, thrombocytopenia, myelosuppression, nausea, vomiting, hypocalcemia and reduced levels of clotting factors.

Plicamycin should be avoided in patients with severe hepatic or renal dysfunction, thrombocytopenia or any cagulopathy, patients concurrently receiving myelotoxic chemotherapy, and patients who are dehydrated. Plicamycin is usually reserved for patients who are unresponsive to other therapy.

As discussed above, current methods of treating hypercalcemia produce many undesired side effects which make treatment difficult and unpleasant for the patient. Additionally, many of the methods also require a significant period of time (24-48 hours or longer) to produce a beneficial effect. Additionally, the methods must be selectively chosen for treatment as many methods are incompatible with other medications, diseases, or the condition of the animal. Thus, there is currently a need for methods that can be used to remove calcium from living systems.

Diabetes is a condition that is characterized by an absolute or relative deficiency of insulin, which leads in more severe cases, to chronic hyperglycemia. The long term complications of diabetes include development
of neuropathy, retinopathy, nephropathy, and an increased susceptibility to infection (Stedman’s Medical Dictionary, 26 ed., William & Wilkins, Baltimore, Md., 1995). There is currently a need for additional methods for treating hyperglycemia, including hyperglycemia that results from diabetes. This is especially the case for hyperglycemia that does not respond to insulin containing regimens.

An excess amount of metal ions is also associated with a variety of diseases and conditions. For example, excess amounts of manganese, iron, mercury, aluminum, and copper have all been associated with Parkinson’s disease. Parkinson’s disease involves the deterioration of specific nerve centers in the brain. It is believed that these excess minerals increase free radical pathology and accelerate cell death. This deterioration changes the chemical balance of two neurotransmitters essential for transmission of nerve signals. The ultimate result is lack of control of physical movements.

Another disease related to high levels of metals in the blood is Alzheimer’s disease. Alzheimer’s disease is a progressive condition that destroys brain cells and structures. Researchers believe the disease is associated with excess zinc, copper, aluminum, and/or iron in the blood and/or brain. People with Alzheimer’s disease slowly lose their ability to learn, remember and function.

Another disease associated with excess metal ions in the body is Wilson’s disease. Wilson’s disease is caused by a build up of excess copper in the liver, brain, and kidneys. The disease is characterized by inflammation and cirrhosis of the liver and brain damage. If untreated, Wilson’s disease is fatal.

The accumulation of copper in the brain has also been associated with various forms of Transmissible Spongiform Encephalopathy (TSE) including Creutzfeldt-Jakob Disease (CJD).

In addition, exposure to heavy metals has been linked to developmental retardation, various cancers, kidney damage. For example, exposure to mercury, gold, and lead has been associated with the development of autoimmunity, a condition in which the immune system attacks its own cells, mistaking them for foreign invaders. Exposure to heavy metals such as mercury, lead, cadmium,
and aluminum is also believed to be associated with increased free radical
damage to the central nervous system and multiple sclerosis.

There is currently a need for methods that are useful for removing
unwanted or deleterious entities from animal systems and from biological
samples.

Summary of the Invention

The present invention provides liposomes that are capable of correcting
an imbalance of one or more entities in an animal. Accordingly, the invention
provides a method for incorporating an entity from an animal into a liposome
comprising administering to an animal in need of such treatment liposomes
capable of incorporating the entity.

The invention also provides a method for incorporating an entity from a
biological sample into a liposome comprising contacting the biological sample
with one or more liposomes capable of incorporating the entity.

The invention also provides a method for removing an entity from animal
serum comprising contacting the serum with one or more liposomes capable of
removing the entity from the serum.

The invention also provides a method for removing an entity from the
cerebral spinal fluid of an animal in need of such treatment comprising
administering (e.g. interthecally) to the animal liposomes capable of
incorporating the entity.

The invention also provides a method for treating Alzheimer’s disease in
an animal comprising administering an amount of a liposome to the animal that
is effective to lower the level of Zn, Al, Fe or Cu, or an ion thereof in the animal.

The invention also provides a method for treating Parkinson’s disease in
an animal comprising administering an amount of a liposome to the animal that
is effective to lower the level of Mn, Fe, Hg, Al, or Cu, or an ion thereof in the
animal.

The invention also provides a method for treating diabetes (e.g.
 hyperglycemia) in an animal comprising administering an amount of a liposome
to the animal that is effective to lower the level of glucose in the animal.
The invention also provides a method for reducing serum calcium load in an animal in need of such treatment comprising administering an effective calcium reducing amount of one or more liposomes.

The present invention also provides methods for removing calcium from living systems and methods of treating diseases associated with high calcium ion concentrations. Accordingly, the invention provides a method for removing calcium from animal serum comprising contacting (in vitro or in vivo) the serum with a liposome that removes the calcium from the serum.

The present invention also provides liposomes capable of sequestering calcium ions as well as methods of treating diseases caused by high calcium ion concentration comprising administering to an animal in need of such therapy a therapeutically effective amount of liposomes containing calcium ion receivers or sequesters.

The invention also provides a liposome as described herein for use in medical therapy.

The invention also provides the use of a liposome as described herein to prepare a medicament useful for treating Alzheimer’s disease in an animal.

The invention also provides the use of a liposome as described herein to prepare a medicament useful for treating Parkinson’s disease in an animal.

The invention also provides the use of a liposome as described herein to prepare a medicament useful for treating diabetes (e.g. hyperglycemia) in an animal.

The invention also provides the use of a liposome as described herein to prepare a medicament useful for reducing serum calcium load in an animal.

The invention also provides a pharmaceutical composition comprising a liposome as described herein and a pharmaceutically acceptable vehicle or carrier.

The invention also provides novel liposomes described herein as well as synthetic processes described herein for preparing liposomes of the invention.

**Brief Description of the Figures**

Figure 1 illustrates the lipid transition temperature dependence of calcium loading for various preparations.
Figure 2 illustrates calcium loading for three formulations prepared using different methods.

Figure 3 illustrates the influence of pH and NaCl on calcium loading efficiency.

Figure 4 illustrates calcium loading for HSPC:cholesterol formulations.

Figure 5 illustrates loading efficiencies for HSPC:cholesterol formulations.

Figure 6 illustrates calcium loading for DOPC:cholesterol formulations.

Figure 7 illustrates loading efficiencies for DOPC:cholesterol formulations.

Figure 8 illustrates calcium loading for DEPC:cholesterol formulations.

Figure 9 illustrates loading efficiencies for DEPC:cholesterol formulations.

Figure 10 illustrates calcium loading for DPPC:cholesterol formulations.

Figure 11 illustrates loading efficiencies for DPPC:cholesterol formulations.

**Detailed Description of the Invention**

**Definitions**

As used herein, the term “animal” refers to mammals, birds, reptiles, and fishes.

As used herein the term “biological sample” includes a tissue, serum, blood, plasma, cerebral spinal fluid, saliva, urine, etc. sample taken from an animal.

As used herein the term “calcium” includes calcium and calcium ions.

As used herein the term “channel/transporter” includes any molecule or set of molecules that allow an entity (e.g. calcium) to enter the liposome (e.g. that can effectively transport the entity to the interior of the liposome).

As used herein, the term “insoluble” or “slightly soluble” refers to inorganic salts (e.g. calcium salts) having a solubility constant (Ksp) in the range of that allows effective removal of an entity (e.g. calcium) from serum.
As used herein, the term "primarily bind" refers to a sequestering agent which either covalently or ionically binds to an entity (e.g. calcium) preferably over one or more other ions within the surroundings.

As used herein, the term "sequestering agent" or "receiver" refers to compounds complexes, molecules, or atoms capable of binding to an entity thereby removing the entity from the immediate surroundings.

Entities

The invention provides methods for removing unwanted entities from animals or from biological samples. Liposomes can be formulated to incorporate a wide variety of entities. Accordingly, the methods of the invention are generally useful for removing a wide variety of materials from an animal or from a biological sample. For example, the methods of the invention can be used to remove metal or a metal ions e.g. an alkali metal, an alkaline earth metal, Fe, Os, Co, Ni, Pd, Cu, Ag, Au, Zn, Al, Cd, Hg, Sn, or Pb, or an ion thereof. In particular, the methods of the invention are useful for removing Zn, Al, Fe or Cu, or an ion thereof from an animal. In a preferred embodiment, the methods are useful for removing calcium from an animal.

The methods are also useful for removing unwanted molecules (e.g. peptides, organic molecules, therapeutic agents, nitrous oxide, or glucose) from an animal. Accordingly, the methods are useful for therapy (i.e. removing peptides or compounds that are associated with a pathological condition). The methods are also useful for treating over exposure to therapeutic agents (i.e. overdoses) or toxic substances (i.e. poisonings). As used herein, the term organic compound includes any compound that comprises one or more carbon atoms. Typically an organic compound has a molecular weight of less than about 450 atomic mass unit (amu). In one preferred embodiment, the organic compound has a molecular weight of less than about 300 amu.

Contact with the liposome

The methods of the invention can be carried out in vitro or in vivo. For example, the methods can typically be carried out by administering liposomes to an animal. In some situations, it may be more convenient to remove a biological
sample (e.g. serum, cerebral spinal fluid, or tissue) from the animal and contact the sample with the liposomes outside the animal. Following removal of the undesirable entity, the sample can be returned into the animal. The methods of the invention are useful for therapeutic applications as well as for diagnostic applications.

**Incorporation into the liposome**

When used to incorporate a metal or a metal ion, the liposomes used in the methods of the invention typically include an ion channel or shuttle to facilitate entry of the metal or the ion into the liposome. For example, A23187 (available from CalBiochem (La Jolla, CA) or from Fermentek Ltd (Jerusalem, Israel) can be used to transport Ca^{2+} or other divalent cations. Such a channel or shuttle, however, is not always required. For example, amphiphilic entities can load in response to a chemical potential gradient (e.g. against a pH gradient).

Additionally, when used to incorporate hydrophobic entities, a channel that facilitates entry of the hydrophobic entity may or may not be necessary. The liposome may be permeable to some hydrophobic entities, but the entity may be trapped or modified inside the liposome after entry, so that it does not immediately pass back out of the liposome.

Once incorporated into the liposome, the entity may simply reside in the hydrophilic environment of the liposome interior, in the hydrophobic bilayer, or the liposome may include a sequestering agent that associates with (e.g. through hydrogen bonding or ionic interactions) the entity and reduces its ability to pass out of the liposome. For example, the liposome may include sequestering agents such as a polyamine, polylentate carboxylic acid (e.g. EDTA), crown ether, lactam, an inorganic compound, or other agents that create chemical gradients to draw the entity into liposomes. The liposomes may also include a reagent or enzyme capable of reacting with the entity so as to reduce its ability to pass out of the liposome. For example glucose could undergo conversion within the liposome to glucose-6-phosphate using hexokinase. If the reaction in the liposome requires an energy source (e.g. ATP), a co-factor, a specific pH, or a specific ion balance to take place, the liposomes can be prepared so that they
include these features. For *in vitro* applications, such features may also be provided from an external source.

**Mechanism of liposome action**

According to the methods of the invention, the liposomes can reduce the available concentration of an entity in an animal in a number of ways. For example, 1) the liposome can incorporate the entity in a reversible manner and then release the entity over time so that the maximum amount or concentration of entity available in the animal over that time is reduced; 2) the liposome can incorporate the entity and then be cleared from the animal thereby eliminating the entity from the animal’s serum; 3) the liposome can incorporate the entity and the entity can be sequestered or modified within the liposome so that it does not pass out of the liposome; or 4) following incorporation of the entity, the liposome can be redirected to other locations in the body (e.g. loaded into a macrophage) where the entity can be released or where the liposome containing the entity can be eliminated from the body. A combination of one or more of 1-4 above may also occur.

**Methods of Treating Hypercalcemia**

The present invention includes methods of treating an animal having hypercalcemia. The methods comprise administering to an animal in need of such treatment a therapeutically effective amount of at least one calcium sequestering liposome thereby reducing calcium ion concentration within the animal. The method typically reduces serum calcium concentration by administering an effective calcium reducing amount of at least one liposome that comprises a calcium sequestering agent to an animal.

In one embodiment, the method comprises administering to an animal in a hypercalcemic state at least one calcium sequestering liposome in an amount effective to lower serum cholesterol levels in the animal. Thereafter, the animal’s calcium ion serum concentration can be monitored about every 4 to about 6 hours for several days. Treatment can be terminated once the desired calcium ion serum concentration is reach, *i.e.* normocalcemia. Optionally, the
levels of magnesium ion, PTH, and phosphate may be monitored either simultaneously or sequentially with calcium ion concentration.

**Combination Therapies**

Use of the liposomes can also be combined with other therapies to treat a given condition (e.g. hyperglycemia, Alzheimer’s disease, Wilson’s disease, heavy metal poisoning, or hypercalcemia). For example, when the methods of the invention are used to treat hypercalcemia, the administration of liposomes can be combined with the administration of one or more therapeutic agents useful for reducing calcium load in an animal. The additional therapeutic agents may be administered, either prior, during, or after administration of the liposomes of the invention.

In one embodiment of the invention, the liposomes can contain one or more additional therapeutic agents within the liposome to treat a given condition (e.g. hyperglycemia, Alzheimer’s disease, Wilson’s disease, heavy metal poisoning, or hypercalcemia). Therapeutic agents contemplated for use in treating hypercalcemia include, but are not limited to, furosemide, calcitonin, bisphosphonates, etidronate, pamidronate, zoledranate, glucocorticoids, gallium nitrate, plicamycin, mithromycin.

**Liposomal Forming Lipids**

The liposomes include some liposome forming lipids (e.g. a phosphatidyl choline or sphingomyelin). Typically, the lipids include at least one phosphatidyl choline, which provides the primary packing/entrapment/structural element of the liposome. Typically, the phosphatidyl choline comprises mainly C₁₆ or longer fatty-acid chains. Chain length provides for both liposomal structure, integrity, and stability. Optionally, one of the fatty-acid chains have at least one double bond.

As used herein, the term “phosphatidyl choline” includes Soy PC, Egg PC dielaidoyl phosphatidyl choline (DEPC), dioleoyl phosphatidyl choline (DOPC), distearoyl phosphatidyl choline (DSPC), hydrogenated soybean phosphatidyl choline (HSPC), dipalmitoyl phosphatidyl choline (DPPC), 1-
palmitoyl-2-oleo phosphatidyl choline (POPC), dibehenoyl phosphatidyl choline (DBPC), and dimyristoyl phosphatidyl choline (DMPC).

As used herein, the term “Soy-PC” refers to phosphatidyl choline compositions including a variety of mono-, di-, tri-unsaturated, and saturated fatty acids. Typically, Soy-PC includes palmitic acid present in an amount of about 12% to about 33% by weight; stearic acid present in an amount of about 3% to about 8% by weight; oleic acid present in an amount of about 4% to about 22% by weight; linoleic acid present in an amount of about 60% to about 66% by weight; and linolenic acid present in an amount of about 5% to about 8% by weight.

As used herein, the term “Egg-PC” refers to a phosphatidyl choline composition including, but not limited to, a variety of saturated and unsaturated fatty acids. Typically, Egg-PC comprises palmitic acid present in an amount of about 34% by weight; stearic acid present in an amount of about 10% by weight; oleic acid present in an amount of about 31% by weight; and linoleic acid present in an amount of about 18% by weight.

**Cholesterol**

Cholesterol typically provides stability to the liposome. The ratio of phosphatidyl choline to cholesterol is typically from about 0.5:1 to about 4:1 by mole ratio. Preferably, the ratio of phosphatidyl choline to cholesterol is from about 1:1 to about 2:1 by mole ratio. More preferably, the ratio of phosphatidyl choline to cholesterol is about 2:1 by mole ratio.

**Calcium Sequestering Agents**

Calcium sequestering agents suitable for use with the liposome of the invention include, but are not limited to, compounds, complexes, ions, molecules, reagents, or combinations thereof capable of binding to calcium ions sufficiently to remove the calcium ions from the immediate surroundings. Calcium removal includes, but is not limited to, forming calcium complexes or insoluble or slightly soluble inorganic compounds within the liposome and thereafter removing the liposome from the serum. In one embodiment of the invention, the calcium sequestering agent preferably binds to calcium ions, however, the
calcium sequester may bind to other ions. For example, the calcium sequestering agent may primarily bind to calcium ions and/or remove other ions such as magnesium ions, among others.

Typical calcium sequestering agents include polyamines, polycarbonates, polydendate carboxylic acids, crown ethers, lactams, inorganic compounds, polyanions, protein fragments and peptides. Particular, calcium sequesters include, but are not limited to, ethylenediaminetetraacetic acid (EDTA), ethylenebis(oxyethylenenitrilo)-tetraacetic acid (EGTA), and calcitonin.

The amount of calcium sequester in the liposome may vary according to the type of calcium sequester desired, the desired time for reduction of hypercalcemia, and the condition of the animal. Typically, the amount of calcium sequester should be sufficient to cause a reduction in calcium ion concentration within minutes or hours from liposome administration. Alternatively, if rapid reduction of hypercalcemia is not desired, the calcium sequester may be selected to provide a steady and prolonged reduction of calcium ion over time, e.g. a day or several days. Typically, the calcium sequester is present in a concentration of about at least 50 mM prior to contact with the animal serum. Preferably, the calcium sequester is present in a concentration of about at least 100 mM, and more preferably, in a concentration of about at least 200 mM prior to contact with the animal serum. In one embodiment, the calcium sequester is capable of removing from about 10% to about 30% of the calcium ion concentration within the animal’s serum which may also cause normalization of the animals EKG.

Preparation of Liposomes

The liposomes typically comprise a lipid layer of phospholipids and cholesterol. Typically, the ratio of phospholipid to cholesterol is sufficient to form a liposome that will not dissolve or disintegrate once administered to the animal. The lipid and cholesterol are dissolved in a suitable solvent or solvent mixture with an appropriate amount of ionophore. After a suitable amount of time, the solvent is removed via vacuum drying or spray drying. The resulting solid material can be stored or used immediately.
Subsequently, the resulting solid material is hydrated in aqueous solution optionally containing a sequestering agent (e.g. a calcium sequestering agent such as EDTA), or optionally containing another therapeutic agent at appropriate temperatures, resulting in multilamelllar vesicles (MLV). The solutions containing MLV are size-reduced via homogenization to form Small Unilamellar Vesicles (SUV). A portion of the calcium sequestering agent is encapsulated in the aqueous compartment of SUV during the process. The unencapsulated sequestering agent is removed via suitable methods, such as dialysis, desalting column, or cross filtration. The resulting liposome solution is filtered and ready for use.

Calcium sequestering agent loading in buffer is formulation dependent. Among the relevant factors is the effective phase transition temperature of the lipid components (Tm). Figure 1 illustrates the lipid transition temperature dependence of calcium loading for various preparations. The loading in buffer may or may not directly relate to loading \textit{in vitro} (serum, plasma or blood) or \textit{in vivo}. Serum proteins or other elements may influence (positively or negatively) the function of the transporter channel. The overall liposome performance will result in part from loading \textit{in vivo} and the biodistribution, pharmacokinetics, and/or pharmacodynamics of the liposome.

Formulations

The lipid-based dispersions of the invention can also be formulated to be administered parenterally. Moreover, the lipid-based dispersions can be formulated for subcutaneous, intramuscular, intravenous, or intraperitoneal administration by infusion or injection. These preparations may also contain a preservative to prevent the growth of microorganisms, buffers, or anti-oxidants in suitable amounts.

Compositions and preparations will typically contain at least 0.1% of the sequestering agent. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of a given unit dosage form. The amount of sequestering agent active in such therapeutically useful compositions is such that an effective dosage level will be obtained.
The liposome may also comprise physiologically acceptable salts to maintain isotonicity with animal serum. Any pharmaceutically acceptable salt that achieves isotonicity with animal serum is acceptable, such as NaCl.

The amount of formulation required for use in treatment will vary not only with particular agent but also with the route of administration, the nature of the condition being treated and the age and condition of the animal; the amount required will be ultimately at the discretion of the attendant physician or clinician.

The desired amount of a formulation may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations. Optionally, the liposomes and/or the formulations may comprise at least one anti-oxidant, buffer, stabilizer, or pharmaceutically acceptable excipient.

Specific and Preferred Embodiments

Specific and preferred values are listed below; they do not exclude other defined values or other values within defined ranges.

In a specific embodiment of the invention the contacting occurs in vivo.

In another specific embodiment of the invention the contacting occurs in vitro

In another specific embodiment of the invention the entity is a metal or a metal ion.

In another specific embodiment of the invention the entity is an alkali metal, an alkaline earth metal, Fe, Os, Co, Ni, Pd, Cu, Ag, Au, Zn, Al, Cd, Hg, Sn, or Pb, or an ion thereof.

In another specific embodiment of the invention the entity is Zn, Al, Fe or Cu, or an ion thereof.

In another specific embodiment of the invention the entity is calcium or an ion thereof.

In another specific embodiment of the invention the entity is a peptide.

In another specific embodiment of the invention the entity is an organic molecule.
In another specific embodiment of the invention the entity is a therapeutic agent.

In another specific embodiment of the invention the entity is glucose.

In another specific embodiment of the invention the animal is a mammal.

In another specific embodiment of the invention the animal is a human.

In another specific embodiment of the invention the liposome comprises one or more liposome forming lipids and cholesterol, wherein the mole ratio of the lipids to cholesterol is from about 0.5 to 1 to about 4:1.

In another specific embodiment of the invention the liposome comprises phosphatidyl choline and cholesterol.

In another specific embodiment of the invention the mole ratio of phosphatidyl choline to cholesterol in the liposome is from about 0.5 to 1 to about 4:1.

In another specific embodiment of the invention the mole ratio of phosphatidyl choline to cholesterol in the liposome is from about 1 to 1 to about 2:1.

In another specific embodiment of the invention the mole ratio of phosphatidyl choline to cholesterol in the liposome is about 2:1.

In another specific embodiment of the invention the phosphatidyl choline is selected from Soy-PC, Egg-PC, DEPC, DOPC, DSPC, HSPC, DMPC, and DPPC, and mixtures thereof.

In another specific embodiment of the invention the phosphatidyl choline is selected from DEPC, DOPC, DSPC, HSPC, DMPC, POPC, DBPC, and DPPC, and mixtures thereof.

In another specific embodiment of the invention the phosphatidyl choline is selected from DEPC, DOPC, DSPC, HSPC, DMPC, and DPPC, and mixtures thereof.

In another specific embodiment of the invention the phosphatidyl choline is selected from DOPC, DSPC, HSPC, DMPC, and DPPC, and mixtures thereof.

In another specific embodiment of the invention the phosphatidyl choline is selected from DOPC, DSPC, HSPC, and DPPC, and mixtures thereof.

In another specific embodiment of the invention the phosphatidyl choline is DPPC.
In another specific embodiment of the invention the liposome comprises a channel or shuttle to facilitate incorporation of the entity into the liposome.

In another specific embodiment of the invention the channel is an ion channel.

In another specific embodiment of the invention the pH of the interior of the liposome is less than about 8 prior to administration or contact with the biological sample or the serum.

In another specific embodiment of the invention the liposome is an SUV or an MLV.

In another specific embodiment of the invention the entity binds with a sequestering agent inside the liposome to form a complex that is incapable of passing out of the liposome.

In another specific embodiment of the invention calcium binds with a sequestering agent inside the liposome to form a calcium complex that is incapable of passing out of the liposome.

In another specific embodiment of the invention the liposome comprises one or more calcium sequestering agents.

In another specific embodiment of the invention the liposome comprises liposome forming lipids and a transporter/channel that allows calcium to pass into the liposome.

In another specific embodiment of the invention the sequestering agent is a polyamine, polydentate carboxylic acid, crown ether, lactam, or an inorganic compound.

In another specific embodiment of the invention the sequestering agent is EDTA.

In another specific embodiment of the invention the concentration of EDTA in the liposome is at least about 10 mM prior to administration or contact.

In another specific embodiment of the invention the concentration of EDTA in the liposome is at least about 20 mM prior to administration or contact.

In another specific embodiment of the invention the concentration of EDTA in the liposome is at least about 25 mM prior to administration or contact.

In another specific embodiment of the invention the concentration of EDTA in the liposome is at least about 50 mM prior to administration or contact.
In another specific embodiment of the invention the concentration of EDTA in the liposome is at least about 80 mM prior to administration or contact.

In another specific embodiment of the invention the concentration of EDTA in the liposome is at least about 100 mM prior to administration or contact.

In another specific embodiment of the invention the concentration of EDTA in the liposome is at least about 200 mM prior to administration or contact.

In another specific embodiment of the invention the sequestering agent is at least 2-fold selective for calcium binding compared to zinc, selenium, or copper.

In another specific embodiment of the invention the entity reacts with a reagent inside the liposome to form a reacted entity. In another specific embodiment of the invention the reacted entity is incapable of passing out of the liposome.

In another specific embodiment of the invention the entity reacts with an enzyme inside the liposome. In another specific embodiment of the invention the reaction between the entity and the enzyme requires ATP.

In another specific embodiment of the invention the liposome comprises the ion channel A23187.

In another specific embodiment of the invention the liposome comprises an ion channel that is at least 2-fold selective for calcium entry into the liposome compared to zinc, selenium, or copper.

In another specific embodiment the methods of the invention utilize liposomes that are capable of removing calcium from an animal's circulation.

In another specific embodiment of the invention the liposomes contain calcium sequestering agents or receivers capable of associating with calcium ions.

In another specific embodiment of the invention the liposomes comprise a lipid layer comprising liposome forming lipids, cholesterol, and a channel/transporter, as well as a calcium sequestering agent or receiver capable of binding to one or more calcium ions.
Figures

Figure 1 illustrates the lipid transition temperature dependence of calcium loading in buffer solution for various liposome preparations. Five different formulations of 2:1 phospholipid-cholesterol preparations are shown. The five phospholipids were HSPC (55°C), DPPC (41°C), DMPC (23°C), DEPC (12°C) and DOPC (-20°C). All samples were prepared at pH 4.5 with 200 mM EDTA. In the DSPC-cholesterol formulation, two additional preparations were made using ammonium sulfate either entrapped or entrapped and in the exterior buffer.

Figure 2 illustrates calcium loading for three formulations prepared in four different manners. The formulations were prepared using 2:1 HSPC:cholesterol (Formulation 2A); 1:1.25:1.5 HSPC:cholesterol:DOPC (Formulation 2B); and 2:1 DPPC:cholesterol (Formulation 2C), at pH 4.5 with and without 140 mM NaCl or at a pH of 7.5 with and without 140 mM NaCl. Each phospholipid:cholesterol preparation in the 4 different preparations were averaged to obtain the loading curves presented.

Figure 3 illustrates the in vitro pH and NaCl dependency of calcium loading efficiency. Four formulations were prepared. A 2:1 DPPC:cholesterol formulation was prepared using 140 mM NaCl and 200 EDTA at a pH of 4.5 (Formulation 3A) and a second at a pH of 7.5 (Formulation 3B). Then a 2:1 DPPC:cholesterol formulation was prepared using 200 EDTA at a pH of 4.5 (Formulation 3C) and a second at a pH of 7.5 (Formulation 3D). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min. The graph indicates that the formulations prepared at pH of 4.5 contained the highest EDTA % saturation.

Figure 4 illustrates the calcium loading for four HSPC:cholesterol formulations. In particular, a 2:1 HSPC:cholesterol formulation was prepared using 140 mM NaCl and 200 mM EDTA at a pH of 4.5 (Formulation 4A) and a second at a pH of 7.5 (Formulation 4B). Then a 2:1 HSPC:cholesterol formulation was prepared using 200mM EDTA at a pH of 4.5 (Formulation 4D) and a second at a pH of 7.5 (Formulation 4D). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min. The graph indicates that the
formulation prepared with 140 mM NaCl and 200 mM EDTA at pH of 4.5 contained the highest serum saturation.

Figure 5 illustrates the loading efficiency for four HSPC:cholesterol formulations. A 2:1 HSPC:cholesterol formulation was prepared using 140 mM NaCl and 200 mM EDTA at a pH of 4.5 (Formulation 5A) and a second at a pH of 7.5 (Formulation 5B). Then a 2:1 DPPC:cholesterol formulation was prepared using 200 mM EDTA at a pH of 4.5 (Formulation 5D) and a second at a pH of 7.5 (Formulation 5C). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min. The graph indicates that the formulation prepared without NaCl and with 200 mM EDTA at pH of 4.5 contained the highest calcium ion concentration.

Figure 6 illustrates the calcium loading for seven HSPC:cholesterol:DOPC formulations. Four 1:1.25:1.5 HSPC:cholesterol:DOPC formulations were prepared: one 140 mM NaCl and 200 mM EDTA at a pH of 4.5 (Formulation 6B), a second at a pH of 7.5 (Formulation 6C), a third without NaCl at a pH of 7.5 (Formulation 6A), and a fourth without NaCl at a pH of 4.5 (Formulation 6D). Three HSPC:cholesterol:DOPC formulations were prepared. One is 1:0.14:0.25 HSPC:cholesterol:DOPC using 200 mM EDTA at a pH of 7.5 (Formulation 6E), a second is 1:0.12:0.05 HSPC:cholesterol:DOPC using 200 mM EDTA at a pH of 7.5 (Formulation 6F), a third is 1:0.17:0.5 HSPC:cholesterol:DOPC using 200 mM EDTA at a pH of 7.5 (Formulation 6G). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min.

Figure 7 illustrates the loading efficiency for seven HSPC:cholesterol:DOPC formulations. Four 1:1.25:1.5 HSPC:cholesterol:DOPC formulation were prepared: one 140 mM NaCl and 200 mM EDTA at a pH of 4.5 (Formulation 7B), a second at a pH of 7.5 (Formulation 7C), a third without NaCl at a pH of 7.5 (Formulation 7A), and a fourth without NaCl at a pH of 4.5 (Formulation 7D). Three HSPC:cholesterol:DOPC formulations were prepared. One is 1:0.17:0.5 HSPC:cholesterol:DOPC using 200 mM EDTA at a pH of 7.5 (Formulation 7E) a second is 1:0.14:0.25 HSPC:cholesterol:DOPC using 200 mM EDTA at a pH of 7.5 (Formulation 7F) a third is 1:0.12:0.05 HSPC:cholesterol:DOPC using
200 mM EDTA at a pH of 7.5 (Formulation 7G). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min.

Figure 8 illustrates the calcium loading for three HSPC:cholesterol:DEPC formulations. Three HSPC:cholesterol:DEPC formulations were prepared. One is 1:0.14:0.25 HSPC:cholesterol:DEPC using 200 mM EDTA at a pH of 7.5 (Formulation 8A) a second is 1:0.12:0.05 HSPC:cholesterol:DEPC using 200 mM EDTA at a pH of 7.5 (Formulation 8B) a third is 1:0.17:0.5 HSPC:cholesterol:DEPC using 200 mM EDTA at a pH of 7.5 (Formulation 8C). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min.

Figure 9 illustrates the loading efficiency for three HSPC:cholesterol:DEPC formulations. The three HSPC:cholesterol:DEPC formulations were prepared. One is 1:0.17:0.5 HSPC:cholesterol:DEPC using 200 mM EDTA at a pH of 7.5 (Formulation 9A) a second is 1:0.14:0.25 HSPC:cholesterol:DEPC using 200 mM EDTA at a pH of 7.5 (Formulation 9B) a third is 1:0.12:0.05 HSPC:cholesterol:DEPC using 200 mM EDTA at a pH of 7.5 (Formulation 9C). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min.

Figure 10 illustrates the calcium loading for four DPPC:cholesterol formulations. In particular, a 2:1 DPPC:cholesterol formulation was prepared using 140 mM NaCl and 200 EDTA at a pH of 4.5 (Formulation 10D) and a second at a pH of 7.5 (Formulation 10C). Then a 2:1 DPPC:cholesterol formulation was prepared using 200 EDTA at a pH of 4.5 (Formulation 10B) and a second at a pH of 7.5 (Formulation 10A). Measurements were taken at 0 min, 30 min, 60 min, 120 min, and 240 min. The graph indicates that the formulation prepared with 140 mM NaCl and 200 mM EDTA at pH of 7.5 contained the highest calcium ion concentration.

Figure 11 illustrates the loading efficiency for four DPPC:cholesterol formulations. A 2:1 DPPC:cholesterol formulation was prepared using 140 mM NaCl and 200 mM EDTA at a pH of 4.5 (Formulation 11D) and a second at a pH of 7.5 (Formulation 11C). Then a 2:1 DPPC:cholesterol formulation was prepared using 200 mM EDTA at a pH of 4.5 (Formulation 11B) and a second at a pH of 7.5 (Formulation 11A). Measurements were taken at 0 min, 30 min, 60
min, 120 min, and 240 min. The graph indicates that the formulation prepared with 140 mM NaCl and 200mM EDTA at pH of 4.5 contained the highest calcium ion concentration.

The invention is further defined by reference to the following examples describing the preparation of the liposomes and methods of treating hypercalcemia using the liposomes. It will be apparent to those skilled in the art, that many modifications, both to materials and methods, may be practiced without departing from the purpose and interest of this invention.

Calcium loading was studied in vitro by diluting liposomes in buffer or biological media (e.g. serum) and incubating (e.g. at 37°C). Over the course of incubation, samples are microfiltered through a small diameter cutoff (< 100kDa) microfiltration device. Filtrates are assayed by high performance liquid chromatography for total calcium concentration. These filtrate concentrations represent the amount of free calcium found in the media. Thus a decrease in filtrate calcium can be attributed to liposome uptake of calcium from the media.

Examples
Example 1: General Liposome Preparation

Phospholipids and cholesterol in a ratio of about 2:1, respectively, are dissolved in suitable solvent or solvent mixtures with appropriate amount of ionophore. The solvent is removed subsequently via vacuum drying or spray drying. The resulting solid material can be stored or used immediately.

Subsequently, the resulting solid material is hydrated in aqueous solution containing EDTA at appropriate temperatures, resulting in multilameller vesicles (MLV). The solutions containing MLV are size-reduced via homogenization to form Small Unilameller Vesicles (SUV). A portion of the EDTA is encapsulated in the aqueous compartment of SUV during the process. The unencapsulated EDTA is removed via suitable methods, such as dialysis, desalting column, or cross filtration. The resulting liposome solution is filtered and ready for use.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference.
Claims

What is claimed is:

1. A method for incorporating an entity from an animal into a liposome comprising administering to an animal in need of such treatment liposomes capable of incorporating the entity.

2. A method for incorporating an entity from a biological sample into a liposome comprising contacting the biological sample with one or more liposomes capable of incorporating the entity.

3. A method for removing an entity from animal serum comprising contacting the serum with one or more liposomes capable of removing the entity from the serum.

4. The method of claim 3 further comprising separating the liposomes from the serum.

5. The method of any one of claims 2-4 wherein the contacting occurs \textit{in vivo}.

6. The method of any one of claims 2-4 wherein the contacting occurs \textit{in vitro}.

7. The method of any one of claims 1-6 wherein the entity is a metal or a metal ion.

8. The method of any one of claims 1-6 wherein the entity is an alkali metal, an alkaline earth metal, Fe, Os, Co, Ni, Pd, Cu, Ag, Au, Zn, Al, Cd, Hg, Sn, or Pb, or an ion thereof.
9. The method of any one of claims 1-6 wherein the entity is Zn, Al, Fe or Cu, or an ion thereof.

10. The method of any one of claims 1-6 wherein the entity is calcium or an ion thereof.

11. The method of any one of claims 1-6 wherein the entity is a peptide.

12. The method of any one of claims 1-6 wherein the entity is an organic molecule.

13. The method of any one of claims 1-6 wherein the entity is a therapeutic agent.

14. The method of any one of claims 1-6 wherein the entity is glucose.

15. A method for treating Parkinson's disease in an animal comprising administering an amount of a liposome to the animal that is effective to lower the level of Mn, Fe, Hg, Al, or Cu, or an ion thereof in the animal's brain.

16. A method for treating Alzheimer's disease in an animal comprising administering an amount of a liposome to the animal that is effective to lower the level of Zn, Al, Fe or Cu, or an ion thereof in the animal's brain.

17. A method for treating diabetes in an animal comprising administering an amount of a liposome to the animal that is effective to lower the level of glucose in the animal's serum.

18. A method for removing an entity from the cerebral spinal fluid of an animal in need of such treatment comprising interthecally administering to the animal liposomes capable of incorporating the entity.

19. The method of claim 18 wherein the entity is a metal or a metal ion.
20. A method for reducing serum calcium load in an animal in need of such treatment comprising administering an effective calcium reducing amount of one or more liposomes to the animal.

21. The method of any one of claims 1 and 3-20 wherein the animal is a mammal.

22. The method of any one of claims 1 and 3-20 wherein the animal is a human.

23. The method of any one of claims 1-22 wherein the liposome comprises one or more liposome forming lipids and cholesterol, wherein the mole ratio of the lipids to cholesterol is from about 0.5 to 1 to about 4:1.

24. The method of any one of claims 1-22 wherein the liposome comprises phosphatidyl choline and cholesterol.

25. The method of claim 24 wherein the mole ratio of phosphatidyl choline to cholesterol is from about 0.5 to 1 to about 4:1.

26. The method of claim 24 wherein the mole ratio of phosphatidyl choline to cholesterol is from about 1 to 1 to about 2:1.

27. The method of claim 24 wherein the mole ratio of phosphatidyl choline to cholesterol is about 2:1.

28. The method of any one of claims 24-27 wherein the phosphatidyl choline is selected from Soy-PC, Egg-PC, DEPC, DOPC, DSPC, HSPC, DMPC, and DPPC, and mixtures thereof.
29. The method of any one of claims 24-27 wherein the phosphatidyl choline is selected from DEPC, DOPC, DSPC, HSPC, DMPC, POPC, DBPC, and DPPC, and mixtures thereof.

30. The method of any one of claims 24-27 wherein the phosphatidyl choline is selected from DEPC, DOPC, DSPC, HSPC, DMPC, and DPPC, and mixtures thereof.

31. The method of any one of claims 24-27 wherein the phosphatidyl choline is selected from DOPC, DSPC, HSPC, DMPC, and DPPC, and mixtures thereof.

32. The method of any one of claims 24-27 wherein the phosphatidyl choline is selected from DOPC, DSPC, HSPC, and DPPC, and mixtures thereof.

33. The method of any one of claims 24-27 wherein the phosphatidyl choline is DPPC.

34. The method of any one of claims 1-33 wherein the liposome comprises a channel or shuttle to facilitate incorporation of the entity into the liposome.

35. The method of claim 34 wherein the channel is an ion channel.

36. The method of any one of claims 1-35 wherein the pH of the interior of the liposome is less than about 8 prior to administration or contact with the biological sample.

37. The method of any one of claims 1-35 wherein the liposome is an SUV or an MLV.

38. The method of any one of claims 1-35 wherein the entity binds with a sequestering agent inside the liposome to form a complex that is incapable of passing out of the liposome.
39. The method of claim 10 or 20 wherein the calcium binds with a sequestering agent inside the liposome to form a calcium complex that is incapable of passing out of the liposome.

5 40. The method of claim 10 or 20 wherein the liposome comprises one or more calcium sequestering agents.

10 41. The method of claim 10 or 20 wherein the liposome comprises liposome forming lipids and a transporter/channel that allows calcium to pass into the liposome.

15 42. The method of any one of claims 38-40 wherein the sequestering agent is a polyamine, polydentate carboxylic acid, crown ether, lactam, or an inorganic compound.

15 43. The method of claim 42 wherein the sequestering agent is EDTA.

20 44. The method of claim 43 wherein the concentration of EDTA in the liposome is at least about 10 mM prior to administration or contact.

25 45. The method of claim 43 wherein the concentration of EDTA in the liposome is at least about 20 mM prior to administration or contact.

30 46. The method of claim 43 wherein the concentration of EDTA in the liposome is at least about 50 mM prior to administration or contact.

30 47. The method of claim 43 wherein the concentration of EDTA in the liposome is at least about 100 mM prior to administration or contact.

30 48. The method of claim 40 wherein the sequestering agent is at least 2-fold selective for calcium binding compared to zinc, selenium, or copper.
49. The method of any one of claims 1-37 wherein the entity reacts with a reagent inside the liposome to form a reacted entity.

50. The method of claim 49 wherein the reacted entity is incapable of passing out of the liposome.

51. The method of any one of claims 1-37 wherein the entity reacts with an enzyme inside the liposome.

52. The method of claim 51 wherein the reaction between the entity and the enzyme requires ATP.

53. The method of claim 41 wherein the liposome comprises the ion channel A23187.

54. The method of claim 40 or 48 wherein the liposome comprises an ion channel that is at least 2-fold selective for calcium entry into the liposome compared to zinc, selenium, or copper.

55. The method of any one of claims 1 and 15-20 further comprising administering another therapeutic agent to the animal.

56. The method of claim 10 or 20 further comprising administering a therapeutic agent useful for reducing calcium load to the animal.

57. The method according to claim 56, wherein the therapeutic agent is pamidronate, furosemide, a diphosphonate, gallium nitrate, a glucocorticoid, zolendranate, etidronate, or calcitonin.

58. The method according to claim 56 or 57 wherein the liposome comprises the therapeutic agent.

59. A liposome capable of incorporating an entity for use in medical therapy.
60. The use of a liposome capable of incorporating calcium or an ion thereof to prepare a medicament useful for reducing serum calcium load in an animal.

5 61. The use of a liposome capable of incorporating Zn, Al, Fe, or Cu, or an ion thereof to prepare a medicament useful for treating Alzheimer's disease in an animal.

10 62. The use of a liposome capable of incorporating glucose to prepare a medicament useful for treating diabetes in an animal.

15 63. The use of a liposome capable of incorporating Mn, Fe, Hg, Al, or Cu, or an ion thereof to prepare a medicament useful for treating Parkinson's disease in an animal.

64. The use of a liposome capable of incorporating an entity to prepare a medicament useful for removing the entity from the cerebral spinal fluid of an animal.

20 65. The use of claim 64 wherein the entity is a metal or a metal ion.

66. A liposome as described herein.

25 67. A pharmaceutical composition comprising a pharmaceutically acceptable vehicle or carrier, and a liposome capable of incorporating an entity as described herein.

68. The liposome of claim 59 wherein the entity is a metal, a metal ion, a peptide, an organic molecule, a toxic substance, nitrous oxide, or glucose.

30 69. The liposome of claim 59 wherein the entity is Ca, Fe, Os, Co, Ni, Pd, Cu, Ag, Au, Zn, Al, Cd, Hg, Sn, or Pb, or an ion thereof.
70. The composition of claim 67 wherein the entity is a metal, a metal ion, a peptide, an organic molecule, a toxic substance, nitrous oxide, or glucose.

71. The composition of claim 67 wherein the entity is Ca, Fe, Os, Co, Ni, Pd, Cu, Ag, Au, Zn, Al, Cd, Hg, Sn, or Pb, or an ion thereof.