

NEW PHARMACEUTICAL COMPOSITIONS COMPRISING GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS

Field of the Invention

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This invention relates to a new formulation for use in, for example, the field of drug delivery and in particular in the treatment of type 2 diabetes and obesity.

10 The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or common general knowledge.

Diabetes is a metabolic disorder characterised by chronic hyperglycaemia with disturbances in carbohydrate, fat and/or protein metabolism that result from defects
15 in insulin secretion, insulin action, or both. It was one of the first diseases ever to be described, in an Egyptian manuscript from c. 1500 BC. However, the importance of insulin to the disease was not determined until the 1920s.

The most prevalent form of diabetes (about 90% of all patients) is type 2 diabetes,
20 which has become something of a global pandemic in recent years, presenting a major healthcare burden. The number of patients diagnosed with the condition is projected to be over half a billion worldwide by 2035.

Type 2 diabetes is characterised by impaired insulin production and secretion by
25 pancreatic beta-cells, as well as peripheral tissue insulin resistance.

Type 2 diabetes typically begins in later years (e.g. middle or older age), although rates are known to be increasing in young people. It is not only associated with a shorter life expectancy in itself, but it also often leads to long-term complications
30 including cardiovascular diseases, end-stage renal disease, lower limb amputation and blindness.

The aetiology of type 2 diabetes is largely thought to be linked to diet, particularly one that includes excessive consumption and/or insufficient energy expenditure through
35 exercise. In this respect, type 2 diabetes and obesity are interdependent, with weight loss being associated with an improved prognosis for overweight type 2 diabetes patients and obese individuals. Indeed, the increased global burden of type 2 diabetes

is thought to be largely due to an increase in obesity, which has itself become something of a global pandemic over recent decades.

5 Glucagon-like peptide-1 (GLP-1) is a peptide hormone that is produced following the tissue-specific posttranslational processing of the proglucagon peptide. It is secreted by intestinal enteroendocrine L-cells and certain neurons within the brainstem upon food consumption. The amino acid sequence of human GLP-1 has been described in *inter alia* Schmidt *et al*, *Diabetologia*, **28**, 704 (1985) as a 37 amino acid residue peptide.

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Alongside glucose-dependent insulintropic peptide (GIP), GLP-1 is an incretin, i.e. it has the ability to decrease blood sugar levels in a glucose-dependent manner by enhancing the secretion of insulin. GLP-1-based treatment has been associated with weight loss and a lower risk of hypoglycemia, making it particularly important in the treatment of patients with type 2 diabetes.

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Endogenous GLP-1 is rapidly degraded, having a half-life of only about 2 minutes. This has led to the substantial research into the development of *inter alia* GLP-1 receptor agonists to increase GLP-1 activity. In this respect, GLP-1 and analogues and fragments thereof (hereinafter referred to together as 'GLP-1 receptor agonists' or 'GLP1RAs') are useful as insulintropic agents, and thus in the treatment of diabetes (including type 1 and, particularly, type 2 diabetes) and/or obesity.

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Approved GLP1RAs include exenatide (Byetta®, Bydureon®; AstraZeneca), liraglutide (Victoza®, Saxenda®; Novo Nordisk), lixisenatide (Lyxumia®, Adlyxin®; Sanofi), albiglutide (Tanzeum®; GlaxoSmithKline), dulaglutide (Trulicity®; Eli Lilly), semaglutide (Ozempic®, Wegovy®; Novo Nordisk) and tirzepatide (Mounjaro®, Eli Lilly).

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30 The above-listed GLP1RA-containing products are administered by injection subcutaneously into fatty tissue, usually into the abdomen or upper thigh. Existing injectable formulations comprising GLP1RAs are typically injected on a once or twice daily basis (classified as short-acting GLP1Ras), or weekly (long-acting GLP1Ras), which is still relatively frequent.

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According to the review article by Sikirica *et al* in *Diabetes Metab. Syndr. Obes.*, **10**, 403 (2017), the percentage of patients reporting that they experience problems with injections of GLP1RAs was higher than mean physicians' estimates. Frequent patient-

reported issues include that injections are painful and/or of inconvenient regularity. The most common complaint reported by patients (56%) and physicians (32.6%) is that oral medications are preferred over injections.

5 These factors are known to lead to patient compliance issues. Despite a general awareness among patients with type 2 diabetes that, in the treatment of their condition, non-compliance can lead to highly detrimental sequelae, in a recent study reported by Weiss *et al* in *Patient Prefer. Adherence*, **27**, 2337 (2020), approximately 50% of patients do not adhere strictly to their prescribed dosage
10 regimen.

Although semaglutide has been formulated as oral medication in the form of Rybelus® (Novo Nordisk), the compound exhibits extremely poor oral bioavailability, and has to be taken on a daily basis along with no more than 120 mL of water, on an empty
15 stomach, and with a recommendation to eat thereafter within 30 and 60 minutes, all of which may also lead to patient compliance issues, as well as an increased risk of adverse events.

In a chronic condition like type-2 diabetes or obesity, it would be advantageous to
20 provide an extended-release composition, in which the active ingredient is released at a desired and predictable rate *in vivo* following injection over several weeks or months, in order to ensure better patient compliance and possibly a more optimal pharmacokinetic profile. Increased patient compliance is expected to contribute to an improved overall treatment effect over time (and so healthier patients in general), as
25 well as savings for healthcare and society.

In any event, a more convenient, less-frequently injected GLP1RA formulation is expected to be an attractive treatment option for all type 2 diabetes patients that are presently treated with GLP1RAs, even for those for whom compliance with their
30 prescribed dosing regimen is not an issue.

In the case of any sustained release composition, it is of critical importance that its release profile shows minimal initial rapid release of active ingredient, that is a large concentration of drug in plasma shortly after administration. Such a 'burst' release will
35 result in unwanted, high concentrations active ingredient, and may be hazardous in the case of drugs that have a narrow therapeutic window or drugs that are toxic at high plasma concentrations.

In the case of an injectable suspension of an active ingredient, it is also important that the size of the suspended particles is controlled so that they can be injected through a needle. If large, aggregated particles are present, they will not only block the needle, through which the suspension is to be injected, but also will not form a stable suspension within (i.e. they will instead tend to sink to the bottom of) the injection liquid.

There is thus an unmet clinical need in the treatment of conditions like type-2 diabetes and obesity for a longer lasting, more effective and/or improved drug delivery system comprising GLP1RAs.

Atomic layer deposition (ALD) is a technique that is employed to deposit thin films comprising a variety of materials, including organic, biological, polymeric and, especially, inorganic materials, such as metal oxides, on solid substrates. It is an enabling technique for atomic and close-to-atomic scale manufacturing (ACSM) of materials, structures, devices and systems in versatile applications (see, for example, Zhang et al. *Nanomanuf. Metrol.* **2022**, <https://doi.org/10.1007/s41871-022-00136-8>). Based on its self-limiting characteristics, ALD can achieve atomic-level thickness that is only controlled by adjusting the number of growth cycles. Moreover, multilayers can be deposited, and the properties of each layer can be customized at the atomic level.

Due to its atomic-level control, ALD is used as a key technique for the manufacturing of, for example, next-generation semiconductors, or in atomic-level synthesis of advanced catalysts as well as in the precise fabrication of nanostructures, nanoclusters, and single atoms (see, for example, Zhang et al, *supra*).

The technique is usually performed at low pressures and elevated temperatures. Film coatings are produced by alternating exposure of solid substrates within an ALD reactor chamber to vaporized reactants in the gas phase. Substrates can be silicon wafers, granular materials or small particles (e.g. microparticles or nanoparticles).

The coated substrate is protected from chemical reactions (decomposition) and physical changes by the solid coating. ALD can also potentially be used to control the rate of release of the substrate material within a solvent, which makes it of potential use in the formulation of active pharmaceutical ingredients.

In ALD, a first precursor, which can be metal-containing, is fed into an ALD reactor chamber (in a so called 'precursor pulse'), and forms an adsorbed atomic or molecular monolayer at the surface of the substrate. Excess first precursor is then purged from the reactor, and then a second precursor, such as water, is pulsed into the reactor.

5 This reacts with the first precursor, resulting in the formation of a monolayer of e.g. metal oxide on the substrate surface. A subsequent purging pulse is followed by a further pulse of the first precursor, and thus the start of a new cycle of the same events (a so called 'ALD cycle').

10 The thickness of the film coating is controlled by *inter alia* the number of ALD cycles that are conducted.

In a normal ALD process, because only atomic or molecular monolayers are produced during any one cycle, no discernible physical interface is formed between these
15 monolayers, which essentially become a continuum at the surface of the substrate.

In international patent application WO 2014/187995, a process is described in which a number of ALD cycles are performed, which is followed by periodically removing the resultant coated substrates from the reactor and conducting a re-dispersion/agitation
20 step to present new surfaces available for precursor adsorption.

The agitation step was done primarily to solve a problem observed for nano- and microparticles, namely that, during the ALD coating process, aggregation of particles takes place, resulting in 'pinholes' being formed by contact points between such
25 particles. The re-dispersion/agitation step was performed by placing the coated substrates in water and sonicating, which resulted in deagglomeration, and the breaking up of contact points between individual particles of coated active substance.

The particles were then loaded back into the reactor and the steps of ALD coating of
30 the powder, and deagglomerating the powder were repeated 3 times, to a total of 4 series of cycles. This process has been found to allow for the formation of coated particles that are, to a large extent, free of pinholes (see also, Hellrup *et al.*, *Int. J. Pharm.*, **529**, 116 (2017)).

35 We have now made a novel, injectable composition comprising one or more GLP1RAs, in which ALD is used to coat microparticles comprising one or more GLP1RAs with specific mixed oxide coating layers, which coated particles are suspended in a vehicle. This composition produces an advantageous pharmacokinetic profile by releasing

active ingredient over an extended period of time to provide a therapeutically-effective level of drug in systemic circulation, without any significant initial burst effect.

Disclosure of the Invention

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According to a first aspect of the invention there is provided a pharmaceutical formulation that is useful in the treatment of metabolic disorders or conditions, comprising a plurality of particles suspended in a carrier system, which particles:

- 10 (a) have a weight-, number-, or volume-based mean diameter that is between about 10 nm and about 700 μm ; and
- (b) comprise solid cores comprising one or more GLP1RAs, or pharmaceutically-acceptable salts thereof, coated, at least in part, by a coating of inorganic material comprising mixture of:
 - (i) zinc oxide (ZnO); and
 - 15 (ii) one or more other metal and/or metalloid oxides, wherein the atomic ratio ((i):(ii)) is at least about 1:10 and up to and including about 10:1,

which formulations are hereinafter referred to as 'the formulations of the invention'.

- 20 In a preferred that the atomic ratio ((i):(ii)) is at least about 1:1 and up to and including about 6:1.

The coating comprising a mixture of zinc oxide and one or more other metal and/or metalloid oxides is referred to hereinafter as the 'mixed oxide' coating or coating material(s).

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The term 'solid' will be well understood by those skilled in the art to include any form of matter that retains its shape and density when not confined, and/or in which molecules are generally compressed as tightly as the repulsive forces among them will allow. The solid cores have at least a solid exterior surface onto which a layer of coating material can be deposited. The interior of the solid cores may be also solid or may instead be hollow. For example, if the particles are spray dried before they are placed into the reactor vessel, they may be hollow due to the spray drying technique.

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- 35 The solid cores of the formulation of the invention comprise one or more GLP1RAs or pharmaceutically-acceptable salts thereof and, in this respect, may consist essentially of said one or more GLP1RAs or said salts thereof, or may include said one or more GLP1RAs or said salts thereof along with other excipients or other active ingredients.

By 'consists essentially' of GLP1RA or pharmaceutically-acceptable salt thereof, we include that the solid core is essentially comprised only of said one or more GLP1RAs or salts thereof, i.e. it is free from non-biologically active substances, such as excipients, carriers and the like (*vide infra*), and from other active substances. This means that the core may comprise less than about 5%, such as less than about 3%, including less than about 2%, e.g. less than about 1% of such other excipients and/or active substances.

10 In the alternative, cores comprising one or more GLP1RAs or pharmaceutically-acceptable salts thereof may include that active ingredient is in admixture with one or more pharmaceutical ingredients, which may include pharmaceutically-acceptable excipients, such as adjuvants, diluents or carriers, and/or may include other biologically-active ingredients, such as those described hereinafter.

15 Non-biologically active adjuvants, diluents and carriers that may be employed in cores to be coated in accordance with the invention may include pharmaceutically-acceptable substances that are soluble in water, such as carbohydrates, e.g. sugars, such as lactose and/or trehalose, and sugar alcohols, such as mannitol, sorbitol and xylitol; or
20 pharmaceutically-acceptable inorganic salts, such as sodium chloride. Preferred carrier/excipient materials include sugars and sugar alcohols.

Such excipients are preferably incorporated into cores comprising GLP1RA or pharmaceutically-acceptable salt thereof *via* a process of 'spray-drying', which will be
25 understood by the skilled person to include any method of producing a dry powder from a liquid, including a solution or a suspension (including a slurry) that involves rapid drying using hot gas to convert a stream of liquid into vaporized solvent and particles of solid, which solid particles comprise the solute that was previously dissolved in a solution, and/or particles that were previously suspended in the evaporated liquid.

30 GLP1RAs and pharmaceutically-acceptable salts thereof may be presented in a crystalline, a part-crystalline and/or an amorphous state. GLP1RAs and pharmaceutically-acceptable salts thereof may be in the solid state, or may be converted into the solid state, at about room temperature (e.g. about 18°C) and about
35 atmospheric pressure, irrespective of the physical form. Active agent (and optionally other pharmaceutical ingredients as mentioned hereinbefore) should also remain in the form of a solid whilst being coated in, for example, an ALD reactor, and also should not decompose physically or chemically to an appreciable degree (i.e. no more than

about 10% w/w) whilst being coated, or after having been covered by the mixed metal oxide coating material.

Pharmaceutically acceptable salts of GLP1RAs include addition salts, particularly acid addition salts. Such salts may be formed by conventional means, for example by reaction of a free acid or a free base form of the active ingredient with one or more equivalents of an appropriate base or, preferably, acid, optionally in a solvent, or in a medium in which the salt is insoluble, followed by removal of said solvent, or said medium, using standard techniques (e.g. *in vacuo*, by freeze-drying or by filtration). Salts may also be prepared using techniques known to those skilled in the art, such as by exchanging a counter-ion of the active ingredient in the form of a salt with another counter-ion, for example using a suitable ion exchange resin.

Particular salts that may be mentioned include acid additional salts of, for example, hydrochloric acid, L-lactic acid, phosphoric acid, (+)-L-tartaric acid, citric acid, propionic acid, butyric acid, hexanoic acid, L-aspartic acid, L-glutamic acid, succinic acid, ethylenediaminetetraacetic acid (EDTA), maleic acid, acid, particularly acetic acid, more particularly trifluoroacetic acid, and the like.

Preferred GLP1RAs include exenatide, lixisenatide, albiglutide, dulaglutide, tirzepatide, more preferably semaglutide, and especially liraglutide, all of which are known in the prior art and each of which may be synthesised by classical solution-phase techniques, solid-phase method or by recombinant peptide production.

Formulations of the invention comprise a pharmacologically-effective amount of said one or more GLP1RA or pharmaceutically-acceptable salt thereof. Preferably, the solid cores of the formulation of the invention comprise said pharmacologically-effective amount of one or more (preferably one) GLP1RA or salt thereof.

The term 'pharmacologically-effective amount' refers to an amount of the relevant GLP1RA (alone or in combination) or salt(s) thereof, which is/are capable of conferring a desired physiological change (such as a therapeutic effect) on a treated patient, whether administered alone or in combination with another active ingredient (which may or may not be another GLP1RA). Such a biological or medicinal response, or such an effect, in a patient may be subjective (i.e. the subject gives an indication of, or feels, an effect), and includes at least partial alleviation of the symptoms of the disease or disorder being treated, or curing or preventing said disease or disorder, or may be objective (i.e. measurable by some test or marker).

Dosages of GLP1RAs/salts thereof that may be administered to a patient should thus be sufficient to affect a therapeutic response over a reasonable and/or relevant timeframe. One skilled in the art will recognize that the selection of the exact dose and composition and the most appropriate delivery regimen will also be influenced by not only the pharmacological properties of the active ingredient, and the constituents of the formulation, but also *inter alia* the route of administration, the nature and severity of the condition being treated, and the physical condition and mental acuity of the recipient, as well as the age, condition, body weight, sex and response of the patient to be treated, and the stage/severity of the disease, as well as genetic differences between patients.

Dosages of GLP1RAs/salts thereof may also be determined by the timing and frequency of administration. In any event, the medical practitioner, or other skilled person, will be able to determine routinely the actual dosage of the relevant GLP1RA(s) and/or salt(s) thereof, which will be most suitable for an individual patient.

GLP1RA(s) is/are released over a prolonged period of time. That period of time may be at least about 3 days, such as about 5, or about 7, days, and up to a period of about a year, such as up to about 10 months, up to about 8 months, up to about 6 months, up to about 4 months, up to about 3 months or up to about 4 weeks, such as up to about 3 weeks (e.g. up to about 2 weeks).

Suitable doses of GLP1RAs or pharmaceutically-acceptable salts thereof in formulations of the invention may thus provide plasma concentration-time profiles that provide an exposure (AUC, defined as, for example AUC_{last} (the area under plasma concentration vs. time curve up to the last detectable concentration over a prolonged period of time) or, more preferably, AUC_{∞} (the area under the plasma concentration vs. time curve up to infinite time)) that provides at least the same therapeutic effect as that/those obtained for current, commercial subcutaneous injections of relevant GLP1RA(s) that is/are used in current clinical practice.

Formulations of the invention may be capable of providing an exposure, in terms of AUC_{∞} , for GLP1RAs in plasma over any one of the above-mentioned time periods that is no more than between about 80% and about 125% of the total exposure (AUC_{∞}) obtained from relevant current standard of care/dosing regimens administered by (e.g. daily or weekly) injections of the relevant GLP1RA, administered by injection, for example intravenously, intramuscularly or, preferably, subcutaneously.

More preferably, the total exposure (e.g. AUC_{∞}) for the relevant GLP1RA over any one of the above-mentioned time periods may be at least about 50% (e.g. at least about 65%), at least about 75% (e.g. at least about 80%), such as at least about 85% of the total exposure (e.g. AUC_{∞}) obtained from the current standard of care/dosing regimen administered by injections of the relevant GLP1RA.

This will enable a dose of the relevant GLP1RA or pharmaceutically-acceptable salt thereof within a formulation of the invention that provides, or is capable of providing, a daily, or weekly, dose (that is the mean dose released per day, or week, from the formulation after injection over any one of the above-mentioned time periods) that is in the range of is between about 10% (e.g. about 15%) and about 80% (e.g. about 70%, such as about 65%) of the daily, or weekly, dose (as appropriate) administered within the current standard of care, that is daily, or weekly, injection treatment (as appropriate) for the relevant GLP1RA compound or salt thereof (calculated in relation to the free compound).

Total doses that may be injected into patients by way of a formulation of the invention comprising liraglutide or a pharmaceutically-acceptable salt thereof within a formulation of the invention may thus be in the range of about 15 mg (such as about 270 mg) up to about 1000 mg.

Total doses that may be injected into patients by way of a formulation of the invention comprising semaglutide or a pharmaceutically-acceptable salt thereof within a formulation of the invention may thus be in the range of about 1 mg (such as about 30 mg) up to about 100 mg.

As formulations of the invention provide a steady state release of the relevant GLP1RA after injection, this means that the average $C_{\max}$ (the maximum concentration observed in the plasma concentration vs. time curve) will be less than that obtained from the current standard of care/dosing regimen administered by injection of the relevant GLP1RA (e.g. 20 ± 15 ng/mL for liraglutide and 210 ± 30 ng/mL for semaglutide). For formulations of the invention comprising liraglutide, the average $C_{\max}$ may be between about 20 and about 100 ng/mL. For formulations of the invention comprising semaglutide, the average $C_{\max}$ may be between about 75 and about 410 ng/mL.

The solid GLP1RA-containing cores of the formulation of the invention are provided in the form of nanoparticles or, more preferably, microparticles. Preferred weight-,

number-, or volume-based mean diameters are between about 50 nm (e.g. about 100 nm, such as about 250 nm) and about 30 μm , for example between about 500 nm and about 100 μm , more particularly between about 1 μm (such as about 5 μm , including about 7 μm , about 9 μm , about 10 μm or about 15 μm) up to about 50 μm , such as
5 about 25 μm , e.g. about 20 μm .

As used herein, the term 'weight based mean diameter' will be understood by the skilled person to include that the average particle size is characterised and defined from a particle size distribution by weight, i.e. a distribution where the existing fraction
10 (relative amount) in each size class is defined as the weight fraction, as obtained by e.g. sieving (e.g. wet sieving). As used herein, the term 'number based mean diameter' will be understood by the skilled person to include that the average particle size is characterised and defined from a particle size distribution by number, i.e. a distribution where the existing fraction (relative amount) in each size class is defined as the number
15 fraction, as measured by e.g. microscopy. As used herein, the term 'volume based mean diameter' will be understood by the skilled person to include that the average particle size is characterised and defined from a particle size distribution by volume, i.e. a distribution where the existing fraction (relative amount) in each size class is defined as the volume fraction, as measured by e.g. laser diffraction. The person
20 skilled in the art will also understand there are other suitable ways of expressing mean diameters, such as area based mean diameters, and that these other expressions of mean diameter are interchangeable with those used herein. Other instruments that are well known in the field may be employed to measure particle size, such as equipment sold by e.g. Malvern Instruments, Ltd (Worcestershire, UK) and Shimadzu
25 (Kyoto, Japan).

Particles may be spherical, that is they possess an aspect ratio smaller than about 20, more preferably less than about 10, such as less than about 4, and especially less than about 2, and/or may possess a variation in radii (measured from the centre of gravity
30 to the particle surface) in at least about 90% of the particles that is no more than about 50% of the average value, such as no more than about 30% of that value, for example no more than about 20% of that value.

Nevertheless, the coating of particles on any shape is also possible in accordance with
35 the invention. For example, irregular shaped (e.g. 'raisin'-shaped), needle-shaped, flake-shaped or cuboid-shaped particles may be coated. For a non-spherical particle, the size may be indicated as the size of a corresponding spherical particle of e.g. the same weight, volume or surface area. Hollow particles, as well as particles having

pores, crevices etc., such as fibrous or 'tangled' particles may also be coated in accordance with the invention.

5 Particles may be obtained in a form in which they are suitable to be coated or be obtained in that form, for example by particle size reduction processes (e.g. crushing, cutting, milling or grinding) to a specified weight based mean diameter (as hereinbefore defined), for example by wet grinding, dry grinding, air jet-milling (including cryogenic micronization), ball milling, such as planetary ball milling, as well as making use of end-runner mills, roller mills, vibration mills, hammer mills, roller
10 mill, fluid energy mills, pin mills, etc. Alternatively, particles may be prepared directly to a suitable size and shape, for example by spray-drying, freeze-drying, spray-freeze-drying, vacuum-drying, precipitation, including the use of supercritical fluids or other top-down methods (i.e. reducing the size of large particles, by e.g. grinding, etc.), or bottom-up methods (i.e. increasing the size of small particles, by e.g. sol-gel
15 techniques, crystallization, etc.). Nanoparticles may alternatively be made by well-known techniques, such as gas condensation, attrition, chemical precipitation, ion implantation, pyrolysis, hydrothermal synthesis, etc.

It may be necessary (depending upon how the particles that comprise the cores are
20 initially provided) to wash and/or clean them to remove impurities that may derive from their production, and then dry them. Drying may be carried out by way of numerous techniques known to those skilled in the art, including evaporation, spray-drying, vacuum drying, freeze drying, fluidized bed drying, microwave drying, IR radiation, drum drying, etc. If dried, cores may then be deagglomerated by grinding,
25 screening, milling and/or dry sonication. Alternatively, cores may be treated to remove any volatile materials that may be absorbed onto its surface, e.g. by exposing the particle to vacuum and/or elevated temperature.

Surfaces of cores may be chemically activated prior to applying the first layer of coating
30 material, e.g. by treatment with hydrogen peroxide, ozone, free radical-containing reactants or by applying a plasma treatment, in order to create free oxygen radicals at the surface of the core. This in turn may produce favourable adsorption/nucleation sites on the cores for (e.g. ALD) precursors.

35 The GLP1RA-containing cores are coated at least in part with a coating material that comprises a mixture of zinc oxide, and one or more other metal and/or metalloid oxides, at an atomic ratio of zinc oxide to the other oxide(s) that is at least about 1:10 (e.g. at least about 1:6 or about 1:4, such as at least about 1:2), preferably at least about

1:1 (e.g. at least about 1.5:1, such as at least about 2:1), including at least about 2.25:1, such as at least about 2.5:1 (e.g. at least about 3.25:1 or least about 2.75:1 (including 3:1)), and is up to (i.e. no more than) and including about 10:1, including up to about 8:1, about 7:1, about 6:1, about 5.5:1, or up to about 5:1, such as up to about 4.5:1, including up to about 4:1 (e.g. up to about 3.75:1).

Preferred methods of applying the coating(s) to the cores comprising biologically-active agents include gas phase techniques, such as ALD or related technologies, such as atomic layer epitaxy (ALE), molecular layer deposition (MLD; a similar technique to ALD with the difference that molecules (commonly organic molecules) are deposited in each pulse instead of atoms), molecular layer epitaxy (MLE), chemical vapor deposition (CVD), atomic layer CVD, molecular layer CVD, physical vapor deposition (PVD), sputtering PVD, reactive sputtering PVD, evaporation PVD and binary reaction sequence chemistry. ALD is the preferred method of coating according to the invention.

When ALD is employed, the above-described mixed oxide coating may be prepared by feeding a first, zinc-, other metal- or metalloid-containing precursor into an ALD reactor chamber (in a so called 'precursor pulse') to form the adsorbed atomic or molecular zinc-, other metal- or metalloid-containing monolayer at the surface of the particle. A second precursor (e.g. water) is then pulsed into the reactor and reacts with the first precursor, resulting in the formation of a monolayer of zinc, metal or metalloid oxide, respectively, on the substrate surface. A subsequent purging pulse is followed by a further pulse of the first precursor, and thus the start of a new cycle of the same events, which is an ALD cycle.

When MLD is employed, an organic, polymeric and/or hybrid organic-inorganic coating may be prepared by using an MLD precursor, for example an organic molecule comprising a difunctional group, such as a diol, diamine, diisocyanate, dichloride, dialdehyde.

In most instances, the first of the consecutive reactions will involve some functional group or free electron pairs or radicals at the surface to be coated, such as a hydroxy group (-OH) or a primary or secondary amino group (-NH₂ or -NHR where R e.g. is an aliphatic group, such as an alkyl group). The individual reactions are advantageously carried out separately and under conditions such that all excess reagents and reaction products are essentially removed before conducting the subsequent reaction.

In order to make a mixed oxide coating with an atomic ratio (in terms of the number of monolayers that are applied) of (for example) between at least about 1:1 and up to and including about 6:1 of zinc oxide relative to the one or more other metal and/or metalloid oxides, the skilled person will appreciate that for every one ALD cycle (i.e. monolayer) of the other oxide(s), between about 1 and about 6 ALD cycles of zinc oxide must also be deposited. For example, for a 3:1 atomic (zinc:other oxide) mixed oxide coating to be formed, 3 zinc-containing precursor pulses may each be followed by second precursor pulses, forming 3 monolayers of zinc oxide, which will then be followed by 1 pulse of the other metal and/or metalloid-containing precursor followed by second precursor pulse, forming 1 monolayer of oxide of the other metal and/or metalloid. Alternatively, 6 monolayers of zinc oxide may be followed by 2 monolayers of the other oxide, or any other combination so as to provide an overall atomic ratio of about 3:1. In this respect, the order of pulses to produce the relevant oxides is not critical, provided that the resultant atomic ratio is in the relevant range in the end.

Metal and/or metalloid elements other than zinc that may be mentioned include alkali metals, alkaline earth metals, noble metals, transition metals, post-transition metals, lanthanoids, etc. Metal and/or metalloids that may be mentioned include aluminium, titanium, magnesium, iron, gallium, zirconium, niobium, hafnium, tantalum, lanthanum, and/or silicon; more preferably aluminium, titanium, magnesium, iron, gallium, and/or zirconium. Particular metal and/or metalloid elements that may be mentioned include aluminium and silicon.

In this respect the mixed oxide coating material preferably comprises one or other or both of aluminium oxide (Al_2O_3) and/or silicon dioxide (SiO_2).

There is provided a method of preparing of plurality of coated particles in accordance with the invention, wherein the coated particles are made by applying precursors of at least two metal and/or metalloid oxides forming a mixed oxide on the solid cores, and/or previously-coated solid cores, by a gas phase deposition technique. Precursors for forming a metal oxide or a metalloid oxide often include an oxygen precursor, such as water, oxygen, ozone and/or hydrogen peroxide; and a metal and/or metalloid compound, typically an organometal compound or an organometalloid compound.

Non-limiting examples of precursors are as follows: Precursors for zinc oxide may be water and di $\text{C}_1\text{-C}_5$ alkylzinc, such as diethylzinc. Precursors for aluminium oxide may be water and tri $\text{C}_1\text{-C}_5$ alkylaluminium, such as trimethylaluminium. Precursors for silicon oxide (silica) may be water as the oxygen precursor and silanes, alkylsilanes,

aminosilanes, and orthosilicic acid tetraethyl ester. Precursors for iron oxide includes oxygen, ozone and water as the oxygen precursor; and di C₁-C₅alkyl-iron, dicyclopropyl-iron, and FeCl₃. It will be appreciated that the person skilled in the art is aware of what precursors are suitable for the purpose as disclosed herein.

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In ALD, layers of coating materials may be applied at process temperatures from about 20°C to about 800°C, or from about 40°C to about 200°C, e.g. from about 40°C to about 150°C, such as from about 50°C to about 100°C. The optimal process temperature depends on the reactivity of the precursors and/or substances (including
10 biologically-active agents, e.g. GLP1RA/salt) that are employed in the core and/or melting point of the core substance(s). It is preferred that a lower temperature, such as from about 30°C to about 100°C is employed. In particular, in one embodiment of the method a temperature from about 20°C to about 80°C is employed, such as from about 30°C to about 70°C, such as from about 40°C to about 60°C, such as about
15 50°C.

We have found that, when coatings comprising zinc oxide are applied using ALD at a lower temperature, such as from about 50°C to about 100°C (unlike other coating materials, such as aluminium oxide, titanium oxide and silicon dioxide, that form
20 amorphous layers) the coating materials are largely crystalline in their nature.

As alluded to above, and without being limited by theory, because zinc oxide is crystalline, if only zinc oxide is employed as coating material, we are of the understanding that interfaces may be formed between adjacent crystals of zinc oxide
25 that are deposited by ALD, through which a carrier system, medium or solvent in which zinc oxide is partially soluble (e.g. an aqueous solvent system) can ingress following suspension therein. It is believed that this may give rise to dissolution that is too fast for the depot-forming composition that it is intended to make.

In addition, previous studies have shown that, when suspended in aqueous media, the relative bioavailability for formulations comprising certain active ingredients coated with zinc oxide is lower than respective uncoated active ingredient. We believe that this lower relative bioavailability is due to degradation of that active ingredient before it can be released into systemic circulation. Penetration of water through crystalline
30 interfaces within a zinc oxide coating as described above is thought to lead to hydrolysis of the active ingredient within the interior of the coated particle.
35

We have now found that these problems may be alleviated by making a mixed oxide coating as described herein. In particular, by forming a mixed oxide coating as described herein, that is predominantly, but not entirely, comprised of zinc oxide, we have been able to coat active ingredients with coatings that appear to be essentially
5 amorphous, or a composite between crystalline and amorphous material and/or in which ingress of injection vehicles such as water may be reduced. In this respect, it appears to us that the presence of the aforementioned perceived interfaces may be reduced, or avoided altogether, by employing the mixed oxide aspect of the invention, in either a heterogeneous manner (in which the other oxide is 'filling in' gaps formed
10 by the interfaces), or in a homogeneous manner (in which a true composite of mixed oxide materials is formed during deposition, in a manner where the interfaces are potentially avoided in the first place).

The gas phase deposition reactor chamber used may optionally, and/or preferably, be
15 a stationary gas phase deposition reactor chamber. The term 'stationary', in the context of gas phase deposition reactor chambers, will be understood to mean that the reactor chamber remains stationary while in use to perform a gas phase deposition technique, excluding negligible movements and/or vibrations such as those caused by associated machinery for example.

20 Additionally, a so-called 'stop-flow' process may be employed for example, as described hereinafter. Using a stop-flow process, once the first precursor has been fed into the reactor chamber and prior to the first precursor being purged from the reactor chamber, the first precursor may be allowed to contact the cores in the reactor
25 chamber for a pre-determined period of time (which may be considered as a soaking time). During the pre-determined period of time there is preferably a substantial absence of pumping that may result in flow of gases and/or a substantial absence of mechanical agitation of the cores.

30 The employment of the stop-flow process may increase coating uniformity by allowing each gas to diffuse conformally in high aspect-ratio substrates, such as powders. The benefits may be even more pronounced when using precursors with slow reactivity as more time is given for the precursor to react on the surface. This may be evident especially when depositing mixed oxide coatings according to the invention. For
35 example, when depositing a mixed zinc oxide/aluminium oxide coating as described herein, we have found that a zinc-containing precursor, such as diethylzinc (DEZ), which has a lower reaction probability towards the surface of a substrate than, for example, aluminium containing precursors, such as trimethylaluminum (TMA).

In addition to generating coatings with good shell integrity and more controlled release profiles, the employment of such a stop-flow process may improve the ability to achieve a particular coating composition.

5

For example, when attempting to employ a gas phase technique to produce a coating comprising an atomic ratio of 3:1 between zinc and aluminium in the resulting shell as described above, we have found that a ratio that is much closed to 3:1 may be achieved using a stop-flow process than when depositing material using a continuous flow of

10

precursors.

Preferably, and/or optionally, a 'multi-pulse' technique may also be employed to feed the first precursor, the second precursor or both precursors to the reactor chamber.

15

Using such a multi-pulse technique, the respective precursor may be fed into the reactor chamber as a plurality of 'sub-pulses', each lasting a short period of time such as 1 second up to about a minute (depending on the size and the nature of the gas phase deposition reactor), rather than as one continuous pulse. The precursor may be allowed to contact the cores in the reactor chamber for the pre-determined period of time, for example from about 1 to 500 seconds, about 2 to 250 seconds, about 3 to 100 seconds, about 4 to 50 seconds, or about 5 to 10 seconds, for example 9 seconds, after each sub-pulse. Again, depending on the size and the nature of the gas phase deposition reactor, this time could be extended up to several minutes (e.g. up to about 30 minutes). The introduction of a sub-pulse followed by a period of soaking time may be repeated a pre-determined number of times, such as between about 5 to 1000 times, about 10 to 250 times, or about 20 to 50 times in a single step.

20

25

Formulations of the invention that may be mentioned include those that are not made by way of such a stop-flow process.

30

The cores may be coated with one or more separate, discrete layers, of mixed oxide coatings as defined herein. Preferably, more than one separate, discrete mixed oxide layer, coating or shell (which terms are used herein interchangeably) is applied (that is 'separately applied') to the solid cores comprising the GLP1RA(s) sequentially.

35

By 'separate application' of 'separate layers, coatings or shells', we mean that the solid cores may be coated with a first layer of coating material, which layer is formed by more than one (e.g. a plurality or a set of) cycles as described herein, each cycle

producing a monolayer of zinc oxide, or other metal and/or metalloid oxide (as appropriate), and then that resultant coated core may be subjected to some form of deagglomeration process.

5 In other words, 'gas-phase deposition (e.g. ALD) cycles' may be repeated several times to provide a 'gas-phase deposition (e.g. ALD) set' of cycles, which may consist of e.g. 10, 25 or 100 cycles. However, after this set of cycles, the coated core may be subjected to some form of deagglomeration process, which is followed by a further set of cycles.

10

This process may be repeated as many times as is desired and, accordingly, the number of discrete layers of coating material(s) produced by sets of cycles that is in a final coating corresponds to the number of these intermittent deagglomeration steps with the option of a final mechanical deagglomeration being conducted prior to the application of a final layer (set of cycles) of coating material.

15

The particles of the formulation may have between 1 and about 100 discrete layers of mixture of oxides (and, if appropriate, of other coating materials as described hereinafter), for example between 2 and about 50 discrete layers, such as between 3 and about 10 discrete layers, for example between 3 and 6 discrete layers.

20

The terms 'disaggregation' and 'deagglomeration' are used interchangeably when referring to the coated particles, and disaggregating coated particles aggregates is preferably done by way of a mechanical sieving technique.

25

Coated cores may be subjected to the aforementioned deagglomeration process internally, without being removed from said apparatus by way of a continuous process. Such a process will involve forcing solid product mass formed by coating said cores through a sieve that is located within the reactor, and is configured to deagglomerate any particle aggregates upon forcing of the coated cores by means of a forcing means applied within said reactor, prior to being subjected to a second and/or a further coating. This process is continued for as many times as is required and/or appropriate prior to the application of the final coating as described herein.

30

35 Having the sieve located within the reactor vessel means that the coating can be applied by way of a continuous process which does not require the particles to be removed from the reactor. Thus, no manual handling of the particles is required, and no external machinery is required to deagglomerate the aggregated particles. This not

only considerably reduces the time of the coating process being carried out, but is also more convenient and reduces the risk of harmful (e.g. poisonous) materials being handled by personnel. It also enhances the reproducibility of the process by limiting the manual labour and reduces the risk of contamination.

5

Alternatively, and/or preferably, coated cores may be removed from the coating apparatus, such as the ALD reactor, and thereafter subjected to an external deagglomeration step, for example as described in international patent application WO 2014/187995. Such an external deagglomeration step may comprise agitation, such as sonication in the wet or dry state, or preferably may comprise subjecting the resultant solid product mass that has been discharged from the reactor to sieving, e.g. by forcing it through a sieve or mesh in order to deagglomerate the particles, for example as described hereinafter, prior to placing the particles back into the coating apparatus for the next coating step. Again, this process may be continued for as many times as is required and/or appropriate prior to the application of the final coating.

In an external deagglomeration process, deagglomeration may alternatively be effected (additionally and/or instead of the abovementioned processes) by way of subjecting the coated particles in the wet or dry state to one or more of nozzle aerosol generation, milling, grinding, stirring, high sheer mixing and/or homogenization. If the step(s) of deagglomeration are carried out on particles in the wet state, the deagglomerated particles should be dried (as hereinbefore described in relation to cores) prior to the next coating step.

However, we prefer that, in such an external process, the deagglomeration step(s) comprise one or more sieving step(s), which may comprise jet sieving, manual sieving, vibratory sieve shaking, horizontal sieve shaking, tap sieving, or (preferably) sonic sifting as described hereinafter, or a like process, including any combination of these sieving steps. Manufacturers of suitable sonic sifters include Advantech Manufacturing, Endecott and Tsutsui.

Vibrational sieving techniques may involve a means of vibrationally forcing the solid product mass formed by coating said cores through a sieve that is located internally or (preferably) externally to (i.e. outside of) the reactor, and is configured to deagglomerate any particle aggregates upon said vibrational forcing of the coated cores, prior to being subjected to a second and/or a further layer of coating material. This process is repeated as many times as is required and/or appropriate prior to the application of a final layer of coating material.

Vibrational forcing means comprises a vibration motor which is coupled to a sieve. The vibration motor is configured to vibrate and/or gyrate when an electrical power is supplied to it. For example, the vibration motor may be a piezoelectric vibration motor comprising a piezoelectric material which changes shape when an electric field is applied, as a consequence of the converse piezoelectric effect. The changes in shape of the piezoelectric material cause acoustic or ultrasonic vibrations of the piezoelectric vibration motor.

10 The vibration motor may alternatively be an eccentric rotating mass (ERM) vibration motor comprising a mass which is rotated when electrical power is supplied to the motor. The mass is eccentric from the axis of rotation, causing the motor to be unbalanced and vibrate and/or gyrate due to the rotation of the mass. Further, the ERM vibration motor may comprise a plurality of masses positioned at different
15 locations relative to the motor. For example, the ERM vibration motor may comprise a top mass and a bottom mass each positioned at opposite ends of the motor. By varying each mass and its angle relative to the other mass, the vibrations and/or gyrations of the ERM vibration motor can be varied.

20 The vibration motor is coupled to the sieve in a manner in which vibrations and/or gyrations of the motor when electrical power is supplied to it are transferred to the sieve.

The sieve and the vibration motor may be suspended from a mount (such as a frame
25 positionable on a floor, for example) *via* a suspension means such that the sieve and motor are free to vibrate relative to the mount without the vibrations being substantially transferred to or dampened by the mount. This allows the vibration motor and sieve to vibrate and/or gyrate without impediment and also reduces noise generated during the vibrational sieving process. The suspension means may comprise
30 one or more springs or bellows (i.e. air cushion or equivalent cushioning means) that couple the sieve and/or motor to the mount. Manufacturers of vibratory sieves or sifters suitable for carrying out such a process include for instance Russell Finex, SWECO, Filtra Vibracion, VibraScreener, Gough Engineering and Farley Greene.

35 Preferably, the vibrational sieving technique further comprises controlling a vibration probe coupled to the sieve. The vibration probe may be controlled to cause the sieve to vibrate at a separate frequency to the frequency of vibrations caused by the vibration motor. Preferably the vibration probe causes the sieve to vibrate at a higher

frequency than the vibrations caused by the vibration motor and, more preferably, the frequency is within the ultrasonic range.

5 Providing additional vibrations to the sieve by means of the vibration probe reduces the occurrence of clogging in the sieve, reduces the likelihood of the sieve being overloaded and decreases the amount of time needed to clean the mesh of the sieve.

10 Preferably, the aforesaid vibrational sieving technique comprises sieving coated particles with a throughput of at least 1 g/minute. More preferably, the vibrational sieving technique comprises sieving coated particles with a throughput of 4 g/minute or more.

15 The throughput depends on the area of the sieve mesh, mesh-size of the sieve, the particle size, the stickiness of the particles, static nature of the particle. By combining some of these features a much higher throughput is possible. Accordingly, the vibrational sieving technique may more preferably comprise sieving coated particles with a throughput of up to 1 kg/minute or even higher.

20 Any one of the above-stated throughputs represents a significant improvement over the use of known mechanical sieving, or sifting, techniques. For example, we found that sonic sifting involved sifting in periods of 15 minutes with a 15-minute cooling time in-between, which is necessary for preserving the apparatus. To sift 20 g of coated particles required 9 sets of 15 minutes of active sifting time, i.e. a total time (including the cooling) of 255 minutes. By comparison, by using the aforementioned
25 vibrational sieving technique, 20 g of coated particles may be sieved continuously in, at most, 20 minutes, or more preferably in just 5 minutes, or less.

30 The sieve mesh size may be determined so that the ratio of the size of the sieved or sonic sifted particles to the sieve mesh size is about 1:>1, preferably about 1:2, and optionally about 1:4. The size mesh size may range from about 20 μm to about 100 μm , preferably from about 20 μm to about 60 μm .

35 Appropriate sieve meshes may include perforated plates, microplates, grid, diamond, threads, polymers or wires (woven wire sieves) but are preferably formed from metals, such as stainless steel.

Surprisingly, using a stainless steel mesh within the vibrational sieving technique is as gentle to the particle coatings as using a softer polymer sieve as part of a mechanical sieving technique such as sonic sifting.

- 5 Also, a known problem with sieving powders is the potentially dangerous generation of static electricity. A steel mesh has the advantage of removing static electricity from the powder while that is not the case with a polymeric mesh, which has to be used in a sonic sifter.
- 10 Further, the mesh size of known sonic sifters is limited to about 100 μm since the soundwaves travel through the mesh rather than vibrating it. That limitation does not exist using for vibrational sieving techniques as there is no reliance on soundwaves to generate vibrations in the sieve. Therefore, the vibrational sieving technique described herein allows larger particles to be sieved than if alternative mechanical sieving
- 15 techniques were used.

If a (e.g. vibrational) sieve is located externally to (i.e. outside of) the reactor, the process for making coated cores of formulations of the invention comprises discharging the coated particles from the gas phase deposition reactor prior to subjecting the coated particles to agitation, followed by reintroducing the deagglomerated, coated

20 particles into the gas phase deposition reactor prior to applying a further layer of at least one coating material to the reintroduced particles.

As stated above, we have found that applying separate layers of coating materials following external deagglomeration gives rise to visible and discernible interfaces that may be observed by analysing coated particles according to the invention, and are observed by e.g. TEM as regions of higher electron permeability. In this respect, the thickness of the layers between interfaces correspond directly to the number of cycles in each series that are carried out within the ALD reactor, and between individual

25 external agitation steps.

30

Because, in an ALD coating process, coating takes place at the atomic level, such clear, physical interfaces are typically more difficult to observe.

- 35 Without being limited by theory, it is believed that removing coated particles from the vacuum conditions of the ALD reactor and exposing a newly-coated surface to the atmosphere results in structural rearrangements due to relaxation and reconstruction of the outermost atomic layers. Such a process is believed to involve rearrangement

of surface (and near surface) atoms, driven by a thermodynamic tendency to reduce surface free energy.

Furthermore, surface adsorption of species, e.g. hydrocarbons that are always present in the air, may contribute to this phenomenon, as can surface modifications, due to reaction of coatings formed with hydrocarbons, as well as atmospheric oxygen and the like. Accordingly, if such interfaces are analysed chemically, they may contain traces of contaminants or the core material, such as active ingredient that forms part of the core, that do not originate from the coating process, such as ALD.

10

Whether carried out inside or outside of the reactor, particle aggregates are preferably broken up by a forcing means that forces them through a sieve, thus separating the aggregates into individual particles or aggregates of a desired and predetermined size (and thereby achieving deagglomeration). In the latter regard, in some cases the individual primary particle size is so small (i.e. $<1\ \mu\text{m}$) that achieving 'full' deagglomeration (i.e. where aggregates are broken down into individual particles) is not possible. Instead, deagglomeration is achieved by breaking down larger aggregates into smaller aggregates of secondary particles of a desired size, as dictated by the size of the sieve mesh. The smaller aggregates are then coated by the gas phase technique to form fully coated 'particles' in the form of small aggregate particles. In this way, the term 'particles', when referring to the particles that have been deagglomerated and coated in the context of the invention, refers to both individual (primary) particles and aggregate (secondary) particles of a desired size.

25 In any event, the desired particle size (whether that be of individual particles or aggregates of a desired size) is maintained and, moreover, continued application of the gas phase coating mechanism to the particles after such deagglomeration *via* the sieving means that a complete coating is formed on the particle, thus forming fully coated particles (individual or aggregates of a desired size).

30

Whether carried out inside or outside of the reactor, the above-described repeated coating and deagglomeration process may be carried out at least 1, preferably 2, more preferably 3, such as 4, including 5, more particularly 6, e.g. 7 times, and no more than about 100 times, for example no more than about 50 times, such as no more than about 40 times, including no more than about 30 times, such as between 2 and 20 times, e.g. between 3 and 15 times, such as 10 times, e.g. 9 or 8 times, more preferably 6 or 7 times, and particularly 4 or 5 times.

35

Whether carried out inside or outside of the reactor, it is preferred that at least one sieving step is carried out and further that that step preferably comprises a vibrational sieving step as described above. It is further preferred that at least the final sieving step comprises a vibrational sieving step being conducted prior to the application of a final layer (set of cycles) of coating material. However, it is further preferred that more than one (including each) of the sieving steps comprise vibrational sieving techniques, steps or processes as described herein.

The preferable repetition of these steps makes the improved throughput of any vibrational sieving technique all the more beneficial.

Formulations of the invention that may be mentioned include those that are not made by a process that includes a vibrational sieving technique.

The total thickness of the coating (meaning all the separate layers/coatings/shells) will on average be in the region of between about 0.5 nm and about 2 μm .

The minimum thickness of each individual layer/coating/shell will on average be in the region of about 0.1 nm (including about 0.5 nm, for example about 0.75 nm, such as about 1 nm).

The maximum thickness of each individual layer/coating/shell will depend on the size of the core (to begin with), and thereafter the size of the core with the coatings that have previously been applied, and may be on average about 1 hundredth of the mean diameter (i.e. the weight-, number-, or volume-based mean diameter) of that core, or core with previously-applied coatings.

Preferably, for particles with a mean diameter that is between about 100 nm and about 1 μm , the total coating thickness should be on average between about 1 nm and about 5 nm; for particles with a mean diameter that is between about 1 μm and about 20 μm , the coating thickness should be on average between about 1 nm and about 10 nm; for particles with a mean diameter that is between about 20 μm and about 700 μm , the coating thickness should be on average between about 1 nm and about 100 nm.

In this respect, coated cores of the formulation of the invention have preferred weight-, number-, or volume-based mean diameters that are preferably between about 50 nm (e.g. about 100 nm, such as about 250 nm) and about 30 μm , for example between

about 500 nm and about 100 μm , more particularly between about 1 μm (such as about 5 μm , including about 7 μm , about 9 μm , about 10 μm or about 15 μm) up to about 50 μm , such as about 25 μm , e.g. about 20 μm .

- 5 We have found that applying coatings/shells followed by conducting one or more deagglomeration step such as sonication gives rise to abrasions, pinholes, breaks, gaps, cracks and/or voids (hereinafter 'cracks') in the layers/coatings, due to coated particles essentially being more tightly 'bonded' or 'glued' together directly after the application of a thicker coating. This may expose a core comprising biologically-active ingredient
10 to the elements once deagglomeration takes place.

As it is intended to provide particles in a suspension prior to administration to a patient, it is necessary to provide deagglomerated primary particles without pinholes or cracks in the coatings. Such cracks will result in an undesirable initial peak (burst) in plasma
15 concentration of active ingredient directly after administration.

We have found that, by conducting one or more of the deagglomeration steps described herein, this gives rise to significantly less pinholes, gaps or cracks in the final layer of coating material, giving rise to particles that are not only completely covered by that
20 layer/coating, but are also covered in a manner that enables the particles to be deagglomerated readily (e.g. using a non-aggressive technique, such as vortexing) in a manner that does not destroy the layers of coating material that have been formed, prior to, and/or during, pharmaceutical formulation.

- 25 In this respect, the mixed oxide coating typically completely surrounds, encloses and/or encapsulates said solid cores comprising active ingredient(s). In this way, the risk of an initial drug concentration burst due to the drug coming into direct contact with solvents in which the relevant active ingredient is soluble is minimized. This may include not only bodily fluids, but also any medium in which such coated particles may
30 be suspended prior to injection.

Thus in a further embodiment of the invention, there are provided particles as hereinbefore disclosed, wherein said coating surrounding, enclosing and/or encapsulating said core covers at least about 50%, such as at least about 65%,
35 including at least about 75%, such as at least about 80%, more particularly at least about 90%, such as at least about 91%, such as at least about 92%, such as at least about 93%, such as at least about 94%, such as at least about 95%, such as at least about 96%, such as at least about 97%, such as at least about 98%, such as at least

about 99%, such as approximately, or about, 100%, of the surface of the solid core, such that the coating essentially completely surrounds, encloses and/or encapsulates said core.

- 5 As used herein, the term 'essentially completely coating completely surrounds, encloses and/or encapsulates said core' means a covering of at least about 98%, or at least about 99%, of the surface of the solid core.

- 10 In the alternative, processes described herein may result in the deagglomerated coated particles with the essential absence of said cracks through which active ingredient can be released in an uncontrolled way.

- 15 Although some minor cracks may appear in the said coating without effecting the essential function thereof in terms of controlling release, in a further embodiment, there are provided particles as hereinbefore disclosed, wherein at least about 90% of the particles do not exhibit cracks in the coating surrounding, enclosing and/or encapsulating said core. In one embodiment at least about 91%, such as at least about 92%, such as at least about 93%, such as at least about 94%, such as at least about 95%, such as at least about 96%, such as at least about 97%, such as at least about 98%, such as at least about 99%, such as approximately 100% of the particles do not exhibit said cracks.

- 25 Alternatively, by 'essentially free of said cracks' in the coating(s), we also mean that less than about 1% of the surfaces of the coated particles comprise abrasions, pinholes, breaks, gaps, cracks and/or voids through which active ingredient is potentially exposed (to, for example, the elements).

- 30 The layers of coating material may, taken together, be of an essentially uniform thickness over the surface area of the particles. By 'essentially uniform' thickness, we mean that the degree of variation in the thickness of the coating of at least about 10%, such as about 25%, e.g. about 50%, of the coated particles that are present in a formulation of the invention, as measured by TEM, is no more than about $\pm 20\%$, including $\pm 50\%$ of the average thickness.

- 35 In addition to the essential mixed oxide coating that is employed in formulations of the invention, other coating materials, which may be pharmaceutically-acceptable and essentially non-toxic coating materials may also be applied in addition, either between separate mixed oxide coatings (e.g. in-between separate deagglomeration steps)

and/or whilst a mixed oxide coating is being applied herein. Such materials may comprise multiple layers or composites of said mixed oxide and one or more different inorganic or organic materials, to modify the properties of the layer(s).

5 Additional coating materials may comprise organic or polymeric materials, such as a polyamide, a polyimide, a polyurea, a polyurethane, a polythiourea, a polyester or a polyimine. Additional coating materials may also comprise hybrid materials (as between organic and inorganic materials), including materials that are a combination
10 between a metal, or another element, and an alcohol, a carboxylic acid, an amine or a nitrile. Such additional organic, polymeric and/or hybrid organic-inorganic coatings are preferably applied using a coating technique that comprises MLD as described hereinbefore. Such polymeric coatings can be polyimides, polyazomethines, polyureas, polyamides, nylons, metalcones, alucones, titanicones, zincones, metal-organic framework polymers, oxycarbides and hybrid nanolaminates.

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However, we prefer that coating materials comprise inorganic materials.

Additional inorganic coating materials (to zinc oxide) may comprise other compounds of metals and/or metalloids, such as oxides, nitrides, sulphides, selenides, carbonates,
20 other ternary compounds, etc. Metal, and metalloid, hydroxides and, especially, oxides are preferred, especially metal oxides.

In addition, oxides of elements other than zinc, aluminium or silicon that may be mentioned include alkali metals, alkaline earth metals, noble metals, transition metals,
25 post-transition metals, lanthanoids, etc. Metal and metalloids that may be mentioned include titanium, magnesium, iron, gallium, zirconium, niobium, hafnium, tantalum and/or lanthanum; more preferably titanium, magnesium, iron, gallium and/or zirconium.

30 Additional coating materials that may be mentioned, thus, include those comprising titanium dioxide (TiO_2), iron oxides (Fe_xO_y , e.g. FeO and/or Fe_2O_3 and/or Fe_3O_4), gallium oxide (Ga_2O_3), magnesium oxide (MgO), niobium oxide (Nb_2O_5), hafnium oxide (HfO_2), tantalum oxide (Ta_2O_5), lanthanum oxide (La_2O_3), zirconium dioxide (ZrO_2) and/or silicon dioxide (SiO_2). More preferred additional coating materials include iron
35 oxide, titanium dioxide, zinc sulphide and, more preferably, one or other or both of aluminium oxide (Al_2O_3) and/or silicon dioxide (SiO_2).

We prefer that the additional inorganic materials comprise one or more layers of single metal oxide (e.g. titanium oxide, silicon oxide or, preferably, aluminium oxide) are applied to the core before and/or after application of layers of mixed oxide as described herein.

5

Although the plurality of mixed oxide coated particles in accordance with the invention are essentially free of the aforementioned cracks in the applied coatings, through which active ingredient is potentially exposed (to, for example, the elements), two further, optional steps may be applied to the plurality of coated particles prior to subjecting it to further pharmaceutical formulation processing.

10

The first optional step may comprise, subsequent to the final deagglomeration step as hereinbefore described, application of a final overcoating layer, the thickness of which outer 'overcoating' layer/coating, or 'sealing shell' (which terms are used herein interchangeably), must be thinner than the previously-applied separate layers/coatings/shells (or 'subshells').

15

The thickness may therefore be on average no more than a factor of about 0.7 (e.g. about 0.6) of the thickness of the widest previously-applied subshell. Alternatively, the thickness may be on average no more than a factor of about 0.7 (e.g. about 0.6) of the thickness of the last subshell that is applied, and/or may be on average no more than a factor of about 0.7 (e.g. about 0.6) of the average thickness of all of the previously-applied subshells. The thickness may be on average in the region of about 0.3 nm to about 10 nm, for particles up to about 20 μm . For larger particles, the thickness may be on average no more than about 1/1000 of the coated particles' weight-, number-, or volume-based mean diameter.

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The role of such as sealing shell is to provide a 'sealing' overcoating layer on the particles, covering over those cracks, so giving rise to particles that are not only completely covered by that sealing shell, but also covered in a manner that enables the particles to be deagglomerated readily (e.g. using a non-aggressive technique, such as vortexing) in a manner that does not destroy the subshells that have been formed underneath, prior to, and/or during, pharmaceutical formulation.

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For the reasons described herein, it is preferred that the sealing shell does not comprise zinc oxide. The sealing shell may on the other hand comprise silicon dioxide or, more preferably, aluminium oxide.

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The second optional step may comprise ensuring that the few remaining particles with broken and/or cracked shells/coatings are subjected to a treatment in which all particles are suspended in a solvent in which the active ingredient thereof is soluble (e.g. with a solubility of at least about 0.1 mg/mL), but the least soluble material in
5 the mixed oxide coating is insoluble (e.g. with a solubility of no more than about 0.1 µg/mL), followed by separating solid matter particles from solvent by, for example, centrifugation, sedimentation, flocculation and/or filtration, resulting in mainly intact particles being left.

10 The above-mentioned optional step provides a means of potentially reducing further the likelihood of a (possibly) undesirable initial peak (burst) in plasma concentration of active ingredient, as discussed hereinbefore.

At the end of the process, coated particles may be dried using one or more of the
15 techniques that are described hereinbefore for drying cores. Drying may take place in the absence, or in the presence, of one or more pharmaceutically-acceptable excipients (e.g. a sugar or a sugar alcohol).

Alternatively, at the end of the process, separated particles may be resuspended in a
20 solvent (e.g. water, with or without the presence of one or more pharmaceutically acceptable excipients as defined herein), for subsequent storage and/or administration to patients.

Prior to applying the first layer of coating material or between successive coatings,
25 cores and/or partially coated particles may be subjected to one or more alternative and/or preparatory surface treatments. In this respect, one or more intermediary layers comprising different materials (i.e. other than the inorganic material(s)) may be applied to the relevant surface, e.g. to protect the cores or partially-coated particles from unwanted reactions with precursors during the coating step(s)/deposition
30 treatment, to enhance coating efficiency, or to reduce agglomeration.

An intermediary layer may, for example, comprise one or more surfactants, with a view to reducing agglomeration of particles to be coated and to provide a hydrophilic surface suitable for subsequent coatings. Suitable surfactants in this regard include well known
35 non-ionic, anionic, cationic or zwitterionic surfactants, such as the Tween series, e.g. Tween 80. Alternatively, cores may be subjected to a preparatory surface treatment if the active ingredient that is employed as part of (or as) that core is susceptible to

reaction with one or more precursor compounds that may be present in the gas phase during the coating (e.g. the ALD) process.

5 Application of 'intermediary' layers/surface treatments of this nature may alternatively be achieved by way of a liquid phase non-coating technique, followed by a lyophilisation, spray drying or other drying method, to provide particles with surface layers to which coating materials may be subsequently applied.

10 Outer surfaces of particles of formulations of the invention may also be derivatized or functionalized, e.g. by attachment of one or more chemical compounds or moieties to the outer surfaces of the final layer of coating material, e.g. with a compound or moiety that enhances the targeted delivery of the particles within a patient to whom the nanoparticles are administered. Such a compound may be an organic molecule (such as PEG) polymer, an antibody or antibody fragment, or a receptor-binding protein or
15 peptide, etc.

Alternatively, the moiety may be an anchoring group such as a moiety comprising a silane function (see, for example, Herrera *et al.*, *J. Mater. Chem.*, **18**, 3650 (2008) and US 8,097,742). Another compound, e.g. a desired targeting compound may be
20 attached to such an anchoring group by way of covalent bonding, or non-covalent bonding, including hydrogen bonding, or van der Waals bonding, or a combination thereof.

25 The presence of such anchoring groups may provide a versatile tool for targeted delivery to specific sites in the body. Alternatively, the use of compounds such as PEG may cause particles to circulate for a longer duration in the blood stream, ensuring that they do not become accumulated in the liver or the spleen (the natural mechanism by which the body eliminates particles, which may prevent delivery to diseased tissue).

30 Cores coated with a mixed oxide coating, whether in the form of separate, discrete layers, coatings or shells or otherwise, as defined herein are referred to hereinafter as 'the coated particles of the formulation of the invention'.

35 According to a further aspect of the invention, coated particles of the formulation of the invention comprise a plurality of particles:

- (a) having a weight-, number-, or volume-based mean diameter that is between about 10 nm and about 700 μm ; and

(b) comprise solid cores comprising one or more GLP1RAs, or pharmaceutically-acceptable salts thereof, coated, at least in part, by a coating of inorganic material comprising mixture of:

(i) zinc oxide (ZnO); and

5 (ii) one or more other metal and/or metalloid oxides,
wherein the atomic ratio ((i):(ii)) is at least about 1:10 and up to and including about 10:1.

10 For the avoidance of doubt, all aspects, including preferred aspects, disclosed and/or claimed herein for formulations of the invention are equally applicable as aspects and/or preferences for the above-described coated particles of the formulation of the invention. For the further avoidance of doubt, such aspects, preferences and features, alone or in combination, are hereby incorporated by reference to this aspect of the invention.

15 Pharmaceutical (or veterinary) formulations of the invention may include particles of different types, for example particles comprising different functionalization (as described hereinbefore), particles of different sizes, and/or different thicknesses of the layers of mixed oxide coating materials, or a combination thereof. By combining, in a
20 single pharmaceutical formulation, particles with different coating thicknesses and/or different core sizes, the drug release following administration to patient may be controlled (e.g. varied or extended) over a specific time period.

Formulations of the invention may be administered systemically, for example by
25 infusion, intravenously or intraarterially (including by intravascular or other perivascular devices/dosage forms (e.g. stents)), intraosseously, intracerebrally, intracerebroventricularly, intrasynovially, intrasternally, intrathecally, intralesionally, intracranially, intratumorally, cutaneously, intracutaneously, transdermally or, most preferably by injection, for example intramuscularly or, preferably, subcutaneously, in
30 the form of a pharmaceutically- (or veterinarily) acceptable dosage form.

The preparation of formulation of the invention comprises incorporation of coated particles as described herein into an appropriate pharmaceutically-acceptable (by which we include physiologically-acceptable) carrier system, and may be achieved with
35 due regard to the intended route of administration and standard pharmaceutical practice. Thus, appropriate excipients (including physiologically-acceptable injectable, e.g. physiologically-acceptable, intramuscularly-injectable or, more preferably, physiologically-acceptable, subcutaneously-injectable excipients as mentioned herein)

should be chemically inert to the active agent that is employed, and have no detrimental side effects or toxicity under the conditions of use. Such pharmaceutically-acceptable carriers may also impart an immediate, or a modified, release of active agent from the particles of the formulations of the invention.

5

Thus, the pharmaceutically-acceptable carrier system in accordance with the invention may be an oleaginous, or oil-based system. Carrier systems may therefore comprise one or more pharmaceutically- or veterinarily-acceptable liquid lipid, which may include fixed oils, such as mono-, di- or triglycerides, including Miglyol™ (e.g. 812N),
10 propylene glycol dicaprylocaprate (Miglyol 840, C8/C10 esters), tricaprylin (Miglyol oil), Gelucire™ 43/01, Kollisolv™ GTA, Labrafil™. The carrier systems may also comprise polysorbates, such as polysorbate 20, polysorbate 60, polysorbate 80, glycols, such as propylene glycol, polyethylene glycol, polyethylene glycol 300, polyethylene glycol 400, polyethylene glycol 600, and/or natural and/or refined pharmaceutically-acceptable
15 oils, such as olive oil, peanut oil, soybean oil, corn oil, cottonseed oil, sesame oil, castor oil, oleic acid, and their polyoxyethylated versions (e.g. sorbitan trioleate, lauroglycol 90, Capryol™ PGMC, PEG-60 hydrogenated castor oil, polyoxyl 35 castor oil). Non-lipid oils such as ethyl lactate, perfluorohexyloctane (F6H8), etc. may also be used. More preferred carrier systems include mono-, di- and/or triglycerides, wherein most
20 preferred is medium chain triglycerides, such as alkyl chain triglycerides (e.g. C₆-C₁₂ alkyl chain triglycerides).

Sterile aqueous suspensions of the particles of the formulation of the invention may be formulated according to techniques known in the art. The aqueous media should
25 contain at least about 50% water, but may also comprise other aqueous excipients, such as Ringer's solution, and may also include polar co-solvents (e.g. ethanol, glycerol, propylene glycol, 1,3-butanediol, polyethylene glycols of various molecular weights and tetraglycol); viscosity-increasing, or thickening, agents (e.g. carboxymethylcellulose, microcrystalline cellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethyl
30 hydroxyethyl cellulose, sodium starch glycolate, Poloxamers, such as Poloxamer 407, polyvinylpyrrolidone, cyclodextrins, such as hydroxypropyl-β-cyclodextrin, polyvinylpyrrolidone and polyethylene glycols of various molecular weights); surfactant/wetting agents to achieve a homogenous suspension (e.g. sorbitan esters, sodium lauryl sulfate; monoglycerides, polyoxyethylene esters, polyoxyethylene alkyl
35 ethers, polyoxylglycerides and, preferably, Tweens (Polysorbates), such as Tween 80 and Tween 20). Preferred ingredients include isotonicity-modifying agents (e.g. sodium lactate, dextrose and, especially, sodium chloride); pH adjusting and/or buffering agents (e.g. citric acid, sodium citrate, and especially phosphate buffers, such

as disodium hydrogen phosphate dihydrate, sodium acid phosphate, sodium dihydrogen phosphate monohydrate, and combinations thereof, which may be employed in combination with standard inorganic acids and bases, such as hydrochloric acid and sodium hydroxide); as well as other ingredients, such as mannitol, croscarmellose sodium and hyaluronic acid.

Formulations of the invention may further be formulated in the form of injectable suspension of coated particles with a size distribution that is both even and capable of forming (or re-forming following an appropriate degree of agitation) a stable suspension within the injection liquid (i.e. without settling), such that it may be injected through a needle. In this respect, the formulations of the invention may comprise inactive ingredients that may prevent premature 'caking' (i.e. forming a solid or semi-solid, non-dispersable residue), or gelling (e.g. hydrogel formation), within the formulations, by which we mean that the formulation is viscous enough to prevent sedimentation, leading to suspensions that are not 'homogeneous' and thus the risk of under or overdosing of active ingredient, or at the minimum that it is possible to redisperse the formulation to form a sufficiently homogenous dispersal prior to administering it.

With the above requirements in mind, formulations of the invention that comprise an aqueous carrier system in which the coated particles are suspended may further comprise:

- i. a physiologically-acceptable injectable (e.g. intramuscularly-injectable and/or, more preferably, subcutaneously-injectable) compound that is capable of reacting with zinc and, in doing so, reduces (e.g. essentially prevents) the reaction of zinc with water to form zinc hydroxide. Such a compound may, for example, comprise a compound that, when dissolved in the aqueous carrier, provides a source of counter-ions, which counter-ions are capable of forming a compound (e.g. salt) with zinc that is, for example, essentially insoluble in said aqueous media (at any given temperature, pressure and pH); and
- ii. a physiologically-acceptable injectable (e.g. intramuscularly-injectable and/or, more preferably, subcutaneously-injectable) buffer system that either:
 1. is essentially incapable of forming a compound (e.g. salt) with zinc; or
 2. comprises a source of counter-ions, which counter-ions are capable of forming a compound (e.g. salt) with zinc that is more soluble in said aqueous media than zinc hydroxide ($\text{Zn}(\text{OH})_2$).

We have found that, when coated particles are presented in aqueous media at medium to high concentrations (e.g. corresponding to between at least about 5 mg (e.g. at least about 10 mg) of the at least one GLP1RA per mL of carrier, for example between about 25 mg and about 200 mg of active agent per mL of carrier, such as between
5 about 30 mg/mL and about 150 mg/mL, e.g. between about 40 mg/mL and about 100 mg/mL), such as about 50 mg/mL) with coatings (and/or with at least the outermost layer of coating material) comprising zinc oxide (for example at least about 1 mg, such as at least about 5 mg, including at least about 10 mg of zinc content in the coated particles per mL of aqueous carrier), viscous agglomerates and/or hydrogels may be
10 formed, which have a tendency to clog needles when attempting to inject formulations e.g. subcutaneously or intramuscularly.

We believe that the unexpected formation of agglomerates and/or hydrogels results from the reaction of zinc in the coating with water to form zinc hydroxide, giving rise
15 to a suspension that is both unstable and uninjectable.

We have found that this unforeseen problem may be solved by the addition of a compound that competes with with water to react with zinc. Such a compound is thus capable of reacting with zinc and, in doing so, reduced and/or substantially prevents
20 (e.g. prevents up to about 75% of, such about 80% of, including up to about 90% of, such as about 95% of, and even up to about 99% of) the reaction of zinc with water to form zinc hydroxide.

Such a compound may for example provide a source of counter-ions to the aqueous carrier system that, when included (e.g. dissolved) in the latter, may form compounds
25 (e.g. salts) with zinc that serve to prevent the above-mentioned reaction of free zinc with water. Such counter-ions may achieve this by, for example, complexing with zinc in some way, and/or by being essentially insoluble in water and/or precipitating out of aqueous solution, which competing reactions may occur at or near the surfaces of
30 coated particles. Whatever the mechanism involved, in competing with water to react with zinc, further reaction of zinc with water (and therefore gelling) is reduced and/or prevented.

The term 'essentially insoluble' in aqueous media (such as pure water) includes
35 compounds (e.g. salts) of zinc that are sparingly soluble in such media, such as those with a solubility that is less than about 33.3 mg/mL, such as less than about 25 mg/mL, including less than about 20 mg/mL and particularly less than about 10 mg/mL, such as less than about 5 mg/mL, down to less than about 1 mg/mL and including less than

about 0.1 mg/mL, at atmospheric pressure (e.g. about 1 bar), room temperature (e.g. about 21°C) and neutral pHs (e.g. pH values between about 5 and about 9, such as about 6 and about 8.5, such as between about 7 and about 8 (e.g. about 7.4).

5 It is preferred that the solubility of the relevant compound (e.g. salt) of zinc in aqueous media (such as pure water) is less (e.g. at least about 10% less, such as at least about 5% less) than that of zinc hydroxide ($\text{Zn}(\text{OH})_2$) at any given temperature, pressure and pH.

10 Appropriate counter-ions that possess the aforementioned properties in this respect include aspartate, tartrate, maleate, fumarate, malate, benzoate and, preferably, phosphate counter-ions. Appropriate sources of such counter-ions include materials that are capable of forming aspartate-, tartrate-, maleate-, fumarate-, malate-, benzoate- and/or phosphate-based buffers, such as: aspartic acid, aspartate salts (e.g.
15 sodium aspartate) and hydrates thereof, and mixtures of these components; tartaric acid, tartrate salts (sodium tartrate) and hydrates thereof (e.g. dibasic hydrate), and mixtures of these components; maleic acid, maleate salts (e.g. sodium maleate) and hydrates thereof, and mixtures of these components; fumaric acid, fumarate salts (e.g. monosodium fumarate) and hydrates thereof, and mixtures of these components;
20 malic acid, malate salts and hydrates thereof, and mixtures of these components; benzoic acid, benzoate salts (e.g. sodium benzoate) and hydrates thereof, and mixtures of these components; and, more especially, phosphate buffers (such as phosphoric acid, disodium hydrogen phosphate dihydrate, sodium acid phosphate, sodium dihydrogen phosphate monohydrate, and combinations thereof).

25 Alternative sources of phosphate counter-ions include salts such as sodium phosphate, potassium phosphate and calcium phosphate. Sources may also include sources of organophosphates (e.g. glycerol phosphate, sodium glycerophosphate and potassium glycerophosphate), as well as pyrophosphates and polyphosphates. Alternative sources
30 of tartrate counter-ions include potassium tartrate, diethyl tartrate and disodium tartrate. Alternative sources of benzoate counter-ions include sodium benzoate, benzyl benzoate, denatonium benzoate and potassium benzoate.

Appropriate concentrations of phosphate counter-ions in the aqueous media of
35 formulations of the invention may be in the range of about 1 mM, such as about 2 mM up to about 50 mM, including about 40 mM, such as about 3 mM up to about 35 mM, e.g. between about 4 mM (e.g. about 5 mM) and about 30 mM (such as about 25 mM, including about 20 mM, about 15 mM and about 10 mM). (The recommended upper

concentration limit for of phosphate buffer in e.g. subcutaneously injectable compositions is 10 mM; see Usach *et al*, *Adv. Ther.*, **36**, 2986 (2019).)

Although the above-mentioned sources of counter-ions capable of forming a zinc compound (e.g. salt) may prevent the gelling issue mentioned herein, we have unexpectedly found that this may result in a change in the pH of the resultant formulation (particularly over time, e.g. during storage). This is thought to arise from the reaction of the above-mentioned counter-ion(s) (e.g. phosphate ions) with zinc depleting the buffering capacity of the relevant sources of counter-ions (e.g. buffer(s)).

10

We have found that this problem may be solved by the inclusion of a physiologically-acceptable injectable (e.g. intramuscularly-injectable or, more preferably, subcutaneously-injectable) buffer system that either:

- comprises a source of counter-ions that are capable of forming a salt with zinc that is more soluble in aqueous media than zinc hydroxide ($\text{Zn}(\text{OH})_2$), or, more preferably,
- is essentially incapable of forming a salt with zinc.

15

We have found that the presence of such buffers, when combined with the aforementioned other source of counter-ions, counter the above-described effect of depletion of the buffering capacity of the latter, and thus serves to maintain a constant (e.g. physiologically-acceptable) pH within a formulation of the invention. By 'maintaining a constant pH' within a formulation of the invention, we have found that, during storage, there is a mean a variation of pH that is less than $\pm 20\%$, such as less than $\pm 10\%$, including less than $\pm 5\%$, when compared to the pH that is as measured immediately following preparation a formulation of the invention.

20

Buffers that comprise a source of counter-ions that are capable of forming a salt with zinc that is more soluble in aqueous media than zinc hydroxide, include citrate buffers (e.g. citric acid, trisodium citrate dihydrate and combinations thereof), acetate buffers (e.g. acetic acid, sodium acetate and combinations thereof), lactate buffers (e.g. lactic acid, magnesium lactate and combinations thereof), gluconate buffers (e.g. gluconic acid, sodium gluconate and combinations thereof), glutamate buffers (e.g. glutamic acid, monosodium glutamate and combinations thereof), succinate buffers (e.g. succinic acid, sodium succinate and combinations thereof), α -ketoglutarate buffers (α -ketoglutaric acid, α -ketoglutarate salts and combinations thereof), ascorbate buffers (e.g. ascorbic acid, sodium ascorbate and combinations thereof), bicarbonate buffers (e.g. carbonic acid, sodium bicarbonate and combinations thereof), ammonium buffers

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(e.g. ammonium chloride, ammonium hydroxide and combinations thereof), glycine buffers (e.g. glycine, sodium glycinate and combinations thereof) or combinations of any of the above.

- 5 Buffers that are essentially incapable of forming a salt with zinc include histidine, diethanolamine (e.g. diethanolamine, magnesium chloride hexahydrate and combinations thereof) or, most preferably, tromethamine ('Tris' or 'Trizma'), buffers.

10 All of the aforementioned buffers may be employed, alone or in combination with along with standard inorganic acids and bases, such as hydrochloric acid and sodium hydroxide, which may be used in order to adjust pH. Preferred pHs for formulations of the invention may be in the pH range of about pH 3 and about pH 10, such as about pH 4 and pH 9, including pH 5 and about pH 8.

- 15 Appropriate concentrations of such buffers (e.g. tromethamine buffers) are in the range of about 0.1 mM (such as about 5 mM, including about 10 mM) up to about 200 mM, such as about 25 mM up to about 175 mM, for example between about 50 mM and about 150 mM, including between about 75 mM and about 125 mM (e.g. about 100 mM).

20

Formulations of the invention may also comprise a carrier system that is a mixture of one or more oils and an aqueous system, for example an emulsion, such as an oil-in-water emulsion or a water-in-oil emulsion.

- 25 In (particularly oil-based) carriers, suitable dispersing or wetting agents (e.g. Tweens, such as Tween 80), and/or suspending agents may be employed.

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In (particularly oil-based) carriers, suitable dispersing or wetting agents (e.g. Tweens, such as Tween 80), and/or suspending agents may be employed.

- 35 Formulations may thus be stored under normal storage conditions, and maintain their physical and/or chemical integrity. The phrase 'maintaining physical and chemical integrity' essentially means chemical stability and physical stability.

By 'chemical stability', we include that any formulation of the invention may be stored (with or without appropriate pharmaceutical packaging), under normal storage conditions, with an insignificant degree of chemical (including stereochemical) degradation or decomposition of any active ingredient (particularly any GLP1RA) and/or inert excipient, and/or the aforementioned changes in pH.

By 'physical stability', we include that the any formulation of the invention may be stored (with or without appropriate pharmaceutical packaging), under normal storage conditions, with an insignificant degree of physical transformation, such as sedimentation as described above, or changes in the nature and/or integrity of the coated particles, for example in the coating itself or the active ingredient (including dissolution, solvatisation, solid state phase transition, etc.).

Examples of 'normal storage conditions' for formulations of the invention include temperatures of between about -50°C and about +80°C (preferably between about -25°C and about +75°C, such as about 50°C), and/or pressures of between about 0.1 and about 2 bars (preferably atmospheric pressure), and/or exposure to about 460 lux of UV/visible light, and/or relative humidities of between about 5 and about 95% (preferably about 10 to about 40%), for prolonged periods (i.e. greater than or equal to about twelve, such as about six months).

Under such conditions, formulations of the invention may be found to be less than about 15%, more preferably less than about 10%, and especially less than about 5%, chemically and/or physically degraded/decomposed, as appropriate. The skilled person will appreciate that the above-mentioned upper and lower limits for temperature and pressure represent extremes of normal storage conditions, and that certain combinations of these extremes will not be experienced during normal storage (e.g. a temperature of 50°C and a pressure of 0.1 bar).

Formulations of the invention may comprise between about 1% to about 99%, such as between about 10% (such as about 20%, e.g. about 50%) to about 90% by weight of the coated particles with the remainder made up by carrier system and/or other pharmaceutically-acceptable excipients.

Formulations of the invention may be in the form of a liquid, a sol or a gel, which is administrable *via* a surgical administration apparatus, e.g. a needle, a catheter or the like, to form a depot formulation.

In any event, the preparation of suitable formulations may be achieved non-inventively by the skilled person using routine techniques. Formulations of the invention and dosage forms comprising them, may thus be formulated with conventional pharmaceutical additives and/or excipients used in the art for the preparation of pharmaceutical formulations, and thereafter incorporated into various kinds of pharmaceutical preparations and/or dosage forms using standard techniques (see, for example, Lachman *et al.*, '*The Theory and Practice of Industrial Pharmacy*', Lea & Febiger, 3rd edition (1986); '*Remington: The Science and Practice of Pharmacy*', Troy (ed.), University of the Sciences in Philadelphia, 21st edition (2006); and/or '*Aulton's Pharmaceutics: The Design and Manufacture of Medicines*', Aulton and Taylor (eds.), Elsevier, 4th edition, 2013), and the documents referred to therein, the relevant disclosures in all of which documents are hereby incorporated by reference.

According to a further aspect of the invention there is provided a process for the preparation of a formulation of the invention which comprises mixing together the coated particles as described herein with the aqueous carrier system, for example as described herein.

For intramuscular or, especially, subcutaneous injections, the formulations of the invention may be presented in the form of sterile injectable dosage forms administrable *via* a surgical administration apparatus (e.g. a syringe with a needle for injection, a catheter or the like), to form a depot formulation.

Particularly preferred administration apparatuses include autoinjectors, which will be known to those skilled in the art as a type of medical device that is specifically designed for a patient to self-administer a pre-determined dose of a drug, which are typically single use, disposal, spring-loaded syringes, and include any of those presently known in the art for the administration of GLP1RAs.

There is thus further provided an injectable dosage form comprising a formulation of the invention, wherein said formulation is contained within a reservoir that is connected to, and/or is associated with, an injection means (e.g. a syringe with a needle for injection or the like).

Alternatively, formulations of the invention can be stored prior to being loaded into a suitable injectable dosing means (e.g. a syringe with a needle for injection), or may even be prepared immediately prior to loading into such a dosing means.

Sterile injectable dosage forms may thus comprise a receptacle or a reservoir in communication with an injection means into which a formulation of the invention may be pre-loaded, or may be loaded at a point prior to use, or may comprise one or more reservoirs, within which coated particles of the formulation of the invention and the aqueous carrier system are housed separately, and in which admixing occurs prior to and/or during injection.

There is thus further provided a kit of parts comprising:

- (a) coated particles of the formulation of the invention; and
 - (b) a carrier system of the formulation of the invention,
- as well as a kit of parts comprising coated particles of the formulation of the invention along with instructions to the end user to admix those particles with a carrier system according to the invention.

There is further provided a pre-loaded injectable dosage form as described herein above, but modified by comprising at least two chambers, within one of which chamber is located the coated particles of the formulation of the invention and within the other of which is located the aqueous carrier system of the formulation of the invention, wherein admixing, giving rise to a suspension or otherwise, occurs prior to and/or during injection.

Formulations of the invention may be used in human medicine. Formulations of the invention are particularly useful in any indication in which GLP1RAs are either approved for use in, or otherwise known to be useful in. In particular, formulations of the invention are useful in the treatment of metabolic conditions or disorders.

The term 'metabolic condition or disorder' will be understood to include endocrine diseases and/or disorders, and thus disorders of glands of the endocrine system (including the adrenal gland, the thyroid gland, the pituitary gland and, in particular, the pancreas), and thus includes all types of diabetes, such as hyperglycaemia, type 1 diabetes, type 2 diabetes, impaired glucose tolerance, non-insulin dependent diabetes, maturity onset diabetes of the young, gestational diabetes; conditions associate with weight and/or over-eating, such as obesity, which can be induced by force of habit, or by extraneous sources, such as drugs such as steroids and antipsychotic, as well as binge eating, bulimia nervosa. The term may also include the medical term 'metabolic syndrome'.

5 A human subject suffering from obesity (an obese subject) may have a body mass index (BMI; calculated as a person's weight in kilograms divided by the square of that subject's height in meters) of at least about 25, such as at least about 27, including at least about 30, for example in the range of about 30 to about 40 (e.g. about 35) or even greater than 40.

10 Preferred metabolic conditions include type 2 diabetes and obesity, which, as alluded to above, are often regarded as co-morbidities. In addition to diabetes (such as type 2 diabetes), excess weight-related comorbidities that may also be treated by formulations of the invention include hypertension, dyslipidaemia, high cholesterol, and obstructive sleep apnoea.

15 Formulations of the invention are indicated in the therapeutic, palliative, and/or diagnostic treatment, as well as the prophylactic treatment (by which we include preventing and/or abrogating deterioration and/or worsening of a condition) of any of the above metabolic disorders or conditions.

20 This may include delaying or preventing diabetic disease progression, such as progression of type 2 diabetes, delaying the progression of impaired glucose tolerance (IGT) to insulin-requiring type 2 diabetes, and/or delaying the progression of non-insulin-requiring type 2 diabetes to insulin-requiring type 2 diabetes. It may also include decreasing food intake, reducing body weight, suppressing appetite, inducing satiety, reduction of gastric motility and/or delaying gastric emptying.

25 Formulations of the invention may also be useful in the treatment and/or the prevention (which, as stated above, may include abrogating deterioration and/or worsening) of conditions, disorders or diseases that may be consequential of a metabolic condition or disorder, including hypertension and more serious adverse cardiovascular events, such as myocardial infarction, stroke, angina pectoris, heart failure and other cardiovascular disorders; non-alcoholic fatty liver diseases, such as non-alcoholic fatty liver and non-alcoholic steatohepatitis; as well as other neurodegenerative conditions such as Alzheimer's disease and Parkinson's disease, and other conditions, including nerve damage, skin disorders such as chronic diabetic ulcers, diabetic ketoacidosis, eye conditions such as retinopathy and/or kidney problems (such as chronic kidney failure), such as nephropathy, gum disease and/or mouth problems, cancer and/or sexual dysfunction.

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In the treatment of any of the above conditions, GLP1RAs may be combined with other treatments that are known to be useful in the treatment of the relevant conditions.

This includes type 2 diabetes receptor analogues, for example GIP, glucagon, sodium glucose co-transporter, amylin and oxyntomodulin/peptide YY, as well as known diabetes treatments, such as insulin, biguanides (such as buformin, phenformin and, particularly, metformin), sulphonylureas (e.g. tolbutamide, acetohexamide, tolazamide, chlorpropamide, glipizide, glibenclamide, glimepiride, gliclazide, glyclopyramide and gliquidone), alpha-glucosidase inhibitors (e.g. miglitol, voglibose and acarbose), prandial glucose regulators (such as repaglinide and nateglinide), peroxysome proliferator activated receptor γ (PPARs or glitazones (such as rosiglitazone, troglitazone or pioglitazone), dipeptidyl peptidase-4 (DPP-4) inhibitors (gliptins, such as vildagliptin, sitagliptin, saxagliptin, linagliptin, sepagliptin, teneligliptin, gemigliptin and alogliptin) and/or SGLT2 inhibitors (such as dapagliflozin, canagliflozin, empagliflozin and remogliflozin).

It also includes known obesity treatments, such as orlistat, cetilistat, lorcaserin, sibutramine, rimonabant, metformin, berberine, forskolin, and combinations such as amylin/pramlinatide, phentermine/topiramate, naltrexone/bupropion, superabsorbent hydrogels (e.g. Gelesis100), and/or statins.

Formulations of the invention (with or without the above co-therapies) may also be used in conjunction with blood glucose meters and continuous glucose monitors, and/or accompany more invasive techniques, such as islet cell transplantation or weight loss surgery.

In addition, as described below, we have found that injection of formulations of the invention may cause a mild inflammatory response. Such a response may be alleviated by co-administration with an antiinflammatory agent that is suitable for injection.

Appropriate antiinflammatory agents that may be employed in this regard include butylpyrazolidines (such as phenylbutazone, mofebutazone, oxyphenbutazone, clofezone, kebuzone and suxibuzone); acetic acid derivatives and related substances (indomethacin, sulindac, tolmetin, zomepirac, diclofenac, alclofenac, bumadizone, etodolac, lonazolac, fentiazac, acetaminophen, difenpiramide, oxametacin, proglumetacin, ketorolac, aceclofenac and buprenorphine); oxycams (such as piroxicam, tenoxicam, droxicam, lornoxicam and meloxicam); propionic acid derivatives (such as ibuprofen, naproxen, ketoprofen, fenoprofen, fenbufen, benoxaprofen, suprofen, piroprofen,

flurbiprofen, indoprofen, tiaprofenic acid, oxaprozin, ibuprofen, dexibuprofen, flunoxaprofen, alminoprofen, dexketoprofen, vedaprofen, carprofen and tepoxalin); fenamates (such as mefenamic acid, tolfenamic acid, flufenamic acid, meclofenamic acid and flunixin), coxibs (such as celecoxib, rofecoxib, valdecoxib, parecoxib, etoricoxib, lumiracoxib, firocoxib, robenacoxib, mavacoxib and cimicoxib); other non-steroidal antiinflammatory agents (such as nabumetone, niflumic acid, azapropazone, glucosamine, benzydamine, glucosaminoglycan polysulfate, proquazone, orgotein, nimesulide, feprazone, diacerein, morniflumate, tenidap, oxaceprol, chondroitin sulfate, pentosan polysulfate and aminopropionitrile); corticosteroids (such as 11-dehydrocorticosterone, 11-deoxycorticosterone, 11-deoxycortisol, 11-ketoprogesterone, 11 β -hydroxypregnenolone, 11 β -hydroxyprogesterone, 11 β ,17 α ,21-trihydroxypregnenolone, 17 α ,21-dihydroxypregnenolone, 17 α -hydroxypregnenolone, 17 α -hydroxyprogesterone, 18-hydroxy-11-deoxycorticosterone, 18-hydroxycorticosterone, 18-hydroxyprogesterone, 21-deoxycortisol, 21-deoxycortisone, 21-hydroxypregnenolone (prebediolone), aldosterone, corticosterone (17-deoxycortisol), cortisol (hydrocortisone), cortisone, pregnenolone, progesterone, flugestone (flurogestone), fluorometholone, medrysone (hydroxymethylprogesterone), prebediolone acetate (21-acetoxypregnenolone), chloroprednisone, cloprednol, difluprednate, fludrocortisone, fluocinolone, fluperolone, fluprednisolone, loteprednol, methylprednisolone, prednicarbate, prednisolone, prednisone, tixocortol, triamcinolone, alclometasone, beclometasone, betamethasone, clobetasol, clobetasone, clocortolone, desoximetasone, dexamethasone, diflorasone, difluocortolone, flucolorolone, flumetasone, fluocortin, fluocortolone, fluprednidene, fluticasone, fluticasone furoate, halometasone, meprednisone, mometasone, mometasone furoate, paramethasone, prednylidene, rimexolone, ulobetasol (halobetasol), amcinonide, budesonide, ciclesonide, deflazacort, desonide, formocortol flucolorolone acetonide (flucoloronide), fludroxycortide (flurandrenolone, flurandrenolide), flunisolid, fluocinolone acetonide, fluocinonide, halcinonide and triamcinolone acetonide); quinolines (such as oxycinchophen); gold preparations (such as sodium aurothiomalate, sodium aurothiosulfate, auranofin, aurothioglucose and aurotioprol); penicillamine and similar agents (such as bucillamine); and antihistamines (such as akrivastin, alimemazin, antazolin, astemizol, azatadin, azelastin, bamipin, bilastin, bromdifenhidramin, bromfeniramin, bukizin, cetirizin, cinnarizine, cyklizin, cyproheptadine, depropine, desloratadin, dexbromfeniramin, dexklorfeniramin, difenylpyralin, dimenhydrinat, dimetinden, doxylamin, ebastin, epinastin, fenindamin, feniramin, fexofenadin, histapyrrodin, hydroxietylprometazin, isotipendyl, karbinoxamin, ketotifen, kifenadin, klemastin, klorcyklizin, klorfenamin, klorfenoxamin, kloropyramin, levocetirizin, loratadin, mebhydrolin, mekitazin,

meklozin, mepyramin, metapyrilen, metdilazin, mizolastin, oxatomide, oxomemazine, pimetixen, prometazin, pyrrobutamin, rupatadin, sekifenadin, talastin, tenalidin, terfenadin, tiazinam, tietylperazin, tonzylamin, trimetobenzamid, tripelennamin, triprolidine and tritokvalin). Combinations of any one or more of the above mentioned
5 antiinflammatory agents may be used.

Preferred antiinflammatory agents include non-steroidal anti-inflammatory drugs, such as diclofenac, ketoprofen, meloxicam, aceclofenac, flurbiprofen, parecoxib, ketoralac tromethamine or indomethacin.

10

Subjects may receive (or may already be receiving) one or more of the aforementioned co-therapeutic and/or antiinflammatory agents, separate to a formulation of the invention, by which we mean receiving a prescribed dose of one or more of those other therapeutic agents, prior to, in addition to, and/or following, treatment with a
15 formulation of the invention.

When GLP1RAs/salts thereof are 'combined' with such other therapeutic agents, the active ingredients may be administered together in the same formulation, or administered separately (simultaneously or sequentially) in different formulations
20 (hereinafter referred to as 'combination products').

Such combination products provide for the administration of GLP1RA in conjunction with the other therapeutic agent, and may thus be presented either as separate formulations, wherein at least one of those formulations is a formulation of the
25 invention, and at least one comprises the other therapeutic agent in a separate formulation, or may be presented (i.e. formulated) as a combined preparation (i.e. presented as a single formulation including GLP1RA/salt and the other therapeutic agent).

In this respect another therapeutic agent may be co-presented with GLP1RA at an appropriate dose in one or more of the cores that form part of a formulation of the invention as hereinbefore described, or may be formulated using the same or a similar process for coating to that described hereinbefore for GLP1RAs, which may allow for the release of the other therapeutic agent over the same, or over a different timescale.
30

35

Thus, there is further provided a pharmaceutical formulation of the invention that further comprises a therapeutic agent that is useful in the treatment of a metabolic

condition, such as type 2 diabetes or obesity as hereinbefore defined, and/or an antiinflammatory agent.

5 Pharmaceutical formulations of the invention that may be mentioned include those that are does not comprise an antiinflammatory agent and/or are essentially free of said antiinflammatory agent. By 'essentially free of antiinflammatory agent', we mean that the formulation comprises less than about 1% by weight (e.g. less than about 0.5%, such as less than about 0.1% or about 0.01% by weight) of one or more of the
10 aforementioned antiinflammatory agents.

In such formulations of the invention, the further therapeutic agent may be included by:

- (1) formulating along with the GLP1RA within the solid cores of a formulation of the invention (which formulation is hereinafter referred to as a 'combined
15 core preparation'); or
- (2) dissolving it, and/or suspending it, within the aqueous carrier system of a formulation of the invention (which formulation is hereinafter referred to as a 'combination preparation').

20 In embodiment (2) above, the other therapeutic agent may be presented in a formulation of the invention in any form in which it is separate to the GLP1RA-containing cores. This may be achieved by, for example, dissolving or suspending that active ingredient directly in the aqueous medium of a formulation of the invention, or by presenting it in a form in which its release can, like the GLP1RA, also be controlled
25 following injection.

The latter option may be achieved by, for example, providing the other therapeutic agent in the form of additional particles suspended in the aqueous carrier system of formulation of the invention, which additional particles have a weight-, number-, or
30 volume-based mean diameter that is between about 10 nm and about 700 μm , and comprise cores comprising the therapeutic agent that is useful in the treatment of a metabolic disorder as hereinbefore defined, and/or the antiinflammatory agent, which cores are coated, at least in part, by one or more coating materials as hereinbefore described (which formulation is hereinafter referred to as a 'combination suspension').

35 There is further provided a pharmaceutical formulation of the invention that is in the form of a kit of parts comprising components:

- (A) a pharmaceutical formulation of the invention; and

(B) a pharmaceutical formulation, comprising a therapeutic agent that is useful in the treatment of a metabolic disorder as hereinbefore defined, and/or an antiinflammatory agent,
which Components (A) and (B) are each provided in a form that is suitable for
5 administration in conjunction with the other.

Although Component (B) of a kit of parts as presented above may be different in terms its chemical composition and/or physical form from Component (A) (i.e. a formulation of the invention), it may also be in a form that is essentially the same or at least similar
10 to a GLP1RA-containing formulation of the invention, that is in the form of a plurality of particles suspended in an (e.g. aqueous) carrier system, which particles:

- (a) have a weight-, number-, or volume-based mean diameter that is between about 10 nm and about 700 μm ; and
- (b) comprise solid cores comprising that other therapeutic agent, which cores are
15 coated, at least in part, by one or more coatings of (e.g. inorganic) material.

In addition, although, in such preferred kits of parts, and the combination suspensions presented under embodiment (2) above, the coated cores comprising the other therapeutic agent may be different in terms of their chemical composition(s) and/or
20 physical form(s), it is preferred that the coating of inorganic material that is employed is the same or similar to that employed in GLP1RA-containing formulations of the invention, which means that the other therapeutic agent is coated by one or more inorganic coatings as hereinbefore described, for example one or more inorganic coating materials comprising one or more metal-containing, or metalloid-containing,
25 compounds, such as a metal, or metalloid, oxide, for example iron oxide, titanium dioxide, zinc sulphide, more preferably zinc oxide, silicon dioxide and/or aluminium oxide, which coating materials may (on an individual or a collective basis) consist essentially (e.g. are greater than about 80%, such as greater than about, 90%, e.g. about 95%, such as about 98%) of such oxides, and more particularly inorganic
30 coatings comprising a mixture of:

- (i) zinc oxide; and
- (ii) one or more other metal and/or metalloid oxides,

wherein the atomic ratio ((i):(ii)) is at least about 1:6 and up to and including about 6:1.
35

Preferably, the atomic ratio ((i):(ii)) is at least about 1:1 and up to and including about 6:1.

In any event, and for the avoidance of doubt, all aspects, including preferred aspects, disclosed and/or claimed herein for in GLP1RA-containing formulations of the invention are equally applicable as aspects and/or preferences for coated cores comprising one or more of the further therapeutic agents described above. For the avoidance of doubt, such aspects, preferences and features, alone or in combination, are hereby incorporated by reference to these aspects of the invention.

All combination products, including combined core preparations, combination suspensions and kits of parts described above may thus be used in human medicine and, in particular, any indication in which GLP1Ras are either approved for use in, or otherwise known to be useful in, such as metabolic disorders or conditions as hereinbefore defined, such as type 2 diabetes and/or obesity.

In certain instances, such additional therapeutic agents, including some of those that are useful in the treatment of e.g. metabolic disorders or conditions as hereinbefore defined, such as type 2 diabetes and/or obesity, may be termed 'standard of care' in relation to a particular condition. The term 'standard of care' will be understood by the skilled person to include a treatment process that a clinician should, and/or is expected to, follow for certain types of patients, illnesses and/or clinical circumstances. In certain new or poorly-understood conditions, standard of care may change and/or develop over time.

According to a further aspect of the invention, there is provided a method of making a kit of parts as defined above, which method comprises bringing Component (A), as defined above, into association with a Component (B), as defined above, thus rendering the two components suitable for administration in conjunction with each other.

By bringing the two components 'into association with' each other, we include that Components (A) and (B) of the kit of parts may be:

- (i) provided as separate formulations (i.e. independently of one another), which are subsequently brought together for use in conjunction with each other in combination treatment; or
- (ii) packaged and presented together as separate components of a 'combination pack' for use in conjunction with each other in combination treatment.

Thus, there is further provided a kit of parts as hereinbefore defined in which Components (A) and (B) are packaged and presented together as separate components

of a combination pack, for use in conjunction with each other in combination treatment, as well as a kit of parts comprising:

(I) one of Components (A) and (B) as defined herein; together with

(II) instructions to use that component in conjunction with the other of the two components.

As alluded to above, the kits of parts described herein may comprise more than one formulation including an appropriate quantity/dose of GLP1RA/salt, and/or more than one formulation including an appropriate quantity/dose of the other therapeutic agent, in order to provide for repeat dosing as hereinbefore described.

In this respect, with respect to the kits of parts as described herein, by 'administration in conjunction with', we include that Components (A) and (B) of the kit are administered, sequentially, separately and/or simultaneously, over the course of treatment of the relevant condition.

Thus, the term 'in conjunction with' includes that one or other of the two formulations may be administered (optionally repeatedly) prior to, after, and/or at the same time as, administration of the other component. When used in this context, the terms 'administered simultaneously' and 'administered at the same time as' include that individual doses of GLP1RA/salt and other therapeutic agent are administered within 48 hours (e.g. 24 hours) of each other.

In respect of any of the above combination products according to the invention, the respective formulations are administered (or, in the case of the kit of parts, the two components are administered, optionally repeatedly, in conjunction with each other) in a manner that may enable a beneficial effect for the subject, that is greater, over the course of the treatment of the condition, than if a formulation (e.g. a formulation of the invention) comprising GLP1RA/salt alone is administered (e.g. repeatedly, as described herein) in the absence of the other component, over the same course of treatment.

Determination of whether a combination product provides a greater beneficial effect in respect of, and over the course of treatment will depend upon the condition to be treated and/or its severity, but may be achieved routinely by the skilled person.

For example, a physician may initially administer a formulation of the invention comprising alone to treat a patient with a metabolic disorder or condition, and then

find that that person exhibits an inflammatory response (which may be caused by the active ingredient *per se* and/or by any other component of the formulation).

The physician may then administer one or more of:

- 5
- Component (B) of a kit of parts as described above,
 - a combined core preparation,
 - a combination preparation, and/or
 - a combination suspension

as described above, any of which comprises an antiinflammatory agent as hereinbefore
10 described.

The other active ingredients/therapeutic agents mentioned above that may be employed in combination products according to the invention may be provided in the form of a (e.g. pharmaceutically-acceptable) salt, including any such salts that are
15 known in the art and described for the drugs in question to in the medical literature, such as *Martindale – The Complete Drug Reference*, 38th Edition, Pharmaceutical Press, London (2014) and the documents referred to therein (the relevant disclosures in all of which documents are hereby incorporated by reference).

The amount of the other active ingredient/therapeutic agent that may be employed in
20 combination products according to the invention must be sufficient so exert its pharmacological effect.

Doses of such other active ingredients that may be administered to a patient should thus be sufficient to affect a therapeutic response over a reasonable and/or relevant timeframe. One skilled in the art will recognize that the selection of the exact dose
25 and composition and the most appropriate delivery regimen will also be influenced by not only the nature of the other active ingredient, but also *inter alia* the pharmacological properties of the formulation, the route of administration, the nature and severity of the condition being treated, and the physical condition and mental acuity of the recipient, as well as the age, condition, body weight, sex and response of
30 the patient to be treated, and the stage/severity of the disease, as well as genetic differences between patients.

As administration of formulations of the invention may be continuous or intermittent (e.g. by bolus injection), dosages of such other active ingredients may also be
35 determined by the timing and frequency of administration.

In any event, the medical practitioner, or other skilled person, will be able to determine routinely the actual dosage of any particular additional active ingredient, which will be most suitable for an individual patient, and doses of the relevant additional active ingredients mentioned above include those that are known in the art and described for the drugs in question to in the medical literature, such as *Martindale – The Complete Drug Reference*, 38th Edition, Pharmaceutical Press, London (2014) and the documents referred to therein, the relevant disclosures in all of which documents are hereby incorporated by reference.

10 The use of formulations of the invention may control the dissolution rate of GLP1RAs and affect their pharmacokinetic profiles by reducing any burst effect as hereinbefore defined (e.g. a concentration maximum shortly after administration), and/or by reducing $C_{\max}$ in a plasma concentration-time profile.

15 Formulations of the invention may also provide a release and/or pharmacokinetic profile that increases the length of release of GLP1RAs from the formulation.

These factors not only reduce the frequency at or over which the formulation needs to be administered to a person suffering from a relevant metabolic condition or disorder, but also allows the sufferer more time as an out-patient, and so to have a better quality of life, as well as providing the advantage of fewer/less frequent inconvenient and/or painful injections.

25 The formulation of the invention also has the advantage that by controlling the release of active ingredient at a steady rate over a prolonged period of time, a lower daily exposure to the relevant active ingredient is provided, which is expected to reduce unwanted side effects.

30 The formulations and processes described herein may also have the advantage that, in the treatment of relevant metabolic conditions, they may be more convenient for the physician and/or patient than, be more efficacious than, be less toxic than, have a broader range of activity than, be more potent than, produce fewer side effects than, or that it may have other useful pharmacological properties over, any similar treatments known in the prior art.

35 Wherever the word 'about' is employed herein, for example in the context of amounts (e.g. numbers, concentrations, dimensions (sizes and/or weights), doses, time periods, pharmacokinetic parameters, etc.), relative amounts (percentages, weight ratios, size

ratios, atomic ratios, aspect ratios, proportions, factors, fractions, etc.), relative humidities, lux, temperatures or pressures, it will be appreciated that such variables are approximate and as such may vary by $\pm 15\%$, such as $\pm 10\%$, for example $\pm 5\%$ and preferably $\pm 2\%$ (e.g. $\pm 1\%$) from the numbers specified herein. This is the case even if such numbers are presented as percentages in the first place (for example 'about 15%' may mean $\pm 15\%$ about the number 10, which is anything between 8.5% and 11.5%).

The invention is illustrated, but in no way limited, by the following examples with reference to the attached figures in which Figure 1 shows coating integrity assay results for two batches of coated liraglutide microparticles, Figure 2 shows decrease in drug load of coated and uncoated particles following gamma irradiation, Figure 3 shows a semilog plot of mean plasma concentration (ng/mL) versus time (days) after single subcutaneous administration of liraglutide to rats in various formulations and dose levels, Figure 4 shows mean plasma concentrations in the same study over the initial 72 hours, Figure 5 shows *in vitro* release results for two batches of coated liraglutide microparticles, Figure 6 shows a semilog plot of mean plasma concentration (ng/mL) versus time (days) after single subcutaneous administration of liraglutide to minipigs in various formulations, Figure 7 shows *in vitro* release results for four batches of coated liraglutide microparticles, and Figures 8 and 9 show semilog plots of mean plasma concentration (ng/mL) versus time (Figure 8: 7 days, Figure 9: 42 days) after single subcutaneous administration of liraglutide to rats in various formulations.

Examples

Example 1

Coated Liraglutide Microparticles

R&D grade liraglutide (MedChemExpress, New Jersey, US) with a purity of 98.5% and a peptide content of 90.8% was suspended in a solution of 0.1% Span 85 (Sigma-Aldrich, MO, USA) in cyclohexane (Merck, Germany).

The particle size distribution was determined by means of laser diffraction (SALD-7500nano (Shimadzu, Japan), 405 nm laser) to be as follows: %D(10): 2.0 μm , %D(50): 7.3 μm , %D(90): 23.8 μm .

The raw material was spray dried in a Mini Spray Drier (B-290, with Dehumidifier B-296 with a Two-fluid nozzle; BÜCHI Labortechnik GmbH, Germany), by dispersing the

raw material in purified water ($0.8\text{--}2\text{ M}\Omega/\text{cm}^2$) to form a milky white liquid with a liraglutide concentration of 15 wt%.

5 The dispersion was spray dried with an inlet temperature of 115°C , aspiration rate 100% ($\sim 35\text{ m}^3/\text{h}$), pump rate 8% ($3.8\text{ mL}/\text{min}$), nozzle clean 2, volume flow 35 mm (N_2 , $\sim 600\text{ L}/\text{h}$), which gave an outlet temperature of 73°C . The mass yield was approximated to 77.5%.

10 The spray-dried material was assayed using a Nexera UPLC-UV-DAD (Shimadzu, Japan) with a SunShell nC18-WP, $4.6\times 100\text{ mm}$, $2.6\text{ }\mu\text{m}$ particle size column (Chromanik Technologies Inc., Japan) and was found to have a $100.1 \pm 0.2\%$ (relative) liraglutide content in the spray-dried material.

15 The particle size of the spray-dried material was determined as above as follows: %D(10): $1.7\text{ }\mu\text{m}$, %D(50): $8.2\text{ }\mu\text{m}$, %D(90): $21.2\text{ }\mu\text{m}$.

The spray-dried liraglutide particles were first coated with three layers of pure aluminium oxide by way of the following process:

- a. A valve on the piping between the pump and the ALD reactor was closed.
- 20 b. A valve on the trimethylaluminium (TMA) precursor bottle was then opened for 1 second, letting evaporated metal containing precursor fill the ALD reactor for 1 second.
- c. The valve to the precursor bottle was closed and before opening to the pump again the chamber rested for 30 seconds (soaking time) to ensure the metal containing
- 25 precursor vapour reacted with the surface of the drug particles.
- d. The ALD reactor was thereafter pumped for 9 seconds.
- e. Steps a-d above were repeated 20 times.

30 The chamber was then purged with nitrogen in a continuous flow to remove non-reacted reagents and organic gases. After that, steps (a) to (e) above were essentially repeated, with the exception that water was used as a second reagent to form a discrete aluminium oxide layer on the surfaces of the active ingredient microparticles. This was followed by a further purging pulse using nitrogen in a continuous flow, which was carried out to remove gaseous water and organic gases.

35

The above procedure was repeated twice to form a total of three initial aluminium oxide atomic layers.

Following this, three layers of zinc oxide were applied by repeating the above ALD steps using diethylzinc (DEZ), and then water, as precursors, in step (b) above to form a total of three zinc oxide layers. That was followed by coating with one layer of aluminium oxide, using the same precursors as mentioned above under (b). This provided a mixed oxide layer with an atomic ratio of 1:3 ($\text{Al}_2\text{O}_3:\text{ZnO}$) of a total of four atomic layers.

This was repeated a total of 9 times to form a mixed oxide (1:3 $\text{Al}_2\text{O}_3:\text{ZnO}$) layer with a total of 40 atomic layers (30 of ZnO and 10 of Al_2O_3).

The powder was then removed from the ALD reactor and deagglomerated using a sonic sifter (Tsutsui Sonic Agitated Sifting Machine SW-20AT) with a 20 μm mesh size sieve.

The resultant deagglomerated powder was re-loaded into the ALD reactor and the same procedure for coating the particles with a mixed oxide (1:3 $\text{Al}_2\text{O}_3:\text{ZnO}$) layer with a total of 40 atomic layers. The deagglomeration and coating steps were repeated twice to create a sample with a priming layer of pure aluminium oxide and 4 discrete coatings of mixed oxide in a 1:3 $\text{Al}_2\text{O}_3:\text{ZnO}$ atomic ratio.

The resultant particles were then divided into two batches. For the first batch, a further outer layer of pure aluminium oxide was applied using the same coating procedure for pure aluminium oxide as that described above.

For the second batch, the procedure described above for making 40 layers of mixed $\text{Al}_2\text{O}_3:\text{ZnO}$ (1:3) oxide was repeated twice with deagglomeration to provide two further discrete coatings of mixed oxide, which was then finished with a further outer layer of pure aluminium oxide as described above.

Batch 1 thus comprised 3 (inner) layers of Al_2O_3 , 4 x 40 layers of 1:3 $\text{Al}_2\text{O}_3:\text{ZnO}$ mixed oxide and 3 (outer) layers of Al_2O_3 , and Batch 2 comprised 3 (inner) layers of Al_2O_3 , 6 x 40 layers of 1:3 $\text{Al}_2\text{O}_3:\text{ZnO}$ mixed oxide and 3 (outer) layers of Al_2O_3 .

Particle sizes of the batches were determined by suspending the coated particles in a solution of 0.1% Span 85 (Sigma-Aldrich, MO, USA) in heptane (Merck, Germany).

Particle size distribution, as measured by means of laser diffraction was:

Batch 1 %D(10): $3.5 \pm 0.3 \mu\text{m}$; %D(50): $10.7 \pm 0.6 \mu\text{m}$; %D(90): $23.6 \pm 0.6 \mu\text{m}$;

Batch 2 %D(10): $3.2 \pm 0.2 \mu\text{m}$, %D(50): $9.7 \pm 0.6 \mu\text{m}$, %D(90): $22.3 \pm 0.8 \mu\text{m}$.

The drug load of the coated batches was determined by etching sample in a solution of 43% acetonitrile (Spectrascan, Scotland), 45% water (PanReac, Spain) and 12% (v/v) phosphoric acid (Merck, Germany) to dissolve the coatings, before diluting to 160 µg/mL with 10% acetonitrile in water + 0.1% trifluoroacetic acid (Merck, Germany) (Mobile Phase A) and injection into an HPLC system for quantification (Prominence-I HPLC-UV-DAD, Shimadzu, Japan), with column Kinetex C18 PS, 150×4.6 mm, 2.6 µm particle size (Phenomenex Ltd., CA, USA); Mobile phase B: 80% acetonitrile in water + 0.1% trifluoroacetic acid; injection volume 10 µL, autosampler temp. 15°C, oven temp. 25°C; gradient elution was used with flow rate of 1.0 mL/min; UV-absorbance detection at 220 nm; single-point calibration used for assay; retention time of liraglutide was 6.8 min; reference material 99.4% gross liraglutide by assay, 99.1% purity by HPLC (Bachem AG, Switzerland)).

Batch 1 had a drug load of $61.9 \pm 0.2\%$ and Batch 2 had a drug load of $53.1 \pm 0.4\%$. The purity of the coated liraglutide in both cases was measured as 98.9%. Thus, no significant decrease in the purity of coated liraglutide was seen, indicating that it tolerates the ALD process without decomposition.

To determine coating integrity, samples of both batches were suspended in dimethylsulfoxide (Rathburn, UK) at a concentration of 0.4 mg liraglutide per mL of solvent and rotated on overhead stirrer for up to 72 hours. Intermittent samples were taken, centrifuged (EBA 20, Hettich, Germany) for 7 min at 6000 rpm, and the supernatant diluted in Mobile Phase A (as above) before injection into the above HPLC system for quantification. The results are shown in Figure 1 (Batch 1 (circles), Batch 2 (squares)), and indicate minimal defects in the coating, so any burst release is expected to be low.

Sample material from Batch 1 and from uncoated spray-dried material was subjected to sterilizing levels of gamma irradiation from a ^{60}Co source (Ionisos Baltics OÜ, Alliku, Estonia) in doses within the ranges of 20.4–20.6 kGy and 23.5–25.3 kGy. The drug load of the coated material was determined, using the same method as described above, and compared to that of the uncoated material. The absolute decrease in drug load of Batch 1 material gamma irradiated at approximately 21 kGy was less than 5 %-units, indicating that this material can be terminally sterilized using this method in a sufficiently non-destructive manner. The results are shown in Figure 2 (error bars show the standard deviation ($n=3$) and ns indicates lack of significant difference ($p>0.05$) as determined by 2-way ANOVA with Dunn–Šidák correction). The purity of liraglutide in the treated materials were determined using the same method as

described above, and were in all samples above 95%, corroborating the results from the drug load assay.

Example 2

5 Formulations of the Invention I

Coated liraglutide particles (Batch 1 and Batch 2 from Example 1 above) are suspended in an appropriate aqueous carrier, such as between 5 and 30 mM of phosphate buffered saline and 100 mM of tromethamine buffer, made up to volume with water for injection,
10 to provide a suspension with a concentration of about 100 mg of liraglutide per mL of aqueous carrier. The pH of the resultant formulation is measured at about 7.4.

Example 3

15 Formulations of the Invention II

Two suspensions of coated microparticles of liraglutide (Batch 1 and Batch 2, prepared according to the processes described in Example 1 above) were prepared by adding an appropriate volume of Hyonate vet. (1 wt% hyaluronic acid solution, in 1 mM phosphate buffer, pH 7.4) to the test item vials to achieve a concentration liraglutide
20 corresponding to 10 mg/mL. The vial was then tapped at least 10 times to dislodge any material which might have settled to the bottom of the test vial. The formulation was then vortexed for *ca.* 60 seconds to ensure a uniform suspension.

Example 4

25 In Vivo Study

Sixteen male Sprague Dawley rats weighing approximately 300 g at the day of administration were supplied by Charles River Labs (UK). The animals were divided randomly into four animals per group.

30

The intended administration area was clipped free from hair prior to injection and the injection site was marked. The suspensions (prepared as described in Example 3 above) were drawn into a 1 mL syringe and single, subcutaneous injections (*ca.* 0.08 mL (Batch 1 and Batch 2, Groups 1 and 3, as identified in Table 1 below, respectively,
35 and 0.17 mL (Batch 2, Group 2, as identified in Table 1 below) were administered subcutaneously through a 23G needle into the flank of each rat. A comparator liraglutide solution (Victoza, Novo Nordisk, Denmark) was diluted in normal saline solution for injection to 0.6 mg/mL (Group 4, as identified in Table 1 below), drawn

into a 1 mL syringe, and administered subcutaneously through a 23G needle into the flank of each rat. Administration was performed no more than 30 minutes after preparation of the formulations.

- 5 The dose site on each animal was marked post-dosing and kept free from hair for the duration of the study. Dose site observations were performed at 1 and 24 hours post dosing, and then daily until the final sample point.

Table 1

10

Group	Description	Dose (mg/kg)
1	Batch 1 particles in Hyonate vet.	2.8
2	Batch 2 particles in Hyonate vet.	5.6
3	Batch 2 particles in Hyonate vet.	2.8
4	Victoza, diluted in normal saline	0.2

- 15 Blood samples (ca. 0.2 mL) were collected from the jugular vein into K₂EDTA (dipotassium ethylenediaminetetraacetic acid) tubes at the following time-points for Group 1 to 3: 1, 3, 6, 12, 24, 48, 72, 120, 168, 251, 384, 480, 576 and 672 hours post-dose, and at the following time-points for Group 4: 1, 2, 3, 6, 9, 12, 24 and 48 hours post subcutaneous dose. Actual sampling times were recorded. As soon as practically possible following blood sampling, plasma was separated by centrifugation (1500 g for 10 min at 4°C), which was stored at -80°C until analysis was conducted.

- 20 Following study completion, all plasma samples were shipped for analysis having been deep frozen on dry ice. Animals were sacrificed on the last day of the study.

- 25 Plasma concentration of liraglutide was determined with LC-MS/MS. Study samples were prepared by pipetting 35 µL of rat plasma into a 96 well plate, adding 75 µL of an internal standard working solution using an EVO-2 liquid handling robot (Tecan, Austria). The 96 well plates were then shaken for 15 minutes and centrifuged. All samples were then injected on a UPLC-MS/MS system (Waters, MA, USA). Separation was obtained with an ACQUITY UPLC Protein BEH C4 Column, 300 Å, 1.7 µm, 2.1 mm x 50 mm (Waters, MA, USA) at 60°C using 0.3% formic acid in water as mobile phase A and acetonitrile as mobile phase B.
- 30

The pharmacokinetic (PK) analysis of liraglutide in plasma was evaluated using non-compartmental analysis (NCA) utilizing the software Phoenix WinNonlin, version 8.3 (Certara, USA). C_{max} , and t_{max} were derived from the observed plasma concentration data. AUC was assessed by integration of the plasma concentration vs time curve using linear interpolation for increasing plasma levels and logarithmic interpolation for decreasing plasma levels (Linear Up Log Down method). For AUC_{∞} , the area was calculated to the last point showing a measurable plasma concentration (AUC_{last}) and then extrapolated to infinity using the concentration in the last quantifiable sample and λ_{dz} , the first order rate constant associated with the terminal portion of the curve.

$t_{1/2,z}$ was calculated by $\ln 2 / \lambda_{dz}$.

Results

Dose-normalised plasma concentrations of liraglutide over four weeks after single subcutaneous administration of the various formulations are presented in Figure 3, with Figure 4 showing the same plasma concentration profiles over the first 72 hours.

The plasma pharmacokinetic parameters are also presented as mean values for the group of 6 rats (with standard deviations provided in parentheses) in Table 2 below, in which:

- ' t_{max} ' is the time to peak concentration expressed in hours
- ' C_{max} ' is the maximum concentration found in analysis expressed in ng/mL
- ' t_{last} ' is the time of the last detectable concentration expressed in hours
- ' $t_{1/2,z}$ ' is the terminal half-life expressed in hours
- ' AUC_{∞} ' is the area under concentration vs. time curve extrapolated up to infinite time expressed in ng*h/mL
- ' F ' is the relative bioavailability expressed as a percentage
- ' C_{max}/D ' is the maximum concentration normalized to 1 mg/kg expressed in ng/mL/mg/kg body weight of the rat
- ' AUC_{last}/D ' is the area under blood concentration vs. time curve up to the last detectable concentration normalized to 1 mg/kg expressed in ng*h/mL/mg/kg body weight of the rat
- ' AUC_{∞}/D ' is the area under concentration vs. time curve up to infinite time normalized to 1 mg/kg expressed in ng*h/mL/mg/kg body weight of the rat
- ' $Fr. Rel. 0-24h$ ' is the fraction released during the first 24 hours of the area under concentration vs. time curve up to infinite time expressed as a percentage.

Table 2

Parameter	Group 1	Group 2	Group 3	Group 4
$t_{\max}$	21 (6.0)	21 (6.0)	18 (6.9)	6 (0)
$C_{\max}$	799 (176)	670 (61.2)	391 (81.6)	343 (49.5)
t_{last}	672	672	672	48
$t_{1/2,z}$	79.4 (14.8)	153 (89.0)	142 (27.6)	4.43 (0.71)
AUC_{∞}	30100 (7089)	48775 (5545)	26325 (4892)	4620 (733.8)
F	47 (11)	38 (4.2)	41 (7.6)	100 (34)
$C_{\max}/D$	285 (62.9)	120 (10.9)	140 (29.2)	1710 (248)
AUC_{last}/D	10738 (2512)	8304 (1155)	9321 (1798)	23090 (3664)
AUC_{∞}/D	10750 (2532)	8710 (990.2)	9045 (1747)	23100 (3669)
Fr. Rel.0-24h	42 (1)	23 (3)	28 (2)	100

It can be seen that the plasma concentration profile was comparable for Groups 1 to 3, with maximal plasma concentration ($C_{\max}$) reached within the first 24 hours followed by a rapid decline until 72 hours post-dose, and then a slow but steady decline in concentrations over the 28 day study period, as compared to Group 4, which showed complete release after just 2 days.

Group 1 had higher initial drug release compared with the thicker coating formulations in Group 2 and Group 3.

Comparing Group 1 to 3 to each other, it can be seen that the systemic exposure to liraglutide was highly proportional to the administered dose, regardless of the coating thickness.

Groups 1 to 3 demonstrated relative bioavailabilities (F) that were lower than liraglutide solution. The trend was proportional to the relative amount of the administered dose released within the first 24 hours, but not statistically significant.

20

$C_{\max}$ was higher for liraglutide administered as mixed oxide coated particles (Groups 1 to 3) compared to liraglutide solution (Group 4), with approximately one fifth of the dose being released during the first day in Group 2, one third in Group 3, and two fifths in Group 1. When normalised for dose, the difference is about an order of magnitude

for the thicker coating (Groups 2 and 3). Also, the residual area (correlating to unreleased drug) following the last sampling time (672 hours post-dosing) was <5%.

5 The results for Group 1 showed a slightly different profile characterized by a larger fraction of the dose released during the first day, a higher $C_{\max}/D$, and a shorter duration.

10 In summary, Groups 1 to 3 demonstrated a prolonged-release profile, which differs from the rapid decline following administration of liraglutide solution. Similarly, advantageous plasma concentration-time profiles were observed for all of the formulations of the invention, but especially the formulation in Group 2 and 3.

Example 5

Coated Liraglutide Microparticles

15

Coated liraglutide microparticles were prepared using a similar method as described for Example 1, resulting in two batches corresponding to Batch 3 and Batch 4.

20 The particle size of the spray-dried liraglutide particles was determined as above as follows: %D(10): $1.5 \mu\text{m} \pm 2.5\%$, %D(50): $5.2 \mu\text{m} \pm 2.9\%$, %D(90): $11.5 \mu\text{m} \pm 2.3\%$.

Batch 3

25 For Batch 3, the spray-dried liraglutide particles were first coated with three layers of pure aluminium oxide by way of the following process:

- a. A valve on the piping between the pump and the ALD reactor was closed.
- b. A valve on the trimethylaluminium (TMA) precursor bottle was then opened for 1 second, letting evaporated metal containing precursor fill the ALD reactor for 1 second.
- 30 c. The valve to the precursor bottle was closed and before opening to the pump again the chamber rested for 30 seconds (soaking time) to ensure the metal containing precursor vapour reacted with the surface of the drug particles.
- d. The ALD reactor was thereafter pumped for 9 seconds.
- e. Steps a-d above were repeated 20 times.

35

The chamber was then purged with nitrogen in a continuous flow to remove non-reacted reagents and organic gases. After that, steps (a) to (e) above were essentially repeated, with the exception that water was used as a second reagent to form a

discrete aluminium oxide layer on the surfaces of the active ingredient microparticles. This was followed by a further purging pulse using nitrogen in a continuous flow, which was carried out to remove gaseous water and organic gases.

- 5 The above procedure was repeated twice to form a total of three initial aluminium oxide atomic layers.

Following this, one layer of zinc oxide was applied by repeating the above ALD steps using diethylzinc (DEZ), and then water, as precursors, in step (b) above to form a
10 total of one zinc oxide layers. That was followed by coating with one layer of aluminium oxide, using the same precursors as mentioned above under (b). This provided a mixed oxide layer with an atomic ratio of 1:1 ($\text{Al}_2\text{O}_3\text{:ZnO}$) of a total of four atomic layers.

- 15 This was repeated a total of 11 times to form a mixed oxide (1:1 $\text{Al}_2\text{O}_3\text{:ZnO}$) layer with a total of 25 atomic layers (11 of ZnO and 14 of Al_2O_3), including the three initial aluminium oxide atomic layers.

The powder was then removed from the ALD reactor and deagglomerated using a sonic
20 sifter (Tsutsui Sonic Agitated Sifting Machine SW-20AT) with a 20 μm mesh size sieve.

The resultant deagglomerated powder was re-loaded into the ALD reactor and the same procedure for coating the particles with three layers of aluminum oxide followed by a mixed oxide (1:1 $\text{Al}_2\text{O}_3\text{:ZnO}$) layer with a total of 25 atomic layers. The
25 deagglomeration and coating steps were repeated four times to create a sample with a priming layer of pure aluminium oxide and 5 discrete coatings of mixed oxide in a 1:1 $\text{Al}_2\text{O}_3\text{:ZnO}$ atomic ratio.

Batch 3 thus comprised 5 x 25 layers, i.e. 5 x (3 layers of Al_2O_3 and 22 layers of 1:1
30 $\text{Al}_2\text{O}_3\text{:ZnO}$ mixed oxide).

Batch 4

For Batch 4, the spray-dried liraglutide particles were first coated with ten layers of pure aluminium oxide by way of the following process:

- 35 a. A valve on the piping between the pump and the ALD reactor was closed.
b. A valve on the trimethylaluminium (TMA) precursor bottle was then opened for 1 second, letting evaporated metal containing precursor fill the ALD reactor for 1 second.

- c. The valve to the precursor bottle was closed and before opening to the pump again the chamber rested for 30 seconds (soaking time) to ensure the metal containing precursor vapour reacted with the surface of the drug particles.
- d. The ALD reactor was thereafter pumped for 9 seconds.
- 5 e. Steps a-d above were repeated 20 times.

The chamber was then purged with nitrogen in a continuous flow to remove non-reacted reagents and organic gases. After that, steps (a) to (e) above were essentially repeated, with the exception that water was used as a second reagent to form a
10 discrete aluminium oxide layer on the surfaces of the active ingredient microparticles. This was followed by a further purging pulse using nitrogen in a continuous flow, which was carried out to remove gaseous water and organic gases.

The above procedure was repeated nine times to form a total of ten initial aluminium
15 oxide atomic layers.

Following this, three layers of zinc oxide were applied by repeating the above ALD steps using diethylzinc (DEZ), and then water, as precursors, in step (b) above to form a total of three zinc oxide layers. That was followed by coating with one layer of
20 aluminium oxide, using the same precursors as mentioned above under (b). This provided a mixed oxide layer with an atomic ratio of 1:3 (Al_2O_3 :ZnO) of a total of four atomic layers.

This was repeated a total of 9 times to form a mixed oxide (1:3 Al_2O_3 :ZnO) layer with
25 a total of 50 atomic layers (30 of ZnO and 20 of Al_2O_3), including the ten initial aluminium oxide atomic layers.

The powder was then removed from the ALD reactor and deagglomerated using a sonic sifter (Tsutsui Sonic Agitated Sifting Machine SW-20AT) with a 20 μm mesh size sieve.
30

The resultant deagglomerated powder was re-loaded into the ALD reactor and the same procedure for coating the particles with ten pure aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) layer with a total of 50 atomic layers was carried out. After the last deagglomeration step the same procedure for coating the particles with ten pure
35 aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) and finished with three pure aluminium oxide with a total of 53 atomic layers. The deagglomeration and coating steps were repeated five times to create a sample with a priming and finishing

layer of pure aluminium oxide and 6 discrete coatings of mixed oxide in a 1:3 Al₂O₃:ZnO atomic ratio.

Batch 4 thus comprised 6 x 50 layers, i.e. 6 x (10 layers of Al₂O₃ and 40 layers of 1:3 Al₂O₃:ZnO mixed oxide) and 3 (outer) layers of Al₂O₃.

Characterisation of Batches

Particle sizes of the batches were determined by suspending the coated particles in a solution of 1% Span 85 (Sigma-Aldrich, MO, USA) in heptane (Merck, Germany).

Particle size distribution, as measured by means of laser diffraction was:

Batch 3: %D(10): 3.7 µm ± 9.9%; %D(50): 9.3 µm ± 3.9%; %D(90): 21.3 µm ± 5.9%;

Batch 4: Not determined.

The drug load of the coated batches was determined by etching sample in a solution of 2M phosphoric acid (Merck, Germany) in water (PanReac, Spain) to dissolve the coatings, before diluting to 160 µg/mL. For Batch 3, the sample diluent was a mixture of 10% Mobile Phase B (90% acetonitrile in water (Spectrascan, Scotland) + 0.1% trifluoroacetic acid (Sigma Aldrich, Germany)) and 90% Mobile Phase A (10% acetonitrile + 0.1% trifluoroacetic acid). For Batch 4, the sample diluent was 0.1% trifluoroacetic acid. The samples were injected into an HPLC system for quantification (Prominence-I HPLC-UV-DAD, Shimadzu, Japan), with column Kinetex F5, 100Å, 150×4.6 mm, 2.6 µm particle size (Phenomenex Ltd., CA, USA); Mobile Phase A: 10% acetonitrile in water + 0.1% trifluoroacetic acid; Mobile Phase B: 90% acetonitrile in water + 0.1% trifluoroacetic acid; injection volume 10 µL, autosampler temp. 20°C, oven temp. 25°C; gradient elution was used with flow rate of 1.0 mL/min; UV-absorbance detection at 220 nm; single-point calibration used for assay; retention time of liraglutide was 6.8 min; reference material 99.4% gross liraglutide by assay, 99.1% purity by HPLC (Bachem AG, Switzerland).

Batch 3 had a drug load of 70.5 ± 0.7% and Batch 4 had a drug load of 46.8 ± 0.2%.

To determine coating integrity, samples of both batches were suspended in dimethylsulfoxide (Rathburn, UK) at a concentration of 0.4 mg liraglutide per mL of solvent and rotated on overhead stirrer for 3 hours. Intermittent samples were taken, centrifuged (EBA 20, Hettich, Germany) for 7 min at 6000 rpm, and the supernatant diluted in Mobile Phase A (as above) before injection into the above HPLC system for quantification. The results after 3 hours show 3.6% (for Batch 3) and 2.2% (for Batch

4) liraglutide released indicating minimal defects in the coating, so any burst release is expected to be low.

In Vitro Study

- 5 To determine in vitro release, materials were suspended in 50 mL 20 mM PIPES pH 7.2 + 1% Tween at a concentration of 0.5 mg liraglutide per mL of solvent in blue cap flasks and incubated at 37 °C for up to 72 h. Intermittent samples were taken, filtered with 0.2 µm PES filter before analysis with HPLC using the same method as described for the drug load determination. The results for Batch 3 (circles) and Batch 4 (squares)
- 10 are shown in Figure 5.

Example 6

Formulations of the Invention III

- 15 Two suspensions of coated microparticles of liraglutide (Batch 3 and Batch 4, prepared according to the processes described in Example 5 above) were prepared by adding an appropriate volume of Hyonate vet. (1 wt% hyaluronic acid solution, in 1 mM phosphate buffer, pH 7.4) to the test item vials to achieve a concentration liraglutide corresponding to 10 mg/mL. The vial was then tapped at least 10 times to dislodge
- 20 any material which might have settled to the bottom of the test vial. The formulation was then vortexed for *ca.* 60 seconds to ensure a uniform suspension.

Example 7

In Vivo Study

- 25 Nine male Göttingen SPF minipigs weighing approximately 10 kg at the day of administration were supplied by Ellegaard Göttingen Minipigs A/S (Denmark). The animals were divided randomly into three animals per group.
- 30 The suspensions (prepared as described in Example 6 above), or a comparator liraglutide solution (Victoza, Novo Nordisk, Denmark) diluted in normal saline solution, were administered subcutaneously into the left side of the neck of each minipig through a 23G needle as a single dose to three Göttingen minipigs per dose group. Minipigs receiving Batch 3, Batch 4 or the comparator liraglutide solution correspond to Groups
- 35 5, 6 and 7, respectively, as identified in Table 3 below.

Table 3

Group	Description	Dose (mg)
5	Batch 3 particles in Hyonate vet.	1.0
6	Batch 4 particles in Hyonate vet.	1.0
7	Victoza, diluted in saline	0.2

- Blood samples for pharmacokinetics were taken from all animals on Day 1 and the following days. Blood sampling was performed for all groups at the following time points: Pre-treatment, and 2, 4, 6, 12, 24, 48, 72, 96, 144 hours post treatment. Furthermore, blood was collected from animals of Groups 2 and 3, 216, 360, 456, 552- and 648 hours post treatment, corresponding to Days 10, 16, 20, 24 and 28.
- 10 Blood samples of approximately 3 mL were drawn from the jugular vein/bijugular trunk. The blood was sampled into vacutainers containing K₂EDTA as anticoagulant. The vacutainer was placed in ice water until centrifugation (10 min, 1270 G, +4°C). Each plasma sample was divided into two aliquots of approx. 0.5 mL and transferred to cryotubes provided by the sponsor and frozen at -70°C or on dry ice within 90
- 15 minutes after collection. The first set of samples was sent on dry ice (approximately -70°C), with thermologger to Contributing Scientist for analysis. The second set of samples was stored at -70°C or below as back-up samples. The back-up samples were shipped within 1 month after receipt of the last set of primary samples.
- 20 Plasma concentration of Liraglutide was determined with LC-MS/MS. Study samples were prepared by pipetting 35 µL of minipig plasma into a 384 well plate, adding 35 µL of an internal standard working solution using a TECAN liquid handling robot. The 384 well plates were then shaken for 1 minute. Protein precipitation solution was added and the samples were shaken for 5 minutes and centrifuged. All samples were
- 25 injected on a UPLC-MS/MS system. Separation was obtained with an ACQUITY Phenyl-Hexyl Column, 1.7 µm, 2.1 × 50 mm, Waters at 60°C using 0.3% Formic Acid as mobile phase A (MP A) and acetonitrile as mobile phase B (MP B).

The PK in Göttingen minipigs was evaluated using non-compartmental analysis (NCA) utilizing the software Phoenix WinNonlin, version 8.3 (Certara, USA).

Nominal plasma sampling timepoints and nominal doses were used for the non-compartmental PK analysis. PK for animals were calculated with the extravascular

dose option within WinNonlin. Plasma concentrations below LLOQ occurring before $C_{\max}$ was treated as zero.

$C_{\max}$ and $T_{\max}$ were derived from the observed plasma concentration data. AUC was assessed by integration of the plasma concentration versus time curve using linear interpolation for increasing plasma levels and logarithmic interpolation for decreasing plasma levels (Linear Up Log Down method). For AUC_{last} , the area was calculated to the last point showing a measurable plasma concentration (AUC_{last}).

Results

Mean plasma concentrations of liraglutide over four weeks after single subcutaneous administration of the various formulations (n=3) are presented in Figure 6.

The plasma pharmacokinetic parameters are also presented as mean values per dose group (with standard deviations provided in parentheses) in Table 4 below, in which $C_{\max}$ and $t_{\max}$ correspond to the definitions as defined above, and:

- ‘ AUC_{0-24h} ’ - The AUC from time 0 to time 24 hours
- ‘ AUC_{last} ’ -The AUC from time 0 to the time of the last detectable plasma concentration

Table 4

Group	$t_{\max}$ (h)	$C_{\max}$ (ng/mL)	AUC_{0-24h} (h*ng/mL)	AUC_{last} (h*ng/mL)
5	24 (24-24)	29.90 (10.4)	519.6 (209)	3583 (1260)
6	12 (12-24)	16.80 (1.40)	306.4 (22.6)	1419 (162)
7	6.0 (6.0-6.0)	183.0 (12.5)	2948 (341)	4696 (ND)

Subcutaneous administration of the two formulations of coated liraglutide microparticles (Groups 5 and 6) exhibited an initial absorption phase of liraglutide followed by a slower and more prolonged absorption phase spanning over at least 28 days compared to Group 7. The observed mean $C_{\max}$ for liraglutide was considerably lower for Groups 5 and 6 (the coated liraglutide microparticle formulations, dose 1.0 mg) than was observed following subcutaneous administration of liraglutide solution (Group 7, dose 0.2 mg).

The plasma exposure to liraglutide appeared to be higher for Group 5 (Batch 3) than for Group 6 (Batch 4), which may be due to the thicker coating formulations in Group 6 compared to Group 5.

- 5 In summary, Groups 5 and 6 demonstrated a prolonged-release profile, which differs significantly from the higher C_{max} , followed by rapid decline in plasma concentration following administration of liraglutide solution.

Example 8

10 Coated Liraglutide Microparticles

Liraglutide Particles

- Batches with larger-sized spray-dried particles of liraglutide were prepared in a similar spray-drying equipment as used in Example 1, and using process parameters as defined below. Otherwise, all other steps were according to the processes described in Example 1.

- For these formulations, liraglutide was dissolved in water for injection (WFI) to 5% (w/w) in the spray-drying process. Batches of 5 g and 30 g were produced in a small-scale spray-drier, PROCEPT SD3, with equipment setting parameters as defined in Tables 5 (setpoint of process parameters) and 6 (range of outcome parameters during the process).

Table 5

25

Process parameter	Setpoint
Inlet gas flow (m ³ /min)	0.3
Inlet temperature (°C)	160.0
Nozzle gas flow (l/min)	3.5 or 4.5
Cyclone gas flow (m ³ /min)	0.4
Chiller temperature (°C)	2.0

Table 6

Critical parameter	Range
Chamber out temperature (°C)	60 - 70
Cyclone in temperature (°C)	30 - 40
Condensor temperature (°C)	0 - 4
Dosing speed (g/min)	4.0 ± 1.0

Batch 5

- 5 For Batch 5 (297_5_LIR), larger-sized spray-dried liraglutide particles were used as prepared by the process described above. The particle size of the larger-sized spray-dried liraglutide particles was determined as above as follows: %D(10): 3.1 μm ± 4.3%, %D(50): 10.9 μm ± 4.0%, %D(90): 30.5 μm ± 6.8%.
- 10 The large spray-dried liraglutide particles for Batch 5 were first coated with ten layers of pure aluminium oxide by way of the following process:
- a. A valve on the piping between the pump and the ALD reactor was closed.
 - b. A valve on the trimethylaluminium (TMA) precursor bottle was then opened for 1 second, letting evaporated metal containing precursor fill the ALD reactor for 1
 - 15 second.
 - c. The valve to the precursor bottle was closed and before opening to the pump again the chamber rested for 30 seconds (soaking time) to ensure the metal containing precursor vapour reacted with the surface of the drug particles.
 - d. The ALD reactor was thereafter pumped for 9 seconds.
 - 20 e. Steps a-d above were repeated 20 times.

The chamber was then purged with nitrogen in a continuous flow to remove non-reacted reagents and organic gases. After that, steps (a) to (e) above were essentially repeated, with the exception that water was used as a second reagent to form a

25 discrete aluminium oxide layer on the surfaces of the active ingredient microparticles. This was followed by a further purging pulse using nitrogen in a continuous flow, which was carried out to remove gaseous water and organic gases.

The above procedure was repeated nine times to form a total of ten initial aluminium

30 oxide atomic layers.

Following this, twenty layers of zinc oxide were applied by repeating the above ALD steps using diethylzinc (DEZ), and then water, as precursors, in step (b) above to form a total of twenty zinc oxide layers. This provided a multilayer structure of pure aluminium oxide and zinc oxide with a total of 30 atomic layers ($\text{Al}_2\text{O}_3:\text{ZnO}$), including the ten initial aluminium oxide atomic layers.

The powder was then removed from the ALD reactor and deagglomerated using a sonic sifter (Tsutsui Sonic Agitated Sifting Machine SW-20AT) with a 45 μm mesh size sieve.

The resultant deagglomerated powder was re-loaded into the ALD reactor and the same procedure for coating the particles with ten pure aluminium oxide followed by twenty pure zinc oxide layer with a total of 30 atomic layers. After the last deagglomeration step the same procedure for coating the particles with seven pure aluminium oxide layers followed by a twenty pure zinc oxide layers and finished by three pure aluminium oxide layers to give a mixed oxide ($\text{Al}_2\text{O}_3:\text{ZnO}:\text{Al}_2\text{O}_3$) layer with a total of 30 atomic layers. The deagglomeration and coating steps were repeated four times to create a sample with 5 discrete coatings of pure aluminium oxide and zinc oxide.

Batch 5 thus comprised 5 x 30 layers (10 layers of Al_2O_3 and 20 layers of ZnO) and 1 x 30 layers (7 layers of Al_2O_3 , 20 layers of ZnO and 3 layers of Al_2O_3).

Batch 6

For Batch 6, spray-dried liraglutide particles were used as described in Example 1.

The spray-dried liraglutide particles for Batch 6 were first coated with ten layers of pure aluminium oxide by way of the following process:

- a. A valve on the piping between the pump and the ALD reactor was closed.
- b. A valve on the trimethylaluminium (TMA) precursor bottle was then opened for 1 second, letting evaporated metal containing precursor fill the ALD reactor for 1 second.
- c. The valve to the precursor bottle was closed and before opening to the pump again the chamber rested for 30 seconds (soaking time) to ensure the metal containing precursor vapour reacted with the surface of the drug particles.
- d. The ALD reactor was thereafter pumped for 9 seconds.
- e. Steps a-d above were repeated 20 times.

The chamber was then purged with nitrogen in a continuous flow to remove non-reacted reagents and organic gases. After that, steps (a) to (e) above were essentially repeated, with the exception that water was used as a second reagent to form a

discrete aluminium oxide layer on the surfaces of the active ingredient microparticles. This was followed by a further purging pulse using nitrogen in a continuous flow, which was carried out to remove gaseous water and organic gases.

- 5 The above procedure was repeated nine times to form a total of ten initial aluminium oxide atomic layers.

Following this, three layers of zinc oxide were applied by repeating the above ALD steps using diethylzinc (DEZ), and then water, as precursors, in step (b) above to form a
10 total of three zinc oxide layers. That was followed by coating with one layer of aluminium oxide, using the same precursors as mentioned above under (b). This provided a mixed oxide layer with an atomic ratio of 1:3 (Al_2O_3 :ZnO) of a total of four atomic layers.

- 15 This was repeated a total of 9 times to form a mixed oxide (1:3 Al_2O_3 :ZnO) layer with a total of 50 atomic layers (30 of ZnO and 20 of Al_2O_3), including the ten initial aluminium oxide atomic layers.

The powder was then removed from the ALD reactor and deagglomerated using a sonic
20 sifter (Tsutsui Sonic Agitated Sifting Machine SW-20AT) with a 20 μm mesh size sieve.

The resultant deagglomerated powder was re-loaded into the ALD reactor and the same procedure for coating the particles with ten pure aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) layer with a total of 50 atomic layers. After the last
25 deagglomeration step the same procedure for coating the particles with ten pure aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) with a total of 50 atomic layers. The deagglomeration and coating steps were repeated three times to create a sample with a priming layer of pure aluminium oxide and 4 discrete coatings of mixed oxide in a 1:3 Al_2O_3 :ZnO atomic ratio.

30

Batch 6 thus comprised 4 x 50 layers, i.e. 4 x (10 layers of Al_2O_3 and 40 layers of 1:3 Al_2O_3 :ZnO mixed oxide).

Batch 7

- 35 For Batch 7, larger-sized spray-dried liraglutide particles were used as prepared by the process described above. The particle size of the larger-sized spray-dried material was determined as above as follows: %D(10): $3.0 \mu\text{m} \pm 1.2\%$, %D(50): $9.7 \mu\text{m} \pm 2.4\%$, %D(90): $27.6 \mu\text{m} \pm 6.1\%$.

The large spray-dried liraglutide particles for Batch 7 were first coated with three layers of pure aluminium oxide by way of the following process:

- a. A valve on the piping between the pump and the ALD reactor was closed.
- 5 b. A valve on the trimethylaluminium (TMA) precursor bottle was then opened for 1 second, letting evaporated metal containing precursor fill the ALD reactor for 1 second.
- c. The valve to the precursor bottle was closed and before opening to the pump again the chamber rested for 30 seconds (soaking time) to ensure the metal containing
- 10 precursor vapour reacted with the surface of the drug particles.
- d. The ALD reactor was thereafter pumped for 9 seconds.
- e. Steps a-d above were repeated 20 times.

The chamber was then purged with nitrogen in a continuous flow to remove non-

15 reacted reagents and organic gases. After that, steps (a) to (e) above were essentially repeated, with the exception that water was used as a second reagent to form a discrete aluminium oxide layer on the surfaces of the active ingredient microparticles. This was followed by a further purging pulse using nitrogen in a continuous flow, which was carried out to remove gaseous water and organic gases.

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The above procedure was repeated two times to form a total of three initial aluminium oxide atomic layers.

Following this, three layers of zinc oxide were applied by repeating the above ALD steps

25 using diethylzinc (DEZ), and then water, as precursors, in step (b) above to form a total of three zinc oxide layers. That was followed by coating with one layer of aluminium oxide, using the same precursors as mentioned above under (b). This provided a mixed oxide layer with an atomic ratio of 1:3 (Al_2O_3 :ZnO) of a total of four atomic layers.

30

This was repeated a total of 5 times to form a mixed oxide (1:3 Al_2O_3 :ZnO) layer with a total of 27 atomic layers (18 of ZnO and 9 of Al_2O_3), including the three initial aluminium oxide atomic layers.

35 The powder was then removed from the ALD reactor and deagglomerated using a sonic sifter (Tsutsui Sonic Agitated Sifting Machine SW-20AT) with a 32 μm mesh size sieve.

The resultant deagglomerated powder was re-loaded into the ALD reactor and the same procedure for coating the particles with three pure aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) layer with a total of 27 atomic layers. After the last deagglomeration step the same procedure for coating the particles with three pure aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) with a total of 27 atomic layers. The deagglomeration and coating steps were repeated three times to create a sample with a priming layer of pure aluminium oxide and 4 discrete coatings of mixed oxide in a 1:3 Al_2O_3 :ZnO atomic ratio.

Batch 7 thus comprised 4 x 27 layers, i.e. 4 x (3 layers of Al_2O_3 and 24 layers of 1:3 Al_2O_3 :ZnO mixed oxide).

Batch 8

For Batch 8, larger-sized spray-dried liraglutide particles were used as prepared by the process described above. The particle size of the larger-sized spray-dried material was determined as above as follows: %D(10): $3.1 \mu\text{m} \pm 4.3\%$, %D(50): $10.9 \mu\text{m} \pm 4.0\%$, %D(90): $30.5 \mu\text{m} \pm 6.8\%$.

The larger-sized spray-dried liraglutide particles for Batch 8 were first coated with three layers of pure aluminium oxide by way of the following process:

- a. A valve on the piping between the pump and the ALD reactor was closed.
- b. A valve on the trimethylaluminium (TMA) precursor bottle was then opened for 1 second, letting evaporated metal containing precursor fill the ALD reactor for 1 second.
- c. The valve to the precursor bottle was closed and before opening to the pump again the chamber rested for 30 seconds (soaking time) to ensure the metal containing precursor vapour reacted with the surface of the drug particles.
- d. The ALD reactor was thereafter pumped for 9 seconds.
- e. Steps a-d above were repeated 20 times.

The chamber was then purged with nitrogen in a continuous flow to remove non-reacted reagents and organic gases. After that, steps (a) to (e) above were essentially repeated, with the exception that water was used as a second reagent to form a discrete aluminium oxide layer on the surfaces of the active ingredient microparticles. This was followed by a further purging pulse using nitrogen in a continuous flow, which was carried out to remove gaseous water and organic gases.

The above procedure was repeated two times to form a total of three initial aluminium oxide atomic layers.

Following this, three layers of zinc oxide were applied by repeating the above ALD steps using diethylzinc (DEZ), and then water, as precursors, in step (b) above to form a total of three zinc oxide layers. That was followed by coating with one layer of aluminium oxide, using the same precursors as mentioned above under (b). This provided a mixed oxide layer with an atomic ratio of 1:3 (Al_2O_3 :ZnO) of a total of four atomic layers.

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This was repeated a total of 5 times to form a mixed oxide (1:3 Al_2O_3 :ZnO) layer with a total of 27 atomic layers (18 of ZnO and 9 of Al_2O_3), including the three initial aluminium oxide atomic layers.

15 The powder was then removed from the ALD reactor and deagglomerated using a sonic sifter (Tsutsui Sonic Agitated Sifting Machine SW-20AT) with a 45 μm mesh size sieve.

The resultant deagglomerated powder was re-loaded into the ALD reactor and the same procedure for coating the particles with three pure aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) layer with a total of 27 atomic layers. After the last deagglomeration step the same procedure for coating the particles with three pure aluminium oxide followed by a mixed oxide (1:3 Al_2O_3 :ZnO) with a total of 27 atomic layers. The deagglomeration and coating steps were repeated three times to create a sample with a priming layer of pure aluminium oxide and 4 discrete coatings of mixed oxide in a 1:3 Al_2O_3 :ZnO atomic ratio.

25

Batch 8 thus comprised 4 x 27 layers, i.e. 4 x (3 layers of Al_2O_3 and 24 layers of 1:3 Al_2O_3 :ZnO mixed oxide).

30 *Characterisation of Batches*

The particle sizes of the batches were determined by suspending the coated particles in a solution of 1% Span 85 (Sigma-Aldrich, MO, USA) in heptane (Merck, Germany). Particle size distribution, as measured by means of laser diffraction was:

Batch 5: %D(10): 7.2 μm $\pm$ 3.9%; %D(50): 17.9 μm $\pm$ 1.6%; %D(90): 36.2 μm $\pm$ 2.1%;

35

Batch 6: not determined;

Batch 7: %D(10): 3.8 μm $\pm$ 1.3%; %D(50): 11.2 μm $\pm$ 1.9%; %D(90): 26.3 μm $\pm$ 6.8%; and

Batch 8: %D(10): $4.5 \mu\text{m} \pm 5.3\%$; %D(50): $13.0 \mu\text{m} \pm 0.8\%$; %D(90): $30.0 \mu\text{m} \pm 0.5\%$.

5 The drug loads of Batch 5, Batch 7 and Batch 8 were determined by etching sample in a solution of 88% water (PanReac, Spain) and 12% (v/v) phosphoric acid (Merck, Germany) to dissolve the coatings, before diluting to $75 \mu\text{g/mL}$ with 75% (Rathburn, UK) in water + 0.1% trifluoroacetic acid (Merck, Germany) and injection into an HPLC system for quantification (Prominence-I HPLC-UV-DAD, Shimadzu, Japan), with column Kinetex F5, $150 \times 4.6 \text{ mm}$, $150 \times 4.6 \text{ mm}$, $2.6 \mu\text{m}$ particle size (Phenomenex Ltd., CA, USA); Mobile phase A: 10% acetonitrile in water 0.1% TFA Mobile phase B: 80% acetonitrile in water + 0.1% trifluoroacetic acid; injection volume $2 \mu\text{L}$, autosampler temp. 20°C , oven temp. 25°C ; gradient elution was used with flow rate of 1.0 mL/min ; UV-absorbance detection at 220 nm ; single-point calibration used for assay; reference material 99.4% gross liraglutide by assay, 99.1% purity by HPLC (Bachem AG, Switzerland).

20 The drug load of Batch 6 was determined by etching sample in a solution of 43% acetonitrile (Spectrascan, Scotland), 45% water (PanReac, Spain) and 12% (v/v) phosphoric acid (Merck, Germany) to dissolve the coatings, before diluting to $160 \mu\text{g/mL}$ with 10% acetonitrile in water + 0.1% trifluoroacetic acid (Merck, Germany) (Mobile Phase A) and injection into an HPLC system for quantification (Prominence-I HPLC-UV-DAD, Shimadzu, Japan), with column Kinetex C18 PS, $150 \times 4.6 \text{ mm}$, $2.6 \mu\text{m}$ particle size (Phenomenex Ltd., CA, USA); Mobile phase B: 80% acetonitrile in water + 0.1% trifluoroacetic acid; injection volume $10 \mu\text{L}$, autosampler temp. 15°C , oven temp. 25°C ; gradient elution was used with flow rate of 1.0 mL/min ; UV-absorbance detection at 220 nm ; single-point calibration used for assay; retention time of liraglutide was 6.8 min ; reference material 99.4% gross liraglutide by assay, 99.1% purity by HPLC (Bachem AG, Switzerland).

30 The drug load as measured by means of HPLC-UV was $79.1 \pm 0.5\%$ for Batch 5 (297_5_LIR), $62.8 \pm 0.7\%$ for Batch 6 (278_4_LIR), $80.9 \pm 0.2\%$ for Batch 7 (293_4_LIR) and $80.2 \pm 0.3\%$ for Batch 8 (295_4_LIR).

35 To determine coating integrity, materials were suspended in dimethylsulfoxide (Rathburn, UK) at a concentration of 0.4 mg liraglutide per mL of solvent and rotated on an overhead stirrer for 3 hours. Intermittent samples were taken, centrifuged (EBA 20, Hettich, Germany) for 7 min at 6000 rpm , and the supernatant diluted in Mobile Phase A (as above) before injection into the above HPLC system for quantification. The

results after 3 hours show 4.0% liraglutide released for Batch 5, 4.6% liraglutide released for Batch 6, 5.2% liraglutide released for Batch 7 and 7.8% liraglutide released for Batch 8, indicating minimal defects in the coating, so any burst release is expected to be low.

5

In Vitro Study

To determine *in vitro* release, materials were suspended in 50 mL 20 mM PIPES pH 7.2 + 1% Tween at a concentration of 0.5 mg liraglutide per mL of solvent in blue cap flasks and incubated at 37 deg C for up to 72 h. Intermittent samples were taken, filtered with 0.2 µm PES filter before analysis with HPLC using same method as described for the drug load determination. The results for Batch 5 (circles), Batch 6 (squares), Batch 7 (diamonds) and Batch 8 (triangles) are shown in Figure 7.

10

Example 9

15 Formulations of the Invention IV

Four suspensions of coated microparticles of liraglutide (Batch 5, Batch 6, Batch 7 and Batch 8, prepared according to the processes described in Example 8 above) were prepared by adding an appropriate volume of Hyonate vet. (1 wt% hyaluronic acid solution, in 1 mM phosphate buffer, pH 7.4) to the test item vials to achieve a concentration liraglutide corresponding to 10 mg/mL. The vial was then tapped at least 10 times to dislodge any material which might have settled to the bottom of the test vial. The formulation was then vortexed for ca. 60 seconds to ensure a uniform suspension.

20

25

Example 10

In Vivo Study

Sixteen (16) male Sprague-Dawley rats were divided into four groups of four animals/group, according to Table 7 below. Animals was weighed and subjected to a single subcutaneous injection of test item using 23G needles. Blood samples for isolation of plasma were collected at the following time-points post-administration: 3 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h, 120 h, 168 h, 251 h, 384 h, 480 h, 576 h and 672 h. Visual inspections of the administration site were performed within one hour of dosing and at blood sampling time points at 24 h and forward. Animals were euthanised following collection of the final blood sample.

30

35

Table 7

Group	Coating	%D(50) Liraglutide Particle Size (µm)	Description	Dose (mg/kg)
8	Al + Zn ^a	14	Batch 5 particles in hyonate vet.	2.8
9	1:3 AZO ^b	5	Batch 6 particles in hyonate vet.	2.8
10	1:3 AZO ^b	10	Batch 7 particles in hyonate vet.	2.8
11	1:3 AZO ^b	14	Batch 8 particles in hyonate vet.	2.8

a. Al + Zn refers to particles having a multilayer structure of pure Al₂O₃ and ZnO.

b. 1:3 AZO refers to particles having a mixed oxide layer with three layers of ZnO and one layer of Al₂O₃.

Blood samples (ca. 0.2 mL) were collected from the jugular vein into K₂EDTA (dipotassium ethylenediaminetetraacetic acid) tubes at the following time-points for Group 1 to 3: 1, 3, 6, 12, 24, 48, 72, 120, 168, 251, 384, 480, 576 and 672 hours post-dose, and at the following time-points for Group 4: 1, 2, 3, 6, 9, 12, 24 and 48 hours post subcutaneous dose. Actual sampling times were recorded. As soon as practically possible following blood sampling, plasma was separated by centrifugation (1500 g for 10 min at 4°C), which was stored at -80°C until analysis was conducted.

Following study completion, all plasma samples were shipped for analysis having been deep frozen on dry ice. Animals were sacrificed on the last day of the study.

Plasma concentration of liraglutide was determined with LC-MS/MS. Study samples were prepared by pipetting 35 µL of rat plasma into a 96 well plate, adding 75 µL of an internal standard working solution using an EVO-2 liquid handling robot (Tecan, Austria). The 96 well plates were then shaken for 15 minutes and centrifuged. All samples were then injected on a UPLC-MS/MS system (Waters, MA, USA). Separation was obtained with an ACQUITY UPLC Protein BEH C4 Column, 300 Å, 1.7 µm, 2.1 mm x 50 mm (Waters, MA, USA) at 60°C using 0.3% formic acid in water as mobile phase A and acetonitrile as mobile phase B.

The pharmacokinetic (PK) analysis of liraglutide in plasma was evaluated using non-compartmental analysis (NCA) utilizing the software Phoenix WinNonlin, version 8.3 (Certara, USA). C_{max} and t_{max} were derived from the observed plasma concentration data. AUC was assessed by integration of the plasma concentration vs time curve

using linear interpolation for increasing plasma levels and logarithmic interpolation for decreasing plasma levels (Linear Up Log Down method). For AUC_{∞} , the area was calculated to the last point showing a measurable plasma concentration (AUC_{last}) and then extrapolated to infinity using the concentration in the last quantifiable sample and λ_{z} , the first order rate constant associated with the terminal portion of the curve. $t_{1/2,z}$ was calculated by $\ln 2 / \lambda_{z}$.

Results

Dose-normalised plasma concentrations of liraglutide over one week (Figure 8) and 42 days (Figure 9) after single subcutaneous administration of the various formulations are presented in Figures 8 and 9.

The plasma pharmacokinetic parameters are also presented as mean values for the four groups (with standard deviations provided in parentheses) in Table 8 below, in which definitions for the parameters correspond to the definitions above, and:

- 'Fr. Rel._{0-72h}' is the fraction released during the first 72 hours of the area under concentration vs. time curve up to infinite time expressed as a percentage.

Table 8

Parameter	Group 8	Group 9	Group 10	Group 11
t_{max}	15.0 (12-24)	10.5 (6-12)	9.00 (6-12)	10.5 (6-12)
C_{max}	487 (59.1)	1080 (312)	707 (179)	1140 (174)
t_{last}	1008 (0)	1008 (0)	1008 (0)	1008 (0)
AUC_{∞} (h*ng/mL)	74800 (13100)	47700 (7030)	71700 (6630)	86900 (28700)
C_{max}/D	174 (21.1)	386 (111)	253 (63.9)	407 (62.1)
AUC_{last}/D	25929 (4320)	16321 (2220)	24721 (2610)	31036 (10300)
AUC_{∞}/D	26714 (4680)	17036 (2510)	25607 (2370)	31036 (10300)
Fr. Rel. _{0-72h}	30 (7)	56 (6)	26 (3)	56 (6)

As can be seen in Figure 8, subcutaneous administration of the four formulations exhibited an initial absorption phase of liraglutide followed by a slower and prolonged absorption phase spanning over at least 7 days. In particular, Figures 8 and 9 show that the formulations administered to Group 11 exhibited a higher release during the initial 7 days, followed by a lower release between days and 42, compared to Groups

8 to 10 which exhibited a prolonged release over 42 days. Group 8 (corresponding to Batch 5, i.e. the Al + Zn coating) showed a lower $C_{\max}$ compared with Group 11 (corresponding to Batch 8, i.e. the 1:3 AZO coating). Both these formulations were produced using 10.9 μm (%D50) liraglutide particles as raw material.

Claims

1. A pharmaceutical formulation that is useful in the treatment of metabolic disorders
5 or conditions, comprising a plurality of particles suspended in a carrier system,
which particles:
 - (a) have a weight-, number-, or volume-based mean diameter that is between
amount 10 nm and about 700 μm ; and
 - (b) comprise solid cores comprising at least one glucagon-like peptide-1 receptor
10 agonist, or a pharmaceutically-acceptable salt thereof, coated, at least in part,
by a coating of inorganic material comprising mixture of:
 - (i) zinc oxide; and
 - (ii) one or more other metal and/or metalloid oxides,
wherein the atomic ratio ((i):(ii)) is at least about 1:10 and up to and including
15 about 10:1.
2. A formulation as claimed in Claim 1, wherein the atomic ratio ((i):(ii)) is at least
about 1:1 and up to and including about 6:1.
- 20 3. A formulation as claimed in Claim 1 or Claim 2, wherein the coated particles
comprise one or more discrete layers surrounding said cores, in which at least one of
said discrete layers comprises a mixture of zinc oxide and one or more other metal
and/or metalloid oxides.
- 25 4. A formulation as claimed in any one of the preceding claims, wherein coated
particles comprise more than one discrete layers surrounding said cores.
5. A formulation as claimed in Claim 4, wherein at least one of said discrete layers
consists essentially of a single metal and/or metalloid oxide.
- 30 6. A formulation as claimed in Claim 5, wherein at least one of said discrete layers of
single metal and/or metalloid oxide is applied directly to the core prior to and/or after
application of said one or more layers comprising said mixture of zinc oxide and one
or more other metal and/or metalloid oxides.
- 35 7. A formulation as claimed in any one of Claims 4 to 6, wherein the number of discrete
layers is up to four layers of single metal and/or metalloid oxide.

8. A formulation as claimed in any one of Claims 4 to 7, wherein said single metal and/or metalloid oxide is aluminium oxide.
9. A formulation as claimed in any one of the preceding claims, wherein the cores
5 consist essentially of at least one glucagon-like peptide-1 receptor agonist, or a pharmaceutically-acceptable salt thereof.
10. A formulation as claimed in any one of the preceding claims, wherein the weight-, number-, or volume-based mean diameter of the particles is between amount 1 μm
10 and about 50 μm .
11. A formulation as claimed in any one of the preceding claims, wherein more than one discrete layer of the mixture of oxides is applied to the core sequentially.
- 15 12. A formulation as claimed in Claim 11, wherein between 3 and 10 discrete layers of the mixture of oxides are applied.
13. A formulation as claimed in any one of the preceding claims, wherein the total thickness of the mixed oxide coating is between about 0.5 nm and about 2 μm .
20
14. A formulation as claimed in any one of Claims 4 to 13, wherein the maximum thickness of an individual discrete layer of oxide coating is about 1 hundredth of the weight-, number-, or volume-based mean diameter of the core, including any other discrete layers that have previously been applied to the core.
25
15. A formulation as claimed in any one of the preceding claims, wherein the ratio of zinc oxide to other metal and/or metalloid oxides is between about 2:1 and about 5:1.
16. A formulation as claimed in any one of the preceding claims, wherein the one or
30 more other metal and/or metalloid oxides are selected from aluminium oxide and/or silicon dioxide.
17. A formulation as claimed in any one of the preceding claims in the form of a sterile injectable dosage form.
35
18. A formulation as claimed in Claim 17 in a form that is administrable *via* a surgical administration apparatus that forms a depot formulation.

19. A formulation as claimed in any one of the preceding claims, wherein the at least one glucagon-like peptide-1 receptor agonist is liraglutide.

20. A formulation as claimed in any one of Claims 1 to 18, wherein the at least one
5 glucagon-like peptide-1 receptor agonist is semaglutide.

21. A process for the preparation of a formulation as defined in any one of the preceding claims, wherein the coated particles are made by applying the layer(s) of mixed oxide coating material to the cores, and/or previously-coated cores, by atomic
10 layer deposition.

22. A process as claimed in Claim 21, wherein:

- (i) solid cores are coated with a first discrete layer of mixed oxide coating material;
- (ii) the coated cores from step (i) are then subjected to a deagglomeration process
15 step;
- (iii) the deagglomerated coated cores from step (ii) are then coated with a second discrete layer of mixed oxide coating material;
- (iv) repeating steps (ii) and (iii) to obtain the required number of discrete layers.

20 23. A process as claimed in Claim 22 wherein the deagglomeration step that takes place between applications of coatings comprises sieving.

24. A process as claimed in Claim 23, wherein the sieving comprises vibrational sieving.
25

25. A process as claimed in Claim 24, wherein the vibrational sieving comprises controlling a vibration probe coupled to the sieve.

26. A process as claimed in Claim 23, wherein the sieving comprises sonic sifting.
30

27. A process for the preparation of a formulation as defined in any one of Claims 1 to 20 wherein the coated particles are mixed with the carrier system after coating.

28. An injectable dosage form comprising a formulation as defined in any one of Claims
35 1 to 20 contained within a reservoir that is connected to, and/or is in association with, an injection means.

29. A dosage form as claimed in Claim 28, which is a surgical administration apparatus that forms a depot formulation.

30. A dosage form as claimed in Claim 28 or Claim 29, which is an autoinjector.

5

31. A formulation as defined in any one of Claims 1 to 20, or a dosage form as defined in any one of Claims 28 to 30, for use in the treatment of a metabolic disorder or condition.

10 32. The use of a formulation as defined in any one of Claims 1 to 20, or a dosage form as defined in any one of Claims 28 to 30, for the manufacture of a medicament for the treatment of a metabolic disorder or condition.

15 33. A method of treatment of a metabolic disorder or condition, which method comprises administering a formulation as defined in any one of Claims 1 to 20, or a dosage form as defined in any one of Claims 28 to 30, to a patient in need of such treatment.

20 34. A formulation for use as claimed in Claim 31, a use as claimed in Claim 32, or a method as claimed in Claim 33, wherein the metabolic disorder or condition is selected from the type 2 diabetes, obesity and comorbidities or either.

25 35. A formulation for use, a use or a method as claimed in any one of Claims 31 to 34, wherein, following injection, the formulation provides a depot formulation from which the at least one glucagon-like peptide-1 receptor agonist is released over a period of time that is between 3 months and about 1 year.

30 36. A formulation for use, a use or a method as claimed in Claim 35, wherein the total exposure for the at least one glucagon-like peptide-1 receptor agonist is at least about 80% to about 125% of the total exposure obtained from a dosing regimen comprising administering daily or weekly injections of said at least one glucagon-like peptide-1 receptor agonist over the same time period.