release of the drug to its target, and 4) liposome size that permits its extravasation from the blood stream across leaky tumor micro-vasculature permitting targeting of chemotherapeutic drugs to a tumor site.

[0011] An additional important design issue discovered by the inventors with liposomal formulations of doxorubicin (*e.g.* disclosed in U.S. Patent No. 7,901,709) is the stabilizing effect of doxorubicin complex (co-crystal or salt) formation on the stability of the finished drug product. Successful control of degradation rates will result in significant impact on the storage temperature and long term stability of the drug product. Two degradation products of interest are 8-desacetyl-8-carboxy daunorubicin and impurity A.

[0012] The present invention solves a persistent problem with drug degradation in doxorubicin liposomal formulation that results in a citrate complex (co-crystal or salt). It has been found that the citrate complex plays a significant role in doxorubicin instability and formation of degradates. More specifically, the formation of 8-desacetyl-8-carboxy daunorubicin and impurity A can be significantly reduced by changing the formation of a citrate complex to a sulfate complex (co-crystal or salt). In addition, the present invention maintains the key design principles listed above for an efficacious hyperthermically activated liposomal formulation containing an active agent.

BRIEF SUMMARY OF THE INVENTION

[0012A] In one aspect, there is provided a liposomal preparation, comprising a suspension of liposomes having a gel-phase lipid bilayer and an active agent entrapped inside the liposomes; said lipid bilayer comprising:

(i) one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositols, and phosphatidyl ethanolamines;

(ii) one or more phospholipids derivatized with a hydrophilic polymer; and

(iii) one or more lysolipids selected from the group consisting of monoacylphosphatidyl cholines,

monoacylphosphatidylglycerols, monoacylphosphatidylinositols, and monoacylphosphatidyl-ethanolamines;

wherein the active agent is selected from the group consisting of doxorubicin, bleomycin, dacarbazine, daunorubicin, dactinomycin, fludarabine, gemcitabine, idarubicin, methotrexate, mitomycin, mitoxantrone, vinblastine, vinorelbine, and vincristine, and wherein the lipid bilayer constituents are provided in a molar ratio of about 80-90:2-8:2-18; and wherein the size of the liposomes in the suspension is between about 50 and about 150 nm.

[0013] ~~In one aspect, the invention provides~~Also disclosed is a pharmaceutical composition, comprising a suspension of liposomes having a gel-phase lipid bilayer and doxorubicin entrapped inside the liposomes; said lipid bilayer comprising:

(i) one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositols, and phosphatidyl ethanolamines;

(ii) one or more phospholipids derivatized with a hydrophilic polymer; and

(iii) one or more lysolipids selected from the group consisting of monoacylphosphatidyl cholines, monoacylphosphatidylglycerols,

monoacylphosphatidylinositols, and monoacylphosphatidyl-ethanolamines;

wherein the lipid bilayer constituents are provided in a molar ratio (i):(ii):(iii) of about 80-90:2-8:2-18; wherein the liposomes in the suspension have an average particle size of between about 50 and about 150 nm; and

wherein the relative concentration of impurity A after 6 months of storage at about less than or equal to 8 °C is less than 0.5%, and wherein impurity A is a peak with a relative retention time of about 1.4 when separation is achieved using high performance liquid chromatography (HPLC) with a C18 reverse phase column and acetic acid/methanol solvent gradient elution buffer.

[0013A] In another aspect, there is provided a method for loading an active agent into temperature sensitive liposomes, comprising:

(a) preparing a suspension of liposomes having a gel-phase lipid bilayer and a greater concentration of ammonium ions inside the liposomes than outside the liposomes, said lipid bilayer comprising:

(i) one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositols, and phosphatidyl ethanolamines;

(ii) one or more phospholipids derivatized with a hydrophilic polymer; and

(iii) one or more lysolipids selected from the group consisting of monoacylphosphatidyl cholines, monoacylphosphatidylglycerols, monoacylphosphatidylinositols, and monoacylphosphatidyl-ethanolamines;

wherein the lipid bilayer constituents are provided in a molar ratio of about 80-90:2-8:2-18; and

where said preparing includes reducing the size of the liposomes in the suspension to an average particle size of between about 50 and about 150 nm;

(b) adding a solution of the active agent to the suspension of liposomes, wherein the active agent is taken up into the liposomes,

wherein the active agent is selected from the group consisting of doxorubicin, bleomycin, dacarbazine, daunorubicin, dactinomycin, fludarabine, gemcitabine, idarubicin, methotrexate, mitomycin, mitoxantrone, vinblastine, vinorelbine, and vincristine.

[0014] ~~In another aspect, the invention provides~~Also disclosed is a method for loading doxorubicin into temperature sensitive liposomes, comprising:

(a) preparing a suspension of liposomes having a gel-phase lipid bilayer and a greater concentration of ammonium ions inside the liposomes than outside the liposomes, said lipid bilayer comprising:

(i) one or more phospholipids selected from the group consisting of phosphatidyl cholines, phosphatidyl glycerols, phosphatidyl inositols, and phosphatidyl ethanolamines;

(ii) one or more phospholipids derivatized with a hydrophilic polymer; and

(iii) one or more lysolipids selected from the group consisting of monoacylphosphatidyl cholines, monoacylphosphatidylglycerols, monoacylphosphatidylinositols, and monoacylphosphatidyl-ethanolamines;

wherein the lipid bilayer constituents are provided in a molar ratio (i):(ii):(iii) of about 80-90:2-8:2-18; and

where said preparing includes reducing the size of the liposomes in the suspension to an average particle size of between about 50 and about 150 nm;

(b) adding a doxorubicin solution to the suspension of liposomes, wherein the doxorubicin is taken up into the liposomes.

[0015] In another aspect, the invention comprises a liposomal preparation made by the method set forth above.

[0016] ~~In another aspect, the invention comprises~~Also disclosed is a liposomal preparation comprising doxorubicin and an imaging agent. ~~In yet another aspect, the invention comprises~~Also disclosed is a liposomal preparation comprising doxorubicin and another drug.

[0017] These and other aspects and advantages of the invention are set forth in detail hereinbelow.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0018] FIG. 1 schematically represents a liposome having a bilayer membrane containing dipalmitoylphosphatidylcholine (DPPC) as a phospholipid, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethyleneglycol) 2000 (DSPE-MPEG), and monosteroyl-phosphatidylcholine (MSPC) as a lysolipid. The orientation of the lysolipid monomers and their presence in both the inner and outer layers of the lipid bilayer is indicated.

[0019] FIG. 2 is a schematic representation of the ammonium loaded doxorubicin liposomes manufacturing process.

[0020] FIG. 3 is a particle size distribution of NH_4^+-loaded doxorubicin liposomes formed by the extrusion process.

[0021] FIG. 4 depicts a differential scanning calorimetry graph (scan rate of 2°C per minute) of pH loaded liposomes prepared using known methods in the art.

[0022] FIG. 5 depicts a differential scanning calorimetry graph (scan rate of 2°C per minute) of NH_4^+-loaded liposomes of the present invention.

[0023] FIG. 6 depicts a tunneling electron micrograph of pH loaded liposomes prepared according to known methods in the art.

[0024] FIG. 7 depicts a tunneling electron micrograph of NH_4^+-loaded liposomes prepared according to the present invention.

[0025] FIG. 8 shows a comparison of doxorubicin release profiles as a function of solution temperature for the pH-loaded and NH_4^+-loaded liposomes.

[0026] FIG. 9a and 9b show a comparison of the levels of 8-desacetyl-8-carboxy daunorubicin and Impurity A respectively in the pH –loaded or NH_4^+-loaded doxorubicin

liposomes. The "A" and “B” bars denote impurity levels for a formulation prepared using excipients sourced from different suppliers. FIG. 9a and 9b also show the levels of