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(54) **Title**: LIPOSOMAL FORMULATIONS COMPRISING THYMOQUINONE AND TAXANE, AND METHODS OF TREATING CANCER USING SAME

(57) **Abstract**: The present disclosure relates to liposomal pharmaceutical compositions comprising a taxane and thymoquinone, and methods of treating cancer by administering therapeutically effective amounts taxane and thymoquinone in the form of liposomes. The liposomal pharmaceutical compositions have a synergistic anti-cancer effect, significantly enhance the encapsulation efficiency of the taxane into liposomes, significantly enhance the stability of the liposomes, and provide more consistent taxane release patterns.

LIPOSOMAL FORMULATIONS COMPRISING THYMOQUINONE AND TAXANE, AND METHODS OF TREATING CANCER USING SAME

This application is being filed on 11 July 2014, as a PCT International Patent application.

FIELD

The present disclosure relates to pharmaceutically acceptable liposomal formulations comprising a taxane and thymoquinone, and methods of treating cancer by administering therapeutically effective amounts of such formulations.

BACKGROUND

Taxanes constitute a family of diterpenes isolated from yew trees, including the Pacific Yew (*Taxus brevifolia*), the European Yew (*Taxus baccata*), and the Himalayan Yew (*Taxus wallichiana*). Examples of taxanes include paclitaxel, docetaxel, cabazitaxel, larotaxel, ortataxel, and tesetaxel. Taxanes exhibit anti-cancer properties and are used as chemotherapy agents. However, taxanes are generally poorly water-soluble, which makes them difficult to include in various pharmaceutical formulations.

Taxanes are useful for treating cancer because these compounds interfere with cell division by disrupting the cell's microtubule function. In normal cell growth, microtubules are formed when a cell divides. Once the cell stops dividing, the microtubules are broken down or destroyed. Taxanes stop microtubules from breaking down by stabilizing GDP-bound tubulin, thereby preventing mitosis and crowding the cancer cells with microtubules that inhibit further growth and division of the cells.

One of the first taxanes to be identified was paclitaxel (Taxol®) (chemical name: 5β,20-Epoxy-l,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine), which exhibits antitumor activity against ovarian and breast cancers, malignant melanoma, colon cancer, leukemias, and lung cancer. Another known taxane is docetaxel (Taxotere®) (chemical name: (2R,3S)-N-carboxy-3-phenylisoserine,N-tert-butyl ester, 13-ester

with 5β-20epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate), which is a semi-synthetic analogue of paclitaxel. Docetaxel is useful in treating at least breast, ovarian, prostate, gastric, and non-small-cell lung cancers, as well as advanced or metastatic cancers. Docetaxel exerts its anti-mitotic action by directly binding to β–tubulin proteins. This disruption of microtubule assembly results in an increase in microtubule concentration, which leads to inhibition of BCL-2 and cell death by apoptosis. The anti-tumor efficacy of docetaxel has been shown to be at least comparable to the anti-tumor efficacy of paclitaxel and other taxanes. Further, the anti-mitotic cytotoxic efficacy of docetaxel surpasses that of many other anti-tumor drugs, including doxorubicin, paclitaxel, and fluorouracil.

Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone) is a phytochemical compound that can be extracted from the seeds of *Nigella Sativa*, also known as black seed or black cumin. *Nigella Sativa* is a flowering plant found around the Mediterranean Sea, including Jordan and Levant. Black seed has been used as a natural remedy for over 2,000 years due to its positive effects on general health. Thymoquinone is a main active ingredient in volatile oils of black seed.

Thymoquinone exhibits anti-oxidant, anti-inflammatory, and anti-tumor activities both *in vivo* and *in vitro*. In particular, thymoquinone has anti-tumor activity against breast, ovarian, colon, lung, leukemic, and other cancer cell lines. Animal model experiments have also confirmed thymoquinone's anti-tumor activity. The mechanism by which thymoquinone exerts its activities is not completely understood, but thymoquinone has been shown to down-regulate the expression of pro-inflammatory and proliferative mediators such as COX-2, inducible NOS, 5-lipooxygenase, tumor necrosis factor (TNC), and cyclin D1. Thymoquinone has also been found to inhibit the activation of transcription factor NF-kB, Akt, and extracellular signal-regulated kinase (ERK) signaling pathways. There are also studies indicating that thymoquinone is an angiogenesis inhibitor as well as an HDAC inhibitor, and that thymoquinone affects important genes such STAT3, p53, Bax, BCL-2, and p21.

Liposomes are useful for delivery of pharmaceutical drugs by encapsulating pharmaceutically active ingredients in the intravesicular space. Liposomes are generally spherical synthetic vesicles with a lipid bilayer surrounding a hollow

center that is typically filled with an aqueous liquid (also called the intravesicular fluid), thus forming an aqueous cavity within the liposome. Lipids have a hydrophilic head and a hydrophobic tail. When lipids are dispersed by stirring in an aqueous medium, liposomes spontaneously form as hydrophilic heads of the lipids aggregate and are drawn toward the water molecules in the medium, forming an aqueous cavity and leaving the hydrophobic tails pointing outward. This forms the inner layer of the lipid bilayer. Another layer of lipids forms around the outer layer such that the hydrophilic heads of additional lipids point outward toward the aqueous medium while the hydrophobic tails, repelled by water, point inward toward the hydrophobic tails of the inner layer. Liposomes are made mainly of phospholipids but may also contain mixed lipid chains with surfactant properties (e.g., phosphatidylethanolamine). Other phospholipids, such as phosphatidylcholine, phosphatidylserine and phosphatidylglycerol, are commonly used in liposomes, as are sphingolipids such as sphingomyelin and cholesterol.

It would be beneficial to provide taxane-containing liposomal pharmaceutical compositions with improved pharmacological characteristics, such as enhanced encapsulation efficiency, enhanced liposomal stability and more consistent taxane release patterns. It would also be beneficial to provide methods of treating cancer due to the synergistic effect provided by administering liposomal pharmaceutical compositions comprising a taxane and thymoquinone.

SUMMARY

In one aspect, the present disclosure relates to a liposomal pharmaceutical composition comprising thymoquinone, a taxane, and at least one pharmaceutically acceptable lipid.

In another aspect, the liposomal pharmaceutical composition is in the form of a liposome having a lipid bilayer, and wherein the taxane is encapsulated in the liposome and the thymoquinone is incorporated into the lipid bilayer

In another aspect, the liposomal pharmaceutical composition comprises a taxane selected from the group consisting of paclitaxel, docetaxel, cabazitaxel, larotaxel, ortataxel, and tesetaxel.

In another aspect, the liposomal pharmaceutical composition comprises docetaxel.

In another aspect, the liposomal pharmaceutical composition comprises a molar ratio of thymoquinone to taxane of about 5:1 to about 7:1.

In another aspect, the liposomal pharmaceutical composition comprises about 3-8 wt% taxane and about 3-10 wt% thymoquinone, based on the total weight of the composition.

In another aspect, the liposomal pharmaceutical composition comprises at least one pharmaceutically acceptable lipid selected from the group consisting of phospholipids, sphingolipids, and cholesterol.

In another aspect, the encapsulation efficiency of the taxane the liposomal pharmaceutical composition is at least 70%.

In another aspect, less than about 2% of the taxane is released from the liposomal pharmaceutical composition after 120 hours in a dispersion comprising phosphate buffered saline.

In another aspect, the present disclosure relates to a method for treating cancer, comprising administering to a subject in need thereof a liposomal pharmaceutical composition comprising a therapeutically effective amount of a taxane, a therapeutically effective amount of thymoquinone, and at least one pharmaceutically acceptable lipid.

In another aspect, the cancer is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, lung cancers, leukemia, malignant melanoma, prostate cancer, gastric cancer, carcinoma, sarcoma, melanoma, glioma, glioblastoma, thyroid follicular cancer, pancreatic cancer, anaplastic astrocytoma, bladder cancer, myelodysplasia, testicular cancer, rectal cancer, lymphoma, and mycosis fungoides.

In another aspect, the cancer is breast cancer.

In another aspect, the breast cancer comprises a cell line selected from the group consisting MCF-7, T47D, MDA-231, and ZR-751 breast cancer cell lines.

In another aspect, the liposomal pharmaceutical composition is a lyophilized powder that is reconstituted prior to administration.

In another aspect, the liposomal pharmaceutical composition is in a form suitable for oral, parenteral, or topical administration.

In another aspect, the parenteral administration is selected from the group consisting of intramuscular, intravenous, and subcutaneous administration.

In another aspect, the present disclosure relates to a method for providing a cytotoxic effect on tumor cell lines comprising contacting the tumor cells with a liposomal pharmaceutical composition comprising a taxane, thymoquinone, and at least one lipid.

In another aspect, the thymoquinone enhances the liposomal encapsulation efficiency of the taxane in the liposomal pharmaceutical composition.

In another aspect, the liposomal pharmaceutical composition provides a synergistic anti-cancer effect.

In another aspect, the present disclosure relates to the use of a liposomal pharmaceutical composition comprising thymoquinone, a taxane, and at least one pharmaceutically acceptable lipid for the preparation of a medicament for the treatment of cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing a drug dose-effect curve for the cytotoxic effects of thymoquinone, docetaxel, and a thymoquinone-docetaxel combination on the breast cancer cell line MCF-7.

Figure 2 is a graph showing the combination index (CI) of a liposomal pharmaceutical composition of the present disclosure.

Figure 3 is an isobologram graph illustrating the synergistic effect of a liposomal pharmaceutical composition comprising thymoquinone and docetaxel according to at least one embodiment of the present disclosure.

Figure 4 is a graph showing the release pattern of docetaxel from a liposomal pharmaceutical composition of the present disclosure over time.

DETAILED DESCRIPTION

The present disclosure describes a liposomal pharmaceutical composition comprising thymoquinone and a taxane to be used as an anticancer therapeutic. Any taxane, such as paclitaxel, docetaxel, cabazitaxel, larotaxel, ortataxel, or tesetaxel, may be encapsulated in the liposome. Preferably, the taxane is docetaxel, paclitaxel, or cabazitaxel. More preferably, the taxane is docetaxel. The liposomal pharmaceutical composition exhibits enhanced liposomal encapsulation efficiency for the taxane, enhanced liposomal stability, more consistent taxane release patterns, and a synergistic effect in treating cancer. The present disclosure further provides a method for treating cancer, comprising administering a liposomal pharmaceutical composition comprising to a subject in need thereof a therapeutically effective amount of a taxane, a therapeutically effective amount of thymoquinone, and at least one lipid.

Cancers that may be treated with the liposomal pharmaceutical composition include, but are not limited to, breast cancer, ovarian cancer, colon cancer, lung cancers (including non-small-cell lung cancer), leukemia, malignant melanoma, prostate cancer, gastric cancer, carcinoma, sarcoma, melanoma, glioma, glioblastoma, thyroid follicular cancer, pancreatic cancer, anaplastic astrocytoma, bladder cancer, myelodysplasia, testicular cancer, rectal cancer, lymphoma, and mycosis fungoides. Preferably, the cancer is selected from breast cancers, ovarian cancers, colon cancers, lung cancers, leukemias, malignant melanomas, prostate cancers, and gastric cancers. More preferably, the cancer is breast cancer. In at least one embodiment, the liposomal pharmaceutical composition is administered for the treatment of cancer by providing a cytotoxic effect on tumor cell lines. Preferably, the tumor cell line is selected from MCF-7, T47D, MDA-231, and ZR-751 breast cancer cell lines. More preferably, the tumor cell line is the MCF-7 breast cancer cell line.

The liposomal pharmaceutical composition comprising a taxane and thymoquinone is in a liposomal form. According to at least one embodiment, the thymoquinone is incorporated in the lipid bilayer of the liposome and the taxane is encapsulated inside the liposome. It has been found that encapsulation efficiency of the taxane can be significantly improved by the presence of thymoquinone, which modifies the structure of the liposome by its incorporation into the lipid bilayer. The

liposomal structure can be further modified by including various sizes of lipids, glycolipids, and polymers in the lipid bilayer, and by incorporating other ligands in the lipid bilayer that result in a functionalized surface or "decorated" liposome. In accordance with the present disclosure, incorporating both thymoquinone and a taxane into a liposome increases the encapsulation efficiency of the taxane, enhances the stability of the liposome and improves release patterns of the taxane.

The liposomal pharmaceutical composition may further comprise one or more additional pharmaceutically acceptable anti-cancer agents, including other taxanes. For example, the liposomal pharmaceutical composition may comprise docetaxel and cabazitaxel, or docetaxel and paclitaxel.

The liposomal pharmaceutical composition may further comprise one or more pharmaceutically acceptable excipients including, for example, inert diluents and carriers, such as lactose, calcium carbonate, sodium carbonate, microcrystalline cellulose, kaolin, methyl cellulose, tragacanth, sodium alginate, and talc; granulating and disintegrating agents, such as sodium starch glycolate, croscarmellose sodium, alginic acid, and starch; binders such as PVP, HPMC, starch, gelatin, and acacia; lubricating agents such as magnesium stearate, stearic acid, and talc; glidants, such as colloidal silica; wetting agents, such as lecithin and polyoxyethylene stearate; preservatives, such as ethyl-p-hydroxybenzoate; antioxidants, such as α-tocopherol, CoQ10, glutathione, N-acetyl cysteine, quercetin, and 2,6-di-*tert*-butyl-4-methyl phenol (BHT); and combinations thereof. The excipient(s) should be selected so as to not diminish the therapeutic effects of the active ingredients.

The liposomal pharmaceutical composition may further comprise one or more pharmaceutically acceptable lipids. In at least one embodiment, the lipids may be provided as a pharmaceutically acceptable lipid composition comprising lipids selected from phospholipids, sphingolipids, cholesterol, and mixed lipid chains, including phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, sphingomyelin, and combinations thereof. In an exemplary embodiment, the lipid composition comprises dipalmitoylphosphatidylcholine ("DPPC") and cholesterol. In other exemplary embodiments, the lipid composition comprises 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), 1,2 distearoyl-sn-glycero-3-phosphatidylcholine (DSPC), egg yolk phosphatidylcholine (PC), or a combination thereof.

According to at least one embodiment, the intravesicular space of the liposomal pharmaceutical composition comprises a buffer, such as Phosphate Buffered Saline (PBS) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES).

According to at least one embodiment, the liposomal pharmaceutical composition comprises one or more lipids that is/are present in a total concentration of about 80-95 wt%, about 85-90 wt%, about 87 wt%, or 87.0 wt%, based on the total weight of the composition.

According to at least one embodiment, the liposomal pharmaceutical composition comprises about 3-10 wt%, about 6-8 wt%, about 7 wt%, or 7.0 wt% thymoquinone, based on the total weight of the composition; and about 3-8 wt%, about 5-7 wt%, or about 6 wt% taxane, based on the total weight of the composition. The molar ratio of thymoquinone to taxane in the liposomes may be about 5:1 to about 7:1. Preferably, the molar ratio of thymoquinone to taxane is about 6:1. In one embodiment, the liposomal pharmaceutical composition may comprise about 1-2 μM thymoquinone and about 0.7-1.2 nM docetaxel.

Spontaneous formation of liposomes results in a wide range of sizes and structures. To produce liposomes with more controlled qualities, various techniques can be used. For example, the liposomes of the present disclosure can be formed by thin film evaporation or reverse phase evaporation and lipid hydration, solvent injection, freeze-thawing, pH gradient, or detergent dialysis. Preferably, the liposomes are formed by thin film evaporation. The size of the resulting vesicles can be reduced by sonication, homogenization, extrusion through a microporous membrane, and microfluidization. The liposomes can be classified by size as small, intermediate, or large unilamellar vesicles. In general, small unilamellar vesicles range in size from 25-100 nm in diameter, whereas large unilamellar vesicles are typically more than 100 nm in diameter. According to at least one embodiment, the liposomes may be of any suitable size, such as about 100-600 nm in diameter, about 200-550 nm in diameter, or about 300-500 nm in diameter. The liposomes can further be purified by centrifugation, dialysis, column chromatography, or ultrafiltration.

The liposomal pharmaceutical composition can be in a dry, lyophilized form or in the form of a liquid suspension. The lyophilized form is preferred because it can be more stably stored for up to several months. A lyophilized powder comprising the liposomal composition can be reconstituted prior to administration to a subject. Suitable reconstitution buffers include, for example, PBS and HEPES. In at least one embodiment, the reconstitution buffer is PBS pH 7.4, 1X at 2-25°C without Ca^{++} or Mg^{++} ions. Alternatively, the liposomal pharmaceutical composition may be prepared as a suspension in buffered, neutral pH saline and are stable for a storage period ranging from hours to months, depending on the temperature, taxane content, and phospholipid constituents. Suitable storage buffers include, for example, HEPES.

Drug Synergism for Cancer Treatment

Drug synergism occurs when a combination of drugs interacts in such a way that enhances their effect compared to the effect of either drug administered alone. A combination index (CI) can be used to evaluate the combined effect of active ingredients. A CI of less than 1 indicates synergism. A CI equal to 1 indicates an additive effect. A CI of greater than 1 indicates an antagonistic effect. CI can be calculated according to the following classical isobologram equation (Chou et al., *Trends Pharmacol Sci*, 4:450-54 (1983)):

$$CI = \frac{d1}{D1} + \frac{d2}{D2}$$

In the isobologram equation, D1 and D2 represent the doses of drug 1 and drug 2, respectively, that provide a certain degree of inhibition when each drug is administered alone; d1 and d2 represent the doses of each drug that provide the same degree of inhibition when administered in combination. Liposomal pharmaceutical compositions of the present disclosure comprising a taxane and thymoquinone have a CI of less than 1.

One aspect of the present disclosure provides a method of treating cancer by administering a liposomal pharmaceutical composition comprising therapeutically effective amounts of a taxane and thymoquinone to a subject in need thereof. According to at least one embodiment, the subject is in need of anti-tumor treatment.

Another aspect of the present disclosure provides a method of inhibiting tumor growth in a subject having a tumor that is sensitive to a taxane, thymoquinone, or both, wherein the method includes administering a liposomal pharmaceutical formulation comprising therapeutically effective amounts of a taxane and thymoquinone to a subject in need thereof.

The composition may be in any form of administration appropriate for the desired use, including oral, parenteral (including subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intracranial injection or infusion techniques), or topical. Preferably, the composition is administered intravenously; more preferably, by intravenous infusion.

Dosage forms suitable for parenteral administration include solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions which can be dissolved or suspended in sterile injectable medium immediately before use and may contain suspending or dispersing agents.

Suitable oral dosage forms include, e.g., tablets, dispersible powders, granules, capsules, suspensions, syrups, and elixirs. Tablets may be uncoated or may be coated by known techniques to delay disintegration and absorption. The liposomal pharmaceutical composition may also be incorporated into capsules. Inert diluents and carriers that may be used in such capsules include, e.g., calcium carbonate, calcium phosphate, and kaolin. Suspensions, syrups, and elixirs may contain conventional excipients, such as, methyl cellulose, tragacanth, sodium alginate, wetting agents (e.g., lecithin or polyoxyethylene stearate), preservatives (e.g., ethyl-p-hydroxybenzoate), and combinations thereof.

Liposomal pharmaceutical compositions of the present disclosure may be administered alone or in combination with other anti-cancer treatments. Administration of the liposomal pharmaceutical compositions can be carried out continuously or periodically within the maximum tolerated dose.

As used herein, “therapeutically effective amount” means the amount of an active ingredient that, when administered to a subject for treating a disease or condition, is sufficient to effect such treatment. Actual amounts of taxane and

thymoquinone administered will vary according to the particular compounds, the particular composition formulated, and the mode of application, host and disease being treated. Many factors that modify the action will be taken into account by those skilled in the art; e.g., body weight, sex, diet, time of administration, route of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severities, and severity of disease.

As used herein, "subject in need thereof" means an individual, such as a human or other mammal, that would benefit from the administration of the liposomal pharmaceutical composition.

In at least one embodiment, administering a liposomal pharmaceutical composition comprising a taxane and thymoquinone provides not only anti-cancer efficacy but also improved anti-inflammatory, anti-oxidant, and anti-viral effects. In at least one embodiment, the presence of thymoquinone lessens side effects caused by the taxane, which is significant because fewer and milder side effects can alleviate negative effects generally associated with chemotherapy and can thus make cancer treatment more successful.

Enhanced Taxane Encapsulation Efficiency

The inclusion rate of an active ingredient in the liposome is called the encapsulation efficiency ("EE"), and is calculated as the percentage of the active ingredient that is encapsulated inside the liposomes based on the total amount of active ingredient used during preparation of the liposomes. To calculate EE, either the amount encapsulated or the amount not encapsulated to calculate EE can be determined using various techniques, such as HPLC, spectrophotometry, fluorescence spectroscopy, enzyme-based methods, and electrochemical techniques. Measuring the encapsulated amount of active ingredient may be more suitable when low concentrations of components are used; and measuring the not encapsulated amount may be more suitable when higher concentrations are used.

EE may be calculated using either of the following equations:

$$EE\% = \frac{[encapsulated\ active]}{[original\ amount\ of\ active]} x\ 100\%$$

or

$$EE\% = \frac{[original\ amount\ of\ active] - [not\ encapsulated\ active]}{[original\ amount\ of\ active]} x\ 100\%$$

In the context of the present disclosure, it was surprising to observe that thymoquinone significantly enhances the EE of taxane in the liposomes. That is, incorporating both a taxane and thymoquinone into the liposome significantly increases the EE of the taxane as compared to the EE of a liposome comprising a taxane as the only active ingredient. In at least one embodiment, the EE of taxane in the liposomes of the present disclosure comprising a taxane and thymoquinone is at least 70%, at least 75%, or in the range of about 70%-85%, or about 75%-80%.

Additionally, in at least one embodiment, the EE is about 25-30% higher for liposomes comprising a taxane and thymoquinone. For example, thymoquinone has a noticeable influence on the EE of docetaxel, where EE was observed to increase from about 50% (without thymoquinone) to over 75% (with thymoquinone). This significant increase is largely attributable to the lipophilicity of the thymoquinone, which enables thymoquinone to stack with the lipids, and especially with their hydrophobic tails. In other words, good packing and alignment is achieved due to nonpolar-nonpolar interactions between the thymoquinone and the hydrophobic parts (tail regions) of the lipids. Further, since thymoquinine is a relatively small molecule, compared to the size of the lipids (e.g., phospholipids), it is believed that thymoquinone increases the backing and stacking of the lipid molecules, which decreases the permeability of the lipid bilayer, permitting the encapsulation of more taxane.

According to at least one embodiment, the encapsulation effeciency of the taxane is determined using HPLC-UV-Vis. A dialyzed liposome formulation (i.e., containing only the encapsulated drug without any free drug) is destructed and centrifuged, and the concentration of free drug is measured using calibration curves constructed with the same instrument by measuring standard concentrations of the taxane. HPLC also allows for the separation of various constituents in the liposome formulation as well as the measurement of drug concentration by measuring the absorbance of the desired constituent's λmax using the UV-Vis detector.

Enhanced Liposomal Stability

Stability of the liposomal pharmaceutical composition is important at various stages, including manufacturing, storage, and point of use. Preferably, the composition is stable for at least six months to two years at room temperature (about 20-25°C) or at refrigeration temperature (about 4-10°C). Stability is determined by measuring uniformity in the size of the liposome over time. Unstable liposomes can either fuse together (aggregate) to make larger liposomes or can break down into smaller particles. In general, a liposome that exhibits up to a 20-3% change in size within 2-4 weeks may be considered to have satisfactory stability.

According to at least one embodiment, the liposomal pharmaceutical composition comprising a taxane and thymoquinone has the same or better stability than a liposomal pharmaceutical composition comprising a taxane alone. That is, the presence of the thymoquinone preserves or improves the stability of the formulation. As shown in Table 4 below, at least one embodiment of liposomes of the present disclosure maintained a uniform size for more than 2 weeks with insignificant size change (about 5%), which is indicative of a high level of stability.

Enhanced Taxane Release Patterns

According to at least one embodiment, incorporating both taxane and thymoquinone into the liposome provides a significantly more consistent release pattern of the taxane from the liposomes following administration of the liposomal pharmaceutical compositions of the present disclosure. For instance, a more consistent release pattern was observed for liposomes comprising both a taxane and thymoquinone as compared to liposomes comprising only a taxane. The liposomal pharmaceutical compositions of the present disclosure also provide a sustained release of taxane and thymoquinone, with reduced side effects due to limited free concentrations of the active ingredients.

EXAMPLES

The present invention is next described by means of the following examples. The use of these and other examples anywhere in the specification is illustrative only, and in no way limits the scope and meaning of the invention or of any exemplified form. Likewise, the invention is not limited to any particular preferred embodiments described herein. Indeed, modifications and variations of the invention

may be apparent to those skilled in the art upon reading this specification, and can be made without departing from its spirit and scope. The invention is therefore to be limited only by the terms of the claims, along with the full scope of equivalents to which the claims are entitled.

EXAMPLE 1

This example is directed to the preparation of two liposomal pharmaceutical compositions. The first (Formulation 1) was prepared with docetaxel as the only active ingredient. The second (Formulation 2) was prepared with docetaxel and thymoquinone as the active ingredients. The components of Formulations 1 and 2 are identified in Table 1.

Table 1

Components	Formulation 1	Formulation 2
DPPC	38.5 mg	38.5 mg
Cholesterol	9.5 mg	9.5 mg
DSPE –PEG2000	13.8 mg	13.8 mg
Docetaxel	4 mg	4 mg
Thymoquinone	0 mg	5 mg

In Formulation 1, the concentration of docetaxel was 1.0 mM, and the molar ratio of docetaxel to lipids was 6.04%.

In Formulation 2, the concentration of docetaxel was 1.0 mM, and the molar ratio of docetaxel to lipids was 6.04%.

Formulations 1 and 2 were prepared by mixing the drug(s) and lipids, and dissolving the mixture in 3-4 mL chloroform in a 50 mL round bottom flask. Each mixture was dried under vacuum using a rotary evaporator at 37°C for about 25-30 min until a thin lipid film formed. The lipid film was left for 1-2 hr in a fume hood. Next, the lipid film was hydrated with 5-6 mL of HEPES buffer (pH 7.4) with vigorous shaking, and was left overnight to complete the hydration and form the suspensions of the liposomes.

Instruments and materials:

- Thymoquinone powder (Sigma-Aldrich, USA)
- Docetaxel powder (Sigma-Aldrich, USA)

- DPPC lipid (1,2-dipalmitoyl-sn-glycero-3-phosphocholin) (Avanti, USA)
- Cholesterol (chol.) (Avanti, USA)
- Di Stearoyl Phosphatidyl Ethanolamine methoxy Poly Ethylene Glycol 2000 (DSPE-PEG2000) (Avanti, USA)
- Cellulose ester dialysis membrane (co. MW 20,000 Dalton), (Spectrum labs, USA)
- Triton X surfactant (Sigma-Aldrich, USA)
- Acetonitrile (Tedia, USA)
- Rotary evaporator (IKA, Germany)
- Sonicator (JEO-tech, Korea)
- HPLC (Shimadzu, class-VP, Japan); C18 column (Varian, Australia)
- UV-VIS spectrometer (Varian, CARY 100, Australia)
- Shaking Incubator (Heidolph, Germany)

EXAMPLE 2

This example studied the effect of thymoquinone on the liposomal encapsulation efficiency of docetaxel.

Formulations 1 and 2 were again prepared as detailed in Example 1. Free drug was removed from each formulation, and the remaining amount of docetaxel was measured by HPLC, indicating the amount of docetaxel inside the liposomes of each formulation.

Free drug was then removed from Formulations 1 and 2 by dialysis using a dialysis membrane with 20,000 Dalton molecular weight cutoff. Each formulation was added to a cellulose ester membrane tubing/sack/cassette. The membrane was placed into 400 mL acceptor medium consisting of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (pH 7.4), and was shaken at 150 rpm on a plate shaker overnight at 37°C. The acceptor medium was changed three times.

Portions of the treated formulations were drawn, and intact liposomes were destructed using acetonitrile and sonication, followed by centrifugation at 12,000 rpm. The supernatant was taken and analyzed for docetaxel by HPLC. Encapsulation

efficiency was calculated as a ratio of docetaxel inside the liposomes to docetaxel used to prepare the liposomes.

HPLC assay:

- Mobile phase: water: acetonitrile (25:75 volume ratio), flow rate 1 mL/min
- Column type: Microsorb 100 C18, Part no. CP30714
- Column size: length 250 mm, I.D. 4.6 mm, O.D. 3/8 inch, particle size 5 μm
- Detection wave length: 290 nm

This Example 2 experiment was performed twice (Experiment 1 and Experiment 2), and the results from the HPLC analyses are shown in Table 2 below.

Table 2

	Experiment 1		Experiment 2	
	Formulation 1	**Formulation 2**	**Formulation 1**	**Formulation 2**
Initial docetaxel concentration	1.0 mM	1.0 mM	1.0 mM	1.0 mM
Docetaxel concentration in liposomes	0.51 mM	0.76 mM	0.43 mM	0.68 mM
Encapsulation Efficiency	51.7 %	76.6 %	43.8 %	68.5 %
Improvement	48 %		56 %	

EXAMPLE 3

This example studied the stability of liposomes over time.

The stability of Formulation 1 and Formulation 2 as prepared in Example 1 was studied. The stability of the liposomes for various lengths of time at 4 °C was evaluated using a particle size and zeta potential analyzer (Microtrace, USA; Model NPA152).

The results are shown in Table 3 below.

Table 3

	Time	Particle Size Diameter (nm)	Size Distribution (vol %)
Formulation 1	18 h	402	100
	66 h (2.75 days)	400	100
	402 h (16.75 days)	409	100
Formulation 2	18 h	462	100
	66 h (2.75 days)	436	100
	402 h (16.75 days)	431	100

The size distribution indicates the percentage of liposomes that were found to be in the same size range.

It was concluded that both formulations were highly stable and did not lose significant volume during the assay.

EXAMPLE 4

This example studied the cytotoxic effect of thymoquinone, docetaxel, and the thymoquinone-docetaxel combination on the breast cancer cell line MCF-7. The cytotoxic effect was studied using the MTT assay, which is a colorimetric assay for assessing cell viability and can be used to determine the effect of a drug on cells.

Instruments and materials:

- Biological safety cabinet (Thermo, USA)
- CO_2 incubator (Nuaire, USA)
- Microscope (Olympus, USA)
- Water bath (Witeg, Germany)
- Tissue culture flasks (NUNC, Denmark)
- MTT kit (Promega, USA)
- Serological pipettes (JETBIOFIL, China)
- Tissue culture media (GIBCO, USA) (LONZA, Italy)
- ELISA Plate Reader (TECAN, Sunrise, Austria)

Method:

An MTT assay was performed using the Cell Titer Non-Radioactive Cell Proliferation Assay kit from Promega, USA. Based on manufacturer recommendations, around 7,000 of MCF-7 cells per well were seeded and incubated at 37°C for 24 hours in a humidified CO_2 incubator to make sure the cell lines were attached to the wells to reach the appropriate confluency. The cells were then incubated at 37°C for 72 hours in the humidified CO_2 incubator with different concentrations of thymoquinone, docetaxel, and thymoquinone-docetaxel combination. After the incubation period, 15 µL of a dye solution was added to each well and the plate was incubated at 37°C for 4 hours in the CO_2 incubator. 100µL of Stop Mix solution was then added to each well and the plate was incubated in the dark for 1 hour followed by reading the absorbance at 570 nm using a 96-well ELISA plate reader.

The results are shown in Tables 4, 5 and 6, and the dose-effect curve shown in Figure 1.

Table 4: Growth inhibition of MCF-7 cells by docetaxel (DT)

Sample No.	DT Concentration (nM)	Inhibition (%)
1	0.25	13.7
2	0.49	18.5
3	0.98	23.6
4	1.95	34.3
5	3.9	38.7
6	7.8	54.3
7	15.6	57.6
8	31.25	57.6
9	62.5	61.9
10	125	65.5
11	250	61
12	500	60

Table 5: Growth inhibition of MCF-7 cells by thymoquinone (TQ)

Sample No.	TQ Concentration (µM)	Inhibition (%)
1	3.12	7.4
2	6.25	11.0
3	12.5	16.0
4	25	19.7

5	34	35.0
6	50	61.0
7	75	86.8
8	100	89.1
9	200	93.0

Table 6: Growth inhibition of MCF-7 cells by DT-TQ combinations

Sample No.	TQ Concentration (µM)	DT Concentration (nM)	Inhibition (%)
1	0.39	0.24	17.58
2	0.78	0.49	21.68
3	1.56	0.98	35.55
4	3.13	1.95	45.08
5	6.25	3.91	47.63
6	12.5	7.81	49.76
7	25	15.63	55.84
8	50	31.25	81.13
9	100	62.5	91.55
10	200	125	92.29

The combination index (CI) was calculated for combined docetaxel and thymoquinone. A CI<1 indicates synergism; CI=1 indicates additive effect; and CI>1 indicates antagonism. The results are shown in Tables 7 and 8, and Figures 2 and 3. Fraction Affected (FA) refers to the fraction of cells affected by the concentration of drug. The molar ratios (TQ:DT) in Table 7 reflect the relative concentrations of free drug.

Table 7: Combination Index

Sample No.	TQ Concentration (µM)	DT Concentration (nM)	CI	FA	Molar Ratio (TQ:DT)
1	0.39	0.24	0.842	0.1758	1625:1
2	0.78	0.49	0.925	0.2168	1592:1
3	1.56	0.98	0.407	0.3555	1592:1
4	3.13	1.95	0.364	0.4508	1605:1
5	6.25	3.91	0.598	0.4763	1598:1
6	12.5	7.81	1.017	0.4976	1601:1
7	25	15.63	1.304	0.5584	1599:1
8	50	31.25	0.403	0.8113	1600:1

9	100	62.5	0.255	0.9155	1600:1
10	200	125	0.454	0.9229	1600:1

The dose reduction index (DRI) was also calculated. DRI is a measure of how much the dose of each drug in a synergistic combination may be reduced at a given effect level compared with the doses of each drug alone. The results are shown in Table 8.

Table 8: Dose Reduction Index

FA	DT alone nM	TQ alone nM	DRI	
			DT	TQ
0.1758	0.317	5301.53	1.30	13.59
0.2168	0.598	7079.72	1.23	9.08
0.3555	3.20	1.52E+04	3.28	9.75
0.4508	8.41	2.37E+04	4.31	7.57
0.4763	10.50	2.65E+04	2.76	4.24
0.4976	13.28	2.91E+04	1.70	2.33
0.5584	24.06	3.82E+04	1.54	1.53
0.8113	471.96	1.49E+05	15.10	2.97
0.9155	4467.83	4.14E+05	71.49	4.14
0.9229	5693.76	4.63E+05	45.55	2.31

The results surprisingly indicate that most combinations of docetaxel and thymoquinone have a synergistic effect.

The mean IC_{50} for docetaxel was 1.89 nM, which was calculated from the tested concentration range (0.25-500 nmol/L). The cytotoxic effect of docetaxel increased with concentration from 0.25 to 125 nM after which the fraction of affected cells was stable at 60% at maximal concentration tested of 500 nM.

The mean IC_{50} for thymoquinone was 45.74 µM, which was calculated from the tested concentration range (3.12-200 µmol/l). The cytotoxic effect of thymoquinone increased continuously with increasing thymoquinone concentrations from 3.12 up to 200 µM.

By applying the equation of Combination Index CI = d1/D1 + d2/D2 to the results, a synergistic effect of the docetaxel-thymoquinone combination was observed at a certain window of drug concentrations and inhibition values. If the specific growth inhibition % (x) is considered as 35% for docetaxel alone,

thymoquinone alone and the docetaxel-thymoquinone combination, the relevant single drugs/combination concentrations would be:

D1 (Docetaxel) = 1.95 nM

D2 (Thymoquinone) = 34 μM

d1 = 0.976

d2 = 1.56

CI = (0.976/1.95) + (1.56/34)

= 0.5 + 0.046

= 0.505 ≈ 0.51 < 1

(CI < 1 indicates synergism.)

To calculate the CI of both drugs, Calcusyn software from BioSoft, UK was used. This software is based on the Choup-Talalay method (Chou et al., *Trends Pharmacol. Sci.* 4:450-54 (1983)), and takes into account both the potency and shape of the dose-effect curves of each drug and their combinations. This method has been applied in combinations of anti-cancer agents, anti-HIV agents, purging leukemic cells for autologous bone marrow transplantation (Chang et al., *Cancer Res.*, 47:119-22 (1987)) and combinations of immunosuppressants for organ transplants.

Median effect parameters were calculated for each drug individually and for a combination of free drugs at a ratio of 1600:1, thymoquinone to docetaxel. The parameters are shown in Table 9. In the table, Dm refers to dosage at median effect, m refers to sigmoidicity of the median curve, and refers to linear correlation coefficient of the median plot.

Table 9

Drug	ED50	ED75	ED90	Dm	m	r
Combination (1600:1)	0.45459	0.41487	0.55212	5687	0.64	0.96
Thymoquinone alone	N/A	N/A	N/A	29445	0.90	0.92
Docetaxel alone	N/A	N/A	N/A	13.60	0.41	0.97

The data in Table 9 was plotted in an isobologram shown in Figure 3. An isobologram graph indicates the equipotent combinations of various doses and can be used to illustrate additivity, synergism, or antagonism. For a given effect level such as FA = 0.5, the required doses for $(ED_{50})1$, and $(ED_{50})2$ are drawn on the x-axis and y-axis, respectively. If the combination data point for FA = 0.5 falls on the diagonal, an additive effect is indicated; if it falls on the lower left, synergism is indicated; and if it falls on the upper right, antagonism is indicated. In Figure 3, the combination data point for FA = 0.5 falls on the lower left side – i.e., synergism is indicated for the thymoquinone-docetaxel combination.

EXAMPLE 5

This example studied the synergistic effect of the combination of thymoquinone and docetaxel. Synergism of thymoquinone and docetaxel were estimated by extrapolation from the data in Example 4 using Calcusyn software from BioSoft, UK. The same concentration units (nM) were used for both thymoquinone and docetaxel. The results for the calculation of combination index (CI) are shown in Table 10 below.

Table 10: Calculated Combination Index

DT Concentration (nM)	TQ Concentration (nM)	CI	Estimated St. Dev.
0.00836	13.38	7.967	8.74
0.0365	58.38	3.509	2.84
0.117	186.61	1.868	1.13
0.239	383.16	1.286	0.63
0.412	658.61	0.986	0.40
0.644	1030.21	0.803	0.29
0.952	1522.87	0.682	0.21
1.357	2171.83	0.596	0.17
1.892	3027.41	0.534	0.15
2.602	4162.82	0.488	0.13
3.555	5687.47	0.455	0.13
4.857	7770.52	0.430	0.13
6.678	10685	0.415	0.13
9.309	14894	0.406	0.14
13.276	21241	0.406	0.16
19.624	31399	0.415	0.19

30.697	49115	0.436	0.23
52.763	84421	0.475	0.29
108.339	1.73E+05	0.552	0.40
346.295	5.54E+05	0.737	0.68
4511.299	7.22E+06	1.501	2.08

Dose reduction index (DRI) was calculated using the same software. DRI is a measure of how much the dose of each drug in a synergistic combination may be reduced at a given effect level compared with the doses of each drug alone. Results are shown in Table 11.

Table 11: Calculated Dose Reduction Index

FA	DT alone (nM)	TQ alone (nM)	DRI	
			DT	TQ
0.02	0.0011	392.14	0.126	29.311
0.05	0.0106	1122.04	0.289	19.219
0.1	0.065	2571.05	0.557	13.778
0.15	0.200	4295.95	0.836	11.212
0.2	0.467	6322.91	1.134	9.6
0.25	0.940	8700.77	1.46	8.446
0.3	1.731	1.15E+04	1.82	7.551
0.35	3.017	1.48E+04	2.223	6.821
0.4	5.072	1.88E+04	2.681	6.202
0.45	8.346	2.36E+04	3.208	5.661
0.5	13.597	2.94E+04	3.825	5.177
0.55	22.151	3.68E+04	4.561	4.734
0.6	36.451	4.62E+04	5.458	4.322
0.65	61.275	5.85E+04	6.583	3.929
0.7	106.751	7.54E+04	8.041	3.549
0.75	196.704	9.96E+04	10.024	3.174
0.8	395.973	1.37E+05	12.9	2.792
0.85	923.739	2.02E+05	17.507	2.391
0.9	2845.616	3.37E+05	26.266	1.945
0.95	1.75E+04	7.73E+05	50.576	1.395
0.99	9.70E+05	4.83E+06	215.056	0.668

EXAMPLE 6

This example studied the cytotoxic effects of free docetaxel and liposome-encapsulated docetaxel on MCF-7 breast cancer cell line using the MTT assay.

Liposomes of Formulation 2 were prepared as detailed in Example 1. Free docetaxel was dissolved in DMSO at different concentrations as shown in Table 14 below.

The MTT assay was performed using the Cell Titer Non-Radioactive Cell Proliferation Assay kit from Promega, USA. Based on manufacturer recommendations, around 7,000 of MCF-7 cells per well were seeded and incubated at 37°C for 24 hours in a humidified CO_2 incubator. The cells were treated with drug formulations and incubated at 37°C for 72 hours in the humidified CO_2 incubator. After the incubation period 15 µL of a dye solution was added to each well and the plate was incubated at 37°C for 4 hours in the CO_2 incubator. 100µL of Stop Mix solution was added to each well and the plate was incubated in the dark for 1 hour followed by reading the absorbance at 570 nm using a 96-well ELISA plate reader. IC_{50} was calculated using GraphPad Prism software (available from GraphPad Softward, San Diego, CA). For purposes of calculations, the concentration of docetaxel in the liposomes was calculated based on a 67% encapsulation efficiency as determined by HPLC.

Optical density (OD) measurements of four replicates of each treatment were obtained and averaged to assay the viability of cells. Cell survival was calculated as a percentage by dividing average OD by average OD of control wells (no drug treatment). Inhibition was calculated as 100-(% survival). The results are shown in Table 12.

Table 12

Free Docetaxel nM	Average OD	Survival %	Inhibition %	Liposomal Docetaxel nM	Average OD	Survival %	Inhibition %
-				1000	0.419	36.83	63.17
-				500	0.421	37.01	62.99
-				250	0.485	42.68	57.32
125	0.311	34.5	65.5	125	0.449	39.51	60.49
62.5	0.344	38.15	61.85	62.5	0.49	43.1	56.9
31.25	0.383	42.45	57.55	31.25	0.483	42.44	57.56
15.6	0.382	42.36	57.64	15.6	0.493	43.38	56.62
7.8	0.413	45.74	54.26	7.8	0.485	42.68	57.32
3.9	0.553	61.26	38.74	3.9	0.529	46.53	53.47
1.95	0.593	65.75	34.25	1.95	0.595	52.35	47.65
0.975	0.689	76.39	23.61	0.975	0.709	62.31	37.69

0.49	0.736	81.54	18.46	0.49	0.864	75.97	24.03
0.244	0.779	86.31	13.69	0.245	1.102	96.9	3.1
-				0.123	1.061	93.34	6.66
-				0.061	1.015	89.25	10.75
0.0001 (control)	0.902	100	0	0 (control)	1.137	100	0

The IC_{50} for free docetaxel was calculated to be 1.89 nM. The IC_{50} for liposomal docetaxel was calculated to be 0.76 nM – a more than 2.5-fold improvement over free docetaxel. It was concluded that liposomal docetaxel provides the same or higher cytotoxic effect against MCF-7 breast cancer cells at a lower concentration than free docetaxel. Further, it was hypothesized that the liposomes are capable of fusing with cell membranes and releasing active ingredient into the cells, thus improving the efficiency of treatment with a liposome encapsulated drug.

EXAMPLE 7

This example studied the release pattern of docetaxel from liposomes containing docetaxel and thymoquinone over time using a dispersion method.

Formulation 2 was prepared as detailed in Example 1, and 5 mL of this formulation was dispersed in a tube containing 25 mL of Phosphate Buffered Saline (PBS) buffer for a total volume of 30 mL. Concentration of docetaxel in the liposomes was calculated based on a 68.7% encapsulation efficiency. The dispersion was mixed by shaking at 150 rpm in a shaker incubator (Heidolph, Germany) maintained at 37°C for the duration of the test, 120 hours. A 0.5 mL aliquot of the dispersion mixture was withdrawn and replaced with 0.5 mL fresh PBS at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 24 and 120 hours.

Each aliquot was centrifuged at 14500 rpm for 20 minutes. The concentration of docetaxel in the supernatant was analyzed using HPLC at an absorption wavelength of 290 nm.

Results are shown in Table 13 and Figure 4. Release was calculated as a percentage of docetaxel in the supernatant as compared to the prepared liposomes. It was observed that docetaxel was released slowly and steadily over the time of the experiment. No burst release was observed.

Table 13: Release percentage (%) of docetaxel over time from DT/TQ-liposomes

Time (hours)	Release (%)
0.5	0.6
1	0.93
1.5	1.1
2.0	0.6
3.0	0.9
4.0	0.8
5.0	0.5
6.0	0.7
24	0.7
120	0.8

It was concluded that the liposome formulation is highly stable and efficient in retaining docetaxel inside liposomal nano-particles for a considerably long time (at least 120 hours). Over the test period, only about 1-2% of the encapsulated docetaxel was released from the liposomes. The results show that, when placed in a cell-free solution mimicking the blood environment, the liposomes only release very small amounts of the drugs. The results further suggest that because the liposomes only release any significant amounts of drug when in contact with human cells in an *in vivo* environment (i.e., in physiological conditions), the liposomes would release minimal amounts of cytotoxic drug into circulation, which would increase the half-life of the composition, as well as minimize toxicity in normal tissues. Because blood vessels within tumors have a higher porosity than elsewhere in the body, this would eventually result in higher levels of accumulation of the liposomal pharmaceutical composition at tumor sites.

Additionally, as shown in Example 6, the higher retention rates of docetaxel within the liposomes did not decrease the efficiency of drug delivery when the liposomes are in direct contact with tumor cells. According to the present disclosure, docetaxel was efficiently released upon contact with MCF-7 breast cancer cells where the liposomes fuse with cell membranes, releasing the docetaxel. This is demonstrated by, for example, the significantly lower IC_{50} for liposome-encapsulated docetaxel as opposed to free docetaxel in Example 6.

All references cited and/or discussed in this specification are incorporated herein by reference in their entireties and to the same extent as if each reference was individually incorporated by reference.

We claim:

1. A liposomal pharmaceutical composition comprising thymoquinone, a taxane, and at least one pharmaceutically acceptable lipid.

2. The liposomal pharmaceutical composition of claim 1, wherein the composition is in the form of a liposome having a lipid bilayer, and wherein the taxane is encapsulated in the liposome and the thymoquinone is incorporated into the lipid bilayer

3. The liposomal pharmaceutical composition of claim 1, wherein the taxane is selected from the group consisting of paclitaxel, docetaxel, cabazitaxel, larotaxel, ortataxel, and tesetaxel.

4. The liposomal pharmaceutical composition of claim 3, wherein the taxane is docetaxel.

5. The liposomal pharmaceutical composition of claim 1, wherein molar ratio of thymoquinone to taxane is about 5:1 to about 7:1.

6. The liposomal pharmaceutical composition of claim 1, wherein the composition comprises about 3-8 wt% taxane and about 3-10 wt% thymoquinone, based on the total weight of the composition.

7. The liposomal pharmaceutical composition of claim 1, wherein the at least one pharmaceutically acceptable lipid is selected from the group consisting of phospholipids, sphingolipids, and cholesterol.

8. The liposomal pharmaceutical composition of claim 1, wherein the encapsulation efficiency of the taxane is at least 70%.

9. The liposomal pharmaceutical composition of claim 1, wherein less than about 2% of the taxane is released from the composition after 120 hours in a dispersion comprising phosphate buffered saline.

10. A method for treating cancer, comprising administering to a subject in need thereof a liposomal pharmaceutical composition comprising a therapeutically effective amount of a taxane, a therapeutically effective amount of thymoquinone, and at least one pharmaceutically acceptable lipid.

11. The method of claim 10, wherein the cancer is selected from the group consisting of breast cancer, ovarian cancer, colon cancer, lung cancers, leukemia, malignant melanoma, prostate cancer, gastric cancer, carcinoma, sarcoma, melanoma, glioma, glioblastoma, thyroid follicular cancer, pancreatic cancer, anaplastic astrocytoma, bladder cancer, myelodysplasia, testicular cancer, rectal cancer, lymphoma, and mycosis fungoides.

12. The method of claim 11, wherein the cancer is breast cancer.

13. The method of claim 12, wherein the breast cancer comprises a cell line selected from the group consisting MCF-7, T47D, MDA-231, and ZR-751 breast cancer cell lines.

14. The method of claim 10, wherein the liposomal pharmaceutical composition is a lyophilized powder that is reconstituted prior to administration.

15. The method of claim 10, wherein the liposomal pharmaceutical composition is in a form suitable for oral, parenteral, or topical administration.

16. The method of claim 15, wherein the parenteral administration is selected from the group consisting of intramuscular, intravenous, and subcutaneous administration.

17. A method for providing a cytotoxic effect on tumor cell lines comprising contacting the tumor cells with a liposomal pharmaceutical composition comprising a taxane, thymoquinone, and at least one lipid.

18. The liposomal pharmaceutical composition as in claim 1, wherein the thymoquinone enhances the liposomal encapsulation efficiency of the taxane.

19. The liposomal pharmaceutical composition of claim 1, wherein the composition provides a synergistic anti-cancer effect.

20. The use of the liposomal pharmaceutical composition of claim 1 for the preparation of a medicament for the treatment of cancer.

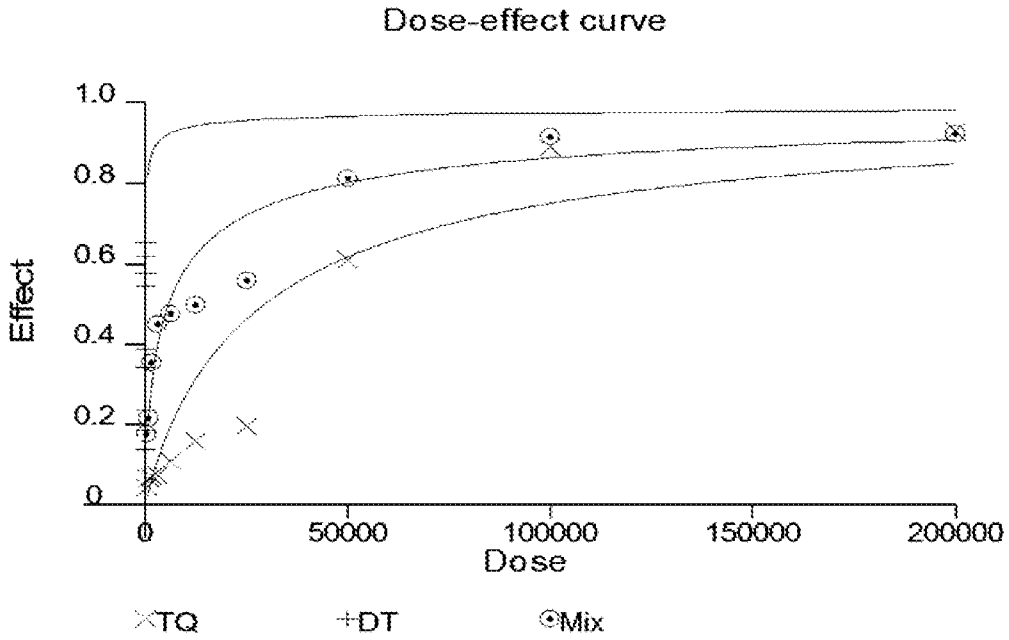

FIGURE 1

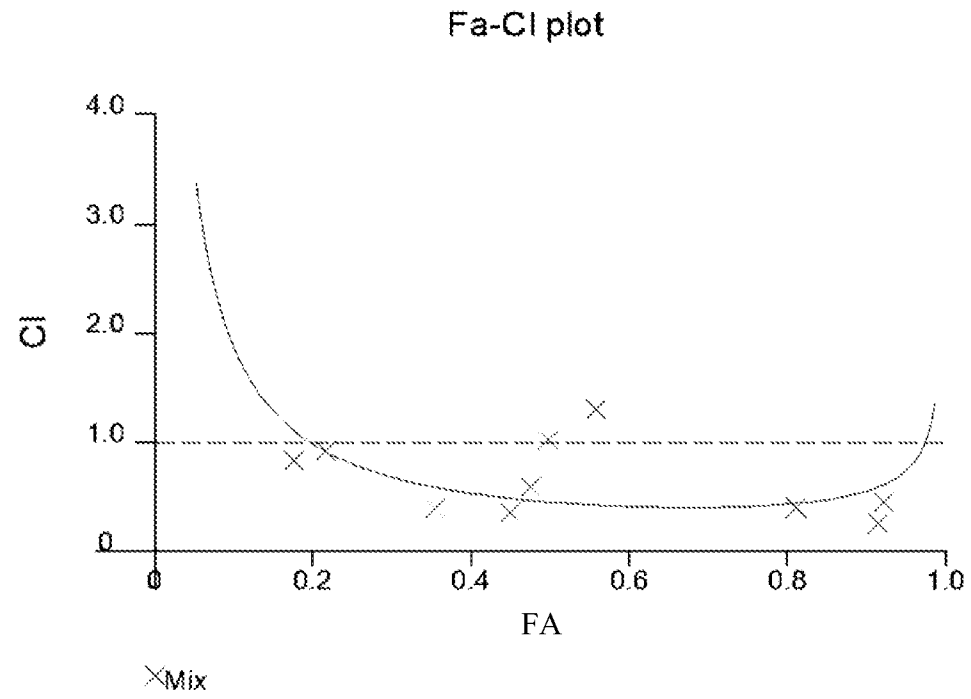

FIGURE 2

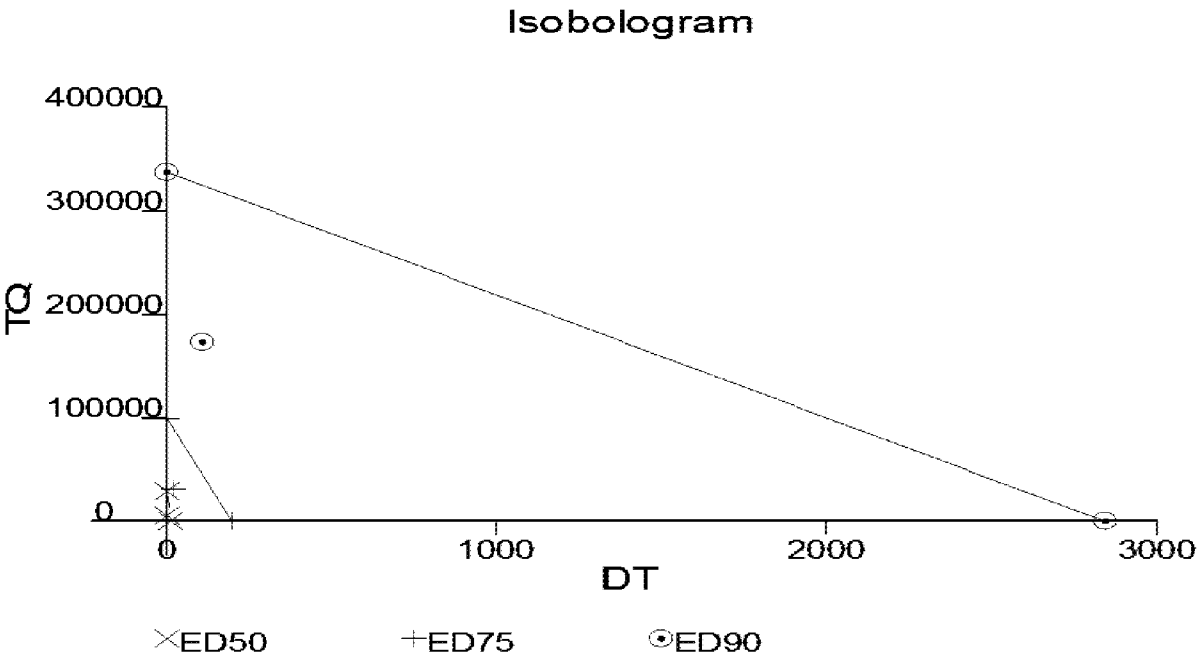

FIGURE 3

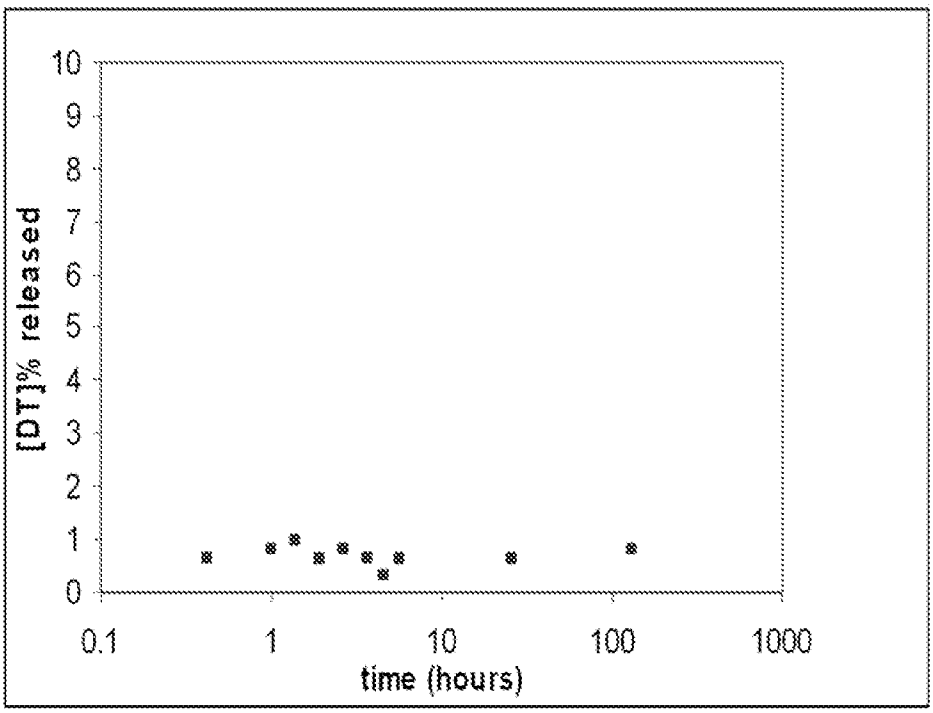

FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/002258

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/127 A61K45/06 A61K31/122 A61K31/337 A61P35/00
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 218 434 B1 (CROOKS PETER A [US] ET AL) 17 April 2001 (2001-04-17) the whole document	1-20

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 February 2015	05/03/2015

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/002258

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	G. SETHI ET AL: "Targeting Nuclear Factor- B Activation Pathway by Thymoquinone: Role in Suppression of Antiapoptotic Gene Products and Enhancement of Apoptosis", MOLECULAR CANCER RESEARCH, vol. 6, no. 6, 1 June 2008 (2008-06-01), pages 1059-1070, XP055171358, ISSN: 1541-7786, DOI: 10.1158/1541-7786.MCR-07-2088 page 1059, right-hand column, paragraph 2 page 1060, left-hand column, paragraph 2-3 page 1063, left-hand column, paragraph 2 - page 1064, left-hand column, paragraph 1 page 1065, left-hand column, last paragraph - right-hand column, last paragraph	1-20
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