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(54) Title: NANOPARTICLE PHARMACEUTICAL CARRIER

(57) Abstract: A nanoparticle pharmaceutical carrier comprising a first polymer, or copolymer, having a plurality of terminal amino-carboxyl groups and a second polymer, or copolymer. Preferably, the second polymer or copolymer is branched or linear. Preferably, the second polymer or copolymer has a plurality of terminal alkyl groups.



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Nanoparticle Pharmaceutical Carrier

15 The present invention relates to a novel nanoparticle pharmaceutical carrier and uses thereof. It further relates to a method of producing a nanoparticle pharmaceutical carrier for the purpose of attaching it to a bioactive agent.

20 Site specific delivery of drug substances to predetermined and localised areas in the body has been a long held pharmacological aspiration. Advances in nanotechnology provide materials in the nanometer range with many potential applications in this field of research. Due to their unique size-dependent properties, nanoparticles offer the possibility to develop both new therapeutic and diagnostic tools however the
25 construction of a drug-loaded nanoparticle delivery system is a multifaceted and complicated procedure.

The desire to maximise therapeutic gain and minimise side effects has been pursued by way of several different strategies such as polymeric
30 immunoconjugates and antibody-containing prodrug constructs. Solid

polymeric nanoparticulate systems allow unimpeded distribution by way of capillary supply. They also proffer sustained drug release capabilities. Their unique form provides simple passive targeting without much modification. For example, a localised injection of drug-loaded
5 nanoparticulate suspensions into discrete anatomical compartments, such as the knee joint or eye by means of minimally invasive procedures.

Active targeting drug delivery systems have been difficult to devise, with a requirement for some element of design related to the intended target.

10 Such approaches are founded on an affinity-based interaction with specific cells, tissues or organs. Actively targeted nanoparticles must be decorated with a ligand that will bind specifically to receptors, either unique or over-expressed, on the intended cells or tissues. These pendant ligands may include antibodies or their fragments, peptides, glycoprotein,
15 carbohydrates, or synthetic polymers. In clinical terms, a good example is the folate ligand, directed towards folate receptors that are known to be over expressed in several human tumour types. The folate-based approach has another advantage in that such receptors will not only facilitate targeting, but enhance endocytosis.

20

An important prerequisite for nanoparticulate targeting is proper ligand attachment. Ideally, some type of covalent bonding is needed to ensure that the ligand resides for the intended duration of use without loss of viability. Biodegradable polyesters, such as poly(lactide) (PLA),
25 poly(glycolide)(PGA), poly(butyl cyanoacrylate)(PBCA) and poly(lactide-co-glycolide) (PLGA), have been extensively studied for a wide variety of pharmaceutical and biomedical applications. PLGA copolymers have been widely used for the preparation of nanoparticles because of their well-documented biodegradation, biocompatibility and high safety. Variations
30 in the ratio and order of lactic acid residues in relation to glycolic acid

residues in the polymer chain provide for and result in a wide diversity of polymeric substances of varying physicochemical characteristics, like mechanical strength, viscosity, degradation rate and molecular weight. In spite of these favourable attributes, one key problem with using PLGA is its relative inertness in terms of lacking the functional groups on its aliphatic backbone for covalently linking to ligands.

Although PLGA possesses terminal carboxyl residues, these represent a small portion of the overall polymer chain and many are buried in the particle matrix. Methods have been described to introduce more carboxylic groups by replacing the nanoparticle stabiliser, poly(vinyl alcohol)(PVA) with one bearing a carboxylic acid side chain, such as poly(ethylene-alt-maleic acid). However, this approach suffers from difficulty in nanoparticle preparation and loss of the targeting effect with time due to desorption or degradation of adsorbed groups as the nanoparticle scaffold erodes.

Thus, according to the first aspect of the present invention, there is provided a nanoparticle pharmaceutical carrier comprising a first polymer, or copolymer, having a plurality of terminal amino-carboxyl groups and a second polymer, or copolymer.

Preferably, the second polymer, or copolymer is branched or linear.

Preferably, the second polymer or copolymer has a plurality of terminal alkyl groups.

In the context of this invention, an alkyl group contains only carbon and hydrogen atoms arranged in a chain. The alkyl groups form a homologous series with the general formula C_nH_{2n+1} .

The first polymer, or copolymer and the second polymer, or copolymer can each have a molecular weight in the range from 10 to 150 kiloDaltons (kDa) and, preferably, the second polymer, or copolymer has a molecular weight higher than the first polymer or copolymer.

5

More preferably, the first polymer, or copolymer, has a low molecular weight in the range 10 to 50 kDa and the second polymer, or copolymer, has a high molecular weight in the range 70 to 150 kDa.

10 Preferably, the second copolymer is poly(lactide-co-glycolide)(PLGA) and the first copolymer is based on PLGA. It is modified as hereinafter described.

15 In a second aspect of the present invention, there is provided a nanoparticle pharmaceutical composition comprising a nanoparticle pharmaceutical carrier, associated pay-load intended to exert a therapeutic or diagnostic effect, and a bioactive agent.

20 Preferably, the bioactive agent is a ligand

Preferably, the nanoparticle pharmaceutical carrier is attached to the bioactive agent, more preferably they are covalently linked.

25 Preferably, the bioactive agent comprises one or more of the following: polyclonal antibody, monoclonal antibody, antibody fragments or single chains, lectins, carbohydrates, amino acids, peptides, proteins, polysorbate 80, folate, aptamers.

30 Preferably, the pay-load is entrapped or adsorbed in the nanoparticle pharmaceutical carrier. The pay-load could comprise one or more of the

group selected from: anti-cancer agents, antibiotics, anti-virals, anti-inflammatory, cytokines, immunomodulators, immunotoxins, anti-tumour antibodies, anti-angiogenic agents, anti-hypertensive, anti-oedema agents, radiosensitizers, DNA, RNA, plasmids, peptides, oligonucleotides and combinations thereof.

Examples of anti-cancer agents are paclitaxel and its derivatives, doxorubicin, deoxydoxorubicin, morpholinodoxorubicin, daunorubicin, 5-fluorouracil, camptothecin and its derivatives, methotrexate and its derivatives, cisplatin and metronidazole.

In a third aspect of the present invention, there is provided a use of a nanoparticle pharmaceutical composition as herein described in the preparation of a medicament for the treatment of a disease.

The present invention also provides a use of said composition for the treatment of a disease comprising administering a therapeutically effective amount of said nanoparticle pharmaceutical composition to a patient. The present invention includes the treatment of cancer and those diseases where the site-specific delivery of a therapeutic agent is judged to be advantageous when compared to administration of said therapeutic agent by conventional means, such as by the oral and intravenous routes.

The term "treatment" includes any regime that can benefit a human or non-human animal. The treatment may be in respect of an existing condition or may be prophylactic (preventative treatment). Treatment may include curative, alleviation or prophylactic effects.

"Treatment of cancer" includes treatment of conditions caused by cancerous growth and includes the treatment of neoplastic growths, metastatic foci or tumours. Examples of tumours that can be treated using

the invention are, for instance, sarcomas, including osteogenic and soft tissue sarcomas, carcinomas, e.g., breast-, lung-, bladder-, thyroid-, prostate-, colon-, rectum-, pancreas-, stomach-, liver-, uterine-, prostate , cervical and ovarian carcinoma, lymphomas, including Hodgkin and non-
5 Hodgkin lymphomas, neuroblastoma, melanoma, myeloma, Wilms tumor, and leukemias, including acute lymphoblastic leukaemia and acute myeloblastic leukaemia, astrocytomas, gliomas and retinoblastomas.

10 Targeting therapies using a nanoparticle pharmaceutical composition may be used to deliver a bioactive agent such as an antibody or cell specific ligand. Targeting therapies can also be used to target certain types of cell. Targeting therapies may be desirable for a variety of reasons, for example if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

15 In a fourth aspect of the present invention, there is provided a method of preparing a nanoparticle pharmaceutical carrier comprising the steps:

20 a) adding a cross-linker and a carbodiimide to a first polymer or copolymer, optionally in an organic solvent, to form an activated first polymer, or copolymer having a plurality of terminal amino-carboxyl groups, and

25 b) admixing the activated first polymer, or copolymer with a second polymer, or copolymer, to generate the nanoparticle pharmaceutical carrier.

30 Carbodiimides or carboxyl activators are known in the art. Their carbodiimide conjugation can react with the hydroxyl of the carboxyl group. Preferably, the organic-soluble carbodiimide is N,N-dicyclohexyl

carbodiimide (DCC). An example of the chemistry involved in the activation of peripheral carboxyl groups is illustrated in Figure 2.

5 The cross-linker may be a succinimide. Succinimides are known to play a stabilising role in the activation of carboxyl groups prior to conjugation when compared to carbodiimide alone. Preferably, the succinimide is any succinimide derivative and more preferably the succinimide is N-hydroxy succinimide (NHS).

10 This method of activating chemical groups on a polymer, or copolymer, uses carbodiimide chemistry to enhance the carboxylic functionality of the first polymer, or copolymer, preferably having a low molecular weight. This approach increases the number of available activated carboxyl groups on the surface of the nanoparticle, thus, enhancing the efficient
15 attachment of a bioactive agent.

Carbodiimides are able to modify carboxylate groups. They create new amide bonds from the carboxyl group, which for the context of this present invention are called terminal amino-carboxyl groups.

20 The cross-linker and carbodiimide can be added simultaneously, or consecutively. Preferably, the first step is the activation of a nanoparticle possessing a number of carboxyl groups on its surface with a carbodiimide to form an *O*-urea intermediate (see accompanying Figure 2).

25 In a second step, the *O*-urea derivative can then react with a stabilising activator ie cross-linker, known in the art such as a succinimide. This reaction produces an intermediate active succinimide ester, which can then act on nucleophilic groups, namely the amino groups of a ligand by
30 formation of a covalent bond.

The present invention extends to succinimide esters of a polymer or copolymer such as PLGA, and their method of manufacture.

5 Some examples of peripheral ligands attachable to the nanoparticulate pharmaceutical carrier are listed in the accompanying Table 1.

10 In the present invention, the first and second polymer or copolymer can be used in the weight ratios of 99/1 to 1/99. More preferably, the high molecular weight polymer or copolymer is between 1% and 80% of the total weight.

15 Generally, the greater the amount of higher molecular weight polymer, or copolymer, the greater the mechanical strength and stability of the structure of said nanoparticle pharmaceutical carrier. In addition, a sustained release profile of the pay-load, or a profile of enhanced duration, is expected.

20 Thus, in a fifth aspect, the present invention provides a method of altering the rate of release of a pay-load from a nanoparticle pharmaceutical carrier wherein the ratio of the first polymer, or copolymer, and the second polymer, or copolymer, is altered. Generally, by increasing the ratio of a first lower molecular weight polymer, or copolymer, an increase in the rate of release will occur. The release of the pay-load will occur over a period of 4 hours to 120 days.

25

In the context of this invention, a nanoparticle pharmaceutical carrier is a nanoparticle which acts as a carrier for any pay-load for delivery, in an biologically available form, to a target cell. Nanoparticle carriers are useable for at least many if not most therapeutic administration routes.

30 The nanoparticle pharmaceutical carrier may combine with a bioactive

agent, which is attached, linked or associated on to its external surface. In the context of this invention, a nanoparticle should be understood to be a particle having one or more diametric axial dimensions of the order of 3000 nm or less, but typically ranging from 80 nm to 650 nm.

5 Polymers and copolymers can exist in many different forms, usually based on their molecular weight. Different molecular weight polymers and copolymers can have different properties and the present invention is able to use suitable polymers and copolymers so as to create a nanoparticle pharmaceutical carrier and a nanoparticle pharmaceutical composition
10 with properties that suit, such as those in terms of rate of biodegradation and rate of pay-load release.

PLGA, an example of copolymer, can be provided by companies like Boehringer Ingelheim in a number of different molecular weights.

15 Examples include Resomer®, a high molecular weight form designated as RG 505 S (80 kDa) and a low molecular weight form coded as RG 502 H (12 kDa). RG 505 S is referred to as being 'endcapped'. This means that that an alkyl group is attached to the carboxyl terminus of each RG 505 S. Alternatively, RG 502 H is referred to as being 'uncapped' indicating that
20 each RG 502 H molecule has a carboxyl group at its terminus(see accompanying Figure 1).

PLGA may also be used because of its well-documented biodegradation, biocompatibility and high safety.

25 Other commonly used commercial biodegradable copolymers used in the art include poly(lactide) (PLA), poly(glycolide)(PGA) and poly(butyl cyanoacrylate (PBCA) (see accompanying Table 1).

In the present invention, a pay-load is any agent which is desired to be
30 delivered to target cells, tissues or organs and made biologically available

to these cells. This includes, but is not limited to, pharmaceuticals, drugs, peptides and oligonucleotides. They can be selected depending on the type of physiological effect required and they can be used in the treatment of a variety of medical conditions and disorders. These pay-loads may
5 relate, but are not limited to, anti-cancer agents, antibiotics, anti-virals, anti-inflammatories, cytokines, immunomodulators, immunotoxins, anti-tumour antibodies, anti-angiogenic agents, anti-hypertensive, anti-oedema agents, radiosensitizers, DNA, RNA, plasmids, peptides, oligonucleotides and combinations thereof.

10

In the present invention, a bioactive agent or ligand can be employed to actively target the bioactive pay-load to specific cells or tissue. The active targeting can be achieved by binding of the nanoparticle pharmaceutical carrier through the linked bioactive agent or ligand to specified receptors
15 or epitopes on the cell or tissue surface, which can be further internalised intracellularly for cytoplasmic delivery through receptor mediated endocytosis. An example of such ligands can be, but not limited to: polyclonal antibody, monoclonal antibody, antibody fragments or single chains, lectins, carbohydrates, amino acids, peptides, proteins,
20 polysorbate 80, folate, aptamers.

The invention will now be described further in the following non-limiting examples. Reference is made to the accompanying tables and drawings in which:

25

Table 1 lists targeting ligands able to direct particulate systems to specific anatomical sites;

Table 2 demonstrates the particle size, zeta potential and celecoxib loading of nanoparticles prepared from different PLGA blends;

Figure 1(a),(b) and (c) illustrate schematic cross-sectional representation of nanoparticle pharmaceutical carriers using both high molecular weight (RG 505 S) and low molecular weight (RG 502 H) and a blend of both high and low molecular weights;

- 5 Figure 2 illustrates the carbodiimide chemistry used in nanoparticle pharmaceutical carrier formation;
- Figure 3 the effect of RG 502 H % w/w in total PLGA blend on the amount of polyclonal antibody conjugated to nanoparticles; and
- Figure 4 illustrates celecoxib release profiles from nanoparticles.

10

Example 1

Materials and Methods

15 Non-activated nanoparticle preparations

PLGA nanoparticles of mixed composition were produced from blends of RG 502 H and RG 505 S ranging from 0–100% w/w RG 502 H in incremental steps of 20 % w/w. Dry powder blends were dissolved in
20 acetone and injected slowly into a 2-morpholino-ethanesulfonic acid monohydrate (MES) buffered continuous phase (MES buffer; pH 5.0; 25 mM) containing 2.5% w/v PVA. An immediate opalescent suspension displaying the Tyndal effect was produced indicating the formation of a colloidal system. Colloidal suspensions were kept stirring overnight to
25 bring about effective evaporation of acetone.

Drug-loaded non-activated nanoparticle preparation

Celecoxib was used as a model drug for incorporation into nanoparticles
30 of mixed polymer blend as previously described. The procedure used a

combination of diffusion and emulsification steps as part of the salting-out procedure. Blends of RG 502 H and RG 505 S, ranging from 0–100% w/w RG 502 H in incremental steps of 20 % w/w, were dissolved in acetone. Celecoxib was dissolved in dichloromethane and added to the acetone solution of PLGA in a ratio of 3:1 acetone:Dichloromethane (DCM) (volume ratio). This organic phase was injected into an aqueous solution of 2.5% w/v PVA in MES buffer (pH 5.0; 25 mM) containing 45% w/w magnesium chloride hexahydrate, as the salting out agent, and sonicated in an ice bath for three minutes (50-55 W). An additional volume of aqueous 2.5% w/v PVA in MES buffer (pH 5.0; 25 mM) was added to initiate acetone diffusion under conditions of moderate stirring. Samples were stored overnight under ambient conditions whilst stirring to allow evaporation of both acetone and DCM. All formulations were performed in triplicate (Table 2 & Figure 4).

Polymeric PLGA activation with NHS using an organic-soluble (of poor aqueous solubility) carbodiimide

Using the method of the present invention, PLGA RG 502 H was activated before particle formulation. RG 502 H, DCC (N,N-Dicyclohexyl carbodiimide hydrochloride) and NHS (N-hydroxy succinimide) were dissolved in anhydrous dioxane and stirred moderately at 15°C for three hours in a molar ratio of 1:1.05:1.05 (RG 502 H-COOH:DCC:NHS). An insoluble urea derivative was formed and removed by filtration. The activated copolymer was collected by precipitation in anhydrous diethyl ether, redissolved in anhydrous dioxane and then precipitated once again in anhydrous diethyl ether. This was repeated a further two times. The recovered copolymer was dried under vacuum at room temperature for 24 hours, ensuring removal of residual solvents.

Drug-loaded nanoparticle preparation using activated PLGA with an organic soluble carbodiimide

The same manufacturing procedure as detailed above was used.

5

Nanoparticle characterisation

Nanoparticle size and zeta potential were measured using photon correlation spectroscopy (ZetaSizer 3000 HS, Malvern instruments, UK) measured at a fixed angle (90°) (Table 2). Determinations were carried out at room temperature (25°C), with each done in triplicate and an average particle size expressed as the mean diameter (Z_{ave}). In Table 2, purified samples were subject to a combination of centrifugation and washing steps while unpurified samples were not. SD refers to the standard deviation and mean of three determinations. (Table 2)

15

Peripheral attachment of polyclonal antibody

Polyclonal antibody (total IgG fraction) was isolated from rabbit serum using affinity chromatography. Serum was applied to a HiTrap Protein A column (GE Healthcare), and non-specifically bound protein removed with 10 column volumes of wash buffer (PBS). The total IgG fraction was eluted from the column using 5 column volumes of 0.1 M glycine HCl, (pH 3.0) and neutralised by addition of 0.1 ml 1.0 M Tris-HCl (pH 8.0) to every 1 ml of elute. The neutralised IgG was dialysed overnight against PBS, prior to purity analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and total protein quantified by a BCA assay.

20

25

Polyclonal (PC) antibody, dissolved in PBS (pH 7.4), was added to the washed activated nanoparticles, which were themselves suspended in

30

PBS (pH 7.4). A molar ratio of 10:1 PC:RG 502 H-COOH was used, with samples then incubated at 4°C and centrifuged at 10°C to remove superfluous antibody. Controls were prepared similarly using nanoparticles without carbodiimide activation. Antibody binding was quantified using the
 5 BCA assay (Micro BCA), with samples incubated for two hours at 37°C and read spectrophotometrically at 562 nm (Biolise, Thermo Spectra III, Tecan, Austria). (Figure 3)

Celecoxib encapsulation efficiency

10

Samples of freeze-dried, celecoxib-loaded activated nanoparticle were dissolved in acetonitrile, centrifuged to remove precipitated PVA and the supernatant analysed using UV absorption spectroscopy at 255 nm with appropriate calibration and blanking procedures. Measurements were
 15 done in triplicate and the entrapment efficiency was calculated as a percentage, referring to purified samples, using the ratio of the mass of drug determined analytically to that added during the formation process, both per unit mass of nanoparticle, as shown in Equation 1 (Table 2).

20

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Mass of drug determined (mg)}}{\text{Mass of drug added (mg)}} \times 100$$

Celecoxib release studies

25

The rate of celecoxib release was determined using a diffusion cell with an integral semi-permeable membrane (20 nm pore size membrane filters) to separate both donor and receiver phases. Both phases comprised methanol/water mixes (50:50) to ensure solubility dependent, sink
 30 conditions across the membrane, particularly in the receiver medium. A

mass of nanoparticles, equivalent to 250 µg celecoxib, was dispersed in the donor medium and 2.0 ml samples were withdrawn from the receiver medium at defined time points and replaced with an equivalent volume of fresh medium. Samples were measured using fluorescence spectroscopy (LS 45, Perkin Elmer, UK) employing excitation at 272 nm and emission at 355 nm (Figure 4).

Figure 1 illustrates a schematic cross-sectional representation of nanoparticles using (a) high molecular weight PLGA (RG 505 S) with endcapping, (b) a nanoparticle containing a blend of both low and molecular weight PLGA (RG 505 S and RG 502 H) and (c) a nanoparticle made using low molecular weight PLGA (RG 502 2) and displaying a surface presenting COOH groups.

Results for nanoparticles prepared from blends of RG 502 H and RG 505 S using a combination of diffusion and emulsification steps and activated with NHS using DCC are listed in Table 2. Activation of RG 502 H prior to nanoparticle carrier formation with PLGA blends resulted in a discernable upward trend in particle size. Changes in both size and zeta potential were resistant to effects arising from activation and centrifugation. This method of preactivation of the RG 502 H nanoparticles results in an activated polymer chain that now carries additional functionality.

It is an important feature of a nanoparticulate pharmaceutical carrier composition to display some degree of sustained release of an entrapped therapeutic pay-load. This sustained action is more pronounced in a nanoparticle carrier composed of high molecular weight PLGA polymers, many of which are available as the end-capped version. The low molecular weight variants erode more quickly and permit a more rapid drug release profile as entrapped drug becomes exposed during the

erosion process. In this example, the effect of the activation process was accessed using celecoxib as a model drug and loaded into nanoparticles composed of blends of RG 502 H and RG 505 S, as detailed in Table 2. Entrapment efficiency was more than 95% in formulations activated using NHS-based procedures with DCC. This indicates that blending activated low and high molecular weight PLGA does not affect entrapment efficiency. This data set also shows that altering the end structure using carbodiimide activation gives a PLGA derivate that does not differ from the loading effectiveness of the parent polymer.

Figure 3 illustrates the effect of increasing the proportion of RG 502 H % w/w in total PLGA blend on amount of polyclonal antibody conjugated to nanoparticles prepared the controllable combination of diffusion and emulsification steps as part of the salting-out procedure activated with NHS using DCC (closed triangle) and with nanoparticles without activation (cross).

The results show an increase in the polyclonal antibody conjugation as the proportion of RG 502 H in the RG 505 S blend was increased. A negative control of corresponding nanoparticles with no conjugation gives rise to no appreciable antibody loading. It can be seen from Figure 3 that up to 20–25 µg per mg nanoparticles at 100% RG 502 H in DCC was achievable.

The results of these experiments show that antibody attachment is an effective process, giving rise to nanoparticles with targeting capabilities. In addition, variations in the ratio of the first and second polymer, or copolymer, produces nanoparticles with adaptable and predetermined pay-load release profiles. The duration of this release profile can be tailored to meet the required therapeutic purpose and adjusted to counteract physiological handling of the nanoparticulate formulation.

Figure 4 illustrates celecoxib release profiles from nanoparticles prepared by a novel and controllable combination of diffusion and emulsification steps as part of the salting-out procedure using different RG 502 H concentrations in PLGA blend (0%= shaded triangle; 20% = asterisk; 60% = closed circles; 100% = dash), diffusion of celecoxib solution between donor and receiver phase was used a control (closed square). The release profiles show an almost linear release, (although this experiment incorporated a permeable membrane that is expected to exert some resistance in the movement of free drug into the receiver phase). The release of celecoxib from solution gives an indication of the maximal drug flux possible across the diffusion cell when all drug is present in its most available form. Slower release implies sustained release exerted by the nanoparticles.

The release profile in this example showed an increase in the initial release with increasing the RG 502 H in PLGA blend. Nanoparticles prepared from RG 505 S showed lowest initial burst release, around 8.5% at 30 minutes, which increased by almost three fold to reach 22% with introducing 20% RG 502 H. It reached 35% in nanoparticles produced from RG 502 H. Such higher initial release with introducing RG 502 H to the PLGA blend is responsible for the faster drug release profiles due to higher cumulative release at any time point for formulations containing 20%, 60% and 100% RG 502 H. The t_{50} value (time for 50% celecoxib release) was 180 minutes for 0% RG 502 H, dropping steadily until 100% RG 502 H nanoparticles are studied, whereupon t_{50} is reached in approximate 60 minutes.

The higher initial release obtained by increasing RG 502 H concentration in PLGA blends can be attributed to the faster hydration of low molecular weight RG 502 H compared to the high molecular weight RG 505 S.

Faster hydration of low molecular weight RG 502 H will also hydrate the closely associated high molecular weight polymer RG 505 S. These results indicate that the proportion of differing molecular weights of PLGA in the nanoparticle matrix can result in a unique and specific regulation of the release profile, either making release more immediate or imposing some degree of sustained effect.

The present invention provides a nanoparticle pharmaceutical carrier system wherein the carboxylic functionality of low molecular weight copolymer can be altered by using carboiimide chemistry, thus creating a wealth of carboxyl groups on the surface of the nanoparticle. The activated copolymer can then be combined with an unactivated high molecular weight copolymer. An increase in the number of carboxyl groups on the low molecular weight copolymer allows a more efficient attachment of a bioactive agent whilst the presence of high molecular weight copolymer provides the carrier with enhanced mechanical strength and stability. The ratio of low and high molecular weight polymers, or copolymers, in combination can be altered so as to regulate the release of any entrapped therapeutic pay-load.

Claims

1. A nanoparticle pharmaceutical carrier comprising a first polymer, or copolymer, having a plurality of terminal amino-carboxyl groups and a second polymer, or copolymer.
2. A nanoparticle pharmaceutical carrier as claimed in Claim 1 wherein said second polymer, or copolymer has a plurality of terminal alkyl groups.
3. A nanoparticle pharmaceutical carrier as claimed in any one of the preceding claims wherein said second polymer, or copolymer, has a molecular weight higher than said first polymer, or copolymer.
4. A nanoparticle pharmaceutical carrier as claimed in Claim 1 and Claim 2 wherein said first and second polymers, or copolymers, each have a preferable molecular weight in the range from 10 to 150 kDa.
5. A nanoparticle pharmaceutical carrier as claimed in any one of the preceding claims wherein the second polymer, or copolymer is poly(lactide-co glycolide) (PLGA) copolymer and the first polymer, or copolymer, is based on a PLGA or a modification of PLGA copolymer.
6. A nanoparticle pharmaceutical composition comprising a nanoparticle pharmaceutical carrier as claimed in any one of the preceding claims, and a pay-load and a bioactive agent.
7. A nanoparticle pharmaceutical composition as claimed in Claim 6 wherein the bioactive agent comprises one or more of the following; polyclonal antibody, monoclonal antibody, antibody fragments or single

chains, lectins, carbohydrates, amino acids, peptides, proteins, polysorbate 80, folate, aptamers.

- 5 8. A nanoparticle pharmaceutical composition, as claimed in Claim 6 and Claim 7, wherein the pay-load comprises one or more of the following; anti-cancer agents, antibiotics, anti-virals, anti-inflammatories, cytokines, immunomodulators, immunotoxins, anti-tumour antibodies, anti-angiogenic agents, anti-hypertensive, anti-oedema agents, radiosensitizers, DNA, RNA, plasmids, peptides, oligonucleotides and combinations thereof.
- 10 9. The use of a nanoparticle pharmaceutical composition as claimed in any one of Claims 6 to 8 in the preparation of a medicament for the treatment of a disease.
- 15 10. The use of a nanoparticle pharmaceutical composition as claimed any one of Claims 6 to 9 for treatment of a disease comprising administering a therapeutically effective amount of said nanoparticle pharmaceutical composition to a patient in need thereof.
- 20 11. A method of preparing a nanoparticle pharmaceutical carrier, as claimed in Claim 1 comprising the steps:
- 25 a) adding a cross-linker and a carbodiimide to a first polymer or copolymer, optionally in an organic solvent, to form an activated first polymer, or copolymer having a plurality of terminal amino-carboxyl groups, and
- b) admixing of the activated first polymer, or copolymer, and second polymer, or copolymer, to generate a nanoparticle pharmaceutical carrier.

12. A method, as claimed in Claim 11 wherein the first and second polymers, or copolymers, have weight ratios ranging from 99/1 to 1/99.
- 5 13. A method, as claimed in Claim 11 wherein said cross-linker is a succinimide, preferably N-hydroxy succinimide.
14. A method, as claimed in Claim 11 wherein the carbodiimide is preferably organic-soluble, more preferably N,N-dicyclohexyl carbodiimide.
- 10 15. A succinimide ester of a polymer, or copolymer, preferably PLGA.
16. A method of altering the rate of release of the pay-load from a nanoparticle pharmaceutical composition as defined in any one of Claims 6 to 8 wherein the ratio of the first polymer, or copolymer and the second
15 polymer, or copolymer, is altered.

1 / 5

Table 1

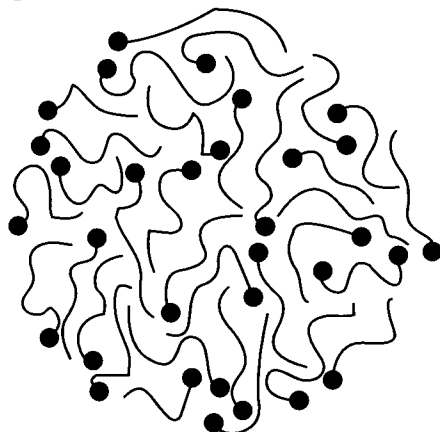
Attached ligand	Particle type	Target
Polysorbate 80	PBCA Nanoparticles	BBB brain
Galactose	PLA Nanoparticles	hepatocytes
Aptamers	PLA Nanoparticles	prostate cancer
Lectin	PLGA Nanoparticles	lungs
Poly(L-Lysine)	PLGA Microspheres	phagocytes
Antibody (Anti-CD3)	Gelatin Nanoparticles	leukemic cells and primary T-lymphocytes
Folate	PLGA-PEG polymeric micelles PAMAM dendrimers	epidermal carcinoma KB tumours
F _{ab} fragment of C225 antibody	immunoliposomes	tumor cells over expressing Epidermal growth factor receptor
Wheat germ agglutinin	PLGA nanoparticles	lung cancer (non-small cell)
Single chain Fv (anti-ErbB2) antibody	immunoliposomes	adenocarcinoma
Anti-HER 2 antibody	PLA nanoparticles	ovarian cancer

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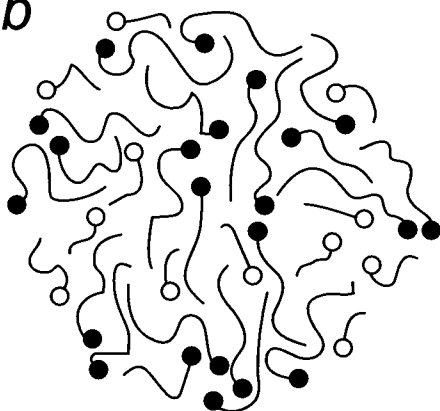
Table 2

Activated before nanoparticle formation (DCC procedure)					
	Unpurified		Purified		
	Mean size (nm)±SD	Zeta (mV)	Mean size (nm)±SD	Zeta (mV)	Celecoxib entrapment
20	232.4 ± 3.3	2.4	235.0 ± 3.78	2.6	98.4 ± 1.2
40	234.3 ± 1.9	3.2	230.7 ± 2.9	3.1	96.1 ± 0.8
60	228.5 ± 2.4	3.4	238.8 ± 3.6	3.4	96.7 ± 1.1
80	226.7 ± 3.0	3.0	225.8 ± 3.5	3.1	96.6 ± 0.5
100	202.6 ± 0.79	3.6	211.8 ± 1.9	3.4	97.5 ± 0.7

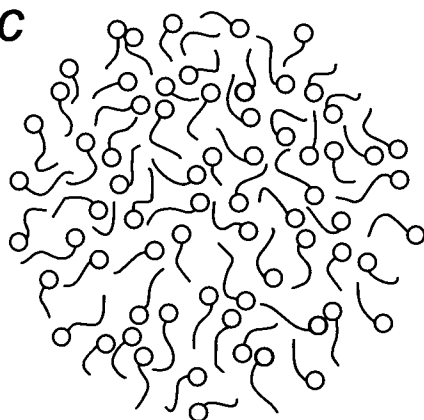
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Fig. 1a

- alkyl endcap
- COOH group

Fig. 1b

- capped high molecular weight PLGA
- uncapped low molecular weight PLGA

Fig. 1c

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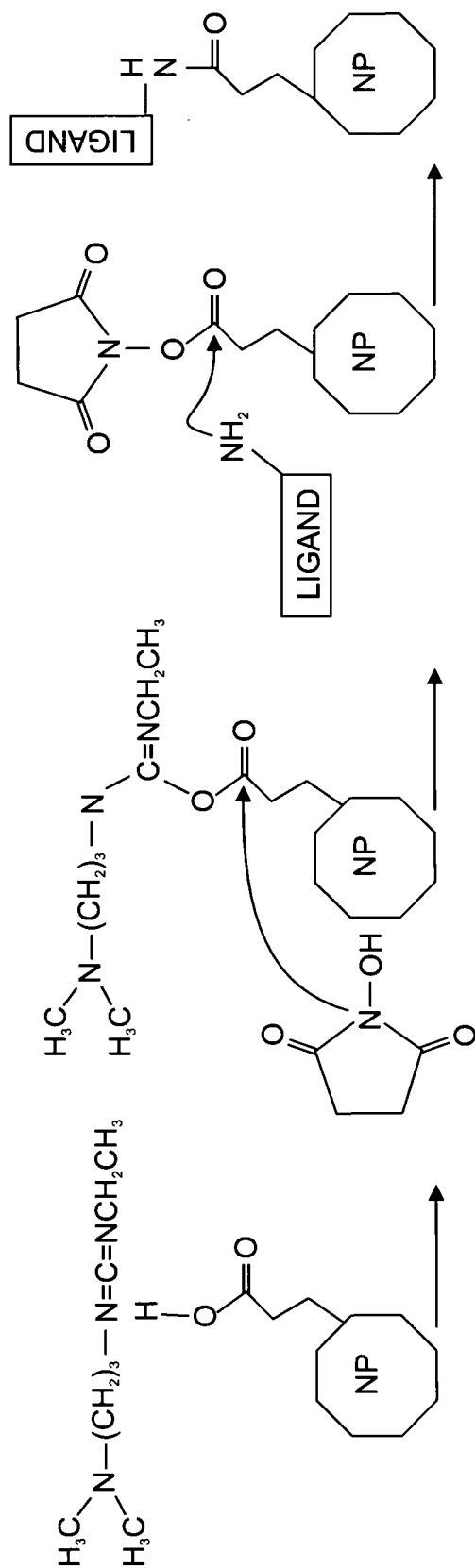


Fig. 2

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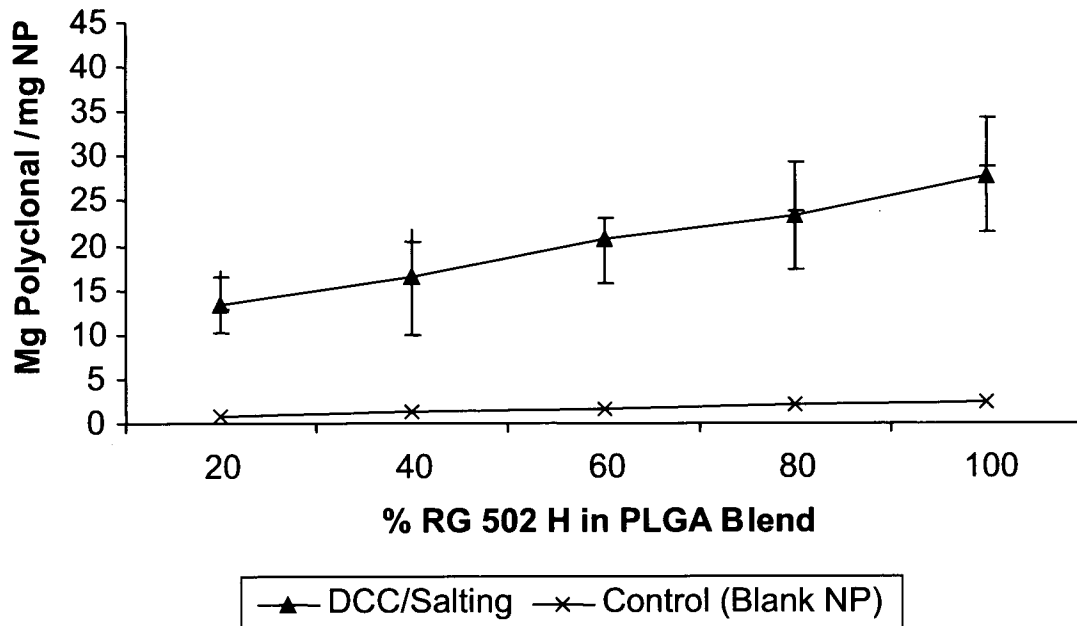


Fig. 3

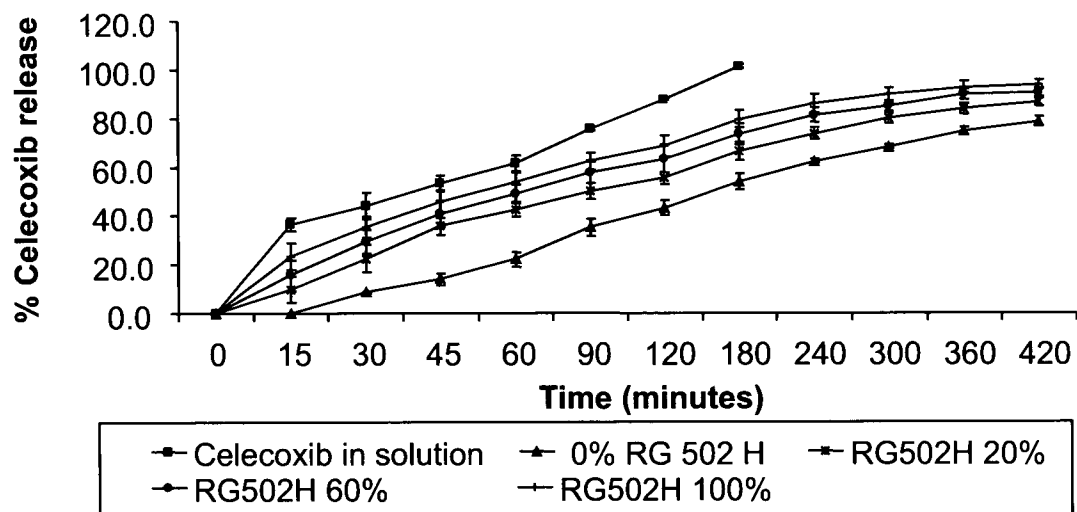


Fig. 4