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(54) **Title**: PHARMACEUTICAL COMPOSITIONS OF POLYMERIC NANOPARTICLES

(57) **Abstract**: The present invention relates to pharmaceutical compositions comprising polymeric nanoparticles of anticancer drugs.

Pharmaceutical Compositions of Polymeric nanoparticles

FIELD OF INVENTION:

The present invention relates to pharmaceutical compositions comprising polymeric nanoparticles of anticancer drugs, a process for preparing such pharmaceutical compositions and their use in the treatment of liver cancer, specifically hepatocellular carcinoma.

BACKGROUND AND PRIOR ART:

Liver cancer is one of the most widespread forms of cancer and the major reason for tumor related mortality. Globally, more than 6,00,000 cases of death per year are associated with hepatic cancer. Although, liver cancer is observed more commonly in the developing countries, its instances are increasing at an alarming rate throughout the world.

Hepatocellular carcinoma comprises of around 95% of the primary liver cancers followed by cholangiocarcinoma (3.4%) and other cancers (1%). Hence, most of the antineoplastic treatments in patients are directed towards hepatocellular carcinoma.

Current treatments for hepatocellular carcinoma include surgical resection, chemotherapy, liver transplant, chemoembolization, trans-arterial embolization, radiation therapy, ethanol injection and cryoablation.

Liver resection is the best suitable treatment for non-cirrhotic liver cancer subjects. The chances of resection and post-operative survival are better in non-cirrhotic patients than those suffering from cirrhosis. However, this therapy holds limitations like suitability for small tumors. Liver transplantation proves to be a boon for patients with small tumor size (<5 cm), but suffers from restricted amount of donors and delay due to the donor non-availability.

Chemotherapy although widely used to treat tumors, results in more side effects and toxicity due to the destruction of normal, non-malignant cells. Drug delivery to the tumors occurs through endothelial junctions, fenestrations and vesicular organelles. Factors like P-glycoprotein mediated drug efflux and changes in enzyme activity add to failure via chemotherapy. Pharmacokinetics is an important parameter to be considered during chemotherapy. Ideally, cancerous cells should be exposed to suitable concentrations of drug over an extended time

period. Current chemotherapy treatments are given over a short period of time with time intervals of 2-3 weeks. This results in speedy growth of tumors and thus diminishes the therapeutic effects of drugs with untoward side effects. Also, it was seen that prolonged drug exposure to tumors produced better results rather than short term exposure to elevated drug concentrations. Currently, the chemotherapy of hepatocellular carcinoma is performed by use of anthracycline antibiotics like doxorubicin, mitomycin, alkylating agent like cisplatin and other drugs like floxuridine, bevacizumab and sorafenib tosylate.

Percutaneous ethanol injection is an excellent option for small tumors; however it cannot be used in case of patients with ascites. Radiofrequency ablation has a curative effect on liver cancer because of lesser sessions required for therapy, but possesses more complications than percutaneous ethanol injection. Therapeutic effects with other treatments prove to be inadequate due to metastasis of tumors, lack of suitable embolizing materials, non-specificity to tumor cells and greater number of complications.

In spite of various therapies available for hepatocellular carcinoma, none have sufficed in complete and side effect free treatment of hepatic cancer. In this respect, many other alternative treatments have been explored for treatment of liver cancer such as nanoparticles.

Nanoparticles are minute, solid particulates in the size range of 10-1000 nm where, an anticancer agent is either entrapped in the core, adsorbed on the surface or both. Nanospheres or nanocapsules could be prepared by varying the methodology for synthesis of these colloidal carriers. The aim of any cancer treatment is to ensure optimum drug delivery to the desired cancerous site. The potential of using nanoparticles for delivery of anticancer drugs is massive and can be applied to various areas of medicine.

Gold Nanoparticles Conjugated with Cisplatin/Doxorubicin/Capecitabine Lower the Chemoresistance of Hepatocellular Carcinoma-Derived Cancer Cells, Tomuleasa *et al*, J Gastrointestin Liver Dis, June 2012 Vol. 21 No 2, 187-196.

Nanotechnology in oncology: Characterization and in vitro release kinetics of cisplatin-loaded albumin nanoparticles: Implications in anticancer drug delivery, Indian J Pharmacol. 2011 Jul-Aug; 43(4): 409–413.

Protein Nanoparticles as Drug Delivery Carriers for Cancer Therapy, BioMed Research International, Warangkana Lohcharoenkal *et al*, Volume 2014 (2014).

Although the prior art discloses various therapies, techniques and formulations for the treatment of hepatocellular carcinoma, each therapy, technique and formulation has a setback due to its side effects, administration type and regime or formulation feasibility and stability. Nanoparticles disclosed in prior art though efficacious would pose to have cost related implications. Hence there exists a need for development of nanoparticulate pharmaceutical compositions comprising anticancer drugs which can alleviate the above problems as well as target such anticancer drugs at the specific site of action.

OBJECT OF THE INVENTION:

An object of the present invention is to provide a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs.

Another object of the present invention is to provide a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs optionally with one or more pharmaceutically acceptable excipients.

Another object of the present invention is to provide a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs exhibiting prolonged release thereby leading to reduced frequency of dosing.

Another object of the present invention is to provide a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs having improved surface area and solubility.

Another object of the present invention is to provide a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs having a reduced dose.

Another object of the present invention is to provide a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs with minimized irritation potential at the site of action thus leading to increased patient compliance.

Another object of the present invention is to provide a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs to deliver the said anticancer drugs to the target site of action as well as attempt to decrease or avoid the side effects.

Another object of the present invention is to provide a process for preparing a pharmaceutical composition comprising homogenous polymeric nanoparticles of anticancer drugs optionally with one or more pharmaceutically acceptable excipients.

Another object of the present invention is to provide the use in the treatment of hepatocellular carcinoma by administering a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs.

Another object of the present invention is to provide the use of a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs and optionally one or more pharmaceutically acceptable excipients in the manufacture of a medicament for treatment of hepatocellular carcinoma.

Another object of the present invention is to provide a method of treating hepatocellular carcinoma by administering a pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs.

SUMMARY OF THE INVENTION:

According to an aspect of the present invention, there is provided a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs, and optionally one or more pharmaceutically acceptable excipients.

According to another aspect of the invention, there is provided a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs, and optionally, one or more pharmaceutically acceptable excipients, wherein the composition is formulated to deliver said anticancer drugs to the target site of action as well as attempt to decrease or avoid the side effects.

According to another aspect of the invention, there is provided a process for the preparation of a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer

drugs, wherein the process comprises formulating the polymeric nanoparticles with one or more pharmaceutically acceptable excipients. Preferably, the process comprises dissolving the one or more anticancer drugs in aqueous solution of human serum albumin or gelatin; adding a desolvating agent for formation of nanoprecipitates; cross linking and neutralization of the formed nanoparticles with other pharmaceutically acceptable excipients; optionally freeze drying the purified nanoparticles; and processing into a dosage form.According to an aspect of the present invention, there is provided a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs and optionally one or more pharmaceutically acceptable excipients for use in the treatment of hepatocellular carcinoma.

According to an aspect of the present invention, there is provided the use of a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs and optionally one or more pharmaceutically acceptable excipients in the manufacture of a formulation for treating hepatocellular carcinoma.

According to an aspect of the invention, there is provided a method of treating hepatocellular carcinoma wherein the method comprises administering a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs and optionally one or more pharmaceutically acceptable excipients. to a patient in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS:

A) Dissolution studies were carried out on Gelatin and HSA nanoparticles of Cisplatin.

Figure 1: Dissolution profiles of Gelatin and HSA nanoparticles of Cisplatin

Plain cisplatin exhibited quick dissolution in phosphate buffer saline (pH 7.4) within 4 hours due to its short half-life after intravenous administration.

In case of HSA nanoparticles, around 23% of Cisplatin was quickly released from the nanoparticles within 1 hour followed by **a** sustained release for a period of 96 hours. HSA-TG nanoparticle formulation exhibited a sustained release pattern for 72 hours.

In case of gelatin nanoparticles, sustained release profile for more than 72 hours was observed. Around 70% of the drug was released in 8 hours followed by a controlled release pattern beyond this point.

B) Sulforhodamine B assay

Figure 2: Sulforhodamine B assay for Gelatin nanoparticles

Plain cisplatin showed cytotoxicity on the liver cancer cell line causing disruption in the cancer cell structure and their shrinkage (GI50 = 3.6 μM).

Cisplatin gelatin nanoparticles exhibited cytotoxicity on the HepG2 cells, but the cytotoxicity was less as compared to pure drug (GI50 = 18.2 μM). This was attributed due to slow release of cisplatin from developed gelatin nanoparticles.

Blank nanoparticles did not exhibit any cytotoxicity (GI50 > 100 μM). C) Biodistribution studies on HSA and Gelatin nanoparticles of Cisplatin

Figure 3: Biodistribution studies of cisplatin gelatin nanoparticles in comparison to plain cisplatin

The concentration of cisplatin gelatin nanoparticles in the liver increased slowly untill 48 hours; however, this concentration was found to reduce over 96 hours. Concurrently, plain cisplatin concentration was found to rise steadily in the lungs over a period of 96 hours. The initial high concentration of cisplatin gelatin nanoparticles in the liver was attributed to the enhanced permeability and retention (EPR) effect leading to rapid distribution in the liver followed by subsequent distribution in lungs. Some amount of cisplatin gelatin nanoparticles after i.v. administration could reach the kidney and brain tissues over a period of 24 hours. However, over a time period of 96 hours, the concentration in the brain was found to reduce and increased in liver and lungs.

Figure 4 & Figure 5: Biodistribution studies on passively targeted and actively targeted cisplatin albumin nanoparticles

The concentration of cisplatin in case of non-targeted cisplatin albumin nanoparticles was maximum in case of liver and was seen to be 4 - 6 times higher as compared to other highly perfused organs. This phenomenon may be related to the preferential uptake of negatively charged albumin nanoparticles by the liver due to enhanced permeability and retention (EPR) effect. Also, the concentration of cisplatin was highest at 48 hours and was maintained throughout the time period of 96 hours in this study, due to slow diffusion of cisplatin from the albumin matrix. High liver concentration was achieved by the targeted galactosamine nanoparticles at all the time points and was significantly elevated as compared to all the other organs.

There was no significant difference between the cisplatin nanoparticle concentrations in the liver for non-targeted as well as the targeted formulations at 48 hours. However, this difference was significant for cisplatin concentration at 72 and 96 hours and it was seen that cisplatin was retained in the liver from galactosamine anchored nanoparticles to a greater extent as compared to the non-targeted albumin nanocarriers.

Figure 6: Biodistribution studies on TG-linked albumin nanocarriers

The highest concentration of cisplatin was attained in the liver with low concentrations in other organs. Concentration of cisplatin was maintained at high levels throughout the time period of 96 hours.

DETAILED DESCRIPTION OF THE INVENTION:

The main aim of anticancer therapy is to ensure specific drug action at the target site/tissue/organ along with reduced distribution to other non-specific organs.

Target oriented drug delivery systems can provide maximum therapeutic benefit through controlled and predetermined release rate kinetics, prevent drug degradation or inactivation

during transit to target sites and prevent the body from adverse reactions. For drugs that possess low therapeutic index, these systems can provide effective treatment at low concentrations.

Nanoparticles due to their colloidal nature, offer numerous advantages over conventional treatment strategies. Small sized particles possess large surface area and hence increase the dissolution properties of antineoplastic agents with poor solubility. Nanoparticles can be tailored to reach specific tumor sites by modulation of their size, surface characteristics, and particle charge.

Delivery of anticancer agents in the form of nanoparticles also ensures reduction in their therapeutic dosage and frequency of administration due to the controlled release properties of the nanosystems.

Additionally, nanoparticulate drug delivery systems reduce the untoward toxicities associated with antitumor agents and prevent their degradation. Site specific targeting is possible with nanoparticles after attachment of certain ligands, antibodies or carriers on the surface by physical adsorption or covalent attachment.

Higher drug loading can be achieved in case of nanocarriers, which is a prime factor for any particulate drug delivery system. Nanoparticles possess the ability to increase the absorption of anticancer agents from the body upon administration, thus causing a concomitant rise in their bioavailability.

These particles can act at cellular levels and can be endocytosed/phagocytosed by cells, with resulting cell internalization of the encapsulated drug. Also, the small size of colloidal carriers makes them suitable for parenteral delivery of drugs. Irritation potential at the site of injection can be minimized with nanocarriers as compared to conventional therapy. Nanoparticles are biocompatible and non-immunogenic and can entrap hydrophilic and hydrophobic moieties, proteins, vaccines and biological macromolecules.

In accordance with the present invention, nanoparticles can be prepared using both non-biodegradable and biodegradable polymers. However, the later are preferred due to their biocompatibility, biodegradability, low cost and safety. These particles can be delivered by various routes of administration like nasal, oral, dermal and ocular, apart from parenteral route.

Biodegradable polymeric nanocarriers are favorable due to their excellent biocompatibility, biodegradability and low toxic potential. Preferred biodegradable polymers include, but are not limited to, natural polymers like gelatin, human serum albumin, sodium alginate, agarose, casein, zein, chitosan, glycol chitosan, N, N trimethyl chitosan, starch, cellulose and wheat gluten, or combinations thereof.

A particularly preferred polymer is Human Serum Albumin (HSA). HSA is the most important soluble protein in human body, which is synthesized in the liver. It is the primary protein in human body controlling the osmotic pressure of serum. Other functions imparted by albumin include binding of toxic products and their transport to the liver, distribution and transport of metal ions and other exogenous components. Albumin is an acidic protein with an isoelectric point of 4.7. This protein is stable in the pH range of 4-9 and exhibits solubility in water and other aqueous solutions. Albumin can be heated at a temperature of around 60°C, without any change in the protein structure. All these properties along with the preferential tumor uptake, biodegradability, non-toxicity and property to accumulate at inflamed tissues, makes it an ideal polymer for cancer drug delivery.

Another preferred polymer is gelatin. Gelatin, a natural macromolecule is commonly employed as a pharmaceutical adjuvant and an encapsulating drug material. Advantages of gelatin for drug delivery include its inexpensiveness, ability to be sterilized, free from contamination with pyrogens and low antigenicity.

Preferably, the polymers may be present in the pharmaceutical composition of the present invention an amount ranging from about 0.1% to about 35% by weight of the composition.

According to the present invention, human serum albumin and gelatin, or combinations thereof are the preferable polymers that have been employed in the pharmaceutical composition comprising polymeric nanoparticles of anticancer drugs.

Preferably, the pharmaceutical composition, according to the present invention, comprises polymeric nanoparticles of one or more anticancer drugs.

According to one aspect of the present invention, the pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs have been engineered to have an affinity for target tissues through passive or active targeting mechanisms.

According to another aspect of the present invention, the pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs deliver the said anticancer drugs to the target site of action as well as attempt to decrease or avoid the side effects.

According to another aspect of the present invention, the pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs exhibits prolonged release.

According to another aspect of the present invention, the pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs which have a reduced dose.

Anticancer drugs according to the present invention include, but are not limited to alkylating agents, antitumor antibiotics, anti-microtubule agents, DNA linking agents, bisphosphonates, topoisomerase inhibitors, antimetabolites, biological agents and nucleoside analogues, or any combinations thereof.

Suitable alkylating agents according to the present invention include, but are not limited to, mechlorethamine, mustine, cyclophosphamide, nitrosoureas, tetrazine, azidine, melphalan, chlorambucil, busulfan, uramustine, bendamustine, ifosfamide, lomustine, semustine, carmustine, fotemustine, streptozotocin, thiotepa, dacarbazine, procarbazine, mitomycin, mitozolomide, temozolomide and the like or combinations thereof.

Suitable DNA linking agents include, but are not limited to, cisplatin, carboplatin, nedaplatin, oxaliplatin, satraplatin and the like or combinations thereof.

Suitable antitumor antibiotics and topoisomerase inhibitors according to the present invention include, but are not limited to, bleomycin, daunorubicin, epirubicin, doxorubicin, idarubicin, mitoxantrone, mitomycin, actinomycin, pirarubicin and the like or combinations thereof.

Suitable antimetabolites according to the present invention include, but are not limited to, fluorouracil, capecitabine, asparaginase, cladribine, nelarabine, decitabine, clofarabine,

pentostatin, methotrexate, pemetrexed, raltitrexed, asparaginase, thioguanine, mercaptopurine and the like or combinations thereof.

Suitable biological agents according to the present invention include, but are not limited to, bevacizumab, cetuximab, regorafenib, ibrutinib, axitinib, crizotinib, dabrafenib, ponatinib, afatinib, dasatinib, nilotinib ruxolitinib, trametinib, erlotinib, sorafenib, gefitinib, imatinib, interferon, lapatinib, ipilimumab, denosumab, panitumumab, rituximab, sunitinib, emsirolimus, trastuzumab and the like or combinations thereof.

Suitable nucleoside analogues according to the present invention include, but are not limited to, didanosine, vidarabine, abacavir, acyclovir, entecavir, cytarabine, lamivudine, emtricitabine, stavudine, telbivudine, idoxuridine, trifluridine, zidovudine and the like or combinations thereof.

Other anticancer agents according to the present invention include, but are not limited to, mesna, octreotide, tamoxifen, anastrozole, amifostine, bexarotene, bicalutamide, buserelin, exemestane, flutamide, folinic acid, cyproterone, degarelix, folinic acid, fulvestrant, goserelin, lanreotide, letrozole, medroxyprogesterone, megestrol, stilbestrol, thalidomide and the like or combinations thereof.

The pharmaceutical composition according to the present invention may comprise human serum albumin nanoparticles as well as gelatin nanoparticles of cisplatin. The term “cisplatin” is used in a broad sense to include not only “cisplatin” *per se* but also its pharmaceutically acceptable derivatives thereof. Suitable derivatives include pharmaceutically acceptable salts, pharmaceutically acceptable solvates, pharmaceutically acceptable hydrates, pharmaceutically acceptable isomers, pharmaceutically acceptable esters, pharmaceutically acceptable anhydrates, pharmaceutically acceptable enantiomers, pharmaceutically acceptable polymorphs, pharmaceutically acceptable prodrugs, pharmaceutically acceptable tautomers and/or pharmaceutically acceptable complexes thereof.

The pharmaceutical composition, according to the present invention, comprises polymeric nanoparticles of one or more anticancer drugs which can be administered by varying routes of administration such as, but not limited to oral, parenteral (subcutaneous, intravenous,

intramuscular, intradermal, intraperitoneal) or topical or localized (directly at the site)and the like.

In one aspect, the pharmaceutical composition, according to the present invention, comprises albumin as well as gelatin nanoparticles of one or more anticancer drugs, which can be provided as but not limited to oral, parenteral or topical dosage forms, localized and the like.

The term "pharmaceutical composition" includes oral dosage forms, such as but not limited to, tablets, soft gelatin capsule, capsules (filled with powders, powders for reconstitution, pellets, beads, mini-tablets, pills, micro-pellets, small tablet units, MUPS, disintegrating tablets, dispersible tablets, granules, sprinkles, microspheres and multiparticulates), sachets (filled with powders, pellets, beads, mini-tablets, pills, micro-pellets, small tablet units, MUPS, disintegrating tablets, dispersible tablets, granules, sprinkles microspheres and multiparticulates) and sprinkles, however, other dosage forms such as liquid dosage forms (liquids, liquid dispersions, suspensions, solutions, emulsions, sprays, spot-on), micro-formulations, nanoformulations may also be envisaged under the ambit of the invention.

The term "pharmaceutical composition" includes parenteral dosage forms such as liquid dosage forms (liquids, liquid dispersions, suspensions, solutions, emulsions, powders for reconstitution), gels, bolus, depots, implants (rods, capsules, rings) biodegradable or non-biodegradable microparticles/microspheres etc. may also be envisaged under the ambit of the invention.

The term "pharmaceutical composition" includes topical dosage forms, such as but not limited to, sprays, solutions, suspensions, ointments, drops, in-situ gel, aerosols, ointments, microspheres, creams, gels, patches, films and the like.

The pharmaceutical composition of the present invention may comprise polymeric nanoparticles of one or more anticancer drugs, in the form of controlled release formulations, lyophilized formulations, delayed release formulations, timed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

Preferably, the pharmaceutical compositions of the present invention comprising polymeric nanoparticles of one or more anticancer drugs are provided in parenteral dosage forms.

Accordingly, the pharmaceutical composition of the present invention comprising polymeric nanoparticles of one or more anticancer drugs can be provided in a lyophilized parenteral dosage form, these dosage forms are to be dissolved or reconstituted in a suitable solvent prior to administration.

Suitable solvents for reconstitution include but are not limited to purified water for injection, sterile saline solution, dextrose solution, Ringer's solution and the like.

The present invention thus provides a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs, wherein the nanoparticles have an average particle size of less than or equal to about 2000 nm, preferably less than or equal to about 1000 nm.

The term average particle size as used herein refers to the average diameter of the particles.

The term "particles" as used herein refers to an individual particle of the polymeric nanoparticle comprising anticancer drug(s), or the polymeric nanoparticles comprising anticancer drug(s).

Suitable processes for preparing polymeric nanoparticles in accordance with the present invention include, but not limited to, milling, precipitation, homogenization, high pressure homogenization, spray-freeze drying, supercritical fluid technology, double emulsion/solvent evaporation, desolvation, PRINT (Particle replication in non-wetting templates), thermal condensation, ultrasonication, interfacial polymerisation, spray drying and combinations thereof. Preferably, the polymeric nanoparticles of the present invention are prepared by the process of desolvation.

Accordingly, the process of desolvation can comprise dissolving the anticancer drug in aqueous solution of one or more polymers, such as human serum albumin or gelatin, and adding a desolvating agent for formation of nanoprecipitates, which are further cross linked and then neutralized. Further, the purified nanoparticles are optionally freeze dried.

Suitable desolvating agents according to the present invention include, but are not limited to, acetone, ethanol or isopropanol and the like or combinations thereof.

Suitable cross linkers for the stabilization of polymers such as human serum albumin or gelatin nanoparticles according to the present invention include, but are not limited to, bifunctional aldehydes like glutaraldehyde and formaldehyde, carbodiimides (EDC), enzymes such as transglutaminase or thermal processes and the like or combinations thereof. Preferably, one or more cross linkers may be present in an amount ranging from about 0.1% to about 20% by weight of the total composition.

Suitable neutralizers according to the present invention include, but are not limited to, glycine, lysine, sodium metabisulphite, sodium sulphate and the like or combinations thereof. Preferably, one or more neutralizers may be present in an amount ranging from about 0.1% to about 20% by weight of the total composition.

Suitable cryoprotectants according to the present invention include,but are not limited to, sucrose, trehalose, dextrose, mannose, glycine, glucose, galactose, lactose and the like or combinations thereof. Preferably, one or more cryoprotectants may be present in an amount ranging from about 0.1% to about 20% by weight of the total composition.

Suitable pH adjusting agents or buffering agents that may be used in the pharmaceutical composition include, but are not limited to, hydrochloric acid, acetic acid, citric acid, tartaric acid, propionic acid, sodium hydroxide, sodium phosphate, ammonia solution, triethanolamine, sodium borate, sodium carbonate, sodium acetate, potassium hydroxide and the like or combinations thereof. Preferably, one or more buffering agent may be present in an amount ranging from about 0.1% to about 10% by weight of the total composition.

Suitable solvents/co-solvents, solubilizer or vehicles, that may be employed, in the pharmaceutical composition include, but are not limited to, dichloromethane, acetonitrile, ethyl acetate, acetone, propylene carbonate, water, glycerine, coconut fatty acid diethanolamide, medium and/or long chain fatty acids or glycerides, monoglycerides, diglycerides, triglycerides, structured triglycerides, soyabean oil, peanut oil, corn oil, corn oil mono glycerides, corn oil di glycerides, corn oil triglycerides, polyethylene glycol, caprylocaproylmacroglycerides, caproyl

90, propylene glycol, polyoxyethylenesorbitan fatty acid esters, polyoxyethylene castor oil derivatives, castor oil, cottonseed oil, olive oil, safflower oil, peppermint oil, coconut oil, palm seed oil, beeswax, oleic acid, methanol, ethanol, isopropyl alcohol, butanol, acetone, methyl isobutyl ketone, methyl ethyl ketone or combinations thereof.

The present invention also provides a method of treating hepatocellular carcinoma by administering a pharmaceutical composition comprising polymeric nanoparticles of one or anticancer drugs to a patient in need thereof..

The present invention also provides the use of a pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs for the treatment of hepatocellular carcinoma..

It may be well acknowledged to a person skilled in the art that the said pharmaceutical composition, according to the present invention, may further comprise one or more active in particular other anticancer drugs, such as, but are not limited to, alkylating agents, anti-metabolites, anti-microtubule agents, topoisomerase inhibitors or antitumor antibiotics, DNA linking agents, biological agents and bisphosphonates.

The following examples are for the purpose of illustration of invention only and are not intended in any way to limit the scope of the present invention.

Example 1:

a) Bolus injection of HSA nanoparticles of Cisplatin

Sr. No	Ingredients	Quantity
1	Human serum albumin	100 - 300 mg (10 ml)
2	Cisplatin	5 - 12 mg
3	Absolute ethanol	10 - 20 ml
4	Glutaraldehyde	50 - 200 µl (8 %w/v)
5	Glycine	40 - 100 mg
6	Vehicle (10 mM NaCl solution)	10 ml

Process:

1. Cisplatin was added to the aqueous human serum albumin solution while stirring after pH adjustment. .
2. Ethanol was added to the solution obtained in step (1) to cause precipitation of the protein molecules leading to the formation of nanoparticles.
3. The nanoparticles obtained in step (2) were cross linked with glutaraldehyde under stirring.
4. Glycine was added under stirring to the nanoparticles obtained in step (3) to remove excess glutaraldehyde.
5. The nanoparticle solution obtained in step (4) was ultracentrifuged, redispersed in phosphate buffered saline (PBS), lyophilized and provided with a sterile solution for reconstitution.

b) Bolus injection of albumin nanoparticles of Cisplatin anchored with galactosamine for liver specific targeting

Sr. No	Ingredients	Quantity
1	Human serum albumin	100 - 300 mg (10 ml)
2	Cisplatin	5 - 12 mg
3	Absolute ethanol	10 - 20 ml
4	Glutaraldehyde	50 - 200 µl (8 %w/v)
5	Glycine	40 - 100 mg
6	Galactosamine	10 - 20 mg
7	EDC (carbodiimide)	10 - 20 mg
8	Vehicle (10 mM NaCl solution)	10 ml

Process:

1. Cisplatin was added to the aqueous human serum albumin solution while stirring after pH adjustment.
2. Ethanol was added to the solution obtained in step (1) to cause precipitation of the protein molecules leading to the formation of nanoparticles.
3. The nanoparticles obtained in step (2) were cross linked with glutaraldehyde under stirring.
4. Glycine was added under stirring to the nanoparticles obtained in step (3) for removal of excess glutaraldehyde.
5. The nanoparticle solution obtained in step (4) was ultracentrifuged, redispersed in chilled phosphate buffered saline (PBS) containing galactosamine and EDC, lyophilized and provided with a sterile solution for reconstitution.

c) Bolus injection (IV) of albumin nanoparticles of Cisplatin anchored with Transglutaminase

Sr. No	Ingredients	Quantity
1	Human serum albumin	100 - 300 mg (10 ml)
2	Cisplatin	5 - 12 mg
3	Absolute ethanol	10 - 20 ml
4	Transglutaminase enzyme	50 - 100 mg
6	Vehicle (10 mM NaCl solution)	10 ml

Process:

1. Cisplatin was added to aqueous human serum albumin solution under stirring after pH adjustment.

2. Ethanol was added to the solution obtained in step (1) to cause precipitation of the protein molecules leading to the formation of nanoparticles.
3. The nanoparticles obtained in step (2)were cross linked with transglutaminase enzyme under stirring
4. The nanoparticle solution obtained in step (4) was ultracentrifuged, redispersed in phosphate buffered saline (PBS), lyophilized and provided with a sterile solution for reconstitution.

d) Bolus injection of gelatin nanoparticles of Cisplatin

Sr. No	Ingredients	Quantity
1	Injectable grade Gelatin	100 - 300 mg (10 ml)
2	Cisplatin	5 - 12 mg
3	Acetone	10 - 30 ml
4	Glutaraldehyde	50 - 200 µl (8 %w/v)
	Glycine	40 - 100 mg
6	Vehicle (10 mM NaCl solution)	10 ml

Process:

Gelatin was dissolved in water and acetone was added.

2. The supernatant containing low molecular weight (LMW) fraction of gelatin discarded by decantation. The remaining high molecular weight (HMW) sediment was re-dissolved in equal volume of distilled water.

3. The pH of gelatin solution obtained in step (2) was adjusted and cisplatin was dissolved in it and gelatin was desolvated again by addition of acetone leading to the formation of nanoparticles.

4. Glutaraldehyde was added to the nanoparticles obtained in step (3) under stirring.

5. Glycine was added under stirring to the nanoparticles obtained in step (4) for removing excess glutaraldehyde.

6. The nanoparticle solution obtained in step (5) was ultracentrifuged, redispersed in phosphate buffered saline (PBS), lyophilized and provided with a sterile solution for reconstitution.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the spirit of the invention. Thus, it should be understood that although the present invention has been specifically disclosed by the preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and such modifications and variations are considered to be falling within the scope of the invention.

It is to be understood that the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of “including,” “comprising,” or “having” and variations thereof herein is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "an excipient" includes a single excipient as well as two or more different excipients, and the like.

Claims

1. A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs ; and optionally one or more pharmaceutically acceptable excipients.

2. A pharmaceutical composition according to claim 1, wherein the one or more anticancer drugs are in the form of a pharmaceutically acceptable derivative.

3. A pharmaceutical composition according to claim 2, wherein the pharmaceutically acceptable derivative comprises a salt, solvate, complex, hydrate, isomer, ester, tautomer, anhydrate, enantiomer, polymorph or prodrug.

4. A pharmaceutical composition according to any preceding claim, wherein the polymeric nanoparticles comprise one or more polymers that are biodegradable, non-biodegradable or a combination thereof.

5. A pharmaceutical composition according to any preceding claim, wherein the one or more anticancer drugs comprise a DNA linking agent.

6. A pharmaceutical composition according to claim 5, wherein the DNA linking agent comprises cisplatin.

7. A pharmaceutical composition according to preceding claim wherein the one or more polymeric nanoparticles comprise one or more biodegradable polymers selected from Human Serum Albumin (HSA) or Gelatin or a combination thereof.

8. A pharmaceutical composition according to any preceding claim, wherein the nanoparticles have an average particle size of less than or equal to about 2000 nm.

9. A pharmaceutical composition according to any preceding claims for parenteral administration.

10. A pharmaceutical composition according to claim 7, wherein the dosage form for parenteral administration is in the form of a liquid, gel, bolus, depots, implant, biodegradable or

non-biodegradable microparticles, microspheres.11. A pharmaceutical composition according to any preceding claims wherein the one or more pharmaceutically acceptable excipients comprise desolvating agents, cross linkers, neutralisers, cryoprotectants, buffering agents, ligands,solvents, or any combination thereof.

12. A pharmaceutical composition according to any preceding claim, further comprising at least one additional active ingredient selected from alkylating agents, anti-metabolites, anti-microtubule agents, topoisomerase inhibitors , antitumor antibiotics, biological agents , bisphosphonates, or any combination thereof.

13. A process for preparing a pharmaceutical compositionaccording to any preceding claim , said process comprising dissolving the anticancer drug in aqueous solution of human serum albumin or gelatin; adding a desolvating agent for formation of nanoprecipitates; cross linking and neutralization of the formed nanoparticles with other pharmaceutically acceptable excipients; optionally freeze drying the purified nanoparticles; and processing into a dosage form.

14. A pharmaceutical composition according to any one of claims 1 to 12 for use in the treatment of hepatocellular carcinoma.

15. Use of a pharmaceutical composition according to any one of claims 1 to12 in the manufacture of a medicament for the treatment of hepatocellular carcinoma.

16. A method of treating hepatocellular carcinoma, wherein the method comprises administering a pharmaceutical composition according to claims 1 to12 to a patient in need thereof.

17. A pharmaceutical composition substantially as described herein with reference to the examples.

18. A process for preparing a pharmaceutical composition substantially as described herein.

Figure 1

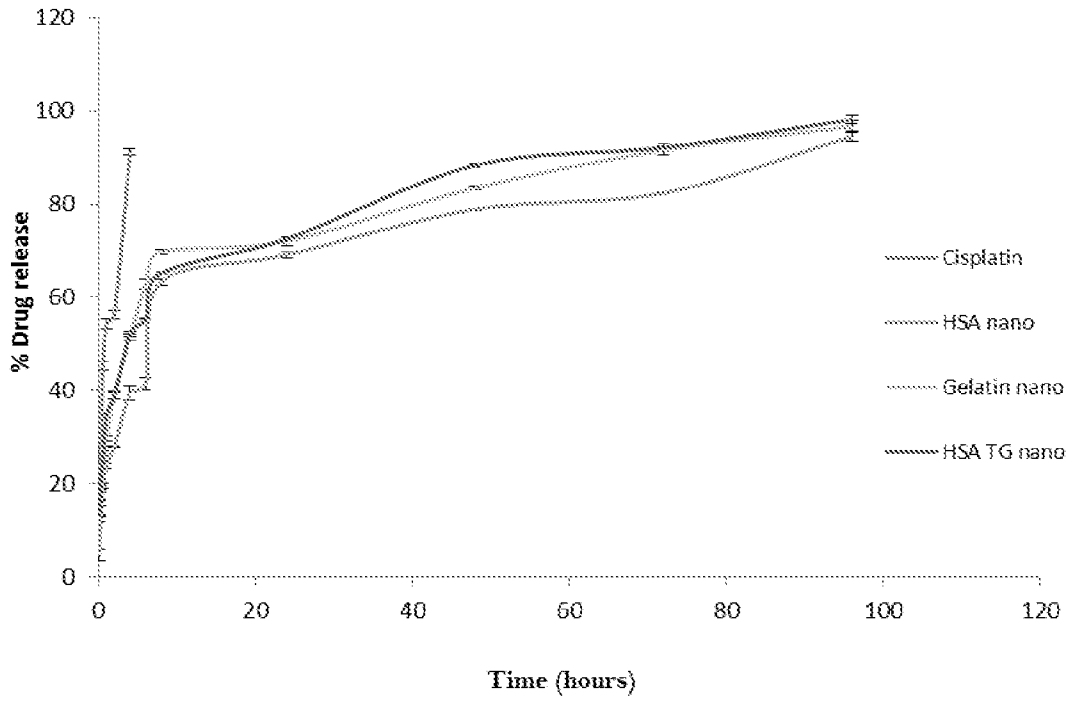

120
100
80
60
40
20
0
% Drug release
0
20
40
60
80
100
120
Time (hours)
Cisplatin
HSA nano
Gelatin nano
HSA TG nano

Figure 2

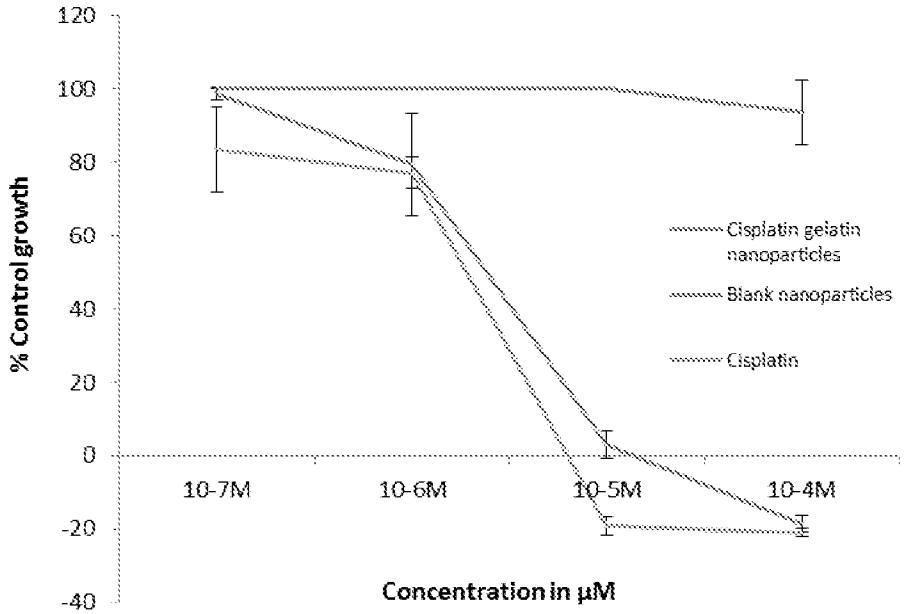

120
100
80
60
40
20
0
-20
-40
% Control growth
10-7M
10-6M
10-5M
10-4M
Concentration in µM
Cisplatin gelatin nanoparticles
Blank nanoparticles
Cisplatin

Figure 3

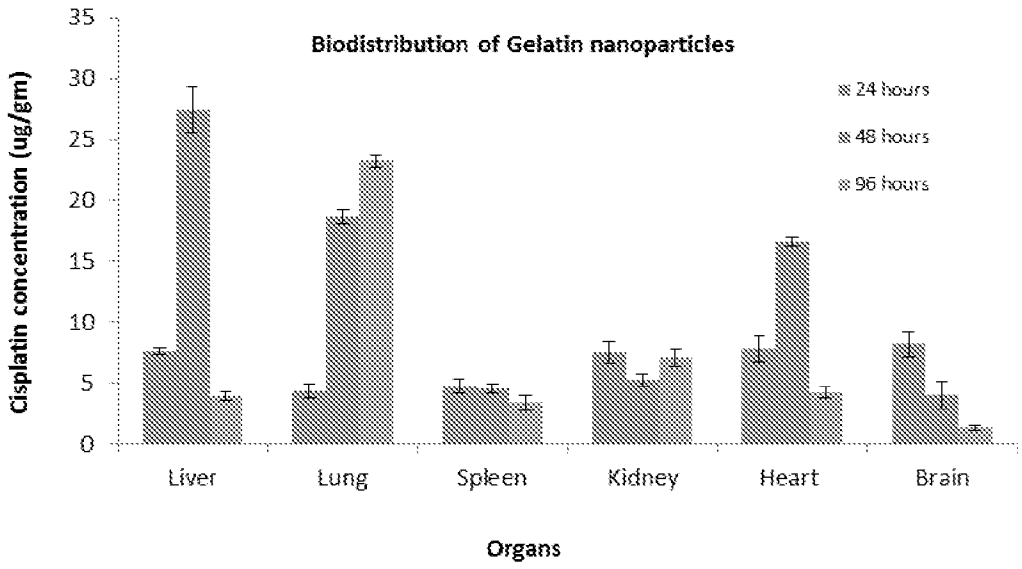

Biodistribution of Gelatin nanoparticles
Cisplatin concentration (ug/gm)
35
30
25
20
15
10
5
0
24 hours
48 hours
96 hours
Liver
Lung
Spleen
Kidney
Heart
Brain
Organs

Figure 4

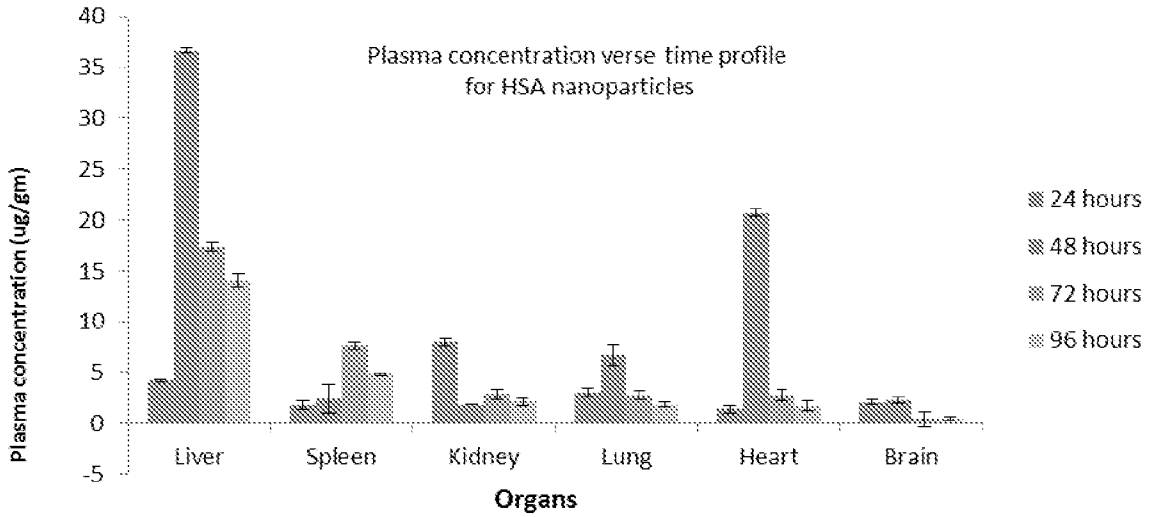

Plasma concentration verse time profile for HSA nanoparticles
Plasma concentration (ug/gm)
40
35
30
25
20
15
10
5
0
-5
Liver
Spleen
Kidney
Lung
Heart
Brain
Organs
24 hours
48 hours
72 hours
96 hours

Figure 5

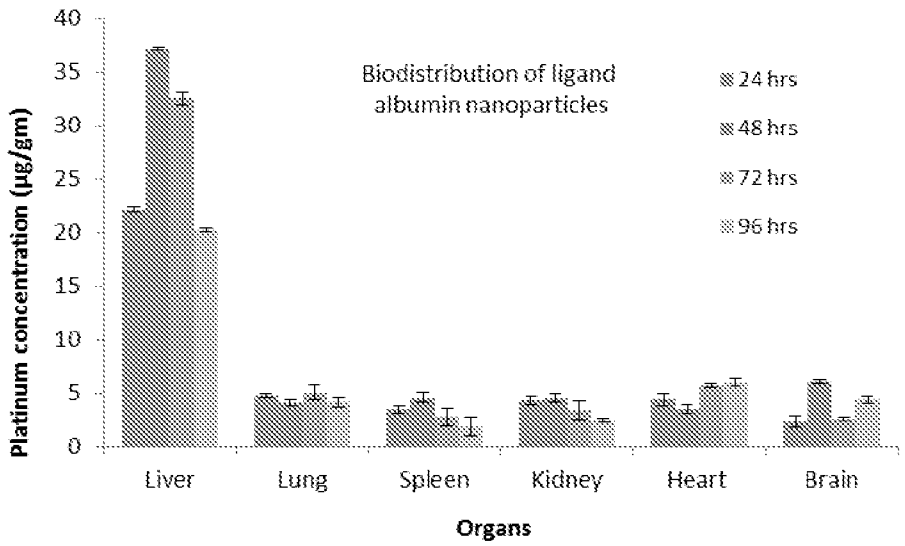
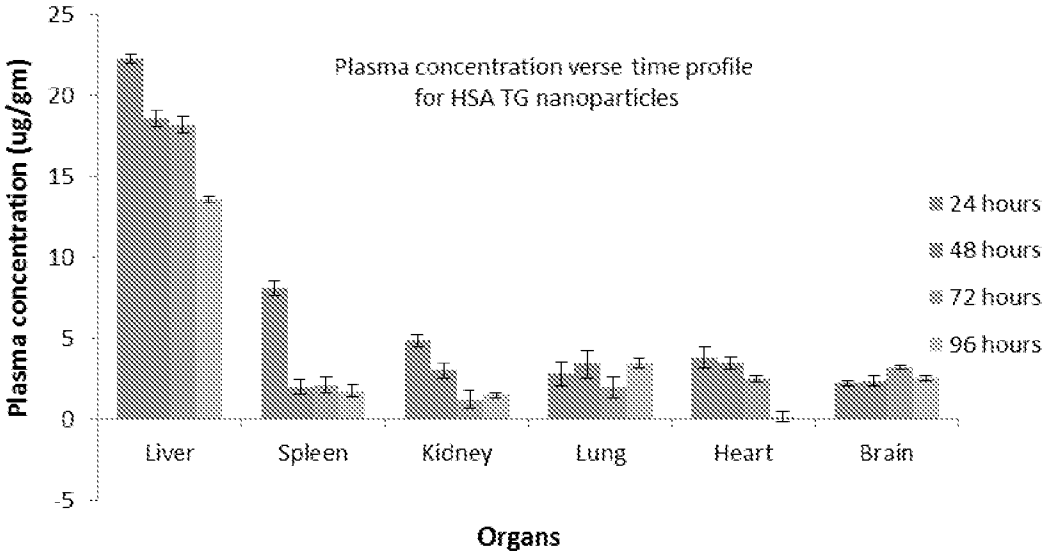

Biodistribution of ligand albumin nanoparticles
24 hrs
48 hrs
72 hrs
96 hrs
Platinum concentration (µg/gm)
40
35
30
25
20
15
10
5
0
Liver
Lung
Spleen
Kidney
Heart
Brain
Organs

Figure 6


Plasma concentration verse time profile
for HSA TG nanoparticles
Plasma concentration (ug/gm)
25
20
15
10
5
0
-5
Liver
Spleen
Kidney
Lung
Heart
Brain
Organs
24 hours
48 hours
72 hours
96 hours

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/052288

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/51 A61K31/00 A61K9/16 A61K9/00 A61K9/19
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, EMBASE, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/059022 A1 (BRIGHAM & WOMENS HOSPITAL [US]; MASSACHUSETTS INST TECHNOLOGY [US]) 17 April 2014 (2014-04-17) page 47, lines 13-28; example 1 page 49, line 6 - page 50, line 26 -----	1-6,8-12
X	US 2009/181090 A1 (DREIS SEBASTIAN [DE] ET AL) 16 July 2009 (2009-07-16) paragraph [0072]; claims 1-5, 7-11 ----- -/--	1-13,17, 18

[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
19 October 2015	27/01/2016

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	Schwald, Claudia

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2015/052288

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.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
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:

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:

see additional sheet

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 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.:

1-18(partially)

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.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHOJIRO KYOTANI ET AL: "A STUDY OF EMBOLIZING MATERIALS FOR CHEMO-EMBOLIZATION THERAPY OF HEPATOCELLULAR CARCINOMA: ANTITUMOR EFFECT OF CIS-DIAMMINEDICHLOROPLATINUM(II) ALBUMIN MICROSPHERES, CONTAINING CHITIN AND TREATED WITH CHITOSAN ON RABBITS WITH VX2 HEPATIC TUMORS", CHEMICAL AND PHARMACEUTICAL BULLETIN, PHARMACEUTICAL SOCIETY OF JAPAN, JP, vol. 40, no. 10, 1 October 1992 (1992-10-01), pages 2814-2816, XP000324875, ISSN: 0009-2363 abstract page 2814, left-hand column, lines 1-23 page 2814, left-hand column, line 24 - page 2814, right-hand column, line 32 -----	1-12, 14-18
X	WO 98/33486 A1 (ANDARIS LTD [GB]) 6 August 1998 (1998-08-06) page 1, line 35 - page 2, line 25 page 3, line 12 - page 4, line 10 page 5, lines 31-34 page 6, line 9; example 1 -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2015/052288

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014059022 A1	17-04-2014	US 2015265716 A1	24-09-2015
		WO 2014059022 A1	17-04-2014
US 2009181090 A1	16-07-2009	AU 2006331030 A1	05-07-2007
		CA 2631003 A1	05-07-2007
		CN 101346131 A	14-01-2009
		DE 102005062440 A1	05-07-2007
		EP 1965769 A2	10-09-2008
		JP 2009521515 A	04-06-2009
		KR 20080081080 A	05-09-2008
		NZ 569898 A	30-06-2011
		US 2009181090 A1	16-07-2009
		WO 2007073932 A2	05-07-2007
		ZA 200804572 A	25-03-2009
WO 9833486 A1	06-08-1998	AR 011099 A1	02-08-2000
		AU 5871898 A	25-08-1998
		EP 0964676 A1	22-12-1999
		JP 2001511775 A	14-08-2001
		US 2002044973 A1	18-04-2002
		US 2002164376 A1	07-11-2002
		WO 9833486 A1	06-08-1998

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-18(partially)

   A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs comprising DNA linking agents; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable polymer is Human Serum Albumin (HSA).

   ---

2. claims: 1-18(partially)

   A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs comprising DNA linking agents; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable polymer is Gelatin.

   ---

3. claims: 1-12, 14-18(all partially)

   A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs comprising DNA linking agents; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable polymer is a combination of HSA and Gelatin.

   ---

4. claims: 1-12, 14-18(all partially)

   A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs comprising DNA linking agents; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable polymer is a polymer other than Human Serum Albumin, Gelatin and combinations thereof.

   ---

5. claims: 1-18(partially)

   A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs other than DNA linking agents; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable polymer is Human Serum Albumin (HSA).

   ---

6. claims: 1-18(partially)

   A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs other than DNA linking agents ; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable

polymer isGelatin .

---

7. claims: 1-12, 14-18(all partially)

A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs other than DNA linking agents; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable polymer is a combination of HSA and Gelatin.

---

8. claims: 1-12, 14-18(all partially)

A pharmaceutical composition comprising polymeric nanoparticles of one or more anticancer drugs other than DNA linking agents; and optionally one or more pharmaceutically acceptable excipients, wherein the one or more biodegradable polymer is a polymer other than Human Serum Albumin, Gelatin and combinations thereof.
