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(54) **Title:** DRUG DELIVERY SYSTEM FOR ADMINISTRATION OF A WATER SOLUBLE, CATIONIC AND AMPHIPHILIC PHARMACEUTICALLY ACTIVE SUBSTANCE

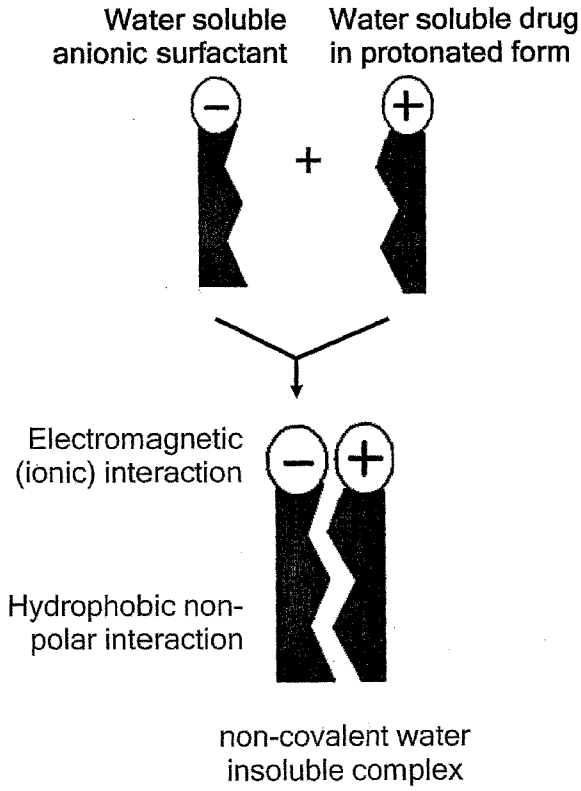

Fig. 1

(57) **Abstract:** A drug delivery system (DDS) for administration of a water soluble, cationic, and amphiphilic pharmaceutically active substance (API) which DDS comprises poorly water soluble nanoparticles formed by the API together with a Na-salt of N-all- trans-retinoyl cysteic acid methyl ester and/or a Na-salt of N-13-cis-retinoyl cysteic acid methyl ester. A pharmaceutical composition comprising such a DDS. Methods for preparation of such a DDS and such a pharmaceutical composition. Use of such a DDS and pharmaceutical composition for treatment of cancer.

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Drug delivery system for administration of a water soluble, cationic and amphiphilic pharmaceutically active substance

Field of the invention

This invention relates to a drug delivery system for administration of amphiphilic cationic pharmaceutically active substances, a pharmaceutical composition comprising such a drug delivery system, and a method for the preparation of such a drug delivery system. The invention also relates to the use of such a drug delivery system for the preparation of a medicament for the treatment of cancer. Furthermore the invention also relates to a method for enhancing the drug efficiency of amphiphilic pharmaceutically active substances, and to a method for increasing the bioavailability of amphiphilic pharmaceutically active substances.

Background

Two important parameters related to the efficaciousness of drugs are the "therapeutic index" (also known as the "therapeutic ratio") and the "therapeutic window". The therapeutic index is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxic effects. Quantitatively, it is the ratio given by the dose required to produce the toxic effect divided by the therapeutic dose. A commonly used measure of therapeutic index LD_{50} divided by ED_{50}. The therapeutic window is a parameter for estimation of drug dosage which can treat disease effectively while staying within the safety range. It is the range between the ED_{50} and the starting point of LD_{50} curve. It is believed that adjustment of this parameter can help to avoid most of the potential side effects.

Pharmaceuticals with narrow therapeutic windows are common and are frequent in groups such as, for instance, antiarrhythmics, anticonvulsants, cardiac glycosides, aminoglycosides, cytotoxics, and immunosuppressants.

A large majority of antitumor agents have a very narrow therapeutic window. One way of improving the therapeutic index of such agents is to use suitable infusion regimens. Ideally, the drug concentration is maintained inside the therapeutical window for a desired time range, after which it quickly leaves the body. Prolonged infusions have in general showed god efficacy with few side effects. For instance prolonged infusion is the most efficient way to reduce cardiotoxicity of doxorubicin,

one of the mostly used anticancer drugs. However, prolonged infusions (sometimes up to 72 hours) are expensive and inconvenient. Accordingly, great efforts have been made to mimic such infusions by the use of drug delivery systems which can ensure slow release of the active ingredient from various kinds of drug depots. Drug delivery systems comprising such depots are usually provided by way of encapsulation of drugs into nanoparticle of various polymers, polymerosomes, liposomes, or microemulsions.

However, in order to protect itself against hostile intruders of different kind (such as viruses, bacteria and fungal spores) human and animal bodies have developed mechanisms to remove or disintegrate particles larger than about 50 nm. The Reticulo-Endothelial System (RES), a part of the immune system, is the most effective destructor of such particles. The probability for a particle to be targeted by RES increases dramatically with increasing particle size.

Many drugs are provided in a cationic amphiphilic form, such as for instance drugs that have one or more amino groups in their structure. In acid environment these drug substances are transformed into salts, e.g. hydrochlorides, sulphates, lactates or tartrates, and exist predominantly in a protonated form. These transformations increase the solubility of the drugs in aqueous solutions and make it possible to use these solutions for i.v. infusions. After infusion the environment is switched to slightly basic as pH of blood is approximately 7.4, which results in deprotonation of the drugs. This in turn reduces the solubility of the substances, which improves the PK/PD properties of the drug by increasing the grade of protein binding, accelerating penetration of the substances into cells as well as decreasing renal clearance. A lot of antineoplastic drugs are provided in a cationic amphiphilic form, and the described way of administration is applied for drugs as, for instance, doxorubicin and its analogues (epirubicin, daunorubicin, idarubicin), vinca alkaloids (vinblastine, vincristine, vinorelbine), amsacrine, mitoxantrone, topotecan and irinotecan.

Short summary of the invention

It would be desirable to be able to create a drug delivery system for administration of water soluble amphiphilic cationic pharmaceutically active substances which system would provide for formation of smaller nanoparticles with decreased water solubility and improved encapsulation capacity. This would give better PK/PD properties and improve the therapeutic indexes of the administered drug.

One object of the present invention is to provide such a drug delivery system.

Thus, one aspect of the invention relates to a drug delivery system for administration of a pharmaceutically active substance that is a cationic amphiphile by itself and has a solubility *per se* in water of at least 4 mg/ml, which drug delivery system comprises nanoparticles having solubility in water below 0.1 mg/ml, said nanoparticles being formed by said substance in association with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof.

The inventive drug delivery system provides for nanoparticles smaller than about 50 nm and an encapsulation capacity of the methyl ester excipient (expressed as the ratio of the weight of the excipient to the weight of encapsulated drug) of about 1.2.

Brief description of the drawings

The present invention will be described in closer detail in the following description, examples and attached drawings, in which

Fig. 1 is a scheme showing the formation of essentially water insoluble nanoparticles by association of cationic amphiphile with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof.

Fig. 2 shows the dependence of the size of the particles formed by sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid and doxorubicin hydrochloride (w/w ratio 2.3:1) on the concentration of doxorubicin. Solvent: aqueous solution of NaCl (130 mmol), $CaCl_2$ (2 mmol) and $MgCl_2$ (0.8 mmol).

Fig. 3 shows the kinetics of dissolving particles after dilution of a formulation of sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid and doxorubicin hydrochloride in w/w ratio 2.1:1. Solvent: aqueous solution of NaCl (5.9 mg/mL), KCl (0.3 mg/mL), $CaCl_2$ (0.295 mg/mL), $MgCl_2$ hexahydrate (0.2 mg/mL), Sodium acetate (4.1 mg/mL). Dilution from 2 to 0.04 mg/mL doxorubicin.

Fig. 4 shows the size distribution by volume of formulation obtained by reconstitution of freeze-dried mixture of doxorubicin, sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid and sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid (w/w/w 1:1.05:1.05) in solution of NaCl (9 mg/mL), doxorubicin concentration 0.5 mg/ml.

Description of embodiments of the invention

Before the present invention is disclosed and described, it is to be understood that this invention is not limited to the particular configurations, process steps, and materials disclosed herein as such configurations, process steps, and materials may vary somewhat. It is also to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

It must be noted that, as used in this specification and the claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

In this specification, unless otherwise stated, the term "about" modifying the quantity of an ingredient in the drug delivery systems or compositions of the invention or employed in the methods of the invention refers to variation in the numerical quantity that can occur, for example, through typical measuring and liquid handling procedures used for making concentrates or use solutions in the real world; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of the ingredients employed to make the drug delivery systems or compositions or carry out the methods; and the like. The term "about" also encompasses amounts that differ due to different equilibrium conditions for a

composition resulting from a particular initial mixture. Whether or not modified by the term "about", the claims include equivalents to the quantities.

In this specification, unless otherwise stated, the term "pharmaceutically acceptable carrier," means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In this specification, unless otherwise stated, the term “drug delivery system” refers to a formulation or device that delivers therapeutic agent(s) to desired body location(s) and/or provides timely release of therapeutic agent(s).

In this specification, unless otherwise stated, the term "pharmaceutically active substance" encompasses any substance that will produce a therapeutically beneficial pharmacological response when administered to a host, including both humans and animals.

In this specification, unless otherwise stated, the term “particle size” refers to the Z-average diameter as measured by dynamic light scattering with the use of red laser with a wavelength of 633 nm.

In this specification, unless otherwise stated, the term “nanoparticle” refers to a microscopic particle whose size is measured in nanometres.

In this specification, unless otherwise stated, the term “solubility” of a substance refers to the ability of that substance to be dissolved in a specified solvent at about room temperature, by which is meant from between about 15 °C to about 38 °C.
In this specification, unless otherwise stated, the term "cytotoxic compound" refers to a compound that has the ability of arresting the growth of, or killing, cells.
In this specification, unless otherwise stated, the term "cytostatic compound" refers to a compound that has the ability of bringing cells, although not necessarily lysed or killed, into a permanent non-proliferative state.

In this specification, unless otherwise stated, the term "derivative" refers to a compound formed from the original structure either directly, by a chemical reaction of

the original structure, or by a "modification" which is a partial substitution of the original structure, or by design and *de novo* synthesis. Derivatives may be synthetic, or may be metabolic products of a cell or an *in vitro* enzymatic reaction.

In one embodiment the nanoparticles of the inventive drug delivery system have solubility in water below 0.01 mg/ml.

In another embodiment the pharmaceutically active substance is non-covalently associated with the sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, the sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof.

The cationic pharmaceutically active substance may, for instance, have one or more amino groups; the counter-anion may, for instance, be chloride, sulphate, lactate, or tartrate. The substance may be of natural, synthetic, or semi-synthetic origin.

In one embodiment the pharmaceutically active substance is a cytotoxic or a cytostatic compound; in one aspect of this embodiment the cytotoxic or cytostatic compound is a protonated form of doxorubicin, mitoxantrone, epirubicin, daunorubicin, idarubicin, topotecan, irinotecan, vinblastine, vincristine, vinorelbine, amsacrine, procarbazine, mechlorethamine, or a combination thereof; in a specific aspect said compound is a protonated form of doxorubicin; in another aspect said compound is a protonated form of mitoxantrone.

According to other embodiments of the present invention there is also provided:

- the use of the inventive drug delivery system for the preparation of a medicament for the treatment of cancer, and to a method for the treatment of cancer wherein the inventive drug delivery system is administered in a therapeutically effective amount to a patient in need of such treatment; and
- the use of the inventive pharmaceutical composition for the preparation of a medicament for the treatment of cancer, and to a method for the treatment of cancer, wherein the inventive pharmaceutical composition is administered in a therapeutically effective amount to a patient in need of such treatment.

Another embodiment of the invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a drug delivery system of this kind. In one aspect of this embodiment the pharmaceutically active substance is a cytotoxic or a cytostatic compound; in one aspect of this embodiment the pharmaceutical composition may be provided in the form of an aqueous solution, a gel, a cream, an ointment, a tablet, a capsule, or a softgel.

Such a composition may be prepared by, for instance, mixing an aqueous solution of a pharmaceutically active substance which comprises one or more protonated amino group(s), e.g. a hydrochloride, sulphate, lactate, or tartrate, with more than one equivalent of sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof, per amino group. This is illustrated by the below formula, showing a hydrochloride example:

$$(\text{Drug-NH}_3)^{n+}\,Cl^-_n + n\ \text{AnSurfact-O}^-\,Na^+ \rightarrow (\text{Drug-NH}_3)^{n+}\,\text{AnSurfact-O}^-_n + n\ \text{NaCl}$$

in which

the term "AnSurfact-O$^-$" denotes an anion of methyl ester of N-all-trans-retinoyl cysteic acid, or methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof; and

the term "(Drug-NH$_3$)$^{n+}$" denotes a pharmaceutically active substance with protonated amino group(s)

As seen, n equivalent(s) of AnSurfact-O$^-$ binds to (Drug-NH$_3$)$^{n+}$ forming an essentially water insoluble complex according to the formula, and the rest amount of AnSurfact-O$^-$ is applied for ensuring the solubility of the complex obtained.

The excess of AnSurfact-O$^-$ can be in the range of 0.2 – 10 equivalents. Pure water or different aqueous solutions can be used as a solvent in this process. These novel composition obtained by mixing of ammonium salts of the drug with AnSurfact-O$^-$ can be used directly or freeze dried for further use.

A further embodiment of the invention relates to the use of a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of the methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof, in the preparation of such a drug delivery system.

A further embodiment of the invention relates to the use of a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of the methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof, for hydrophobation of a cationic amphiphilic substance which has a solubility *per se* in water of at least 4 mg/ml; in one aspect of this embodiment said cationic amphiphilic substance is a cytotoxic or a cytostatic compound.

Another embodiment of the invention relates to a method for the preparation of a drug delivery system for administration of at least one pharmaceutically active substance that is a cationic amphiphile by itself and has a solubility *per se* in water of at least 4 mg/ml, wherein said substance is combined with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof to form nanoparticles having a solubility in water below 0.1 mg/ml; in one aspect of this embodiment said sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof is non-covalently bound to said substance. In a further aspect of this embodiment the substance is combined with an excess of about 0.2-10 equivalents of said sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof. In a specific embodiment of the embodiment the nanoparticles have solubility in water below 0.01 mg/ml.

In one aspect of this embodiment the sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof is mixed in a mol/mol ratio of 1:1 with a hydrochloride, sulphate, lactate, or tartrate of doxorubicin or analogue thereof, such as epirubicin, daunorubicin, or idarubicin; topotecan; irinotecan; or amsacrine to provide nanoparticles that are essentially non-soluble in water.

In a case of pharmaceutically active substances with more than one amino groups, such as for instance mitoxantrone and vinca alkaloids, the amount of methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof should correspond to the number of protonated amino groups.

Another embodiment of the invention relates to a method for the preparation of pharmaceutical composition comprising a pharmaceutically acceptable carrier and a drug delivery system according to any one of claims 1-8, wherein said drug delivery system is combined with an amount of about 0.2-10 equivalents, based on the cationic charge of the amphiphile comprised in the drug delivery system, of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof.

Another embodiment of the invention relates to a method for enhancing the drug efficiency of at least one pharmaceutically active substance that is a cationic amphiphile by itself and has a solubility *per se* in water of at least 4 mg/ml, wherein said substance is combined with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof to form nanoparticles having a solubility in water below 0.1 mg/ml; in one aspect of this embodiment said sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof is non-covalently bound to said substance. In a further aspect of this embodiment said substance is combined with an excess of about 0.2-10 equivalents of said sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof. In a specific embodiment of the embodiment the nanoparticles have solubility in water below 0.01 mg/ml.

Another embodiment of the invention relates to a method for increasing the bioavailability of at least one pharmaceutically active substance that is a cationic amphiphile by itself and has solubility *per se* in water of at least 4 mg/ml,

wherein said substance is combined with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof to form nanoparticles having a solubility in water below 0.1 mg/ml; in one aspect of this embodiment the sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof is non-covalently bound to said substance. In a further aspect of this embodiment said substance is combined with an excess of about 0.2-10 equivalents of said sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof. In a specific embodiment of the embodiment the nanoparticles have solubility in water below 0.01 mg/ml.

The nanoparticles of the inventive drug delivery system provide for lower polarity and decreased water solubility, which in turn lead to improved cell membrane penetration and stronger binding to proteins, leading to increased potency.

The invention will be illustrated in closer detail in the following non-limiting examples.

EXAMPLES

MATERIALS AND METHODS

The formulations used were either freshly prepared or obtained by reconstitution of freeze dried pharmaceutically active substances with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of the methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof, by a specified solution for reconstitution.

The particle size of the formulations was measured by dynamic light scattering method with the use of a red laser (633 nm, Nano-ZS, Malvern Instruments Ltd). Average values of three independent measurements were calculated for plotting of particle size. Y-error bars are composed by +/- standard deviation of the measurements.

For evaluation of cytotoxicity *in vitro* cells of different human tumour cell lines were purchased from American Type Culture Collection (Rockville, Md., USA): Human Breast Adenocarcinoma Cell Line MDA-MB-231 (ATCC-HTB-26, Lot 3576799), Human Ovary Adenocarcinoma Cell Line SKOV-3 (ATCC-HTB-77, Lot 3038337) and Human Lung Non-Small Cancer Cell Line A549 (ATCC-CCL-185, Lot 3244171). MDA-MB-231 cells were propagated in MEM culture medium with 2 mM L-glutamine, 10% fetal bovine serum (FBS) and antibiotics. SKOV-3 cells were cultured in McCoy's 5A culture medium, supplemented with 1,5 mM L-glutamine, 10% FBS and antibiotics. All media and supplements were purchased from Sigma-Aldrich Co. (St. Louis, Mi., USA). Cell propagation of all lines was carried out in BD Falcon™ 25 or 75 cm^2 cultivation flasks (Becton Dickinson Labware). A549 cells were cultured in Ham's F-12 culture medium with 1 mM L-glutamine, 10% FBS and antibiotics. Cell propagation of all lines was carried out in BD Falcon™ 25 or 75 cm^2 cultivation flasks.

Drug cytotoxicity testing was carried out using BD Falcon™ 96-well cultivation plates for adherent cells (Becton Dickinson Labware). These plates were seeded by cells at $8x10^3$ cells/well for MDA-MB-231, at $10x10^3$ cells/well for SKOV-3 or at $6x10^3$ cells/well for A549 in a volume of 200 µl/well. Both flasks and cultivation plates were incubated for cell growth at 37° C in a humidified atmosphere of 95% air and 5% CO_2.

The cell cultures in the cultivation plates were allowed to adhere for 24 hour of incubation. On day 1 after cell seeding 4 µL solutions of the formulations to be tested with different concentrations in appropriate solvents were added to wells with cultures (dose – response experiments). In the control cultures 4 µL of the solvents were added as solvent control. The cells were incubated within 2-4 consecutive days. At the end of the incubation period adherent cells were detached by trypsinization and the number of viable cells was counted using trypan blue exclusion test and a hemocytometer. All experiment were performed at least tree times and data were derived from an average of three determinations each in four replicates. The results were expressed as mean cell number ± SE and the differences between control and test series evaluated by means of Student's t-test. The drug cytotoxicity was

evaluated based on the extent of cell growth inhibition. The cell growth inhibition by the tested drugs was calculated as follows:

$$Cell\ growth\ inhibition\ \% = \frac{Control - Test\ Series}{Control} \times 100$$

In control series 4 µL of different solvents used for drug testing were added to cultures as negative solvent controls. The differences between these control series were insignificant; therefore an average of negative controls was applied for calculations.

Solutions of generic compounds like doxorubicin hydrochloride, mitoxantrone dihydrochloride, topotecan hydrochloride etc., as well as their commercial formulations were used as positive controls. The differences in growth inhibition by these drugs in different solvents were insignificant; therefore an average inhibition of positive controls was applied for calculations.

The mean IC_{50} ± SE was calculated on the basis of at least three separate experiments.

Enhancement factors (EF) were calculated by dividing IC_{50} of the control comparison drug with IC_{50} of the inventive formulation.

Example 1

Transformation of doxorubicin hydrochloride into deprotonated form

20 mg doxorubicin hydrochloride (0.034 mmol), which is soluble in water in an amount of more than 25 mg/ml, was dissolved in 10 ml of water. 3.4 ml of sodium hydroxide (0.01 M) was added to the solution while stirring. During the mixing a fine precipitation emerged. The precipitate was separated by centrifugation of the test tube at 3000 rpm for 10 min. The supernatant was removed and the precipitate was shaken with 10 ml of water followed by a new centrifugation. After three additional washing procedures as described above the supernatant was filtered through a 0.2 mm filter in order to remove possible large aggregates of the product. The solubility

of doxorubicin in amine form was measured by UV method at wavelength 495 nm and was equal to 0.015 mg/ml.

Example 2

Transformation of mitoxantrone dihydrochloride into deprotonated form

26 mg mitoxantrone dihydrochloride (0.05 mmol) was dissolved in 10 ml of water. 10 ml sodium hydroxide (0.01 M) was added to the solution while stirring. During the mixing a fine precipitation emerged. The precipitate was separated by centrifugation of the test tube at 3000 rpm for 10 min. The supernatant was removed and the precipitate was shaken with 10 ml of water followed by a new centrifugation. After three additional washing procedures as described above the supernatant was filtered through 0.2 mm filter in order to remove possible large aggregates of the product. The solubility of mitoxantrone in amine form was measured by UV method at wavelength 660 nm and was equal to 0.03 mg/ml.

Example 3

Transformation of topotecan hydrochloride into deprotonated form

23 mg topotecan hydrochloride (0.05 mmol) was dissolved in 10 ml of water. 5 ml sodium hydroxide (0.01 M) was added to the solution while stirring. During the mixing a fine precipitation emerged. The precipitate was separated by centrifugation of the test tube at 3000 rpm for 10 min. The supernatant was removed and the precipitate was shaken with 10 ml of water followed by a new centrifugation. After three additional washing procedures as described above the supernatant was filtered through 0.2 mm filter in order to remove possible large aggregates of the product. The solubility of topotecan in amine form was measured by UV method at wavelength 385 nm and was equal to 0.09 mg/ml.

Example 4

Formation of particles consisting of doxorubicin in protonated form and methyl ester of N-all-trans-retinoyl cysteic acid in deprotonated form

Aqueous solutions of sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid (2 ml, 5 mg/mL) and doxorubicin hydrochloride (6 ml, 2 mg/ml) were mixed in a 10 ml test tube. During the mixing a fine precipitation emerged. The precipitate was separated by centrifugation of the test tube at 3000 rpm for 10 min. The supernatant

was removed and the precipitate was shaken with 10 ml of water followed by a new centrifugation. After three additional washing procedures as described above the supernatant was filtered through 0.2 mm filter in order to remove possible large aggregates of the product. The solubility of the obtained particles was measured by UV method at wavelength 350 nm and was equal to 0.0002 mg/ml.

Example 5

Formation of particles consisting of mitoxantrone in diprotonated form and two equivalents of methyl ester of N-all-trans-retinoyl cysteic acid in deprotonated form

Aqueous solutions of sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid (2 ml, 5 mg/mL) and mitoxantrone dihydrochloride (5.2 ml, 1 mg/ml) were mixed in a 10 ml test tube. During the mixing a fine precipitation emerged. The precipitate was separated by centrifugation of the test tube at 3000 rpm for 10 min. The supernatant was removed and the precipitate was shaken with 10 ml of water followed by a new centrifugation. After three additional washing procedures as described above the supernatant was filtered through 0.2 mm filter in order to remove possible large aggregates of the product. The solubility of the obtained particles was measured by UV method at wavelength 660 nm and was equal to 0.002 mg/ml.

Example 6

Formation of particles consisting of topotecan in protonated form and methyl ester of N-all-trans-retinoyl cysteic acid in deprotonated form

Aqueous solutions of sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid (2 ml, 5 mg/mL) and topotecan hydrochloride (4.7 ml, 2 mg/ml) were mixed in a 10 ml test tube. During the mixing a fine precipitation emerged. The precipitate was separated by centrifugation of the test tube at 3000 rpm for 10 min. The supernatant was removed and the precipitate was shaken with 10 ml of water followed by a new centrifugation. After three additional washing procedures as described above the supernatant was filtered through 0.2 mm filter in order to remove possible large aggregates of the product. The solubility of the obtained particles was measured by UV method at wavelength 364 nm and was equal to 0.024 mg/ml.

Example 7

Preparation of a formulation of doxorubicin with sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid and sodium salt of methyl ester of 13-cis-retinoyl cysteic acid

50 ml doxorubicin hydrochloride solution (8.6 mg/ml) was added drop-wise under stirring to 200 ml of a solution containing sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid (3 mg/mL) and sodium salt of methyl ester of 13-cis-retinoyl cysteic acid (3 mg/ml) in 500 ml round-bottom flask. Stirring was continued for an additional 20 min. The doxorubicin concentration in the obtained formulation was 1.6 mg/ml. The solution obtained was filtered through 0.2 mm filter and freeze dried. The filtration did not result in reduction of doxorubicin concentration.

Example 8

Preparation of a formulation of topotecan with sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid

Methanol stock-solutions of topotecan hydrochloride (120 ml, 1.09 mg/ml) and sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid (32 ml, 15 mg/ml) were mixed in a 500 ml round-bottom flask and evaporated in vacuo. 120 ml sodium chloride solution (9 mg/ml) was added to the residue obtained after evaporation, and the mixture was stirred until it became clear and transparent (approx. 20 min). The concentration of topotecan in the obtained solution was 1 mg/ml, corresponding to a topotecan hydrochloride concentration of 1.09 mg/ml. The solution obtained was filtered through 0.2 mm filter. The filtration did not result in reduction of topotecan concentration.

Example 9

Preparation of a formulation of irinotecan with sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid

Methanol stock-solutions of irinotecan hydrochloride trihydrate (100 ml, 1.15 mg/ml) and sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid (27 ml, 15 mg/ml) were mixed in a 500 ml round-bottom flask and evaporated in vacuo. 100 ml of water was added to the residue obtained after evaporation and the mixture was stirred until it became clear and transparent (approx. 30 min). The concentration of irinotecan in the obtained solution was 1 mg/ml, corresponding to an irinotecan hydrochloride trihydrate concentration of 1.15 mg/ml. The obtained solution was filtered through 0.2

mm filter and freeze dried. The filtration did not result in reduction of irinotecan concentration.

Example 10

Investigation of the dependence of particle size formed by sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid and doxorubicin hydrochloride (w/w ratio 2.3:1) on the concentration of doxorubicin.

Solutions were prepared by reconstitution of freeze dried samples consisted of the mixture of sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid and doxorubicin with w/w ratio 2.3:1 in aqueous solution containing (130 mmol), $CaCl_2$ (2 mmol) and $MgCl_2$ (0.8 mmol).

Table 1

Concentration of doxorubicin, mg/ml	Average particle size, nm	St. dev.
0.004	31.7	1.2
0.1	31.3	1.7
0.3	40.7	1.2
1	55.7	1.7
3	69.0	4.3

As shown in Table 1 and Fig. 2 a decrease of concentration results in a decrease of particle size in a range of concentrations 0.1-3 mg/ml. Further dilution does not influence on the particle size.

Example 11

Investigation of the kinetics of dissolving of particles.

A starting solution was prepared by dissolving a freeze dried sample of a mixture of sodium salt of methyl ester of N-all-trans-retinoyl cysteic acid and doxorubicin hydrochloride in w/w ratio 2.1:1 in an aqueous solution of NaCl (5.9 mg/mL), KCl (0.3 mg/mL), $CaCl_2$ (0.295 mg/mL), $MgCl_2$ hexahydrate (0.2 mg/mL) and sodium acetate (4.1 mg/mL) to a doxorubicin concentration of 2 mg/ml. The starting solution was diluted 50 times to a doxorubicin concentration of 0.04 mg/ml, the obtained solution was vigorously stirred on the vortex for 10 seconds and used directly for measurements of average particle size.

Table 2

Time after dilution, min	Average particle size, nm	St. dev.
1.5	61.1	5.9
2	50.0	3.3
3	42.7	2.6
7	40.3	3.3
20	40.7	2.5
60	34.6	2.1
120	31.3	0.9
300	30.9	1.6

As shown in Table 2 and Fig. 3 the rate of decreasing of particle size is slowed down with time until almost insoluble particles are formed.

Biological evaluation – Examples 12-14

In vitro experiments on different malignant cell culture lines like breast adenocarcinoma, ovary adenocarcinoma and lung non-small cell cancer showed that the activity of formulations of cationic amphiphilic compounds depends dramatically on the nature of counter ions as well as morphology of nanoparticles. The use of the methyl ester of N-all-trans-retinoyl cysteic acid, the methyl ester of N-13-cis-retinoyl cysteic acid, or combinations thereof reduces the solubility of the cationic amphiphilic compounds, which facilitates the transport of the compounds through cell membrane resulting in increased potency of such formulations.

The following commercial formulations were used as references in the below Examples: DOXIL® (doxorubicin hydrochloride formulated into pegylated liposomes), NOVANTRONE® (mitoxantrone hydrochloride), ADRIAMYCIN® (doxorubicin hydrochloride), HYCAMTIN® (topotecan hydrochloride), and CAMPTO® (irinotecan hydrochloride)

Example 12

Comparative Evaluation of Cytotoxicity of the Formulations in Cultures of Human Breast Adenocarcinoma MDA-MB-231 Cell Line.

Formulations containing mixtures of nanoparticles of the methyl ester of N-all-trans-retinoyl cysteic acid and the methyl ester of N-13-cis-retinoyl cysteic acid were prepared by dissolving freeze dried powder in appropriate aqueous solutions.

Dilutions of commercial formulations were made according to instructions of the manufacturers. The results are set forth in Table 3 below.

Table 3

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day 4	EF day 4
ADRIAMYCIN®	9 mg/ml NaCl	-	$(1.9 \pm 0.13) \times 10^{-7}$	-	$(5.1 \pm 0.17) \times 10^{-8}$	-
DOXIL®	50 mg/ ml glucose	100	$(2.3 \pm 0.15) \times 10^{-6}$	0.08[a]	$(2.8 \pm 0.10) \times 10^{-7}$	0.18[a]
Doxorubicin-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:1.1:1.1 (w/w/w)	9 mg/ml NaCl	34	$(2.0 \pm 0.17) \times 10^{-8}$	9.5[a]	$(1.4 \pm 0.07) \times 10^{-8}$	3.6[a]
NOVANTRONE®	50 mg/ ml glucose	-	$(7.5 \pm 0.38) \times 10^{-8}$	-	$(5.1 \pm 0.21) \times 10^{-9}$	-
Mitoxantrone-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	50 mg/ ml glucose	-	$(8.1 \pm 0.29) \times 10^{-9}$	9.3[b]	$(2.0 \pm 0.12) \times 10^{-9}$	2.6[b]
HYCAMTIN®	9 mg/ml NaCl	-	$(9.2 \pm 1.4) \times 10^{-7}$	-	$(4.4 \pm 0.33) \times 10^{-8}$	-
Topotecan-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	6 mg/ml NaCl, 0.3 mg/ml KCl, calcium chloride hexahydrate 0.4 mg/ml $CaCl_2$ dihydrate, 3.1 mg/ml Na lactate	14	$(1.7 \pm 0.12) \times 10^{-7}$	5.4[c]	$(1.4 \pm 0.19) \times 10^{-8}$	3.1[c]

Table 3

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day 4	EF day 4
CAMPTO®	9 mg/ml NaCl	-	$(3.0 \pm 0.09) \times 10^{-5}$	-	$(3.2 \pm 0.10) \times 10^{-6}$	-
Irinotecan-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	6 mg/ml NaCl, 0.3 mg/ml KCl, calcium chloride hexahydrate 0.4 mg/ml $CaCl_2$ dihydrate, 3.1 mg/ml Na lactate	12	$(8.1 \pm 0.19) \times 10^{-6}$	3.7[d]	$(1.9 \pm 0.11) \times 10^{-6}$	1.7[d]

Enhancement factors were calculated versus: [a]ADRIAMYCIN®, [b]NOVANTRONE®, [c]HYCAMTIN® and [d]CAMPTO®.

Example 13

Comparative Evaluation of Cytotoxicity of the Formulations in Cultures of Human Ovary Adenocarcinoma SKOV-3 Cell Line

Formulations containing mixtures of nanoparticles of the methyl ester of N-all-trans-retinoyl cysteic acid and the methyl ester of N-13-cis-retinoyl cysteic acid were prepared by dissolving of freeze dried powder in appropriate aqueous solutions. Dilutions of commercial formulations were made according to instructions of the manufacturers. The results are set forth in Table 4 below.

Table 4

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day4	EF day 4
ADRIAMYCIN®	9 mg/ml NaCl	-	$(8.5 \pm 0.27) \times 10^{-8}$	-	$(4.8 \pm 0.16) \times 10^{-8}$	-
DOXIL®	50 mg/ ml glucose	100	$(4.8 \pm 0.18) \times 10^{-6}$	0.02[a]	$(8.0 \pm 0.27) \times 10^{-7}$	0.06[a]

Table 4

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day4	EF day 4
Doxorubicin-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:1.1:1.1 (w/w/w)	9 mg/ml NaCl	34	$(5.2 \pm 0.25) \times 10^{-8}$	1.6[a]	$(2.8 \pm 0.1) \times 10^{-8}$	1.7[a]
NOVANTRONE®	50 mg/ ml glucose	-	$(9.6 \pm 0.45) \times 10^{-8}$	-	$(1.8 \pm 0.32) \times 10^{-9}$	-
Mitoxantrone-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	50 mg/ ml glucose	-	$(2.0 \pm 0.09) \times 10^{-9}$	4.8[b]	$(9.2 \pm 0.12) \times 10^{-10}$	2.0[b]
HYCAMTIN®	9 mg/ml NaCl	-	$(3.5 \pm 0.42) \times 10^{-5}$	-	$(1.0 \pm 0.27) \times 10^{-6}$	-
Topotecan-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	6 mg/ml NaCl, 0.3 mg/ml KCl, calcium chloride hexahydrate 0.4 mg/ml $CaCl_2$ dihydrate, 3.1 mg/ml Na lactate	14	$(5.0 \pm 0.22) \times 10^{-7}$	70[c]	$(2.1 \pm 0.08) \times 10^{-8}$	48[c]
CAMPTO®	9 mg/ml NaCl	-	$(4.2 \pm 0.18) \times 10^{-5}$	-	$(4.0 \pm 0.19) \times 10^{-5}$	-

Table 4

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day4	EF day 4
Irinotecan-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	6 mg/ml NaCl, 0.3 mg/ml KCl, calcium chloride hexahydrate 0.4 mg/ml $CaCl_2$ dihydrate, 3.1 mg/ml Na lactate	12	$(1.2 \pm 0.09) \times 10^{-5}$	3.5[d]	$(4.2 \pm 0.27) \times 10^{-6}$	9.5[d]

Enhancement factors were calculated versus: [a]ADRIAMYCIN®, [b]NOVANTRONE®, [c]HYCAMTIN® and [d]CAMPTO®.

Example 14

Comparative Evaluation of Cytotoxicity of the Formulations in Cultures of Human Lung Non-Small Cancer Cell Line A549

Formulations containing mixtures of nanoparticles of the methyl ester of N-all-trans-retinoyl cysteic acid and the methyl ester of N-13-cis-retinoyl cysteic acid were prepared by dissolving of freeze dried powder in appropriate aqueous solutions. Dilutions of commercial formulations were made according to instructions of the manufacturers. The results are set forth in Table 5 below.

Table 5

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day4	EF day 4
ADRIAMYCIN®	9 mg/ml NaCl	-	$(1.2 \pm 0.09) \times 10^{-8}$	-	$(2.7 \pm 0.21) \times 10^{-8}$	-
DOXIL®	50 mg/ ml glucose	100	$(1.9 \pm 0.18) \times 10^{-7}$	0.06[a]	$(1.4 \pm 0.08) \times 10^{-7}$	0.19[a]

Table 5

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day4	EF day 4
Doxorubicin-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:1.1:1.1 (w/w/w)	9 mg/ml NaCl	34	$(2.6 \pm 0.15) \times 10^{-9}$	4.6[a]	$(6.2 \pm 0.15) \times 10^{-9}$	4.4[a]
NOVANTRONE®	50 mg/ ml glucose	-	$(2.1 \pm 0.06) \times 10^{-9}$	-	$(1.1 \pm 0.02) \times 10^{-9}$	-
Mitoxantrone-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	50 mg/ ml glucose	-	$(9.0 \pm 0.34) \times 10^{-10}$	2.3[b]	$(3.7 \pm 0.09) \times 10^{-10}$	3.0[b]
HYCAMTIN®	9 mg/ml NaCl	-	$(2.6 \pm 0.21) \times 10^{-6}$	-	$(7.3 \pm 0.33) \times 10^{-7}$	-
Topotecan-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	6 mg/ml NaCl, 0.3 mg/ml KCl, calcium chloride hexahydrate 0.4 mg/ml $CaCl_2$ dihydrate, 3.1 mg/ml Na lactate	14	$(7.2 \pm 0.22) \times 10^{-7}$	3.6[c]	$(1.0 \pm 0.05) \times 10^{-7}$	7.3[c]
CAMPTO®	9 mg/ml NaCl	-	$(2.5 \pm 0.26) \times 10^{-5}$	-	$(8.5 \pm 0.36) \times 10^{-6}$	-

Table 5

Formulation	Solvent	Particle size, nm	IC_{50} day 3	EF day 3	IC_{50} day4	EF day 4
Irinotecan-Na salt of methyl ester of N-all-trans-retinoyl cysteic acid-Na salt of methyl ester of N-13-cis-retinoyl cysteic acid 1:3.4:3.4 (w/w/w)	6 mg/ml NaCl, 0.3 mg/ml KCl, calcium chloride hexahydrate 0.4 mg/ml $CaCl_2$ dihydrate, 3.1 mg/ml Na lactate	12	$(7.8 \pm 0.53) \times 10^{-6}$	3.2[d]	$(6.7 \pm 0.29) \times 10^{-7}$	12.7[d]

Enhancement factors were calculated versus: [a]ADRIAMYCIN®, [b]NOVANTRONE®, [c]HYCAMTIN® and [d]CAMPTO®.

Although the invention has been described with regard to certain embodiments, including the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention as set forth in the claims appended hereto.

Claims

1. A drug delivery system for administration of a pharmaceutically active substance that is a cationic amphiphile by itself and has a solubility *per se* in water of at least 4 mg/ml, characterized in that the drug delivery system comprises nanoparticles having a solubility in water below 0.1 mg/ml, said nanoparticles being formed by said substance in association with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof.

2. A drug delivery system according to claim 1, characterized in that said nanoparticles have a solubility in water below 0.01 mg/ml.

3. A drug delivery system according to claim 1 or 2, characterized in that said substance is non-covalently associated with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof.

4. A drug delivery system according to any preceding claim, characterized in that said substance is a cytotoxic or a cytostatic compound.

5. A drug delivery system according to claim 4, characterized in that said cytotoxic or cytostatic compound is a protonated form of doxorubicin, mitoxantrone, epirubicin, daunorubicin, idarubicin, topotecan, irinotecan, vinblastine, vincristine, vinorelbine, amsacrine, procarbazine, mechlorethamine, or a combination thereof.

6. A drug delivery system according to claim 5, characterized in that said compound is a protonated form of doxorubicin.

7. A drug delivery system according to claim 5, characterized in that said compound is a protonated form of mitoxantrone.

8. A drug delivery system according to any one of claims 4-7 for use in treatment of cancer.

9. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the drug delivery system according to any preceding claim.

10. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the drug delivery system according to any one of claims 4-7.

11. A pharmaceutical composition according to claim 9 or 10 in the form of an aqueous solution, a gel, a cream, an ointment, a tablet, a capsule, or a softgel.

12. Use of a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of the methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof in the preparation of a drug delivery system according to any one of claims 1-8.

13. Use of a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof for hydrophobation of a cationic amphiphilic substance which has a solubility *per se* in water of at least 4 mg/ml.

14. Use according to claim 13, characterized in that said cationic amphiphilic substance is a cytotoxic or a cytostatic compound.

15. A method for the preparation of a drug delivery system for administration of at least one pharmaceutically active substance that is a cationic amphiphile by itself and has a solubility *per se* in water of at least 4 mg/ml, wherein said substance is combined with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof to form nanoparticles having a solubility in water below 0.1 mg/ml.

16. A method according to claim 15, wherein a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof is non-covalently bound to said substance.

17. A method according to any one of claim 15 or 16, wherein said nanoparticles have a solubility in water below 0.01 mg/ml.

18. A method for the preparation of pharmaceutical composition comprising a pharmaceutically acceptable carrier and a drug delivery system according to any one of claims 1-8, wherein said drug delivery system is combined with an amount of about 0.2-10 equivalents, based on the cationic charge of the amphiphile comprised in the drug delivery system, of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof.

19. A method for enhancing the drug efficiency of at least one pharmaceutically active substance that is a cationic amphiphile by itself and has a solubility *per se* in water of at least 4 mg/ml, wherein said substance is combined with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof to form nanoparticles having a solubility in water below 0.1 mg/ml.

20. A method according to claim 19, wherein a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of the methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof is non-covalently bound to said substance.

21. A method according to claim 19 or 20, wherein said substance is combined with an excess of about 0.2-10 equivalents of said sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof.

22. A method according to any one of claim 19-21, wherein said nanoparticles have a solubility in water below 0.01 mg/ml.

23. A method for increasing the bioavailability of at least one pharmaceutically active substance that is a cationic amphiphile by itself and has a solubility *per se* in water of at least 4 mg/ml,
wherein said substance is combined with a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of the methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof to form nanoparticles having a solubility in water below 0.1 mg/ml.

24. A method according to claim 23, wherein a sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, a sodium salt of the methyl ester of N-13-cis-retinoyl cysteic acid, or a combination thereof is non-covalently bound to said substance.

25. A method according to claim 23 or 24, wherein said substance is combined with an excess of about 0.2-10 equivalents of said sodium salt of the methyl ester of N-all-trans-retinoyl cysteic acid, sodium salt of methyl ester of N-13-cis-retinoyl cysteic acid, or combination thereof.

26. A method according to any one of claim 23-25, wherein said nanoparticles have a solubility in water below 0.01 mg/ml.

27. A method for the treatment of cancer, characterized in that a drug delivery system according to any one of claims 1-8 is administered in a therapeutically effective amount to a patient in need of such treatment.

28. Use of a drug delivery system according to any one of claims 1-8 for the preparation of a medicament for the treatment of cancer.

29. A method for the treatment of cancer, characterized in that a pharmaceutical composition according to any one of claims 9-11 is administered in a therapeutically effective amount to a patient in need of such treatment.

30. Use of a pharmaceutical composition according to any one of claims 9-11 for the preparation of a medicament for the treatment of cancer.

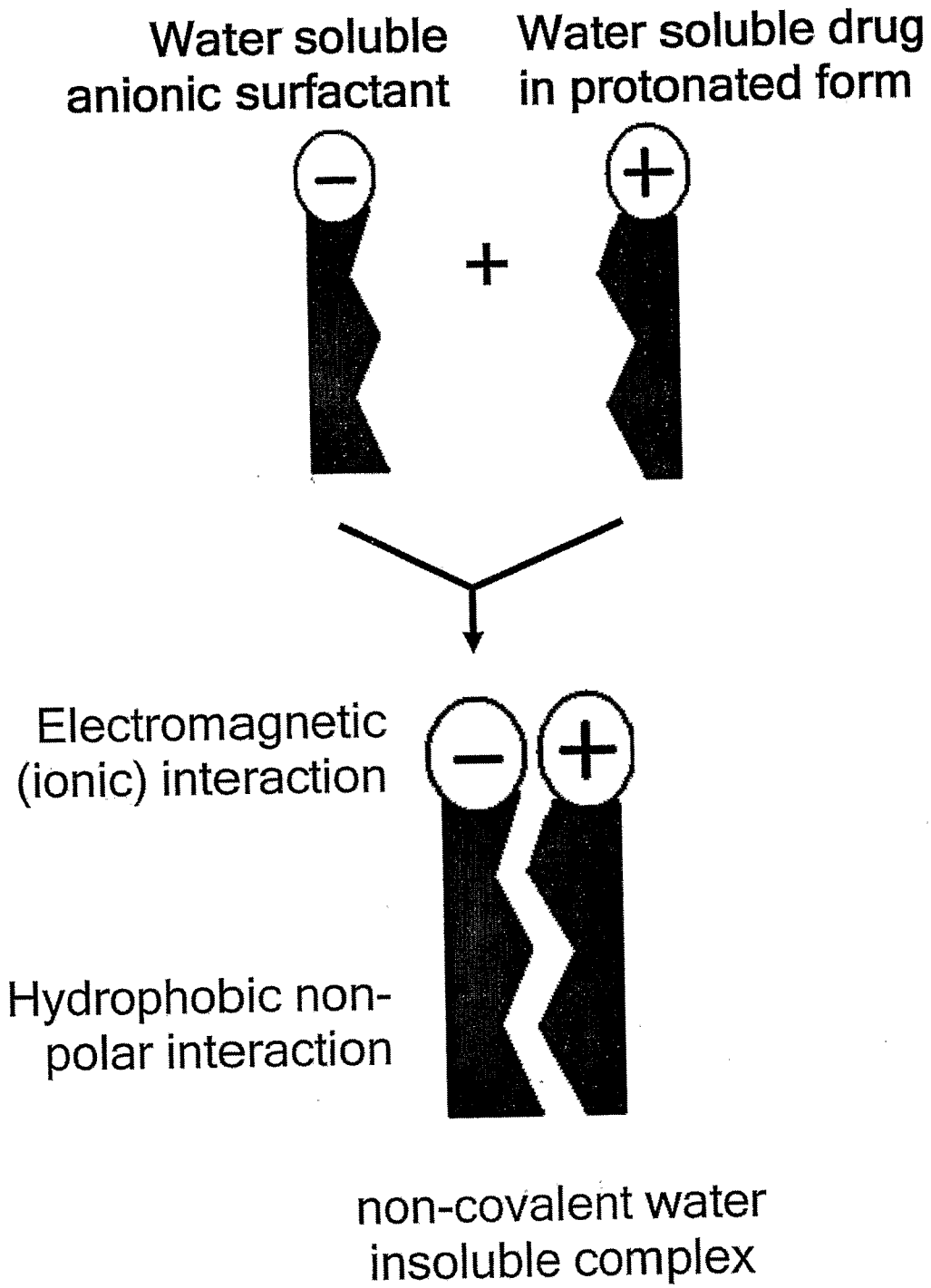

Water soluble anionic surfactant
Water soluble drug in protonated form
Electromagnetic (ionic) interaction
Hydrophobic non-polar interaction
non-covalent water insoluble complex

Fig. 1

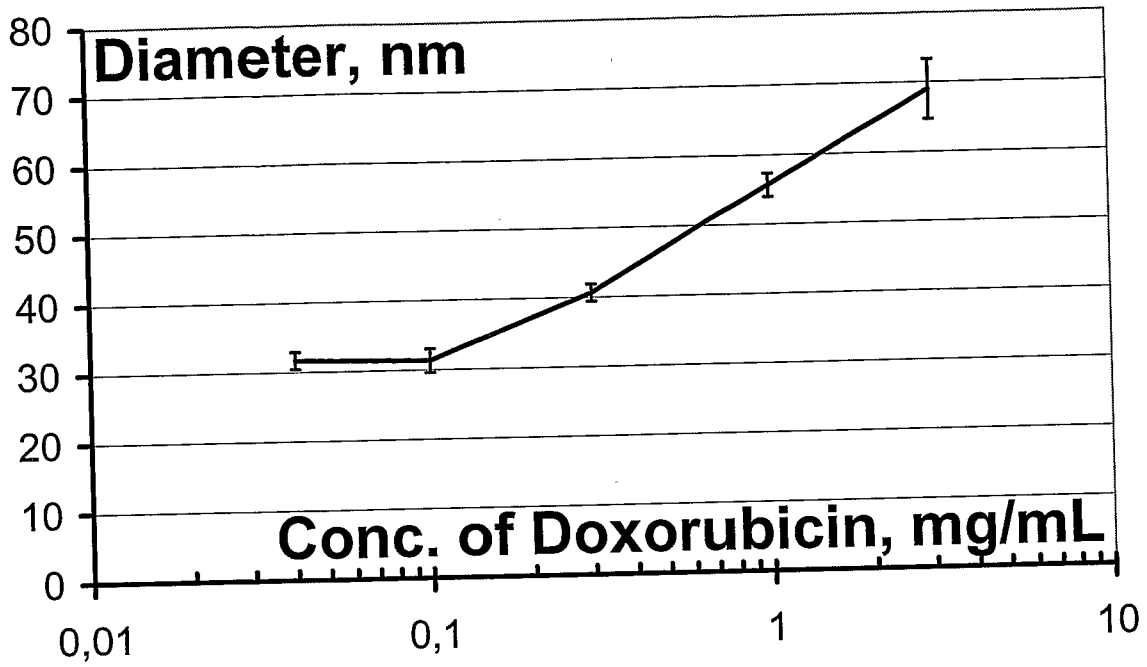
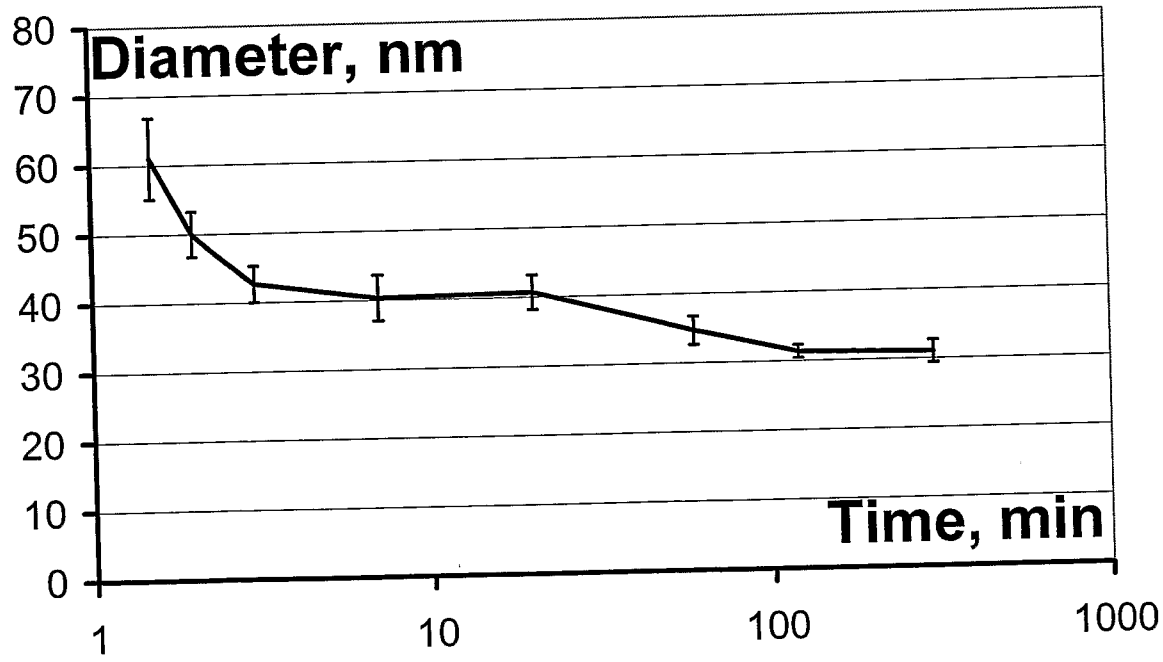

Diameter, nm
80
70
60
50
40
30
20
10
0
Conc. of Doxorubicin, mg/mL
0,01
0,1
1
10

Fig. 2


Diameter, nm
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70
60
50
40
30
20
10
0
Time, min
1
10
100
1000

Fig. 3

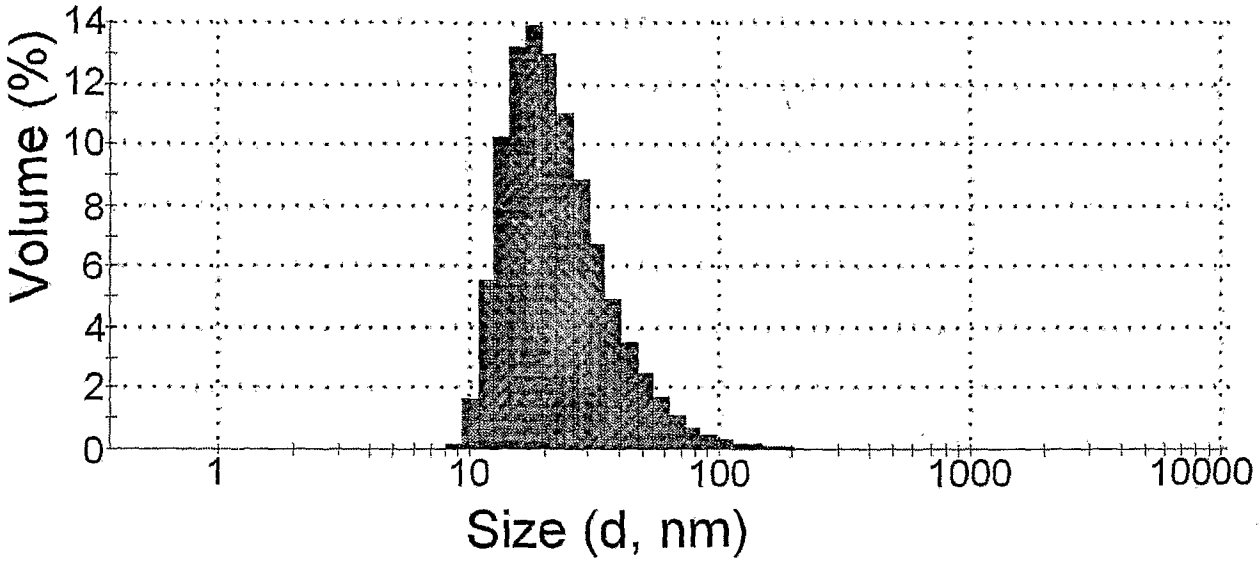

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/001129

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
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)

IPC: A61K

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

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ, EMBASE, MEDLINE, BIOSIS, CHEM. ABS DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 20040048923 A1 (STRELCHENOK, OLEG ET AL), 11 March 2004 (11.03.2004), see paragraphs [0002], [0019]-[0022], [0040]-[0047], [0057]-[0061], [0077]-[0078]; examples 16-17, 24-25, 32-33, 53, 60-62, 64, 66; claims 1-49	1-18,27-30
	--	
X	US 6197809 B1 (STRELCHENOK, OLEG), 6 March 2001 (06.03.2001), see column 2, lines 47-59; column 3, lines 12-67; column 6, lines 20-36; examples 1-26; claims 1-5	13-14
A		1-12,15-18, 27-30
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"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 August 2008	27-08-2008
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Andreas Gustafsson/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/001129

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 27,29
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 27 and 29 relate to a method for treatment of the human or animal body by surgery or by therapy, as well as diagnostic methods, see PCT rule 39.1(iv). Nevertheless, a search has been made for these claims. The search has been directed to the technical content of the claims.

2. ☒ Claims Nos.: 19-26
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See extra sheet

.../...

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Box II.2

Present claims 19-26 relate to methods defined (inter alia) by reference to the following parameters:

P1: Drug efficiency
P2: Bioavailability

The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is very hard to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to the drug delivery system leading to the effects described in claims 19-26.

Thence it follows that a reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability is not established for present claims 19-26.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/001129

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A		1-12,15-18, 27-30
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X	CHAVANPATIL, MAHESH D. et al, "PHARMACEUTICAL NANOTECHNOLOGY, Polymer-Surfactant Nanoparticles for Sustained Release of Water-Soluble Drugs", Journal of Pharmaceutical Sciences, December 2007, Vol. 96, No. 12, page 3379 - page 3389, see the whole document	13-14
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X	WO 02092600 A1 (ARDENIA INVESTMENTS LTD.), 21 November 2002 (21.11.2002), see page 1, lines 1-8; page 5, lines 14-26; page 6, line 14 - page 10, line 9; page 11, lines 1-24; page 13, line 3 - page 15, line 6; examples 5,11,20,21,23,24,37,38; claims 1-32	13-14
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International patent classification (IPC)
A61K 47/48 (2006.01)
A61K 9/14 (2006.01)
A61P 35/00 (2006.01)

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Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

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