Pharmaceutical compositions containing enterokine-releasing substances in multiple dosage forms in combination with gelling agents

The present invention relates to pharmaceutical compositions and pharmaceutical articles comprising such compositions wherein the compositions comprise multiple dosage forms each comprising a core and an enteric coating, wherein the core comprises at least one compound stimulating enteroendocrine cells to release at least one enterokine, wherein the size of the dosage forms, with respect to the largest dimension of the dosage forms, provides for entry of the dosage forms into the intestine of a subject independent of gastric emptying mechanisms, and wherein the composition further comprises one or more gelling agents. The invention also relates to the treatment and/or prevention of conditions amenable to stimulation of enterokine release by enteroendocrine cells.

It has early been recognised that delivery of a nutritional substance to the ileum through use of an enteric dosage form of the nutritional substance leads to satiety of a mammalian subject (US 5753253 A). More recently, specific delivery of a nutritional substance, in particular glucose, to the ileum of a subject, in particular dosage forms of a nutritional substance like glucose, which dosage forms release at least 50 % of the administered substance in the ileum of the subject, was suggested for treatment of metabolic diseases like type 2 diabetes mellitus, metabolic syndrome, insulin resistance, obesity, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatosis (NASH) and other conditions (WO 2010/027498 A2, WO 2012/118712 A2, US 2014/0294951 A1).

Co-pending International Patent Application PCT/EP2019/084530 relates to pharmaceutical oral dosage forms comprising a core and a pH-sensitive enteric coating, wherein the core comprises at least one compound stimulating enteroendocrine cells to release at least one enterokine, and at least one disintegrant providing a burst release of the ingredients of the core when the coating is substantially degraded and/or dissolved, and wherein the coating comprises a pH sensitive polymer being selected such that the coating substantially dissolves and/or is substantially degraded in the jejunum of a subject. Co-pending International Patent Application PCT/EP2019/084531 relates to combinations of at least two pharmaceutical oral dosage forms for use in the prevention and/or treatment of a condition, disorder or disease selected from the group consisting of insulin resistance, type 2 diabetes,

non-alcoholic fatty liver disease, non-alcoholic steatohepatosis, microvascular dysfunction, metabolic syndrome, obesity, osteoporosis, neurodegenerative diseases, cardiovascular diseases, malabsorption conditions and conditions of impaired gastro-intestinal function in a subject wherein the combination comprises at least a first and a second dosage form each comprising a core and a pH-sensitive enteric coating, wherein each core comprises at least one compound stimulating enteroendocrine cells to release at least one enterokine, and at least one disintegrant providing a burst release of the ingredients of the core when the coating is substantially degraded and/or dissolved, and wherein each coating comprises a pH sensitive polymer being selected such that the coating substantially dissolves and/or is substantially degraded in a selected part of the small intestine of a subject, wherein said first and second dosage form are formulated such that said first dosage form releases the at least one compound stimulating enteroendocrine cells to release at least one enterokine in a different part of the small intestine of the subject than the second dosage form and/or the first and second dosage form are formulated such that said first dosage form show a travelling time through the small intestine of the subject being different from the travelling time of the second dosage form through the small intestine of the subject;
wherein the at least one compound stimulating enteroendocrine cells to release at least one enterokine of said first and second dosage form can be the same or different.

The technical problem underlying the present invention is to provide improved preventive and therapeutic measures conditions associated with disturbed enterokine release by enteroendocrine cells and/or amenable to increased enterokine release by enteroendocrine cells.

The solution to the above technical problem is provided by the embodiments of the present invention as disclosed in the claims, the present description and the accompanying figures.

In particular, the present invention provides a pharmaceutical composition comprising multiple dosage forms each comprising a core and an enteric coating, wherein the core comprises at least one compound stimulating enteroendocrine cells to release at least one enterokine, wherein the size of the dosage forms, with respect to the largest dimension of the dosage forms, provides for entry of the dosage forms into the intestine of a subject independent of gastric emptying mechanisms, and wherein the composition further comprises one or more gelling agents.

A “dosage form” according to the invention is an entity which represents in itself a pharmaceutical formulation typically, and most preferably suitable for oral administration to a

subject, preferably a human subject. The multiple dosage forms present in the pharmaceutical composition of the invention are dimensioned, i.e. have size with respect to their largest dimension, such that, when the pharmaceutical composition of the invention is administered orally to the patient, the multiple dosage forms enter the intestine independent of gastric emptying mechanisms, in particular peristaltic waves, contractions of the Antrum, and shrinkage of the size of the stomach. This means that the multiple dosage forms behave, as regards their transition from the stomach into the intestine, in particular the most proximal part of the small intestine, namely, the duodenum, as a fluid.

The skilled person understands that the parameters of the multiple dosage forms, in particular the size of the multiple dosage forms, their number in the inventive composition, in particular in a unit dose of the composition according to the invention, are interdependent and usually depend on further parameters, such as in particular the specific type and amount of the active pharmacological ingredient(s) (API), specifically the at least one compound stimulating enteroendocrine cells to release at least one enterokine.

That being said, the size of each of the dosage forms present in the composition of the invention, with respect to the largest dimension of the dosage forms, is typically below about 3 mm, preferably from about 0.6 mm to about 3.0 mm, more preferably from about 0.6 mm to about 2.6 mm, even more preferred from about 0.6 mm to about 1.7 mm, and most preferred from about 0.8 mm to about 1.2 mm. The multiple dosage forms contained in the composition according to the invention are typically present in form of pellets, beads or granules, with beads, particularly round-shaped beads, being preferred forms.

The composition of the invention contains a multitude of said dosage forms. The term “multiple dosage forms” typically means that the composition contains at least about1000, more preferably at least about 2000, more preferably at least about 3000, particular preferred at least about 5000, even more preferred at least about 10000 dosage forms. Preferred ranges are from about 1000, about 2000, about 3000, about 5000, or about 10000 to about 20000, about 30000, about 40000 or about 50000 dosage forms in the composition of the invention, preferably per unit dose of the composition according to the invention. Especially preferred ranges are about 10000 to about 40000 dosage forms, and most preferred compositions of the invention contain about 20000 to about 30000 dosages forms. In particular with respect to such embodiments, the size of the dosage forms preferably ranges, according to the above definition, from about 0,6 mm to 1,7 mm, and most preferred from about 0,8 mm to about 1,2 mm.

According to particularly preferred embodiments of the invention, the size and number of the multiple dosage forms contained in the inventive pharmaceutical composition is controlled such that the surface area of the multiple dosage forms in the composition, especially in a unit dose of the composition, is selected such that it covers at least 15 %, more preferably at least 20 %, particularly preferred at least about 25 % of the target area where the at least one active compound as defined herein interacts with the enteroendocrine cells so as to induce a release of the enterokine. In this context, it is to be understood that said target area is not necessarily confined, and in certain embodiments of the invention cases preferably not confined, to the area where the core of the multiple dosage forms releases the active compound. The target area of the active compound is typically dependent on the type of enteroendocrine cells to be triggered to release the enterokine(s). As a preferred example, L cells which are preferably induced by the active compound(s) in the core of the multiple dosage forms are, as further outlined below, present in humans in the small intestine from duodenum to ileum, but their enterokine release capacity, specifically GLP-1 release capacity, particularly due to the increased density and differentiation of the enteroendocrine cells, increases from proximal to distal small intestine, and is highest in the terminal ileum In preferred embodiments of the invention, especially for triggering L cells to release GLP-1 and/or PYY, preferably GLP-1, the enteric coating of the multiple dosage forms is selected such that the at least one active compound is released from the core of the multiple dosage forms in the jejunum, preferably the terminal jejunum of a subject, and the surface area of the multiple dosage forms of the composition of the invention, preferably a unit dose of the composition, is selected such that said surface area makes up between from about 10 % to about 50 %, preferably from about 15 about 35 %, more preferred about 20 to about 30 % of the targeted part of the intestine for the active compound where the targeted cells, preferably L cells, have their highest concentration and/or enterokine release capacity, which is for L cells the ileum, in particular the terminal ileum. In this contest, the term “surface area of the target part of the intestine” is approximated as taken the intestine as a tube having a mean diameter of about 0.25 cm and a length of the terminal ileum of about 60 cm.

The pharmaceutical composition of the invention further contains, as essential ingredient, at least one gelling agents. Gelling agents, also known as thickeners, are gel-forming agents when dissolved in a liquid phase as a colloidal mixture forms a weakly cohesive internal structure. They are typically organic hydrocolloids or hydrophilic inorganic substances. Typical examples include tragacanth, pectin, starch, carbomers, polysaccharides, gelatin, cellulose derivatives, polyvinyl alcohol clays, polyethylene glycols and others (for a review, see, e.g. Kar et al. “Current Developments in Excipient Science, Section 2.2.2.5 “Gelling Agents” in: R. Tekade (ed.) Basic Fundamentals in Drug Delivery, Academic Press,

Cambridge, MA, USA, 2019). The term "at least one gelling agent" as used herein also includes compositions of one or more gelling agents, optionally in combination with other components.

Preferred gelling agents, either for use alone or for use in gelling compositions containing combinations of such gelling agents, include, but are not limited to, modified celluloses such as preferably substituted methylcelluloses, such as hydroxypropylmethylcelluloses (HPMCs), polysaccharides such as preferably alginates, e.g. sodium alginate, and Xanthan gum, and polyethylene glycols (PEGs), preferably PEGs having a mean molecular weight of from about 10000 Da to about 30000 Da, more preferably 15000 Da to about 25000 Da, most preferred about 20000 Da. In preferred embodiments of the invention, the composition contains more than one gelling agents in order to fine tune the properties of the composition, in particular with respect to a specific design of the viscosity properties so as to greatly improve swallowability of the composition in use, when containing or when reconstituted, respectively, with a, preferably pharmaceutically acceptable, liquid medium, e.g. water or an aqueous solution. Preferred combinations of gelling agents, typically in form of a gelling composition, include 2, 3 or 4 gelling agents. Particularly preferred combinations of gelling agents for use in the inventive composition are selected from combinations of modified celluloses, polysaccharides and PEGs, more preferably one or more of the above-mentioned preferred examples.

The pharmaceutical composition of the invention can be provided in various forms. In one preferred embodiment, the pharmaceutical composition according to the invention contains a liquid medium so that the multiple dosage forms and the one or more gelling agent are present in a liquid medium, preferably water or an aqueous solution, more preferably a buffered aqueous solution. In other preferred embodiments of the invention, it is also possible that the at least one gelling agent or, if the at least one gelling agent is present in a gelling composition, the gelling composition and the multiple dosage forms form a heterogeneous mixture. For use of the latter embodiment of the pharmaceutical composition, a liquid medium, as described above and further disclosed herein, is preferably added to the composition. For a unit dose of the composition, a volume of the liquid is selected typically based on the number of the multiple dosage and/or their size. For preferred values of the multiple dosage forms, the dry embodiment of the pharmaceutical composition is typically admixed with about 5 ml to about 40 ml, preferably about 10 to about 35 ml, most preferred about 15 to 30 ml of the liquid medium.

In order to provide stabilization of the inventive pharmaceutical composition upon oral administration, in which form it typically comprises a suitable liquid medium, in particular for stabilization of the gel or gel-like formulation of the inventive composition in the milieu of mouth, throat and/or oesophagus, the composition of the invention preferably comprises one or more pH modifiers, such as preferably citric acid, a salt of citric acid such as tri-potassium citrate monohydrate, adipic acid, sodium hydrogen carbonate, tartaric acid and combinations thereof. In further preferred embodiments of the invention, the one or more pH modifiers can also be included in the gelling composition as described above.

An “enteroendocrine cell” (EEC) as used in the present invention, is a cell present in a subject’s, preferably, human subject’s, intestine, in particular in the small intestine’s mucosa, and secretes one or more enterokines upon receiving an appropriate trigger by a nutritional component. There are several types of enteroendocrine cells which can be addressed by the active compound or compounds released by the pharmaceutical composition of the invention (for a review of enteroendorine cells of the lower gastrointestinal tract, see, for example, Gunawardene et al. (2011) Int. J. Exp. Pathol. 92, 219-231, and Latorre et al. (2017) Neurogastrol. Motil 28 (5), 620-630). In particular, preferred enteroendocrine cells in the context of the invention are I cells, K cells and L cells. with L cells being most preferred.

According to the invention, an “enterokine”´ is a hormone secreted by EECs in the gastro-intestinal system. Preferred enterokines are the incretins, i.e. hormones regulating the blood sugar level upon intake of nutrition, preferably GLP-1, GLP-2 GIP and PYY, more preferably GLP-1 and PYY. Other preferred enterokines secreted by the EEC(s) through release of the active compound(s) in the pharmaceutical composition are CCK and neurotensin.

I cells are predominantly present in the proximal small intestine, in particular in the lower duodenum and in the jejunum and secrete cholecystokinin (CCK) upon sensing of nutrients such as amino acids and fatty acids. CCK effects the release of bile acids from the gall bladder into the small intestine, but also promotes the release of digestive secrete from the pancreas.

K cells are found in the complete small intestine and release GIP (glucose-dependent insulinotropic peptide; also known as gastric inhibitory peptide) leading to inhibition of gastric motility and enhancement of insulin production and secretion.

L cells are present throughout the small intestine, i.e. duodenum, jejunum, and ileum, and release GLP-1 (glucagon-like peptide 1), GLP-2 (glucagon-like peptide 2) and PYY (peptide

YY) in response to various nutrients. The concentration of L cells raises from duodenum to jejunum to ileum, and the GLP-1 release from L cells shows a gradient sloping from proximal to distal small intestine with highest GLP-1 release capacity in the terminal ileum. The gradient is not only a function of increasing number of L cells, but also a function of their maturation and differentiation state as well as production rate of GLP-1, which also increase from proximal to distal ileum. In particular, GLP-1 is released from L cells in the crypts and on the villi of the mucosa. L cells mature from crypt to villi, and GLP-1 release capacity is highest when the L cells reach the top of the villi. L cells further secrete PYY (peptide YY) which is predominantly released in the terminal ileum. GLP-1 enhances nutrient-stimulated insulin secretion and inhibits glucagon secretion, gastric emptying and feeding. GLP-1 also has proliferative, neogenic and antiapoptotic effects on pancreatic β-cells. As an intestinal trophic peptide, GLP-2 stimulates cell proliferation and inhibits apoptosis in the intestinal crypt compartment, it also regulates intestinal glucose transport, food intake and gastric acid secretion and emptying. Furthermore, GLP-2 improves intestinal barrier function. PYY inhibits gastric motility and increases water and electrolyte absorption in the colon. PYY also suppresses pancreatic secretion and has been shown to reduce appetite. PYY slows gastric emptying; whereby it increases efficiency of digestion and nutrient absorption after a meal.

EECs, in particular L cells, are triggered to release enterokines such as GLP-1 and PYY through a variety of mechanisms being affected by the one or more active compound(s) in the pharmaceutical composition of the invention.

L cells release GLP-1 and PYY (and also GLP-2; note that GLP-1 and GLP-2 are derived from a common mRNA so that these hormones are essentially co-released; cf., for example, the review of Baggio and Drucker (2004) Best Practice & Research Clinical Endocrinology & Metabolism 18 (4), 531-554) in response to various mechanisms triggered by compounds such as nutrients. One mechanism includes intake of a carbohydrate such as glucose through glucose transporters GLUT2 and/or SGLT1. Other mechanisms rely on the binding to specialized G protein-coupled receptors such as taste receptors, fatty acid receptors, bile acid receptors, peptide receptors and amino acid receptors.

These signals, glucose transport and binding to G protein-coupled receptor, are typically transmitted in the cells by one or more of three mechanisms and lead ultimately to the release of the enterokine, in the case of L cells GLP-1 and PYY: transmembrane calcium influx, intracellular calcium release and/or intracellular cAMP increase.

Accordingly, the active compound(s) triggering the release of an enterokine through an EEC are preferably selected from nutrients, and more preferred active compounds include carbohydrates, fatty acids, bile acids, peptides (including oligopeptides, polypeptides and proteins), amino acids, alcohol amides and anthocyanins. Preferred fatty acids are fatty acids having 2 to 6 carbon atoms. Ethanolamides such as oleoylethanolamide, anandamide (N-arachidonoylethenolamide, AEA), palmitoylethanolamide, steaorylethanolamide, and derivatives of anandaminde such as prostamides, can also preferably be used as alcohol amide compounds in the inventive oral dosage forms. A particularly preferred ethanolamide is oleoylethanolamide (OEA). A preferred example of a peptidic active compound is the protein bovine serum albumin (BSA). Carbohydrates are preferably selected from glucose and sucralose, with glucose being most preferred. In a particular preferred embodiment of the invention, the multiple dosage forms in the pharmaceutical composition contain about 1 % (w/w) to about 99 % (w/w), such as 60 to 70 % (w/w) of active compound triggering the EECs, preferably a carbohydrate, most preferred glucose (dextrose). Particularly preferred glucose contents in the core of the multiple dosage forms are in the range of form about 5 % (w/w) to about 95 % (w/w), preferably from about 25 % (w/w) to about 75 % (w/w), more preferably from about 40 % (w/w) to about 70 % (w/w).

In a preferred embodiment, the active compound triggering the release of an enterokine through an EEC is an anthocyanin, more preferably one or more anthocyanins from *Vaccinium myrtilloides* Michx. A particularly preferred anthocyanin to be included in the core of the pharmaceutical oral dosage forms of the invention is delphinidin 3-rutinoside.

In a preferred embodiment of the invention, one or more of the above active compounds triggering enterokine release by EECs are combined in preferably synergistic combinations.

Preferred combinations are combinations of a carbohydrate, preferably glucose, with sucralose and/or one or more fatty acids having 2 to 6 carbon atoms and/or oleic acid and/or one or more bile acids and/or one or more alcohol amides, preferably one or more ethanolamides, more preferably oleoylethanolamide, and/or one or more peptides (including oligopeptides, polypeptides and proteins, such as preferably BSA) and/or one or more amino acids and/or one or more anthocyanins (preferably delphinidin 3-rutinoside).

Further preferred combinations are combinations of another carbohydrate, preferably sucralose, with glucose and/or one or more fatty acids having 2 to 6 carbon atoms and/or oleic acid and/or one or more bile acids and/or one or more alcohol amides, preferably one or more ethanolamides, more preferably oleoylethanolamide, and/or one or more peptides

(including oligopeptides, polypeptides and proteins) such as preferably BSA, and/or, one or more amino acids and/or one or more anthocyanins (such as preferably delphinidin 3-rutinoside).

Other preferred combinations are combinations of a fatty acid having 2 to 6 carbon atoms with one or more carbohydrates, preferably glucose and/or sucralose, and/or oleic acid and/or one or more bile acids and/or one or more alcohol amides, preferably one or more ethanolamides, more preferably oleoylethamolamide, and/or one or more peptides (including oligopeptides, polypeptides and proteins), preferably BSA) and/or one or more amino acids and/or one or more anthocyanins such as preferably delphinidin 3-rutinoside.

According to a further preferred embodiment, the present invention provides combinations of oleic acid with one or more fatty acids having 2 to 6 carbon atoms and/or one or more carbohydrates, preferably glucose and/or sucralose, and/or one or more bile acids and/or one or more alcohol amides, preferably one or more ethanolamides, more preferably oleoylethanolamide and/or one or more peptides (including oligopeptides, polypeptides and proteins, such as preferably BSA) and/or one or more amino acids and/or one or more anthocyanins such as preferably delphinidin 3-rutinoside.

Further preferred combinations of active compounds are combinations of one or more bile acids with one or more carbohydrates, preferably sucralose and/or glucose, and/or one or more fatty acids having 2 to 6 carbon atoms and/or oleic acid and/or one or more alcohol amides, preferably one or more ethanolamide, more preferably oleoylethanolamide, and/or one or more peptides (including oligopeptides, polypeptides and proteins, preferably BSA, and/or one or more amino acids and/or one or more anthocyanins such as preferably delphinidin 3-rutinoside.

Still further preferred combinations of active compounds are combinations of one or more alcohol amides, preferably one or more ethanolamides, more preferably oleoylethanolamide, with one or more carbohydrates, preferably sucralose and/or glucose, and/or one or more fatty acids having 2 to 6 carbon atoms and/or oleic acid and/or one or more bile acids and/or one or more peptides (including oligopeptides, polypeptides and proteins) preferably BSA, and/or one or more amino acids and/or one or more anthocyanins such as preferably delphinidin 3-rutinoside.

Other preferred combinations of active compounds are combinations of one or more peptides (including oligopeptides, polypeptides and proteins), preferably BSA, with one or more

carbohydrates, preferably sucralose and/or glucose, and/or one or more fatty acids having 2 to 6 carbon atoms and/or oleic acid and/or one or more bile acids and/or one or more alcohol amide, preferably one or more ethanolamide, more preferably oleoylethanolamide, and/or one or more amino acids and/or one or more anthocyanins such as preferably delphinidin 3-rutinoside.

In other preferred embodiments of the invention, the core of the multiple dosage forms contains a combination of one or more amino acids with one or more carbohydrates, preferably sucralose and/or glucose, and/or one or more fatty acids having 2 to 6 carbon atoms and/or oleic acid and/or one or more bile acids and/or one or more ethanolamides, more preferably oleoylethanolamide, and/or one or more peptides (including oligopeptides, polypeptides and proteins); preferably BSA, and/or one or more anthocyanins such as preferably delphinidin 3-rutinoside.

In still further preferred combinations of active compounds in the core part of the multiple dosage forms used in the invention, one or more anthocyanins, preferably delphinidin 3, is/are combined with one or more carbohydrates, preferably sucralose and/or glucose, and/or one or more fatty acids having 2 to 6 carbon atoms and/or oleic acid and/or one or more bile acids and/or one or more alcohol amides, preferably one or more ethanolamides, more preferably oleoylethanolamide, and/or one or more peptides (including oligopeptides, polypeptides and proteins), preferably BSA, and/or one or more amino acids.

The active compound(s) in the core part of the multiple dosage forms are preferably further combined with active ingredients having various functions.

Preferred examples of additional active ingredients are EEC maturation agents. As exemplified before with L cells such maturation agents typically enhance the release capacity of the EECs, such as L cells, for the relevant enterokine, in the case of L cells GLP-1 and/or PYY and/or GLP-2. Preferred EEC maturation agents, in particular L cell maturation agents, include human milk oligosaccharides (HMO) and inhibitors of NOTCH signalling such as γ-secretase inhibitors, preferably dibenzazepine. Various HMOs are commercially available (Jennewein Biotechnologie GmbH, Rheinbreitbach, Germany) and preferred HMOs in the context of the invention include, but are not limited t, e.g. 2'-fucosyllactose, 3-fucosyllactose, 6'-sialyllactose and lacto-N-neotetraose as well as mixtures thereof.

The pharmaceutical composition of the invention preferably contains multiple oral dosage forms designed to burst release the active ingredient(s) at a selected targeted area of the

small intestine of a subject, preferably the jejunum, more preferably, the terminal (= distal) jejunum) of the, preferably human, subject. For providing a burst release of the ingredients of the core of the multiple dosage forms, the core of the multiple dosage forms contains at least one disintegrant. Disintegrants for use in the present invention are generally known in the art as rapidly expanding and dissolving when coming into contact with the targeted environment, typically, an aqueous environment as in the present invention, namely, the small intestine of a subject, preferably a human. Disintegrants lead to rapid breakdown of the core of the pharmaceutical oral dosage form of the invention when the core comes into contact of the aqueous medium present in the subject's small intestine. Preferably, the disintegrant in the multiple dosage forms of the composition of the invention is selected such that more than 70 % of the core is released within two minutes or less or more than 85 % of the core is released within 5 minutes or less, following contact with water or an aqueous medium like the small intestine of a human subject. Preferred disintegrants in the context of the invention are crosslinked polyvinylpyrrolidones, crosslinked carboxymethyl celluloses and modified starchs. Particularly preferred crosslinked polyvinylpyrrolidones for use in the invention include Polyplasdones, in particular Polyplasdone XL, Polyplasdone XL-10 and Polyplasdone INF-10 (commercially available from Ashland Inc., Covington, KY, USA). Other useful disintegrants include, but are not limited to, polyvinylpolypyrrolidone, croscarmellose and carboxymethylcellulose (preferably, the sodium salt thereof).

A burst release of the core's ingredients, in particular the active compound(s) such as glucose, establishes a steep gradient between intracellular and extracellular levels of the respective active compound(s), preferably glucose, sucralose, ethanolamides, BSA and/or an anthocyanine such as delphinidin 3-rutinoside, in the targeted EEC, preferably L cells, at the site of release of the core ingredients.

Compared to previous technologies, in particular WO 2010/027498 A2, WO 2012/118712 A2 and US 2014/0294951 A1, the pharmaceutical composition of the invention shows a combination of highly improved properties: on the one hand side, the pharmaceutical composition of the invention provides for an improved release of enterokine release, in particular GLP-1 and/or PYY release, in patients, especially highly reproducible strong enterokine release combined with very low intra-patient and inter-patient variability, since every dose of the composition of the invention provides for a single peak of enterokine, in particular GLP-1, release at the selected time point after oral administration (see, in particular, Fig. 1 of the present application in comparison to the results obtained with prior art compositions such as, e.g. in US 2014/0294951 A1; cf. Fig. 6a to 6f thereof). On the other hand, due to the combination of the multiple dosage forms with the gelling agent(s),

preferably present in a gelling composition, the pharmaceutical composition described herein is a very safe formulation of the comparatively small multiple dosage forms, which formulation, in particular when reconstituted with a liquid medium such as water or an aqueous solution, provides a suspension of the multiple dosage forms in a gel or gel-like formulation as disclosed herein, which can be swallowed substantially more easily by the subject (as compared, for example, to comparatively large capsules or tablets of 10 g of API (glucose) disclosed in WO 2010/027498 A2 and WO 2012/118712 A2) in need thereof contributing to improved compliance. Furthermore, due to the presence of the at least one gelling agent, it is prevented that the multiple dosage forms, small in size, may be aspired by the subject. This is of high importance both for safety and patient compliance, since oral administration of large tablets or capsules containing, e.g. 7 to 10 g glucose, as taught in WO 2010/027498 A2 and WO 2012/118712 A2 in order to obtain any effect, is particularly difficult for children, such as preferably pediatric patients having an age of up to about 6 years of age, and elderly patients, preferably elder patients in the age ranges outlined below. It is most important for the subjects preferably addressed by the present composition (in particular, subjects suffering from obesity, insulin resistance, type 2 diabetes mellitus (T2DM), metabolic syndrome, but also severe forms of certain viral infections such as COVID-19) are typically elderly subjects such as subjects being of an age of at least about 50, more preferably at least about 55, even more preferred at least about 60, which in general show a statistically high incidence of swallowing problems, up to severe dysphagia and even achalasia, and the specific formulation of the present can be easily and safely taken up be such subjects, even in case of dysphagia, which will provide for a much higher compliance rate and consequently highly improved positive effects in the course of treatment of patients compared to prior art technologies.

Moreover, and of particular relevance, the inventive combination of multiple dosage forms and gelling agent(s) (preferably present in a gelling composition), and optionally further agents such as, in particular pH modifying agents, shows an improved viscosity and flow properties on mucosal surface to optimize ingestion in particular in subjects of the elderly and pediatric populations, whereby preferred age ranges of subjects of these populations are given above. In order to further improve the flow properties of the pharmaceutical composition, in particular when containing or when reconstituted with a liquid medium as disclosed above, respectively, one or more viscosity modifiers (in particular other than gelling agents) and/or one or more flow aids (in particular other than gelling agents) can be included into the pharmaceutical compositions of the invention. Preferred examples of substances useful as viscosity modifiers and/or flow aids are lubricants such as preferably polyalcohols,

more preferably glycerol, colloidal silicon dioxides and vegetable oils, more preferably one or more vegetable oils selected from olive oil, sunflower seed oil, almond oil and sesame oil.

In preferred embodiments of the invention, it is preferred that the multiple dosage forms release the active compound(s) in the jejunum, more preferably in the terminal jejunum, of a subject, preferably a human subject, through a specific design of the enteric coating, typically a pH sensitive coating. Preferably, the coating substantially degrades and/or dissolves in the jejunum, preferably the terminal jejunum, such that the core is released into the jejunum, preferably the terminal jejunum, of the subject, by specific selection of the enteric coating which is preferably chosen from pH sensitive polymers substantially degrading and/or dissolving at a pH value of about 5.5 to about 7.5, preferably about 7.2 to about 7.3. Such pH sensitive polymers are preferably selected from hydroxypropylmethyl celluloses (also called hereinafter “hypromelloses”) and anionic copolymers of methacrylic acid and methacrylmethacrylate. Most preferably, the pH sensitive enteric coating containing or being made of hydroxypropylmethyl cellulose is hydroxypropylmethyl cellulose acetate succinate. A highly preferred commercially available product of this kind is AQOAT®, particularly preferred AQOAT®-HF (Shin-Etsu Chemical Co., Chiyoda, Japan). In other preferred embodiments of the type of anionic copolymers of methacrylic acid and methcrylmethacrylate various forms of Eudragit® polymers may also be used. Eudragit® is commercially available from Evonik Healthcare & Nutrition GmbH, Essen, Germany. In preferred embodiment, Eudragit® FS30D and/or Eudragit® L30D is/are used as the pH sensitive polymer(s) of the coating, or at least a part thereof.

In further preferred embodiments of the invention, different coatings can be applied in combination. According to one embodiment, the coating comprises or is made of a combination of a hydroxypropylmethyl cellulose and an anionic copolymer of methacrylic acid and methacrylmethacrylate. Preferably, a combination of coatings is applied such that typically a sub-coating of one pH sensitive polymer is applied as a first layer and a coating of a second pH sensitive polymer is applied on the sub-coating as a second layer. For example, the pH sensitive coating can comprise a sub-coating of or comprising, respectively, a hydroxypropylmethyl cellulose as a first layer, and a second coating comprising or being made of an anionic copolymer of methacrylic acid and methacrylmethacrylate provided as a second layer on the sub-coating. In a further preferred embodiment, the coating of the pharmaceutical oral dosage form of the invention comprises a coating comprising a first layer (sub-coating) comprising or being made of an anionic polymer of methacrylic acid and methacrylmethacrylate such as an Eudragit®, more preferably Eudragit® FS30D, and a second layer comprising or being made of a hydroxypropylmethyl cellulose such as

AQOAT®, more preferably AQOAT®-HF. More preferably, the anionic copolymer of methacrylic acid and methacrylmethacrylate, e.g. an Eudragit®, preferably Eurdragit® FS30D, is present in less amount than the hydroxypropylmethyl cellulose such as AQOAT®, more preferably AQOAT®-HF. In other words, the thickness of the first layer of this type of combination is lower than the thickness of the second layer in this combination. More specifically, the ratio of amount or thickness, respectively, between first layer and second layer typically ranges from about 1:10 to about 1:50, particularly preferred from about 1:20 to about 1:30.

For further improving the enterokine release exerted by the inventive composition, it is preferred that the multiple dosage forms, in particular their core, further contain enteroendocrine release improvement agents, i.e. a substance enhancing enterokine release by the enteroendocrine cells effected by the compound stimulating enteroendocrine cells to release at least one enterokine, particularly preferred at least one substance enhancing release of GKLP-1 and/or PYY by L cells. For preferred embodiments of the invention where L cells are targeted to release GLP-1 such agents are preferably selected from inhibitors of GLP-1 degradation such as DPP-4 inhibitors, substances enhancing L cell maturation (e.g. human milk oligosaccharides, inhibitors of NOTCH signalling such as γ-secretase inhibitors, preferably dibenzazepine), stimulating agents and combinations thereof. A preferred stimulating agent for use in the present invention is caffeine. One example of a preferred combination of active compound and stimulating agent is glucose and caffeine, preferably in a weight ratio of glucose to caffeine of from about 5:1 to about 50:1, preferably about 8 to 1 to about 40:1. In other embodiments, preferred combinations of glucose and caffeine in the core of the multiple oral dosages forms are about 60 to about 70 % (w/w) glucose and about 2 to about 4 % (w/w) caffeine, most preferred about 67 % (w/w) glucose and about 3.2 % (w/w) caffeine, based on the total weight of the core.

Caffeine and other known substances can also be included for controlling and monitoring the appropriate release and also the spreading of the ingredients of the core of the multiple oral dosage forms contained in the composition of the invention in the intestinal tract of the subject, preferably a human. The release of the tracer substance from the multiple dosage forms of the pharmaceutical composition in the small intestine can be conveniently monitored by analytical methods generally known in the art. In the case of caffeine, blood samples are taken from the subject before and at suitable time intervals after oral administration of the oral dosage form. After centrifugation of the blood sample, the caffeine content in the serum fraction of the sample is measured by ELISA testing using commercially available test kits

(e.g. Caffeine ELISA Kit, BioVision Inc., Milpitas, CA, USA) according to the manufacturer's instructions.

The pharmaceutical composition forms of the invention are preferably fine-tuned in various further ways so as to further improve targeted release and triggering of EECs in the small intestine of a subject, preferably a human subject. Moreover, different multiple dosage forms having a variety of release patterns in the subject's small intestine can be combined in the composition of the invention so as to synergistically act on the subject's EECs and their enterokrine output.

As outlined above, the present invention is directed to specific pharmaceutical compositions comprising preferable multiple dosage forms as outlined above which are small in dimension, preferably below 3 mm in the largest dimension, more preferably about 0.6 mm to about 1.7 mm in the largest dimension, particularly preferred from about 0.8 mm to about 1.2 mm in the largest dimension. As already outlined above, such small dosage forms may conveniently take the form of beads, granules or pellets. As has been explained in detail above, the small dosage forms of the invention have the benefit of behaving like a fluid in a subject's stomach causing a fast and constant entry of the multiple oral dosage forms into the intestinal tract, and therefore to evenly transport it to the targeted area, preferably a targeted burst release area, preferably in the subject's jejunum, more preferably the subject's terminal (i.e. distal part) jejunum.

In addition to the above components the pharmaceutical composition of the invention may contain further ingredients typically present in oral dosage forms such as tablets, capsules, granules and pellets, for providing and/or improving various parameters. Typical additional ingredients for use in the present invention include excipients, carriers, fillers, glidants, dispersants, plasticizers, wetting agents, anti-tacking agents, neutralization agents, colorants, pigments, opacifiers, flavours, taste improvement agents such as sweeteners, and the like. It is to be understood that these and other benefit agents can be included in the multiple dosage forms and/or added to the pharmaceutical composition, for example, and preferably, in the gelling composition, separate from the multiple dosage forms. The person skilled in the art of formulating pharmaceutical compositions and the multiple oral dosage forms is readily able to identify specific compounds and substances of the above and other types as well as their combinations and amounts to be used. Further guidance can be found in Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, in particular pages 1289-1329.

Further subject matter of the invention is a pharmaceutical article comprising multiple dosage forms as defined herein and at least one gelling agent as defined herein, wherein the multiple dosage forms and the at least one gelling agent are physically separated.

As mentioned above with respect to the inventive pharmaceutical composition, the at least one gelling agent can preferably be present in a gelling composition which can contain further gelling agents and/or other pharmaceutically acceptable agents such as preferably one or more pH modifiers, whereby preferred examples of pH modifiers for use in the invention are already outlined above.

In other embodiments of the invention, the pharmaceutical article is comprised of a pharmaceutical composition as defined herein and, physically separated therefrom, of water or an aqueous solution. Preferably, the physical separation is affected by providing the pharmaceutical composition (without water/aqueous solution) in one container, and the water or aqueous solution in a further, second, container. For use, the water or aqueous solution is combined with the pharmaceutical composition in one of the two containers, or in a different container.

In preferred embodiments, the pharmaceutical article further comprises water and/or an aqueous solution physically separated from the multiple dosage forms and from the at least one gelling agent or gelling composition, respectively.

The “physical separation” according to the invention is any means whereby the multiple dosage forms and the one or more gelling agents or the gelling composition, respectively, are separated from each other such that they do not come into contact, typically until used by the subject for which the pharmaceutical article is envisaged. For example, the multiple dosage forms and the gelling agent(s)(gelling composition can be provided in two separate containers which together make up the pharmaceutical article, optionally, and preferably, in combination with a suitable package and, more preferably, further together with a pharmaceutical leaflet containing instructions for use. The article can also comprise water or an aqueous solution which is also physically separated from the multiple dosage forms and the gelling agent(s)/gelling composition. Preferably, the water or aqueous solution, respectively, is provided in a third container.

In other preferred embodiments of the inventive pharmaceutical article the multiple dosage forms and the at least one gelling agent and, optionally, the water and/or aqueous solution, are provided in a single container comprising at least one compartment containing the

multiple dosage form, at least one compartment containing the at least one gelling agent or gelling composition and, optionally, at least one compartment containing the water or aqueous solution, wherein said compartments are separated by an at least partially removable, preferably breakable, physical separation.

Removable physical separations, preferably partially removable physical separations, for use in the pharmaceutical article are known to the skilled person. In one preferred embodiment, the separation is provided by a breakable foil, preferably between each compartment, which foil may preferably embodied as a blister foil typically used for pharmaceuticals. In a preferred embodiment, containing multiple dosage forms, gelling agent(s)/gelling composition, and water or aqueous solution, the compartments are provided in the container such that a first lowest compartment contains the liquid medium, and the two upper compartments contain the multiple dosage forms. The user may then press the contents of the uppermost compartment through the foil such that multiple dosage forms and gelling agents or gelling composition are first mixed, and then by further pressure application, are pressed through the breakable separation, preferably a blister foil, into the lower liquid medium compartment such that the final pharmaceutical composition is reconstituted, typically as a gel or gel-like suspension of the multiple oral dosage forms, and can be administered orally to the subject in need of the treatment preferably by self-administration.

In other preferred embodiments, the pharmaceutical article contains a pharmaceutical composition of the invention (without water or aqueous solution) and water or aqueous solution, respectively, in a single container having two compartments, whereby the pharmaceutical composition is provided in one and the water or aqueous solution, respectively, is provided in a second compartment, and the two compartments are separated by an at least partially removable, preferably, breakable physical separation, more preferred a breakable foil, particularly preferred a blister foil as outlined above for the three-compartment embodiment. For reconstitution, the user, preferably a subject in need of treatment, will at least partially remove the separation between the compartments, preferably by pressing the pharmaceutical composition (without water/aqueous solution) through a blister foil into the compartment containing the liquid medium, preferably water or an aqueous solution.

As regards the pharmaceutical articles as described herein, it is preferred that a container or compartment comprising the multiple dosage forms contains a unit dose of the multiple dosage forms for one administration to the subject. Preferred unit dosages, in particular numbers of multiple dosage forms, are as outlined above.

The present invention also relates to methods of preparing pharmaceutical compositions as defined and described herein. In one embodiment, the method for preparing an inventive pharmaceutical composition comprises combining multiple dosage forms as described above, preferably having sizes and/or numbers as outline before, with at least one gelling agent or a gelling composition respectively. The method preferably comprises the further step of combining the resulting mixture of multiple dosage forms and at least one gelling agent or gelling composition, respectively, with a liquid medium, preferably water or an aqueous solution. In a different aspect of the invention, there is also provided a method for producing a pharmaceutical composition of the invention comprising combining a composition comprising multiple dosage forms as described above and at least one gelling agent or a gelling composition, respectively, with a liquid medium, preferably water or an aqueous solution. As will be understood by the skilled person, the result of this method is typically a gel or gel-like formulation of the multiple dosage forms.

According to the invention, the water for providing a mixture of multiple dosage forms and at least one gelling agent or gelling composition, respectively, in a liquid medium may be tap water or sterilized water. Aqueous solutions for that purpose are preferably buffered or unbuffered saline solution such as physiological sodium chloride solutions, Ringer or Ringer lactate. The liquid medium may also be selected from other embodiments such as flavoured and/or pigmented or dyed aqueous solutions or even suspensions such as juices, e.g. fruit or vegetable juices, as long as such liquids do not interfere with the formation of a gel or gel-like formulation containing the multiple dosage forms.

The pharmaceutical compositions and/or pharmaceutical articles as defined and described herein are particular useful in the prevention and/or treatment in a subject suffering from conditions and/or disorders and/or diseases associated with disturbed enterokine release by enteroendocrine cells and/or conditions and/or disorders and/or diseases amenable to increased enterokine release by enteroendocrine cells.

Preferred conditions, disorders or diseases to which the pharmaceutical compositions and/or pharmaceutical articles of the invention can be applied for treatment and/or prevention are metabolic disorders, vascular disorders, neurodegenerative diseases, skeletal diseases and gastroenterological disorders.

Preferred metabolic disorders are selected from insulin resistance, type 2 diabetes mellitus, non-alcoholic fatty liver disease, non-alcoholic steatohepatosis, metabolic syndrome, hyperlipidemia and obesity.

Especially in the context of diabetes, in particular T2DM, and prediabetic conditions, preferably insulin resistance and/or obesity, the therapeutic and/or preventive regimen of the invention has tremendous benefits, since it is known that increased enterokine levels, in particular GLP-1, exerted by the administration of inventive pharmaceutical composition have a beneficial effect of increasing the Adiponectin/Leptin ratio (cf., for example, Unamuno et al. (2019) Nutrients, 11, 2069-2079) which diminishes known inflammation of adipose tissues frequently encountered in patients suffering from T2DM and/or pre-diabetic conditions such as insulin resistance and obesity.

In contrast to prior art applications of enterokine stimulating compositions, the range of indications for which the inventive compositions and/or articles, respectively, can be applied to successfully is broadened tremendously according to the invention, since the inventors have detected and recognized that enterokines released by enterendocrine cells, in particular GLP-1 released by L cells, have various effector cites in the organism, in particular humans, which is particularly displayed by the expression profiles of enterokine receptors, in particular GLP-1 receptors.

For example, GLP-1 shows effects on vascular tissues, in particular a vasodilatory and cardioprotective function (see, e.g. Ban et al. (2008) Circulation 117, 2340-2350) such that the pharmaceutical compositions and/or pharmaceutical articles of the invention can preferably applied for treatment and/or prevention of vascular disorders such as preferably microvascular dysfunction, cardiovascular diseases, cerebrovascular diseases and pulmonary vascular diseases.

Pulmonary diseases are a preferred target for therapy and/or prevention by the inventive embodiments, since expression of GLP-1 receptors is highest in lungs. As preferred classes of pulmonary vascular diseases, pulmonary diseases associated with pneumonia are contemplated, more preferably in pneumonia associated with or caused by, respectively, viral infection.

Since severe forms of coronavirus infection, in particular by coronaviruses causing respiratory pneumoniae caused by or associated with, respectively, infections with SARS-COV-1, SARS-COV-2 and/or MERS, are associated with severe damage of pulmonary

vessels, it is proposed by the invention that induction of enterokines, in particular GLP-1, by administration, in particular oral administration, of the pharmaceutical composition, e.g. by use of a pharmaceutical articles as disclosed herein. Particularly preferred therapeutic target of the invention is SARS-COV-2, whereby the administration of the pharmaceutical composition is preferably taken place before the onset of the COVID-19 manifestation, or at least before the occurrence of severe forms comprising development of respiratory conditions such as in particular pneumonia.

In this context, it is to be noted that Glucagon-like-peptide 1 (GLP-1) is best known as an enteroendocrine hormone that orchestrates insulin release in response to ingested nutritional stimuli (Paternoster et al. (2018) Front. Endocrinol. 9, 1-26). GLP-1 has also emerged as an important homeostatic element within the cardiovascular system, where it possesses significant endothelial-protective functions (Helmstädter et al. (2020) Arterioscler. Thromb. Vasc. Biol. 40, 145–158; Sun, Y.-H. et al. (2017) Mol. Med. Rep. 16, 929–936). In the lung, GLP-1 tightens barriers via the upregulation of tight junction proteins in barrier-forming cells ; in alveolar type 2 pneumocytes, GLP-1 stimulates the production of surfactant that, by reducing surface tension, helps minimize fluid accumulation within alveolar spaces (Vara et al. (2001) Am. J. Respir. Crit. Care Med. 163, 840–846). In endothelial cells, GLP-1 inhibits TNF Alpha Converting Enzyme (TACE) expression and activity (Ku et al. (2014) Pharmacol. Res. 84, 18–25): It therefore directly opposes key mechanisms that SARS-CoV-2 commandeers to augment inflammation and compromise barrier function. Accordingly, GLP-1 signalling attenuates TACE-dependent Endothelial Protein C Receptor (EPCR) shedding (Ku et al. (2014), supra); GLP-1 signalling also increases deficient Angiotensin Converting Enzyme 2 (ACE2) levels in pathological settings (Romaní-Pérez. et al. (2015) Endocrinology 156, 3559–3569), presumably by reduced ACE2 shedding. Consistent with this anti-inflammatory role, GLP-1 receptor agonists possess several desirable actions, including (i) antagonizing NF-κB signalling (Helmstädter et al. (2020), supra), (ii) reducing immune cell adhesion molecule expression on endothelial cell surfaces (e.g., ICAM-1 and VCAM-1) (Helmstädter et al. (2020), supra; Arakawa et al. (2010) Diabetes 59, 1030–1037), (iii) reducing immune cells cytokine production (Arakawa et al. (2010), *supra*) and (iv) attenuating endothelial cell oxidative stress (Ceriello et al. (2013) Diabetes Care 36, 2346–2350). In fact, several of the risk factors associated with severe COVID-19 (e.g., obesity, diabetes) also associate with reduced GLP-1 secretion and circulating GLP-1 levels.

The present invention therefore expands the repertoire of interventions yielding positive therapeutic effects of GLP-1 effects in COVIDF-19 patients. The high anti-COVID-19 potential of the embodiments of the invention is underscored by previous findings according

to which agonists or supraphysiological levels of native GLP-1 have been utilized to elicit desirable effects. However, normal post-prandial levels of endogenous GLP-1 clearly suffice to stimulate nitric oxide production in the forearm, which increases blood flow, microvascular blood volume, and interstitial oxygen uptake in skeletal muscle cells (Chai et al. (2012) Diabetes 61, 888–896); Dong et al. (2013) Am. J. Physiol. - Endocrinol. Metab. 304, E222–E228) .

A preferred skeletal disease for which the embodiments of the invention can be applied to is osteoporosis.

A further therapeutic aspect of the invention is based on the known proliferative and antiapoptotic effect of enterokines as described herein, specifically GLP-1 and GLP-2, on cells of the gastro-intestinal tract, especially of the gastro-intestinal mucosa (see, for example, Sigalet (2012) J. Anim. Sci. 90, 1224-1232; Aw et al. (2017) Asia-Pac. J. Clin. Oncol. 14, 23-31; and Kissow et al. (2012) Cancer Chemother. Pharmacol. 70, 39-48). By administration of the pharmaceutical compositions of the invention, which may be prepared by use of the pharmaceutical articles as disclosed above, it is possible not only to prevent and/treat malabsorption disorders in affected subjects, but also to treat subjects, in particular human subjects, suffering from disorders, diseases and/or conditions of impaired gastro-intestinal function, in particular due to irregular gastro-intestinal growth including reduced intestinal length and impaired/reduced formation of intestinal mucosa and villi, especially, and preferably, in neonatal and infant children, and/or due to disorders of the intestinal mucosa such as mucositis, preferably resulting from chemotherapy, radiotherapy and/or infections, especially in tumor and/or cancer patients. This treatment aspect of the invention also comprises the prevention of the above gastro-intestinal disorders and diseases.

As disclosed above, the pharmaceutical composition, in particular pharmaceutical gel or gel-like formulations of the multiple dosage forms as described herein, are for use in patients suffering from metabolic disorders, in particular diseases involving aberrant energy household such as diabetes and pre-diabetic conditions as outlined above. In particular in such patients, uptake and transport of conventional oral formulations such as disclosed in the prior art (in particular tablets or capsules containing substantial amounts of active compounds such as glucose) is frequently impaired and/or slowed down considerably under conditions such as gastroparesis (delayed gastric emptying), in particular in patients having infections by *Heliobacter pilori*, neuromuscular dysfunction, and advanced age, and diabetes, in particular T2DM, where in up to 50% of diabetic patients the dysfunction of autonomic nerves, specifically vagal efferent nerves that govern gastric and small intestine peristalsis, is

a comorbidity. Therefore, the pharmaceutical compositions and articles of the invention are particularly useful for treatment of subjects, in particular humans, in which one or more of the indications as disclosed herein is/are associated with one or more of the described comorbidities.

Accordingly, the pharmaceutical compositions and/or pharmaceutical articles are particularly useful in cases wherein the above conditions, disorders or diseases are accompanied by at least one condition selected from the group consisting of swallowing difficulty, dysphagia, achalasia, impaired esophageal peristalsis, gastroparesis and impaired intestinal peristalsis.

The present invention also relates to a method of treatment of the above-described conditions, disorders and/or diseases comprising the oral administration of an effective amount of a pharmaceutical composition of the invention, in particular the inventive formulation of the multiple dosage forms in a gel or gel-like suspension, to a, preferably human, subject in need thereof, wherein the administration pharmaceutical composition preferably leads to the release of GLP-1 and/or GLP-2, in particular a substantial increase of GLP-1 and/or GLP-2 levels, in said subject.

In certain embodiments, it is also preferred that the pharmaceutical composition, before administration to the subject, preferably by self administration is reconstituted, preferably by use of the pharmaceutical articles as disclosed herein, by combining a pharmaceutical composition comprising multiple dosage forms as outlined herein and at least one gelling agent or a gelling composition, respectively, as disclosed above, and a suitable liquid as described herein.

In general, the administration of the pharmaceutical composition of the invention leads to a substantial increase of the addressed at least one enterokine, preferably GLP-1, GLP-2, GIP, PYY, CCK and/or neurotensin, particularly preferred GLP-1 and/or GLP-2 and/or PYY, above the respective base-line level of the enterokine, in particular the level of the enterokine in the blood serum/plasma of the subject being treated. In preferred embodiments of the invention, the level of the respective enterokine in the subject's blood serum/plasma increases by at least about 50 % or more, preferably about 50 % to about 200 % or even more such as about 300 %, as compared to the level in the blood plasma/serum of the subject before administration of the pharmaceutical composition of the invention. The increase in enterokine concentration in the subject's blood plasma/serum typically occurs within about 2 to about 6 hours post administration of the composition of the invention, and lasts for a time period of

typically about 1 to about 5 hours, preferably about 2 to about 4 hours, most preferred from about 3 to about 4 hours.

According to the invention, the term “effective amount of the pharmaceutical composition” depends on various factors such as the specific condition, disorder or disease to be treated and/or prevented, the age and sex of the subject as well as the general condition of the subject. Furthermore, the “effective amount of the pharmaceutical composition” depends on the type of active compound(s) used. Typically, however, pharmaceutical oral compositions of the invention are preferably administered once daily in one or more unit dosages, more preferably in the fasted state of the subject, particularly preferred at least about 30 min, more preferred at least about 45 min, even more preferred at least 1 hour before the first meal of the day. In preferred embodiments of therapeutic regimens of the invention, one or more unit doses of a pharmaceutical composition as defined herein are administered orally at a time point synchronized with the circadian rhythm of the enteroendokine which release is to be increased by the pharmaceutical composition of the invention. For such circadian synchronization of the administration of the pharmaceutical compositions of the invention, it is preferred that one more unit doses of the pharmaceutical composition are administered in a time period of from about 2 to about 4 hours before the respective enterokine, in particular GLP-1, reaches its circadian maximum in the serum or blood, respectively. In the case of GLP-1 as a particularly preferred enterokine for the purposes of the invention, the serum or blood, respectively, level of this enterokine shows a maximum at or around 11 hrs am. In preferred embodiments of the invention, the one or more unit dosages, particularly for exerting optimal GLP-1 release, are therefore administered at a time point from about 7 hrs am to about 9 hrs am. In the context of the time points given herein as hrs, it is to be understood that these time points refer to the local time zone where the pharmaceutical composition of the invention is to be administered.

With glucose as a preferred active compound, the effective amount is preferably a daily dose of about 0.5 to about 20 g glucose orally administered in one or more unit doses, preferably in the fasted state of the subject as outlined before. A dose of about 1 to about 15 g once daily is more preferred, and about 5 g to about 12 g, most preferred about 10 g glucose once daily may be given as a particularly preferred regimen. The above amounts of the active ingredient, preferably glucose, may be administered in one or more-unit doses of the pharmaceutical compositions, which preferred unit doses, in particular as regards the multiple dosage forms, are already outlined above. A typical amount of glucose contained unit dose of the pharmaceutical composition of the invention is in the range of from about 0.5

g to about 30 g glucose, preferably from about 5 g to about 20 g glucose, more preferably from about 7 g to 15 g glucose, such as ca. 10 g glucose per unit dose of the composition.

As regards the pharmaceutical composition of the invention, its use and treatment method of the invention as described above, it is of particular benefit when the composition is formulated, in particular with respect to the sizes, amounts and forms of the multiple dosage forms, such that the at least one compound stimulating enteroendocrine cells to release an enterokine stimulates said cells present in the intestine of the subject from the jejunum to the ileo-cecal valve of the, preferably human, subject.

The therapeutic uses and treatment methods of the invention as generally defined above also entail a combination of different pharmaceutical compositions wherein the individual composition contain multiple dosage forms exhibiting different travelling profiles in the subject's gastro-intestinal tract and/or contain different compounds stimulating enteroendocrine cells to release at least one enterokine. For example, one composition contains multiple dosage forms comprising glucose and a further composition contains multiple dosage forms comprising an anthocyanine (further combinations and specific examples of active compounds and preferred combinations have already been elaborated above). It is also contemplated that a single pharmaceutical composition contains multiple dosage forms comprising different active compounds as outlined above. A combination of different multiple dosage forms using different active compounds is specifically of benefit when the different compounds are not easily compatible (e.g. as regards their chemical properties) for inclusion in a single dosage form.

In other embodiments of this combinatorial approach, the treatment method of the invention comprises administering a first pharmaceutical composition comprising multiple dosage forms as defined herein having a small size as described herein, and administering a second pharmaceutical composition comprising one or more oral dosage forms as otherwise defined herein, but having a size of 3 mm or more, preferably 3 to 10 mm, based on the largest dimension of said first dosage form, wherein the active compound in said first and said second compositions stimulating enteroendocrine cells to release at least one enterokine may be the same or different.

It is, of course, also possible to administer or use, respectively, more than two different sizes and/or more than two different active compounds.

For preparing the multiple dosage forms as described herein, a production method typically comprising the steps of:

(a) preparing a mixture comprising at least one compound stimulating enteroendocrine cells to release at least one enterokine, preferably in combination with at least one disintegrant as defined above,

(b) compressing the mixture obtained in step (a); and

(c) applying to the compressed mixture at least one enteric coating, preferably comprising at least one pH sensitive polymer being preferably selected such that the coating substantially dissolves and/or is substantially degraded in the jejunum of a subject,

wherein the compression and application steps (a) and (b) respectively are selected such that multiple dosage forms having a size as disclosed herein are obtained.

Optionally, the mixture of step (a) and/or the at least one coating of step (c) may comprise further ingredients as outlined above for the pharmaceutical composition *per se*.

Preferred embodiments of the constituents as defined in steps (a) and (c) have already been described in detail above.

According to a further preferred embodiment of the preparation method of the invention, more than one coating is applied to the compressed mixture obtained in step (b), i.e. the core component of the pharmaceutical oral dosage form of the invention.

In particularly preferred embodiments of the preparation method, a combination of at least two coatings is applied in step (c). Preferably, a sub-coating of one pH sensitive polymer is applied as a first layer and a coating of a second pH sensitive polymer is applied on the sub-coating as a second layer. For example, the pH sensitive coating can comprise a sub-coating of or comprising, respectively, a hydroxypropylmethyl cellulose as a first layer, onto which a second coating comprising or made of an anionic copolymer of methacrylic acid and methacrylmethacrylate as a second layer. In a further preferred embodiment, a first layer (sub-coating) comprising or made of an anionic polymer of methacrylic acid and methacrylmethacrylate such as an Eudragit®, more preferably Eudragit® FS30D, is applied to the core obtained in step (b) and a second layer comprising or made of a hydroxypropylmethyl cellulose such as AQOAT®, more preferably AQOAT®-HF, is applied onto the first layer. More preferably, the anionic copolymer of methacrylic acid and methacrylmethacrylate, e.g. an Eudragit®, preferably Eurdragit® FS30D, is applied in less amount than the the hydroxypropylmethyl cellulose such as AQOAT®, more preferably

AQOAT®-HF, of the second layer. In other words, the thickness of the first layer of this type of combination is lower than the thickness of the second layer in this combination. More specifically, the ratio of amount or thickness, respectively, between first layer and second layer typically ranges from about 1:10 to about 1:50, particularly preferred from about 1:20 to about 1:30. In preferred embodiments of the invention, the one or more coatings are applied by spray-coating in step (c).

For preparing the pharmaceutical composition, the multiple dosage forms are then admixed with the gelling agent or gelling agents, preferably a gelling composition, preferably containing further ingredients such as those as outlined above, such as preferably one or more formulation aids, preferably selected from pH modifiers, flavors, taste improvers, lubricants and pigments.

The Figure shows:

Fig. 1 shows a graphical representation of results of time-dependent GLP-1 blood level measurements (pmol/l) in subjects (n= 20) from 1 hour before to 10 hours after oral administration of a composition according to the invention. Data shown are means +/- SEM.

The following non-limiting example further illustrates the invention:

EXAMPLE

An exemplary pharmaceutical composition was prepared by mixing the following components (a) and (b):

(a) Multiple dosage forms: Eudragit®-coated beads of ca. 1 mm diameter having a core containing 40 to 70 % (w/w) glucose and a disintegrant in a carrier substance Total weight of glucose in a unit dose of the composition: 10 g. Number of coated beads ca. 20000 to 30000.

(b) Gelling composition:
Gelling agent mixture (Polysaccharide, modified cellulose, PEG) (84 % (w/w) based on the total weight of the gelling composition)
pH modifier
Flavors

Lubricant

Pigments

Before oral administration, the pharmaceutical composition was mixed with 25 ml of water per unit dose.

The reconstituted composition was administered orally to 20 subjects. Blood GLP-1 values were measured at the following time points (in relation to time point of oral administration = 0 h): -1.0 h, -0.5 h, 0 h, 0.5 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, 4.0 h, 4.5 h, 5.0 h, 5.5 h, 8.0 h, and 10.0 h

The results are shown in Fig. 1. The pharmaceutical composition exerts a remarkably reproducible (low intra patient and inter-patient variability) and sharp rise in GLP-1 blood concentration (maximum at 2.88 +/- 0.46 fold increase with respect to base line GLP-1 blood level at 1.5 h post administration) as compared to prior art approaches, and the GLP-1 increase over base line value at 1.5 h lasts about 4 h. The sharp rise in GLP-1 blood level starting after 1.5 hours post administration is consistent with a burst release of the active compound (here: glucose) in the terminal jejunum of the subjects as demonstrated by the following data as obtained by Evans et al. (1988) Gut 29, 1035-1041).

Tab.1: Localisation of pH sensitive telemetry capsule in the gastro-intestinal tract (GI) in dependency of time after intake

GI region	**Time (hours) after intake**
Duodenum	0.75 to 1.8
Jejunum	1.8 to 3.8
Ileum	3.8 to 5.4
Colon	$\geq$ 5.4

Applying the data of Evans et al. (1988), the burst release of the components of the multiple dosage forms contained in the composition of the invention occurred in the (terminal) jejunum of the subjects at a pH environment of > pH 7.0, and in particular ca. 7.3.