rosuvastatin by hybrid hydration.

FIG. 4 shows the cryo-electron micrographs (A, B and C) and hydrated particle size results (D, E and F) of nanoliposomes HA_H-LP/R_{A150}, HA_M-LP/R_{A150} and HA_L-LP/R_{A150} that actively encapsulate rosuvastatin.

FIG. 5 shows the cryo-electron micrograph (A) and hydrated particle size result (B) of the targeted liposome nanocarrier delivery system HA@LP/R_C that actively encapsulates rosuvastatin.

FIG. 6 shows the cryo-electron micrograph and hydrated particle size (Dynamic Light Scattering, DLS) of the targeted liposome HA-LP/A_C that actively encapsulates atorvastatin.

FIG. 7 shows the cryo-electron micrograph and DLS of the targeted drug liposome HA-LP/Asp_C that actively encapsulates aspirin.

FIG. 8 shows the cryo-electron micrograph and DLS of the targeted liposome HA-LP/P_C that actively encapsulates pitavastatin.

FIG. 9 shows the cryo-electron micrograph and DLS of the targeted liposome HA-LP/L_C that actively encapsulates lovastatin.

FIG. 10 shows the cryo-electron micrograph and DLS of the targeted liposome HA-LP/S_C that actively encapsulates simvastatin.

FIG. 11 shows the cryo-electron micrograph and DLS of the targeted liposome HA-LP/P_C that actively encapsulates pravastatin.

FIG. 12 shows the cryo-electron micrograph and DLS of the targeted liposome HA-LP/B_C that actively encapsulates bezafibrate.

The reagents and instruments used in the following examples are as follows:

reagents:

DSPC, DSPE, cholesterol and DSPE-PEG2000, purchased from AVT Company;

chloroform, anhydrous ethanol, sodium bicarbonate, EDC, NHS, calcium acetate and rosuvastatin calcium, purchased from J&K Scientific;

HA, purchased from Bloomage Biotech; DSPE-PEG2000-NH_2, purchased from Sigma;

an ultrafiltration membrane pack, purchased from Merck; polycarbonate membrane, purchased from Whatman;

atorvastatin, purchased from Lunarsun;

aspirin, pitavastatin, lovastatin, simvastatin, pravastatin, bezafibrate, ciprofibrate, acemetacin, ozagrel, and tirofiban, purchased from J&K Scientific; 2-hydroxypropyl-β-cyclodextrin, purchased from Sigma-Aldrich;

instruments:

an extruder (ATS, AE001);

a laser particle size analyzer, purchased from Malvern Company (model: Nano-ZS).

HPLC, purchased from Waters Company (model: e2695).

EXAMPLE 1 Synthesis of plaque-targeted hyaluronic acid-based targeting materials (HPD)

Distearoylphosphatidylcholine (DSPC), DPN, cholesterol, and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:0.5:1:1) were weighed and the above lipid materials were dissolved with chloroform. The organic solvent was removed by means of slow rotary evaporation (55°C water bath, 90 r/min, 30 min) to form a lipid membrane on the wall of the container. A calcium acetate aqueous solution (200 mM) was added to fully hydrate the lipid membrane in a constant-temperature water bath kettle at 45°C to form a crude liposome suspension. Extrusion was performed through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected and then mixed well with 25 mL of rosuvastatin calcium aqueous solution (133 mg), and then the mixture was incubated at 65°C for 30 min to obtain drug-actively encapsulating liposomes LP/R_C with a particle size of 160 nm. FIG. 5 shows the particle size distribution diagram of HA@LP/R_C in Example 1 of the present disclosure.

The prepared nanoliposome solution loaded with rosuvastatin calcium was mixed with a pre-dissolved HA aqueous solution, and coupling agents EDC and NHSS were added. Reaction was performed while maintaining the temperature at 25°C or above for 2 hours or more. After incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate the uncoupled HA by dialysis to obtain the drug-loaded targeted liposomes HA@LP/R_C with an HA concentration of 10-200 μg/mL in the final product.

EXAMPLE 6 Preparation of targeted liposome nanocarriers actively encapsulating atorvastatin

In this example, the liposomes HA-LP/A_C loaded with a therapeutic agent was prepared by ethanol injection-active encapsulation method and an insertion method.

DSPC, cholesterol, and DSPE-PEG2000 (in a mass ratio of 3:1:1) were weighed and dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid materials in a constant-temperature water bath kettle at 65°C to form a crude liposome

suspension. Extrusion was performed through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder to control the final particle size to 130 nm. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 1 ml of the inner solution was mixed well with 2 mL of atorvastatin calcium aqueous solution (2.5 mg), and then the mixture was incubated at 60°C for 10 min to obtain liposomes LP/A_c actively encapsulating drug.

The prepared nanoliposome solution loaded with atorvastatin calcium was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 min or more. After incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate the uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes with an HPD concentration of 10-200 μg/mL in the final product.

FIG. 6 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/A_C actively encapsulating atorvastatin in this example.

EXAMPLE 7 Preparation of targeted liposome nanocarriers actively encapsulating aspirin

In this example, HA-LP/Asp_C was prepared by ethanol injection-active encapsulation method and a post-insertion method. An aspirin aqueous solution (2 mg/mL) was dissolved by adjusting the pH with NaOH. DSPC, cholesterol and DSPE-PEG2000 (in a mass ratio of 3:1:1) were weighed, and dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of aspirin aqueous solution, and then the mixture was incubated at 40°C for 10 min to obtain

liposomes LP/Asp_C actively encapsulating drug.

The prepared aspirin-loaded nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 7 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/Asp_C actively encapsulating aspirin in this example.

EXAMPLE 8 Preparation of targeted liposome nanocarriers actively encapsulating pitavastatin

In this example, HA-LP/P_C was prepared by ethanol injection-active encapsulation method and a post-insertion method.

A pitavastatin aqueous solution (1 mg/mL). DSPC, cholesterol and DSPE-PEG2000 (in a mass ratio of 3:1:1) were weighed, and dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of pitavastatin aqueous solution, and then the mixture was incubated at room temperature for 10 min to obtain liposomes LP/P_C actively encapsulating drug.

The prepared pitavastatin-loaded nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration

membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/P_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 8 shows the cryo-electron micrograph and DLS of the targeted HA-LP/P_C liposomes actively encapsulating pitavastatin in this example.

EXAMPLE 9 Preparation of targeted liposome nanocarriers actively encapsulating lovastatin

In this example, HA-LP/L_C was prepared by ethanol injection-active encapsulation method and a post-insertion method. A lovastatin aqueous solution (Lova, 1 mg/mL) was dissolved by adjusting the pH with NaOH. DSPC, cholesterol and DSPE-PEG2000 (in a mass ratio of 3:1:1) were weighed, and dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of lovastatin aqueous solution, and then the mixture was incubated at 65°C for 10 min to obtain liposomes LP/L_C actively encapsulating drug.

The prepared lovastatin-loaded nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/L_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 9 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/L_C

actively encapsulating lovastatin in this example.

EXAMPLE 10 Preparation of targeted liposome nanocarriers actively encapsulating simvastatin

In this example, HA-LP/S_C was prepared by ethanol injection, active encapsulation, and post-insertion method. A simvastatin aqueous solution (Simv, 1 mg/mL) was dissolved by adjusting the pH with NaOH and adding 10% 2-hydroxypropyl-β-cyclodextrin. DSPC, cholesterol and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed, and dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of simvastatin aqueous solution, and then the mixture was incubated at room temperature for 30 min to obtain liposomes LP/S_C actively encapsulating drug.

The prepared simvastatin-loaded nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/S_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 10 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/S_C actively encapsulating simvastatin in this example.

EXAMPLE 11 Preparation of targeted liposome nanocarriers actively encapsulating

pravastatin

In this example, HA-LP/P_C was prepared by ethanol injection-active encapsulation method and post-insertion method. A pravastatin aqueous solution (Pravas, 1 mg/mL). DSPC, cholesterol and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed, and dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of pravastatin aqueous solution, and then the mixture was incubated at 40°C for 30 min to obtain liposomes LP/P_C actively encapsulating drug.

The prepared pravastatin-loaded nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/P_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 11 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/P_C actively encapsulating pravastatin in this example.

EXAMPLE 12 Preparation of targeted liposome nanocarriers actively encapsulating bezafibrate

In this example, HA-LP/B_C was prepared by ethanol injection-active encapsulation and post-insertion method. A bezafibrate aqueous solution (Beza, 1 mg/mL) was dissolved by adjusting the pH with NaOH and adding 10% 2-hydroxypropyl-β-cyclodextrin. DSPC,

cholesterol and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed, and the lipid materials were dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of bezafibrate aqueous solution, and then the mixture was incubated at 40°C for 30 min to obtain liposomes LP/B_{C130} actively encapsulating drug.

The prepared bezafibrate-loaded nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/B_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 12 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/B_C actively encapsulating bezafibrate in this example.

EXAMPLE 13 Preparation of targeted liposome nanocarriers actively encapsulating ciprofibrate

In this example, HA-LP/C_C was prepared by ethanol injection, active encapsulation and post-insertion method. A ciprofibrate aqueous solution (Cipro, 1.5 mg/mL) was dissolved by adjusting the pH with NaOH to 6.0. Distearoylphosphatidylcholine (DSPC), cholesterol and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed, and the lipid materials were dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a

constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of ciprofibrate aqueous solution, and then the mixture was incubated at room temperature for 30 min to obtain liposomes LP/C_C actively encapsulating drug.

The prepared ciprofibrate nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/C_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 13 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/C_C actively encapsulating ciprofibrate in this example.

EXAMPLE 14 Preparation of targeted liposome nanocarriers actively encapsulating acemetacin

In this example, HA-LP/Am_C was prepared by ethanol injection, active encapsulation and post-insertion method. An acemetacin aqueous solution (Acemet, 1 mg/mL) was dissolved by adding 10% 2-hydroxypropyl-β-cyclodextrin. Distearoylphosphatidylcholine (DSPC), cholesterol and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed, and the lipid materials were dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was

separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of acemetacin aqueous solution, and then the mixture was incubated at room temperature for 30 min to obtain liposomes LP/Am_C actively encapsulating drug.

The prepared acemetacin nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/Am_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 14 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/Am_C actively encapsulating acemetacin in this example.

EXAMPLE 15 Preparation of targeted liposome nanocarriers actively encapsulating ozagrel

In this example, LP/O_C was prepared by ethanol injection, active encapsulation and post-insertion method. An ozagrel aqueous solution (Ozagrel, 2 mg/mL) was dissolved by adding 10% 2-hydroxypropyl-β-cyclodextrin. Distearoylphosphatidylcholine (DSPC), cholesterol and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed, and the lipid materials were dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of ozagrel aqueous solution, and then the mixture was incubated at room temperature for 30 min to obtain liposomes LP/O_C actively

encapsulating drug.

The prepared ozagrel nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/O_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 15 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/O_C actively encapsulating ozagrel in this example.

EXAMPLE 16 Preparation of targeted liposome nanocarriers actively encapsulating tirofiban

In this example, LP/T_C was prepared by ethanol injection, active encapsulation and post-insertion method. A tirofiban aqueous solution (Tirofiban, 2 mg/mL) was dissolved by adding 10% 2-hydroxypropyl-β-cyclodextrin. Distearoylphosphatidylcholine (DSPC), cholesterol and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed, and the lipid materials were dissolved with ethanol. A calcium acetate aqueous solution (250 mM) was added to fully hydrate the lipid material solution in a constant-temperature water bath kettle at 65°C to form a crude liposome suspension. Extrusion was performed 10 times through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected, and 2 mL of the inner solution was mixed well with 1 mL of tirofiban aqueous solution, and then the mixture was incubated at 65°C for 30 min to obtain liposomes LP/T_{C130} actively encapsulating drug.

The prepared tirofiban nanoliposome solution was mixed with a pre-dissolved HPD aqueous solution, and the mixture was incubated while maintaining the temperature at 25°C or above

for 5 minutes or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes (HA-LP/T_C) with a HPD concentration of 10-200 μg/mL in the final product.

FIG. 16 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/T_C actively encapsulating tirofiban in this example.

EXAMPLE 17 Preparation of the targeted liposome nanocarrier delivery system (HA@LP/R_d) actively encapsulating rosuvastatin

Hydrogenated soybean phosphatidylcholine, DPN, cholesterol, and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:0.5:1:1) were weighed and the above lipid materials were dissolved with chloroform. The organic solvent was removed by means of slow rotary evaporation (55°C water bath, 90 r/min, 30 min) to form a lipid membrane on the wall of the container. A copper gluconate aqueous solution (200 mM) was added to fully hydrate the lipid membrane in a constant-temperature water bath kettle at 45°C to form a crude liposome suspension. Extrusion was performed through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected and mixed well with 25 mL of rosuvastatin calcium (133 mg), and then the mixture was incubated at 65°C for 30 min to obtain liposomes LP/R_d actively encapsulating drug.

Coupling agents EDC and NHSS were added to an HA aqueous solution, and the resulting solution was mixed with the LP/R_d lipid solution to realize coupling. Reaction was performed while maintaining the temperature at 25°C or above for 2 hours or more. After completion, a dialysis bag or an ultrafiltration membrane pack was used to separate uncoupled HA by dialysis to obtain the drug-loaded targeted liposomes HA@LP/R_d with an HA concentration of 10-200 μg/mL in the final product.

FIG. 17 shows the cryo-electron micrograph and DLS of the targeted liposomes HA@LP/R_d actively encapsulating rosuvastatin in this example.

EXAMPLE 18 Preparation of targeted liposome nanocarrier delivery system (HA@LP/A_d) actively encapsulating atorvastatin

Dipalmitoylphosphatidylcholine, DPN, cholesterol, and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:0.5:1:1) were weighed and the above lipid materials were dissolved with chloroform. The organic solvent was removed by means of slow rotary evaporation (55°C water bath, 90 r/min, 30 min) to form a lipid membrane on the wall of the container. A calcium acetate aqueous solution (300 mM) was added to fully hydrate the lipid membrane in a constant-temperature water bath kettle at 45°C to form a crude liposome suspension. Extrusion was performed through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium acetate was separated using an ultrafiltration membrane pack. The inner solution was collected and then mixed well with 25 mL of atorvastatin calcium, and the mixture was incubated at 65°C for 30 min to obtain drug-actively encapsulating liposomes LP/A_d with a particle size of 130 nm.

Coupling agents EDC and NHSS were added to an HA aqueous solution, and the resulting solution was mixed with the LP/A_d lipid solution to realize coupling. Reaction was performed while maintaining the temperature at 25°C or above for 2 hours or more. After completion, a dialysis bag or an ultrafiltration membrane pack was used to separate uncoupled HA by dialysis to obtain the drug-loaded targeted liposomes HA@LP/A_d with an HA concentration of 10-200 μg/mL in the final product.

FIG. 18 shows the cryo-electron micrograph and DLS of the targeted liposomes HA@LP/A_d actively encapsulating atorvastatin in this example.

EXAMPLE 19 Preparation of targeted liposome nanocarrier delivery system

(HA@LP/C_d) actively encapsulating ciprofibrate

Sphingomyelin, cholesterol, and polyethylene glycol-distearoylphosphatidylethanolamine (DSPE-PEG2000) (in a mass ratio of 3:1:1) were weighed and the above lipid materials were dissolved with chloroform. The organic solvent was removed by means of slow rotary evaporation (55°C water bath, 90 r/min, 30 min) to form a lipid membrane on the wall of the container. A calcium bicarbonate aqueous solution (250 mM) was added to fully hydrate the lipid membrane in a constant-temperature water bath kettle at 45°C to form a crude liposome suspension. Extrusion was performed through polycarbonate membranes of 200 nm and 100 nm respectively using an extruder. The unencapsulated calcium bicarbonate was separated using an ultrafiltration membrane pack. The inner solution was collected and then mixed well with 25 mL of ciprofibrate, and the mixture was incubated at 65°C for 30 min to obtain drug-actively encapsulating liposomes LP/C_d with a particle size of 130 nm.

The HPD aqueous solution prepared in Example 1 was mixed and incubation was performed while maintaining the temperature at 25°C or above for 30 min or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes HA@LP/C_d with an HPD concentration of 10-200 μg/mL in the final product.

FIG. 19 shows the cryo-electron micrograph and DLS of the targeted liposomes HA@LP/C_d actively encapsulating ciprofibrate in this example.

EXAMPLE 20 Preparation of targeted liposome nanocarrier delivery system (HA-LP/B_d) actively encapsulating bezafibrate

In this example, bezafibrate-loaded nanoliposomes were prepared by ethanol injection & active encapsulation method.

Distearoylphosphatidylserine (DOPS), cholesterol and DSPE-PEG2000 (in a mass ratio of 3:1:1) were weighted, and the above lipid materials were dissolved with ethanol. A magnesium citrate aqueous solution (200 mM) was added and the ethanol phase was injected into the magnesium citrate aqueous solution while maintaining the temperature at 40-60°C and stirring to fully hydrate the lipid material solution so as to form a crude liposome suspension (the volume ratio of the magnesium citrate aqueous phase to the ethanol phase solution was greater than 2). A dialysis bag or an ultrafiltration membrane pack was used to separate the unencapsulated magnesium citrate by dialysis. The inner solution was collected and mixed well with a rosuvastatin calcium solution or a mixed solution of rosuvastatin calcium and sucrose (the mass ratio of rosuvastatin calcium to the total lipid materials was less than 0.25), and then incubation was performed at 4-66°C for 15 minutes or more to obtain nanoliposomes actively encapsulating rosuvastatin calcium. The prepared nanoliposomes have an encapsulation rate of greater than 20% for the API, and the final formulation has a concentration of API of less than or equal to 20 mg/mL.

The HPD aqueous solution prepared in Example 1 was mixed and incubation was performed while maintaining the temperature at 25°C or above for 30 min or more. After the incubation, a dialysis bag or an ultrafiltration membrane pack was used to separate uninserted HPD by dialysis to obtain the drug-loaded targeted liposomes HA-LP/B_d with an HPD concentration of 10-200 μg/mL in the final product.

FIG. 20 shows the cryo-electron micrograph and DLS of the targeted liposomes HA-LP/B_d actively encapsulating bezafibrate in this example.

Assay Example 1 Stable and controllable properties of the nanocarrier delivery systems of the present disclosure

The prepared nanocarrier delivery systems loaded with the therapeutic agents, as disclosed in the present disclosure, are taken as examples to show that the delivery system of the present

HA-LP/P_C	Ethanol injection-active encapsulation method and post-insertion method	150	95.1±0.9
HA-LP/B_C	Ethanol injection-active encapsulation method and post-insertion method	155	95.7±0.8
HA-LP/C_C	Ethanol injection-active encapsulation method and post-insertion method	148	93.4±1.4
HA-LP/Am_C	Ethanol injection-active encapsulation method and post-insertion method	146	94.7±1.1
HA-LP/O_C	Ethanol injection-active encapsulation method and post-insertion method	147	91.9±1.5
HA-LP/T_C	Ethanol injection, active encapsulation, and post-insertion method	132	92.7±1.3
HA@LP/R_d	Film hydration, active encapsulation, chemical coupling, and copper gluconate	169	91.3±0.9
HA@LP/A_d	Film hydration, active encapsulation, chemical coupling, and calcium acetate	165	94.3±1.6
HA@LP/C_d	Film hydration, active encapsulation, post-insertion method, and calcium bicarbonate	139	91.3±2.2
HA-LP/B_d	Ethanol injection, active encapsulation method, post-insertion method, and magnesium citrate	142	91.7±1.7

Note:

The above data are all expressed in the form of "average + standard deviation" of the results of 3 determinations in parallel.

5. Investigation of long-term stability

The nanocarrier delivery systems of the present disclosure were stored at 4°C, and sampled at different time points. The changes in the hydrated particle sizes thereof were detected by a laser particle analyzer. The HPLC method was used to detect the changes in the content and

Furthermore, the assay animals were randomly divided into the following groups, each including 6 animals:

rosuvastatin oral administration group: treatment was performed by oral administration at a dose of 0.5 mg rosuvastatin/kg body weight; and

rosuvastatin intravenous injection group: treatment was performed by intravenous administration at a dose of 0.5 mg rosuvastatin HA@LP/R_C/kg body weight;

the treatment group was treated once every other day for a total of 11 times of administration.

The curve of animal body weight change with treatment time is shown in FIG. 28. It can be seen from FIG. 28 that the animal body weight increased during the period of treatment, and no death was observed, indicating that the drug safety is good.

Assay Example 4 *In vivo* assay of the effect of targeted liposome nanocarrier delivery system of the present disclosure on atherosclerosis

The purpose of this test example is to verify the *in vivo* therapeutic effect of the targeted liposome nano drug carrier delivery systems of the present disclosure, such as the HA_M-LP/R_{A150} carrier delivery system, on arterial plaques.

1. Establishment of animal models and grouping of assay animals

(1) A normal saline solution of free rosuvastatin was prepared, and a nanodelivery system loaded with the therapeutic agent was prepared by the method described in the above Example 2.

(2) Establishment of ApoE−/− mouse models of atherosclerosis: