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(54) Title: NON-COVALENT SOLUBLE COMPLEXES OF TERIPARATIDE WITH POLYSACCHARIDES AND A DOSAGE FORM OF TERIPARATIDE FOR ORAL ADMINISTRATION

(57) Abstract: The present invention relates to stabilization of teriparatide for oral administration by forming of water-soluble complexes with soluble polysaccharides, e.g. β -glucan, chitosan, alginic acid, or their salts. Orally administered peptide medicaments such as teriparatide exhibit very low biological availability, which is mainly caused by rapid degradation in the digestive tract by proteolytic enzymes, as well as low efficiency of transport through the intestinal wall to the blood circulation. It is an advantage of the pharmaceutical composition according to the invention that teriparatide in the complex form is protected from being digested by proteases, the complex not preventing absorption of teriparatide from the gastrointestinal tract to the blood at the same time.



Non-covalent soluble complexes of teriparatide with polysaccharides and a dosage form of teriparatide for oral administration

Technical Field

The invention relates to stabilization of teriparatide by formation of soluble complexes with polysaccharides and to pharmaceutical composition enabling its oral administration. Teriparatide is a generic name for the therapeutically active portion of the human parathyroid hormone, consisting of 34 N-terminal amino acids of the original peptide hormone (referred to as PTH(1-34)), which can be used for the treatment of osteoporosis. Peptide drugs, including teriparatide, are administered by injection at present as their oral application is limited by the degradation in the digestive tract and by the difficult transport from the gastrointestinal system to the blood circulation and thus an insufficient concentration in the blood. An advantage of this invention consists in increasing the stability of teriparatide against digestive enzymes thanks to complexation with a suitable polysaccharide. In addition, a complex of the drug with a polysaccharide may support transfer to the blood circulation. This approach enables oral administration of teriparatide, which was not possible in the past.

Background Art

Parathyroid hormone

The human parathyroid hormone (PTH) plays an important role in regulation of homeostasis of calcium and phosphates in the human organism. PTH ensures mobilization of calcium and phosphates deposited in bones and increases intake of calcium in the small intestine and excretion of phosphates by the kidneys. PTH also activates cAMP-dependent protein kinase in some populations of bone cells carrying PTH receptors, which stimulates proliferation of osteoblasts. PTH is secreted by the parathyroid glands as a peptide consisting of 84 amino acids (PTH(1-84)) (Bonn, 1996; Kronenberg et al., 1993). The peptide fragment consisting of 34 N-terminal amino acids of PTH (PTH(1-34)) is fully biologically active (Bonn, 1996; Hodsman et al., 2005; Whitfield and Morley, 1995).

Teriparatide and teriparatide treatment

Teriparatide is the amino-terminal sequence of 34 amino acids of the human parathyroid hormone, commercially produced with the use of recombinant technology [rhPTH(1-34)] by Eli Lilly and Company. Unlike continuous hypersecretion of PTH,

intermittent subcutaneous application of teriparatide leads to preferential stimulation of osteoblasts, i.e. new bone formation. The result is an anabolic effect on the bone documented by a considerable improvement of quality parameters of the trabecular and cortical bone. This is related to the evidence of a significant reduction of occurrence of vertebral as well as non-vertebral fractures in postmenopausal women suffering from osteoporosis treated with teriparatide. The increase of the volume and quality of bone matter is manifested by a progressive reduction of the occurrence of fractures during the treatment and continuation of the protective effect against fractures after the end of the treatment. Teriparatide treatment reduces back pain as compared to placebo as well as to anti-resorption drugs. Teriparatide treatment in men results in a reduction of the occurrence of medium and severe fractures of vertebrae. In patients with osteoporosis induced by corticosteroids, application of teriparatide leads to a considerable reduction of the occurrence of fractures of vertebral bodies as compared to patient treated with alendronate. Teriparatide treatment is indicated for patients with severe osteoporosis and/or a high risk of fractures: for women with postmenopausal osteoporosis, for men with primary and hypogonadal osteoporosis and for patients with osteoporosis induced by corticosteroids. A 20 µg dose of teriparatide is administered once a day in a subcutaneous injection with the use of a special injector. The treatment is safe and well tolerated. Infrequent side effects of the treatment include lower limb pains and dizziness; however, interruptions of treatment due to side effects are as frequent as in the case of placebo. The duration of the treatment is limited to 18 months (Rosa, 2008).

Pharmacokinetic properties of teriparatide

After subcutaneous injection teriparatide is excellently absorbed; its absolute biological availability is high. The speed of its absorption and elimination is high as well. Teriparatide achieves the serum concentration peak in 30 minutes after subcutaneous application of a 20 µg dose. Serum concentrations drop to non-measurable values after 3 hours. In clinical studies the top limit of the normal range of serum values of the endogenous PTH are quickly exceeded twice to four times. Peripheral metabolism of teriparatide by non-specific enzymatic mechanisms occurs in the liver and bone, teriparatide, including its amino and carboxy terminal fragments, is eliminated in the kidneys. Teriparatide does not accumulate in bones or other tissues. The inter-individual variability of the systemic clearance and of the distribution volume is 25–50%. The plasmatic half-life of teriparatide is 5 minutes after *i.v.* administration and about 1 hour after subcutaneous administration. The longer half-life after subcutaneous administration reflects the time necessary for absorption from the point of puncture (Daugaard, 1996; Rosa, 2008).

Although teriparatide administered by injection exhibits excellent pharmacokinetic properties, oral administration of the drug is the clearly preferred choice of the general public.

However, oral administration of peptide drugs, just as teriparatide, brings a number of difficulties that are described in the text below.

Oral administration of peptide drugs, including teriparatide

Orally administered peptide drugs have very low biological availability (max. 1 – 2 %) (Pauletti et al., 1996), which is mainly caused by their susceptibility to degradation in the digestive tract and their difficult transport through the intestinal wall to blood. Peptides are very quickly metabolized in the digestive tract by a number of digestive enzymes including various peptidases and proteases, such as pepsin in the stomach, trypsin and chymotrypsin in the small intestine and many others (Humphrey and Ringrose, 1986; Semalty et al., 2007; Siegel and Langer, 1984; Singh et al., 2008). Werle et al. (2006) in their study researched into degradation of teriparatide in the gastrointestinal tract (GIT) by proteolytic enzymes under physiological conditions. The results of their work show that teriparatide is primarily split by trypsin, chymotrypsin and pepsin as quickly as in 5 minutes, which leads to its zero biological availability. The results of this study are summarized in Table I.

Table I: Degradation of teriparatide by various digestive enzymes (adopted from Werle et al., 2006).

Proteolytic enzyme	Protease/teriparatide molar ratio (mol/mol)	Percent of remaining intact teriparatide				
		5 min	30 min	60 min	120 min	180 min
Aminopeptidase N	1:3000	n.a.	n.a.	89 ± 4	78 ± 23	50 ± 5
Trypsin	1:12	0	0	0	0	0
Chymotrypsin	1:6	0	0	0	0	0
Elastase	1:100	94 ± 4	95 ± 2	92 ± 2	86 ± 3	84 ± 5
Pepsin	1:1.3	0	0	0	0	0
Membrane bound peptidases	n.a.	n.a.	94 ± 3	84 ± 11	59 ± 8	51 ± 19

Transport of substances through the intestinal epithelium is mainly influenced by their molecular weight and solubility. Lipophilic substances pass from the intestine to blood on the basis of passive diffusion, hydrophilic substances on the basis of active transport, often using “carrier proteins”. However, active transport is only limited to small molecules with a molecular weight up to 200 Da (Donovan et al., 1990; Humphrey and Ringrose, 1986; McMartin et al., 1987). But an absolute majority of peptides are hydrophilic and their molecular weight exceeds 700 Da (Humphrey and Ringrose, 1986; Semalty et al., 2007; Singh et al., 2008).

Teriparatide, as other peptide drugs, is administered by injection just for the above mentioned reasons. Parenteral administration of teriparatide is described e.g. in the patent document WO 2011/011675, where a diol or diol ether is used to increase the biological availability of teriparatide. But other alternative methods of administration of peptide drugs that avoid the digestive system have been tested (Antosova et al., 2009), namely transdermal (Amsden and Goosen, 1995), intranasal (Ugwoke et al., 2001), pulmonary (Scheuch and Siekmeier, 2007) and buccal (Shojaei, 1998). The transdermal method of administration of teriparatide has been tested by the scientific groups of Suzuki et al. (2001), Ameri et al.

(2010), and Cosman et al. (2010). A method of intranasal administration of teriparatide is described, e.g., in the patent documents US 2007/0173447, US 2006/0189533 and US 2006/0127320. Agu et al. (2004) investigated intranasal application of teriparatide in rats. The patent document WO 1996/019206 describes preparation of inhalable particles containing the parathyroid hormone. Pulmonary application of teriparatide was further tested by the scientific group of Pfützner et al. (2003). Buccal administration of the parathyroid hormone is described in the patent document WO 2006/076692.

However, the main factor that limits the success of these alternative administration methods for therapeutic applications is easy degradation of peptides by proteases present in the blood plasma as after penetration of the peripheral blood circulation the peptide drugs often appear to be immunogenic (Semalty et al., 2007). In addition, unfavourable effects of the drug during long-term application through the skin, lungs, etc. may also limit these administration methods. On the other hand, oral administration of drugs has the advantage that from the intestine the drug directly gets to the portal vein, which brings blood to the liver from where the drug is distributed to the main blood circulation. The advantages of oral administration of drugs such as its easiness, comfort for the patient and affordability, for which this administration method is preferred everywhere it can be used, represent the reason for looking for a dosage form of teriparatide suitable for oral administration.

In spite of considerable difficulties of oral administration of peptide drugs, which are described above, literature provides several strategies that can be employed to increase their biological availability. These procedures mainly include stabilization of the peptide against proteolytic enzymes as well as increasing the efficiency of transport of the peptide drug through the intestinal wall.

Many such methods use, to increase absorbability of peptides, various stabilizers and substances that improve permeability through biological membranes, such as salts (Arakawa et al., 2001; Parkins and Lashmar, 2000; Sikkink and Ramirez-Alvarado, 2008), metal ions (Jorgensen et al., 2009), phenol-like ligands (Huus et al., 2006), polyols (Arakawa et al., 2001; Parkins and Lashmar, 2000; Sathish et al., 2007), chelating agents, or they try to limit degradation of the peptide drug in the digestive tract by simultaneous administration of various protease inhibitors (Bernkop-Schnurch, 2000; Semalty et al., 2007). However, the above mentioned substances do not solve the problem of biological availability of peptides comprehensively, but only partially. For example, addition of protease inhibitors increases stability of the peptide against proteolytic degradation on the one hand, but it does not improve its permeability through the intestinal epithelium; conversely, surfactants increase permeability of the intestinal wall, but do not protect the peptide from the attack of proteases, and the like. Another problem is that in order to achieve the desired effect a number of these substances must be applied in such an amount that may pose their own side effects. For example, a number of traditionally used compounds to increase penetration through biological

membranes (“penetration enhancers”) may cause damage of the digestive tract (Guarini and Ferrari, 1985).

Another approach to increase the stability of the peptide consists in attaching a suitable polymer by a covalent bond, producing polymeric conjugates. Attachment of polymers, such as polyethylene glycol (PEG), poly(vinylpyrrolidone) (PVP), albumin, or oligosaccharides such as cyclodextrin may lead to reduction of immunogenicity and thus to increase of stability of the polypeptide while maintaining its biological activity (Abuchowski and Davis, 1979; Burnham, 1994; Davis et al., 1981; Jorgensen et al., 2009; Semalty et al., 2007; Singh et al., 2008). For example, Nobex have developed so-called “hexyl insulin monoconjugate 2” (HIM2) for oral administration where a short PEG oligomer is attached to insulin to the free Lys- β -29 amino group via an amide bond. Such a conjugate of insulin with a short polymer exhibits an increased stability against proteolytic degradation in the gastrointestinal tract (GIT) and is transported from GIT to blood (Kipnes et al., 2003). Although covalent conjugates of peptides with polymer manifest promising properties that may lead to oral administration of peptide drugs, it should be noted that these are in fact entirely new active substances that must be subjected to further investigation and clinical tests.

Also, various chemical modifications of peptides, such as substitution of certain amino acids (Wyvratt and Patchett, 1985) or reduction of carboxyls (Brewster and Waltham, 1981) can increase stability of a peptide drug in the organism by preventing its degradation by specific peptidases and proteases that recognize a certain amino acid sequence. For example, the patent document US 2004/0197323 A1 describes amidation of peptides, including teriparatide, which increases stability of an orally administered peptide drug against proteolytic enzymes and enhances penetration through the intestinal wall to blood. However, such modifications of peptides, similarly to preparation of covalent conjugates, lead to an entirely new active substance from the point of view of drug approval, which the responsible authority cannot approve without a proof of its safety and activity by carrying out complete clinical trials.

Another approach to increasing stability of the peptide in the GIT and enhancing transfer of the peptide drug through the intestinal wall to the blood circulation, namely without any chemical modification of the peptide, is represented by encapsulation of such a drug in a liposomic or polymeric particle (Antosova et al., 2009). The drug is protected in the particle from the attack of proteases, the particle supports transport through the intestinal epithelium and the intact drug is then gradually released in the blood circulation on the basis of hydrolysis/enzymatic degradation of the particle and the like (Gombotz and Pettit, 1995). Liposomes as particles for oral administration of insulin have been described, e.g., in the studies of Hashimoto and Kawada (1979) and Patel and Ryman (1976). The patent document WO 87/00750 describes oral administration of teriparatide and calcitonin, either separately or in mutual combination, using liposomic particles. However, the use of liposomes for oral

administration of peptide drugs still requires further research, mainly in the sphere of increasing stability and achieving reproducible preparation and standard characteristics of these particles. The most frequently tested polymer used for encapsulation of peptide drugs is a lactic acid polymer (PLGA, poly(lactic-co-glycolic) acid) (van der Walle et al., 2009). PLGA has been used, e.g., to increase stability of insulin for oral administration (Singh et al., 2008). A shortcoming of PLGA for the formulation of peptide drugs is mainly that during the preparation of the particles the peptide gets damaged (Perez and Griebenow, 2003; Schwendeman, 2002). The patent document US 4,925,673 describes the use of so-called protenoids that consist of polypeptides. These polymers are composed of natural or synthetic amino acids or short peptide chains in a random or controlled way. Protenoid micro-particles can then be used for encapsulation of peptide hormones, such as insulin, which increases its biological availability in the case of oral administration. Other patent documents, e.g., US 5,629,020, US 5,643,957, US 5,650,386, US 5,776,888 and US 7,744,910, similarly describe the use of modified amino acids or peptides that non-covalently interact with active substances such as peptide drugs, thus helping to increase the efficiency of their oral administration.

Formulation of teriparatide for oral administration has been tested by several scientific groups. The patent documents US 5,773,647, US 7,744,910 B2, US 5,776,888 and US 5,866,536 describe a number of various compounds, mainly various modified amino acids or peptides, which may be used to deliver active biological and chemical substances, including teriparatide, to the target location in the organism. These substances include, e.g., N-(5-chlorosalicyloyl)-8-aminocaprylic acid (5-CNAC), N-10-[2-hydroxybenzoyl] aminodecanoic acid (SNAD), N-8-[2-hydroxybenzoyl] aminocaprylic acid (SNAC), their disodium salts, hydrates, and the like. These compounds form non-covalent complexes with the given active substance and help to overcome biological obstacles in the organism; therefore they are useful for, inter alia, oral administration of such drugs. These patent documents also describe methods of preparation and administration of the complexes of compounds with the given active substance.

The patent documents US 2006/0217313 A1 and EP 1 420 827 B1 describe the use of N-(5-chlorosalicyloyl)-8-aminocaprylic acid (5-CNAC) for oral administration of teriparatide. The presence of teriparatide in blood after its oral administration was confirmed in an *in vivo* monkey model. The 5-CNAC compound was also used for formulation of calcitonin for oral administration. This is described in the patent document US 7,569,539 B2. The patent documents US 2008/0019411 A1, US 2004/0186050 A1 and EP 1 397 156 B1 describe the use of 5-CNAC, SNAD, SNAC and other similar compounds for oral administration of teriparatide with simultaneous application of calcitonin.

For example, N-[8-(2-hydroxy-4-methoxy)benzoyl]aminocaprylic acid (4-MOAC) has also been selected for increasing biological availability of orally administered teriparatide

(Leone-Bay et al., 2001). Leone-Bay et al. (2001) assume non-covalent interaction of teriparatide with 4-MOAC on the basis of an NMR analysis. 4-MOAC probably changes the physical and chemical properties of teriparatide, e.g. increases its hydrophobicity, thus enabling transfer of the compound through the intestinal epithelium. Efficiency of orally administered teriparatide/4-MOAC was confirmed in *in vivo* mouse and monkey models.

In spite of the first success in oral administration of peptide drugs, including teriparatide, alternative, cheap and non-toxic systems for oral administration of peptides have still to be sought. A number of the above mentioned approaches often i) change the peptide itself through a chemical modification, ii) do not achieve the required stabilization characteristics, or iii) may damage the gastrointestinal system due to toxicity. Therefore, alternative compounds have to be further looked for that meet the requirements for oral administration of peptide drugs and are harmless for humans. Soluble polysaccharides could find application in an oral formulation of teriparatide as they are generally non-toxic pharmaceutically acceptable substances, which are either not metabolized and/or absorbed after oral administration, or are degraded in the organism on the basis of enzymatic hydrolysis to non-toxic monomers (monosaccharides).

Disclosure of Invention

The invention provides non-covalent complexes, soluble in an aqueous environment, of soluble polysaccharides with the peptide drug teriparatide, obtainable by interaction of teriparatide with a soluble polysaccharide in an aqueous solution, the use of these complexes for the protection of teriparatide from the effect of proteases and a pharmaceutical composition for oral administration containing such a complex. The invention further provides a method for the preparation of the complexes and pharmaceutical compositions according to the invention. A soluble polysaccharide is to be understood, in the present application, as a polysaccharide soluble in an aqueous environment, either as such or in a form where dissociated functional groups carried by the polymeric chain are either partly or completely transformed to a salt with a suitable acid or base. An interaction, or complex with a soluble polysaccharide, is to be understood as an interaction or complex with the polysaccharide itself or with any form thereof, soluble in an aqueous environment. An aqueous environment refers to both water and solutions of low-molecular substances dissolved in water where water amounts to at least three fifths of the total weight of such an aqueous environment. An aqueous solution of a soluble polysaccharide means a solution of a polysaccharide in an aqueous environment.

It has been surprisingly found out that soluble polysaccharides, such as β -glucan, alginic acid, chitosan, which are known as non-toxic and pharmaceutically acceptable substances, can be used for stabilization of teriparatide on the basis of formation of a soluble

non-covalent complex, which increases stability of the peptide against proteolytic degradation. Another surprising finding is that although these complexes protect teriparatide from degradation in the gastrointestinal tract, they do not hinder its absorption from the gastrointestinal tract into the blood circulation or even support it by a so far unknown mechanism.

Detailed Description of the Invention

The research was guided by the idea that possible formation of a non-covalent complex of teriparatide with a suitable soluble polymer could increase resistance of teriparatide to proteolytic enzymes that are present in the digestive tract and cause degradation of this peptide before it can even get to the blood circulation. Therefore, it was first tested whether soluble polysaccharides form complexes with teriparatide in an aqueous environment under physiological conditions and can thus lead to protection of this peptide from the attack of proteases. Interactions of teriparatide and soluble polysaccharides in an aqueous environment were investigated with the use of spectrophotometry in the ultraviolet and visible range (UV-Vis), electron circular dichroism (ECD) and fluorescence spectroscopy. A detailed description of the experiments is presented in Examples 1-3; the results are documented in Figs. 1-9. A strong non-covalent interaction of soluble polysaccharides with teriparatide is well evidenced by the difference spectra, which clearly demonstrate changes of the teriparatide spectrum caused by the interaction with the polysaccharide. Formation of complexes of soluble polysaccharides with teriparatide was also confirmed by the determination of stability constants of the teriparatide- β -glucan, teriparatide-alginic acid and teriparatide-chitosan complexes (Example 4, Table II). Although the existence of these non-covalent complexes is indisputable, no more precise conclusions about the structure and bond conditions can be drawn on the basis of the obtained data. Neither can be ruled out more complicated stoichiometry of the formation of complexes than assumed in the calculation of association constants from the measured data, or formation and presence of several complexes with a different stoichiometric ratio of teriparatide and the polysaccharide in the same solution.

For the polysaccharides such as chitosan, carrying basic functional groups, water-soluble acids are suitable low-molecular components facilitating dissolution of the polysaccharide in the aqueous environment. For the polysaccharides carrying acidic functional groups, such as alginic acid, water-soluble bases are suitable low-molecular components facilitating dissolution of the polysaccharide in the aqueous environment. However, low-molecular substances present in an aqueous environment suitable for dissolution of the polysaccharide and formation of the teriparatide - polysaccharide complex may also include salts of inorganic or organic acids and bases, or, on the other hand, non-dissociating water-soluble low-molecular substances such as monosaccharides and oligosaccharides, C1-C4

aliphatic alcohols or C2-C8 aliphatic or alicyclic polyols. After mixing the constituents of the complex in an aqueous environment a certain incubation period is necessary to form the complex, which generally varies in the range of 0.5-72 hours depending on the conditions. Preparation of the complexes according to the invention can be carried out at a temperature of 0–40 °C, preferably in the range of 5–35 °C. Most frequently, preparation of the complex has been carried out at a temperature in the range of 20–30 °C in terms of easiness of performing.

Having proved the formation of soluble non-covalent complexes of teriparatide with soluble polysaccharides in an aqueous environment we proceeded to testing the stability of teriparatide to proteolytic enzymes *in vitro*. As it is known that the principal digestive enzymes that participate in the metabolism of teriparatide in the digestive tract are pepsin, trypsin and chymotrypsin (Werle et al., 2006), the influence of the formation of the complexes according to the invention on proteolysis of teriparatide was tested with the use of these particular enzymes. Teriparatide alone, as well as teriparatide in the form of complexes with the given polysaccharides, was incubated with pepsin, trypsin and chymotrypsin, respectively, *in vitro* and samples were taken in certain time intervals (Example 5). These samples were subjected to qualitative analysis by means of mass spectrometry (MS) with ionization by the matrix assisted laser desorption method and detection of ions by the time of flight (MALDI-TOF) (Example 6) and to quantitative analysis by means of high-performance liquid chromatography (HPLC) (Example 7). The results of the MALDI-TOF MS experiment are summarized in Table IV. An example of the mass spectrum of a sample after 1-hour incubation of teriparatide with trypsin is presented in Fig. 10. The results of the HPLC experiment are summarized in Table V, an example of an HPLC chromatogram after 1-hour incubation of teriparatide with trypsin is shown in Fig. 11. Although teriparatide alone was degraded by the enzymes in a few minutes, teriparatide added in the form of a complex with a polysaccharide was even detected in the samples after several hours (see Tables IV, V). Thus, the *in vitro* stability test of teriparatide against digestive enzymes confirmed that teriparatide in a complex with a soluble polysaccharide, e.g. with β -glucan, a salt of alginic acid or a salt of chitosan exhibited a higher stability in the presence of proteases as compared to the unprotected peptide.

In vivo experiments followed, where complexes of teriparatide with the given polysaccharides were orally administered to laboratory animals (rats) (Example 8). For the determination of teriparatide in the blood plasma two methods are used, namely RIA (radioimmunoassay) (Massfelder et al., 2002; Song et al., 2008) and ELISA (enzyme-linked immunosorbent assay) (Chen K. et al., 2006), both the methods being currently commercially available as kits. With regard to reference data, where the ELISA method had been found problematic in a number of cases, we employed the RIA method for our determination of teriparatide in the blood plasma.

RIA is a quantitative radioimmunoassay, useful for measurements of concentrations of an intact amino-terminal teriparatide in the human serum. RIA is based on the competitive radioimmunoassay technique. A known amount of labelled teriparatide (^{125}I) and an unknown amount of non-labelled teriparatide compete for a limited number of high-affinity binding sites of a polyclonal antibody against teriparatide. A secondary antibody against murine IgG is used for separation of the teriparatide bound to the antibody and the free teriparatide. Radioactivity of the bound labelled antigen (radioindicator) is measured in a gamma counter. The amount of labelled teriparatide in a sample tube is indirectly proportional to the amount of teriparatide in the sample. The concentrations of teriparatide in unknown samples are read on the calibration curve. We used a kit commercialised by DiaSorin (USA) for our determination of teriparatide in the blood plasma.

Teriparatide, orally administered in the form of the complexes according to the invention, was detected by RIA in the blood of laboratory animals (Example 8, Table VI), which confirmed that teriparatide in a complex with polysaccharides is not only stabilized against degradation by digestive enzymes, but is also absorbed from the intestine and released in the blood plasma. Accordingly, the results of the *in vivo* experiments have showed that:

- a) complexes of teriparatide with soluble polysaccharides exhibit an increased stability in the digestive tract as compared to administration of free, non-complexed teriparatide;
- b) absorption of teriparatide through the intestine wall and release thereof into the blood circulation can be achieved by administration of teriparatide in the form of a water-soluble, non-covalent complex with a soluble polysaccharide.

Protection of peptide drugs from degradation in the digestive tract and increase of the success of drug transfer through the intestinal epithelium into the blood are essential for successful administration thereof. The above mentioned experiments have proved that complexes of teriparatide with soluble polysaccharides, e.g. β -glucan, alginic acid, chitosan, meet both these conditions.

Brief Description of Drawings

Fig. 1

Changes of the UV-Vis spectrum of teriparatide during formation of a non-covalent complex with β -glucan in an aqueous environment. As the concentration of β -glucan increases, the absorption maximum of teriparatide increases. This behaviour can be explained on the basis of rearrangement of aromatic amino acids in the teriparatide molecule due to interaction between teriparatide and β -glucan.

Fig. 2

Changes of the UV-Vis spectrum of teriparatide during formation of a non-covalent complex with an alginate in an aqueous environment. As the concentration of alginic acid increases, the

absorption maximum of teriparatide increases. This behaviour can be explained on the basis of rearrangement of aromatic amino acids in the teriparatide molecule due to interaction between teriparatide and alginic acid.

Fig. 3

Changes of the UV-Vis spectrum of teriparatide during formation of a non-covalent complex with chitosan in an aqueous environment. As the concentration of chitosan increases, the absorption maximum of teriparatide decreases. This behaviour can be explained on the basis of rearrangement of aromatic amino acids in the teriparatide molecule due to interaction between teriparatide and chitosan.

Fig. 4

Changes of the ECD spectrum of teriparatide during formation of a non-covalent complex with β -glucan in an aqueous environment. As β -glucan is being added, the ECD signal of teriparatide increases in the far UV range. This behaviour can be explained by an increase of the proportion of α -helical structures of teriparatide due to interaction between teriparatide and β -glucan.

Fig. 5

Changes of the ECD spectrum of teriparatide during formation of a non-covalent complex with an alginate in an aqueous environment. As alginic acid is being added, the ECD signal of teriparatide increases in the far UV range. This behaviour can be explained by an increase of the proportion of α -helical structures of teriparatide due to interaction between teriparatide and alginic acid.

Fig. 6

Changes of the ECD spectrum of teriparatide during formation of a non-covalent complex with chitosan in an aqueous environment. As chitosan is being added, the ECD signal of teriparatide decreases in the far UV range. This behaviour can be explained by a decrease of the proportion of α -helical structures of teriparatide due to interaction between teriparatide and chitosan.

Fig. 7

Changes of the emission fluorescence spectrum of teriparatide during formation of a non-covalent complex with β -glucan in an aqueous environment. As the concentration of β -glucan increases, the fluorescence signal of teriparatide decreases. This behaviour can be explained by extinguishing of fluorescence of tryptophan in the teriparatide molecule due to rearrangement of amino acids in the surroundings of tryptophan, namely due to interaction between teriparatide and β -glucan.

Fig. 8

Changes of the emission fluorescence spectrum of teriparatide during formation of a non-covalent complex with an alginate in an aqueous environment. This behaviour can be explained by extinguishing of fluorescence of tryptophan in the teriparatide molecule due to rearrangement of amino acids in the surroundings of tryptophan, namely due to interaction between teriparatide and alginic acid.

Fig. 9

Changes of the emission fluorescence spectrum of teriparatide during formation of a non-covalent complex with chitosan in an aqueous environment. This behaviour can be explained by extinguishing of fluorescence of tryptophan in the teriparatide molecule due to rearrangement of amino acids in the surroundings of tryptophan, namely due to interaction between teriparatide and chitosan.

Fig. 10

MALDI-TOF mass spectra. (A) Spectrum of teriparatide fragments after 1-hour incubation with trypsin. No intact teriparatide was detected. (B) Spectrum of teriparatide and its fragments after 1-hour incubation with trypsin; in this case teriparatide was in a complex with chitosan. Intact teriparatide was detected, which demonstrates an increase of its stability after complexation with chitosan.

Fig. 11

HPLC chromatogram of teriparatide at the beginning of the experiment (A) and after 1-hour incubation of teriparatide with trypsin without the presence of alginic acid (B) and in the presence thereof (C). The conditions are mentioned in Example 5.

Working Examples**Example 1**

The UV-Vis spectra were measured with a Varian Cary 400 SCAN spectrophotometer (Varian, Japan) in a 1cm cuvette in the range of 250 to 320 nm with the increment of 1 nm. First, the spectrum of teriparatide alone was measured (2 ml of teriparatide at the concentration of 0.1 mg/ml in distilled water). Then, various amounts (2, 5, 10, 20, 40, 60, 80, 100, 150, 300, 450, 600 and 1000 μ l) of 2% (w/v) aqueous solution of β -glucan, 1% (w/v) aqueous solution of alginic acid and 1% (w/v) aqueous solution of chitosan, respectively, were added to teriparatide and the UV-Vis spectra of teriparatide were measured after each addition of the particular polysaccharide. The spectra of the polysaccharides alone were also measured

and these spectra were deducted from the spectra of teriparatide with the corresponding polysaccharides. The resulting UV-Vis spectra of teriparatide and the differential UV-Vis spectra of teriparatide with the addition of β -glucan, alginic acid and chitosan, respectively, are shown in Figs. 1, 2 and 3.

Example 2

The ECD spectra were measured with a J-810 spectropolarimeter (Jasco, Japan) in a 1cm cuvette in the range of 220 to 300 nm with the increment of 1 nm. The resulting ECD spectra represent an average of 3 measurements. First, the spectrum of teriparatide alone was measured (2 ml of teriparatide at the concentration of 0.1 mg/ml in distilled water). Then, 100 μ l of 2% (w/v) aqueous solution of β -glucan, 1% (w/v) aqueous solution of alginic acid and 1% (w/v) of aqueous solution of chitosan, respectively, were added to teriparatide and the ECD spectra of teriparatide with the additions of the particular polysaccharides were measured. The spectra of the polysaccharides alone were also measured and these spectra were deducted from the spectra of teriparatide with the corresponding polysaccharides. The resulting ECD spectra of teriparatide and the differential ECD spectra of teriparatide with the addition of β -glucan, alginic acid and chitosan, respectively, are shown in Figs. 4, 5 and 6.

Example 3

The emission fluorescence spectra (excitation 282 nm) were measured by means of an ISA Jobin Yvon-SPEX Fluoromax-2 fluorescence spectrophotometer (Instrument S.A., USA) in a 1cm cuvette in the range of 300 to 800 nm with the increment of 1 nm. First, the spectrum of teriparatide alone was measured (2 ml teriparatide at the concentration of 0.04 mg/ml in distilled water). Then, various amounts (2, 5, 10, 20, 40, 60, 80, 100, 150, 300, 450, 600 and 1000 μ l) of 2% (w/v) aqueous solution of β -glucan, 1% (w/v) aqueous solution of alginic acid and 1% (w/v) aqueous solution of chitosan, respectively, were added to teriparatide and the fluorescence spectra of teriparatide were measured after each addition of the particular polysaccharide. The spectra of the polysaccharides alone were also measured and these spectra were deducted from the spectra of teriparatide with the corresponding polysaccharides. The resulting fluorescence spectra of teriparatide and the differential fluorescence spectra of teriparatide with the addition of β -glucan, alginic acid and chitosan, respectively, are shown in Figs. 7, 8 and 9.

Example 4

The measured fluorescence spectra from Example 3 were evaluated by means of the LETAGROP program by Silén and Warnquist (1969), which serves for optimization of stability constants on the basis of measured values during titration. The stability constants were calculated for the teriparatide to polysaccharide ratio of 1:1 and the uncertainty of this

constant was also calculated. The obtained values are presented in a logarithmic representation in Table II.

Table II: Decimal logarithm of the stability constants of the complexes (1:1): teriparatide – β -glucan, teriparatide – alginic acid, teriparatide – chitosan.

	teriparatide – β -glucan	teriparatide – alginic acid	teriparatide – chitosan
Decimal logarithm of the stability constant for the 1:1 complex	6.36 ± 0.10	4.67 ± 0.59	3.99 ± 0.17

Example 5

Stabilization of teriparatide in a complex with a soluble polysaccharide against the action of proteolytic enzymes was demonstrated by comparison of the degrees of hydrolysis of complexed and free teriparatide *in vitro* in the presence of pepsin, trypsin and chymotrypsin. The enzyme pepsin was supplied by Princeton Separations, USA (catalogue number EN-180), the enzyme trypsin by Promega (catalogue number V5111) and the enzyme chymotrypsin by Sigma-Aldrich (catalogue number C4129). First, a solution of teriparatide was prepared at the concentration of 1 mg/ml a) in a PBS buffer (4.3mM of Na_2HPO_4 , 1.47mM of KH_2PO_4 , 0.137mM of NaCl, 2.7mM of KCl) with pH 7.3 for the reaction with trypsin and chymotrypsin and b) in 10mM of HCl with pH ~ 2.2 for the reaction with pepsin. The concentration of the stock solutions of individual enzymes was 1 mg/ml and the solutions were prepared by dissolution of the enzyme in the corresponding buffer in accordance with the manual of the given manufacturer. Then, the solution of the corresponding enzyme was added to the teriparatide solution. The prepared enzyme-teriparatide mixtures were incubated at the temperature of 37 °C and in the time intervals of 0.5, 1, 3, 8 and 24 h after the incubation samples were taken for analysis by means of a) MALDI-TOF mass spectrometry and b) HPLC. In parallel trials stability of teriparatide was similarly studied in complexes with the polysaccharides β -glucan, alginic acid and chitosan, respectively, prepared by mixing of the solution of teriparatide and the stock solution of the corresponding polysaccharide, followed by 24-hour incubation with stirring of the prepared mixture of teriparatide with the polysaccharide at the laboratory temperature. For the experiment stock solutions of the polysaccharides prepared in the following way were used:

- i) 2% (w/v) aqueous solution of β -glucan

β -glucan was obtained by fermentation of *Claviceps viridis* in accordance with the study of Flieger et al. (2003). Preparation of β -glucan is also described in the patent document CZ 296475. β -glucan was identified as 1-3,1-6- β -glucan with the molecular weight of

3 ± 0.5 MDa. The molecular weight was determined by means of the MALS (Multiangle Light Scattering) and LALS (Low Angle Light Scattering) methods (Flieger et al., 2004).

- ii) 1% (w/v) aqueous solution of alginic acid (supplied by Sigma-Aldrich, catalogue number 05550, mean molecular weight 117,000 g/mol)

0.5 g of alginic acid was first dissolved in a small amount of a concentrated solution of NaOH, pH was then adjusted to 8.5 and finally the solution volume was filled-up with water to 50 ml.

- iii) 1% (w/v) aqueous solution of chitosan (supplied by Sigma-Aldrich, catalogue number 448869, mean molecular weight 24,000 g/mol)

0.5 g of chitosan was first dissolved in a small amount of concentrated HCl, then pH was adjusted to 6.0 and finally the solution volume was filled-up with water to 50 ml.

The volumes of the stock solutions employed in the individual experiments are presented (in microlitres) in Table III. The evaluation of the experiment was done in accordance with a) of Example 6 and b) of Example 7.

Table III: Volumes of the stock solutions in individual experiments.

		Teriparatide in a PBS buffer, pH 7.3	Polysaccharide in an aqueous environment	Enzyme in the corresponding buffer
Proteolysis of teriparatide by trypsin	Teriparatide/trypsin	50 μ l	---	2.5 μ l
	Teriparatide / β - glucan/trypsin	50 μ l	600 μ l	2.5 μ l
	Teriparatide /alginic acid/trypsin	50 μ l	141 μ l	2.5 μ l
	Teriparatide /chitosan/trypsin	50 μ l	29 μ l	2.5 μ l
Proteolysis of teriparatide by chymotrypsin	Teriparatide /chymotrypsin	50 μ l	---	2.5 μ l
	Teriparatide/ β -glucan /chymotrypsin	50 μ l	600 μ l	2.5 μ l
	Teriparatide /alginic acid/chymotrypsin	50 μ l	141 μ l	2.5 μ l
	Teriparatide /chitosan/chymotrypsin	50 μ l	29 μ l	2.5 μ l
		Teriparatide in 10mM of HCl, pH 2.2	Polysaccharide in an aqueous environment	Enzyme in the corresponding buffer
Proteolysis of teriparatide by pepsin	Teriparatide /pepsin	50 μ l	---	2.5 μ l
	Teriparatide / β -glucan /pepsin	50 μ l	600 μ l	2.5 μ l
	Teriparatide /alginic acid/pepsin	50 μ l	141 μ l	2.5 μ l
	Teriparatide /chitosan/pepsin	50 μ l	29 μ l	2.5 μ l

Example 6

The samples of teriparatide after digestion by the digestive enzymes from Example 5 were qualitatively analyzed by means of MALDI-TOF mass spectrometry (MS). This method was used to test the sample for the presence of intact teriparatide. The samples were first purified and concentrated using a ZipTip[®] commercial pipette tip (Millipore, USA), which contains the C18 chromatographic media. Pre-purified samples were mixed with the matrix solution in the volume ratio of 1:1. 2,3-Dihydroxybenzoic acid (DHB) at the concentration of 20 mg/ml in a solution of 30% acetonitrile/0.1% TFA (TFA = trifluoroacetic acid) was used as the matrix. The samples were analyzed with a Biflex IV mass spectrophotometer (Bruker Daltonics, Germany). The samples were measured in a reflector mode with the detection range of 0.6 - 4.5 kDa. The device was externally calibrated with a standard supplied by Bruker Daltonics, containing peptides with a known molecular weight. The spectra were evaluated by the mMass program (Strohalm et al., 2010). The results of the experiments are summarized in Table IV. An example of the mass spectrum of a sample after 1-hour incubation of teriparatide with trypsin is shown in Fig. 10.

Table IV: Results of the qualitative analysis of stability of teriparatide in the presence of selected digestive enzymes by MALDI-TOF mass spectrometry. + means that intact teriparatide was detected with MALDI-TOF MA; – means that no intact teriparatide was detected with MALDI-TOF MS.

	digestion by pepsin					digestion by trypsin					digestion by chymotrypsin				
	0.5 h	1 h	3 h	8 h	24 h	0.5 h	1 h	3 h	8 h	24 h	0.5 h	1 h	3 h	8 h	24 h
PTH(1-34)	+	+	–	–	–	+	–	–	–	–	+	+	–	–	–
PTH(1-34) + β -glucan	+	+	+	–	–	+	+	+	+	+	+	+	+	+	–
PTH(1-34) + alginic acid	+	+	+	+	+	+	+	–	–	–	+	+	+	–	–
PTH(1-34) + chitosan	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Example 7

The HPLC analysis was performed in an INGOS LC 5000 HPLC system (Czech Republic) with a Nucleosil C18 column (250 x 4.6 mm). The mobile phase solutions used for the gradient elution were: A) 0.1 % TFA in acetonitrile and B) 0.1 % TFA in distilled water. The flow rate was 0.5 ml/min with the gradient conditions: 0-1 min (0 % B), 1-7 min (0-90 % B), 7-9 min (90-70 % B), 9-12 min (70-40 % B), 12-14 min (40-100 % B), 14-27 min (100-0 % B) and 27-30 min (0 % B). The concentration of teriparatide was calculated from absorbance at 220 nm. For this evaluation a set of calibration solutions was prepared and the absorbance dependence was found to be linear in the range from 0.01 mg/ml to 0.15 mg/ml

(the concentration of teriparatide used). The limit of determination was set out as 7% of the teriparatide concentration at the beginning of the experiment. The samples of teriparatide after digestion by the digestive enzymes from Example 5 were analyzed using the HPLC method under the above mentioned conditions.

Table V: Results of the quantitative analysis of stability of teriparatide in the presence of selected digestive enzymes by HPLC expressed as the ratio of the current concentration of teriparatide to the initial concentration at the beginning of the experiment.

	digestion by pepsin					digestion by trypsin					digestion by chymotrypsin				
	0.5 h	1 h	3 h	8 h	24 h	0.5 h	1 h	3 h	8 h	24 h	0.5 h	1 h	3 h	8 h	24 h
PTH(1-34)	0,42	0,28	0,00	0,00	0,00	0,46	0,30	0,00	0,00	0,00	0,59	0,34	0,08	0,00	0,00
PTH(1-34) + β -glucan	0,57	0,33	0,10	0,00	0,00	0,80	0,76	0,45	0,00	0,00	0,64	0,41	0,21	0,00	0,00
PTH(1-34) + alginic acid	0,98	0,89	0,56	0,39	0,09	0,96	0,87	0,57	0,36	0,00	0,95	0,90	0,82	0,58	0,00
PTH(1-34) + chitosan	0,96	0,87	0,61	0,41	0,13	0,97	0,90	0,67	0,53	0,22	0,89	0,71	0,62	0,43	0,00

Example 8

Materials:

Dosage forms for intravenous (i.v.) and subcutaneous application (s.c.): (Eli Lilly and Company)

Recombinant teriparatide produced by *Escherichia coli* bacteria is used. The dosage form is a sterile, colourless, clear isotonic solution in a glass cartridge that is incorporated in an injector (pen) designed for subcutaneous application with a thin needle. The pre-filled injector contains 3.3 ml of the solution out of which 3 ml can be applied. The concentration of the active substance is 250 $\mu\text{g/ml}$. 1 ml of the solution further contains 0.41 mg of glacial acetic acid, 0.10 mg of sodium acetate, 45.4 mg of Mannitol and 3.0 mg of meta-cresol. To achieve pH 4 a 10% solution of hydrochloric acid and/or 10% solution of sodium hydroxide are added. The amount of *s.c.* and *i.v.* applied teriparatide was 5 $\mu\text{g/kg}$ using the commercial formulation of Eli Lilly and Company.

Dosage form for oral administration (Bachem and Sanofi-Aventis)

A synthetic formulation containing 99.8% of API was used. Teriparatide was prepared by linear synthesis in the solid phase followed by HPLC purification. A stock solution of teriparatide at the concentration of 1 mg/ml in re-distilled water was prepared. The complexes of teriparatide with the polysaccharides were prepared by incubation of the stock solution of teriparatide with 2% aqueous solution of β -glucan, 1% aqueous solution of alginic acid and 1% aqueous solution of chitosan, respectively (stirred at the laboratory temperature for 24

hours). Aqueous solutions of the polysaccharides were prepared by dissolution of a lyophilized soluble form of the polysaccharide (prepared in accordance with Example 5) in the selected amount of water. An optimum weight ratio of teriparatide to the polysaccharide for the formation of the complex has been found at 1:9, i.e. 10% (w/w) content of teriparatide in the polymeric saccharide matrix, namely on the basis of the primary experiments of biological availability. Biological availability of the drug was tested after administration of 100 µg of the active substance in the form of polysaccharide complexes with the 3%, 10% and 20% (w/w) content of teriparatide in the polymeric saccharide matrix. The complexes of teriparatide with the polysaccharides were finally lyophilized. Thus prepared dosage form of teriparatide was used for oral administration. The results presented in Table VI were obtained at the dose of administered teriparatide of 50 µg of API /kg of animal using a complex with the 10% (w/w) content of teriparatide in the polysaccharide matrix (i.e. 500 µg of lyophilizate per 1 kg of animal were administered). Teriparatide in the polymeric matrix was weighed on an XS3DU micro-balance from Mettler Toledo (Switzerland) for the oral administration. Administration of teriparatide in the solid form was performed using a probe to the stomach of the laboratory animals to ensure a qualitative value of the measurement.

Experiments:

Laboratory rats of the Wistar strain were randomly divided into 6 groups of 6 animals each. During the experiment the animals were on the standard laboratory diet and *ad libitum* water.

Group 1: control (without administration of the drug)

Group 2: *i.v.* administration of commercial teriparatide (Eli Lilly and Company): teriparatide dose 5 µg/kg of animal (biological availability 100 %)

Group 3: *s.c.* administration of commercial teriparatide (Eli Lilly and Company): teriparatide dose 5 µg/kg of animal

Groups 4–6: single administration of an oral dosage form in the form of a complex of teriparatide (Bachem and Sanofi-Aventis) with β-glucan (Group 4), alginic acid (Group 5), and chitosan (Group 6), respectively: dose of 50 µg teriparatide/kg of animal in the form of lyophilizate containing 10% (w/w) of teriparatide in the polymeric matrix.

Blood samples (100 µl) were taken from the tail vein into micro-tubes with 15% of EDTA as an anticoagulant before administration of teriparatide and at 5, 10, 20, 30, 45, 60, 90, 180, 240 and 300 min after administration of teriparatide. To prevent degradation of teriparatide, the blood samples were processed immediately. Blood plasma was obtained by centrifugation at 6,000 rpm and 4 °C for 10 minutes. For 100 µl of blood plasma 4.8 µl of a protease inhibitor (Sigma-Aldrich, USA) were added and the sample was stored at –80 °C until the quantitative

analysis by RIA was performed. A commercial kit from DiaSorin (USA) was used for RIA, following the instructions of the manual.

Results:

Table VI: Results of *in vivo* experiments in laboratory animals: determination of biological availability of an oral form of teriparatide in a polysaccharide matrix as compared to intravenous (*i.v.*) and subcutaneous (*s.c.*) application of the commercial drug form. The results were obtained by the RIA bioanalytical method.

Time (min)	Concentration of teriparatide in the blood plasma (pg/ml)				
	<i>i.v.</i> administration (5 µg of teriparatide/kg of animal)	<i>s.c.</i> administration (5 µg of teriparatide /kg of animal)	Oral administration (50 µg of teriparatide/kg of animal, 10% (w/w) content of teriparatide in the polymeric matrix)		
			β-glucan	chitosan	alginate acid
5	310	20	24	31	16
10	195	35	40	49	28
20	160	60	70	109	50
30	111	190	186	178	152
45	99	131	164	101	103
60	89	100	118	86	81
90	78	71	70	57	57
180	64	60	62	50	48
240	48	41	39	40	41
300	35	32	31	29	32

The results are presented as the arithmetic average of the measured values in six different animals. The variability among the animals was found to be lower than 9 %.

It could be demonstrated for all the three tested types of teriparatide complexes that they not only stabilize teriparatide from the action of digestive enzymes, but also enable its absorption when administered orally *in vivo*. All the doses of teriparatide were quickly absorbed and showed different time dependencies on the basis of the method of administration. T_{max} for the *s.c.* form was determined to be 30 min, for the oral form, from 25 min (chitosan) to 35 min (β-glucan). The biological availability of the *s.c.* administration was determined as 71 %. The biological availability of our oral formulation was lower as compared to the commercial *s.c.* form as expected, namely in the range from 2.2 to 17 %. While this is a lower biological availability in comparison to the commercial *s.c.* form, this is very satisfactory from the practical point of view for the particular case. Biological availability of orally administered unprotected teriparatide, as mentioned by Werle et al. (2006), is negligible, while the

biological availability of orally administered teriparatide in a polysaccharide complex achieves as high values as 17 %. The best results were achieved with teriparatide in the chitosan matrix, namely containing 10% (w/w) teriparatide in the polymeric matrix. Data from reference literature (Bernkop-Schnurch, 2000) suggest that chitosan might facilitate absorption of peptides in the intestine. Our study has demonstrated that chitosan efficiently stabilizes teriparatide against digestive enzymes. Whether the higher biological availability of teriparatide from the orally administered positively charged chitosan complex as compared to the availability from the complexes with a neutral polysaccharide soluble in water (beta glucan) or negatively charged polysaccharide (alginic acid) can be ascribed to the effect of chitosan as an enhancer of transfer through the intestinal wall is still subject to investigations. Oral administration of teriparatide in the complex with chitosan has been found to exhibit faster elimination than the *s.c.* administration. The values of maximum concentration $C_{\max}$ and areas under the curve of the time dependence of concentration AUC_0 nearest to the values for *s.c.* administration of a 20 μg dose of commercial teriparatide (Eli Lilly and Company) were recorded in the case of oral administration of 0.2 mg of teriparatide in the form of a complex with 1.8 mg of chitosan.

Pharmaceutical compositions for oral administration of teriparatide

The complexes of teriparatide with the soluble polysaccharides β -glucan, chitosan and alginic acid according to the present invention are suitable for the preparation of an oral dosage form of teriparatide in combination with at least one excipient, enabling a more comfortable and cheaper method of administration of the medicament to patients than the hitherto used injection administration. The excipient may also include the excessive soluble polysaccharide not participating in the formation of the complex with teriparatide. Other excipients may include any suitable solid or liquid excipients used in pharmacy. The complex of a soluble polysaccharide with teriparatide required for the preparation of the dosage form can be either prepared in a solid form in advance and then directly used for the preparation of the final dosage form as, e.g., a powder, or the complex can be processed together with further excipients into a semi-finished product, in the form of, e.g., granules or a gel. The final dosage form can principally include any solid or liquid form for oral administration, such as tablets, pellets, capsules, solution or suspension. An alternative of the method of preparation of the dosage form consisting in processing of a solid complex prepared in advance is represented by preparation of the complex of teriparatide with a soluble polysaccharide in a solution and using this solution directly for the preparation of the final dosage form. Another option consists in preparing the solution of the complex right during the process of preparation of the dosage form or the semi-finished product for its preparation; thus, it is possible to let the complex form in the solution already in the presence of at least another excipient, which will become part of the final dosage form.

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Sequence Listing**Identification number: 1**

Characteristic: amino acid sequence of teriparatide

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

Claims

1. A non-covalent complex of teriparatide according to the sequence of id. no. 1, soluble in an aqueous environment, obtainable by reaction of teriparatide with a soluble polysaccharide in an aqueous solution.
2. The complex according to claim 1, characterized in that the polysaccharide is selected from the group consisting of beta-glucan, chitosan and its salts and alginic acid and its salts.
3. Use of the complex according to claim 1 or 2 for the protection of teriparatide from enzymatic hydrolysis.
4. A method for the preparation of the complex according to claim 1 or 2, characterized in that an aqueous solution of teriparatide is mixed with an aqueous solution of the polysaccharide and the mixture is incubated at 0 – 40 °C.
5. The method according to claim 4, characterized in that the incubation is carried out for 0.5 to 72 hours.
6. A pharmaceutical composition for oral administration, characterized in that it comprises a complex of teriparatide soluble in an aqueous environment according to claim 1 or 2 and at least one pharmaceutically acceptable excipient.
7. A method for the preparation of the composition according to claim 6, characterized in that a complex according to claim 1 or 2 is prepared and processed with at least one pharmaceutically acceptable excipient.
8. The method according to claim 7, characterized in that the complex is prepared in a solid form.
9. The method according to claim 8, characterized in that the complex is prepared in the form of a solution.
10. A method for the preparation of the composition according to claim 6, characterized in that a complex according to claim 1 or 2 is prepared in the presence of at least one another excipient pertaining to the formulation of the final dosage form.

1/7

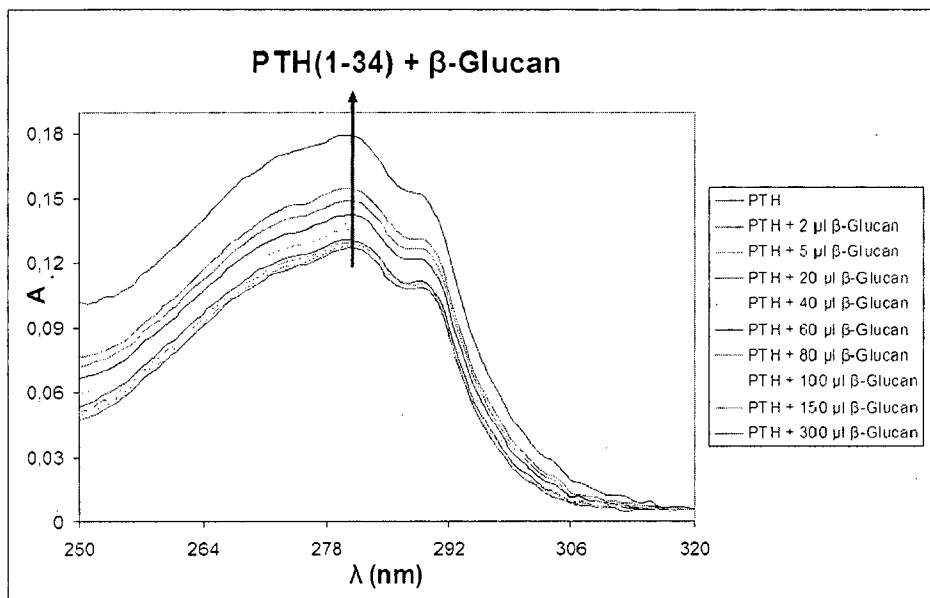


Fig. 1

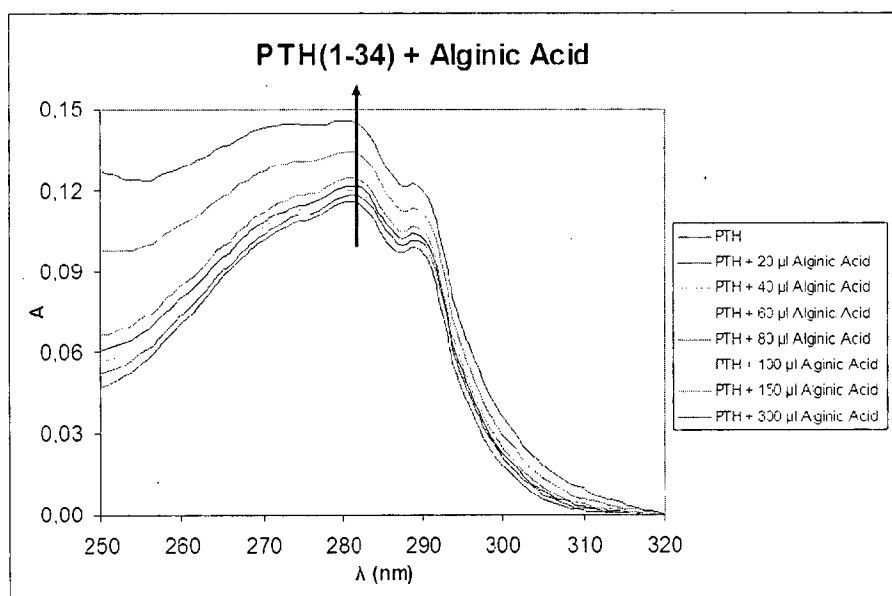
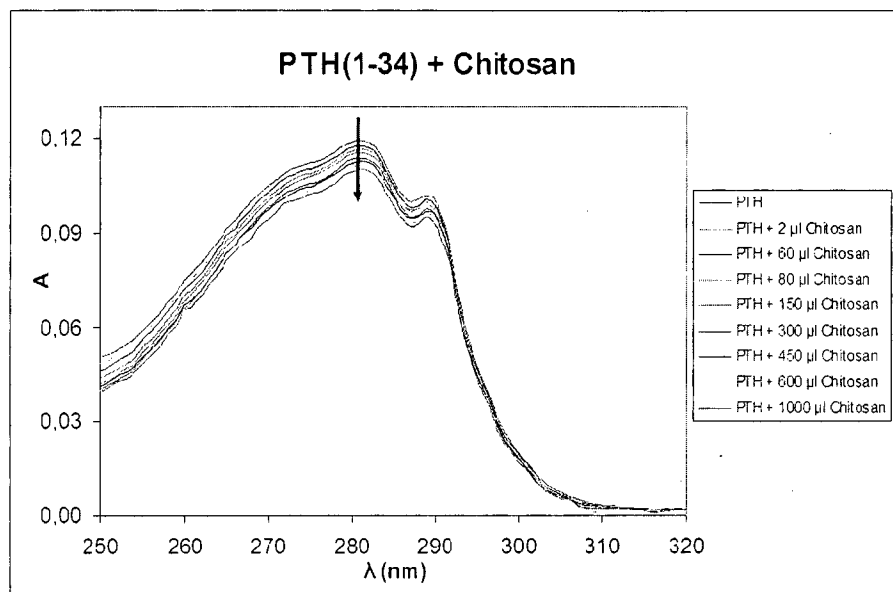
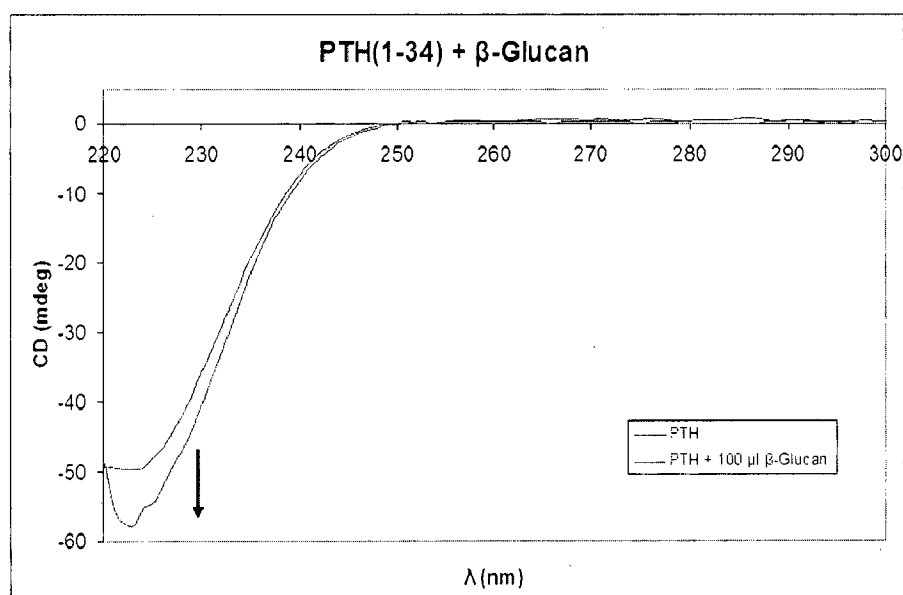


Fig. 2

2/7

**Fig. 3****Fig. 4**

3/7

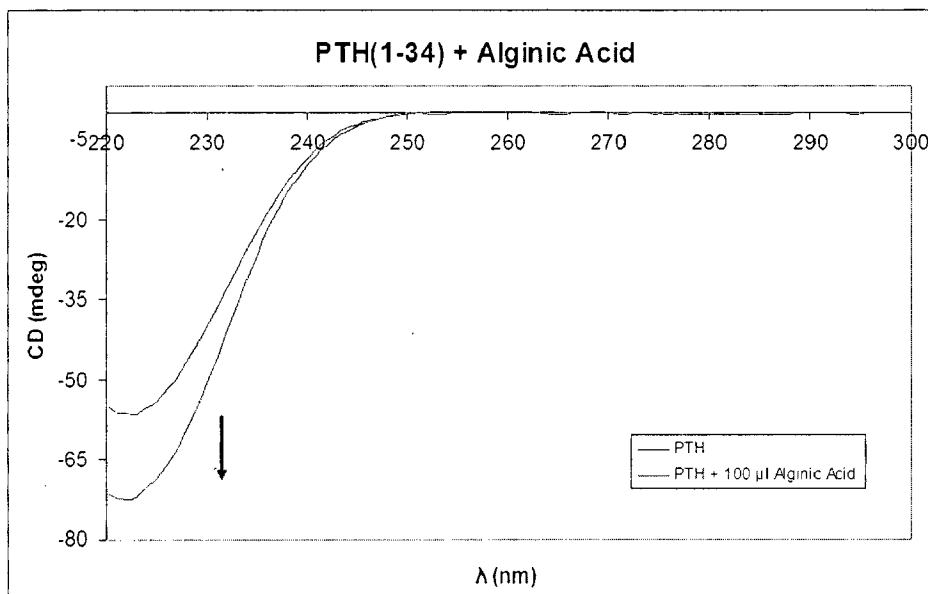


Fig. 5

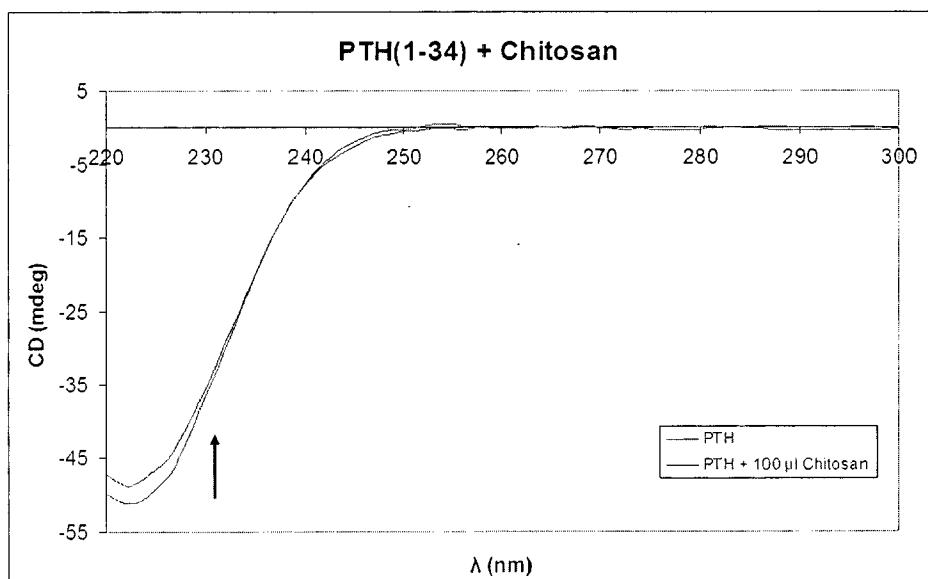


Fig. 6

4/7

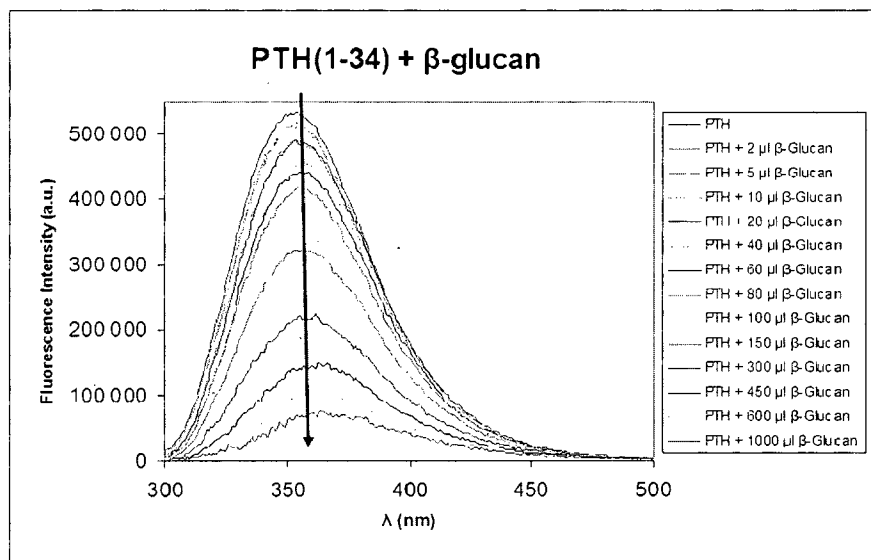


Fig. 7

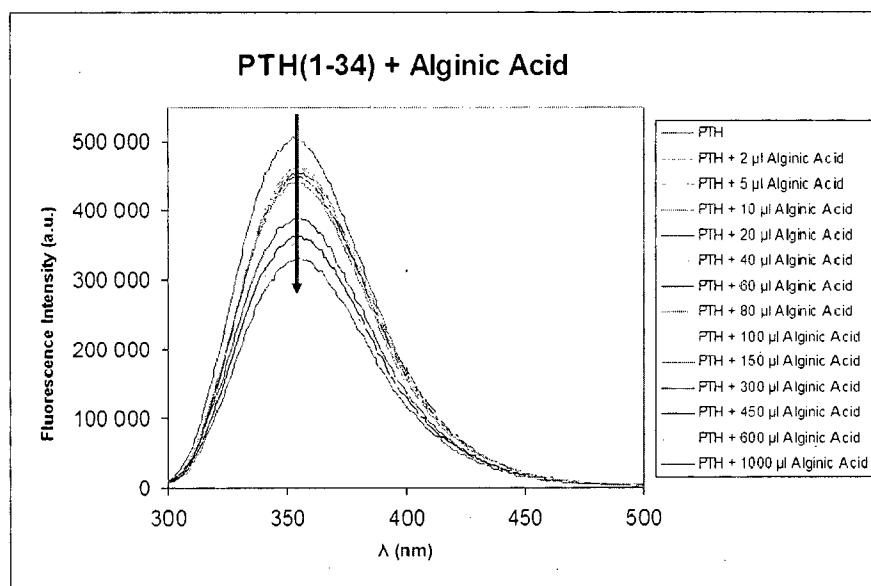
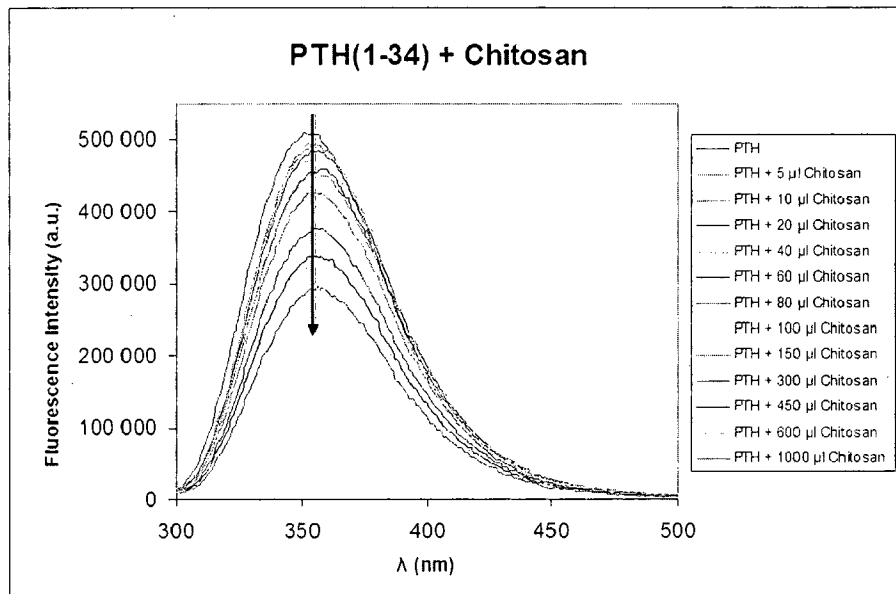


Fig. 8

5/7

**Fig. 9**

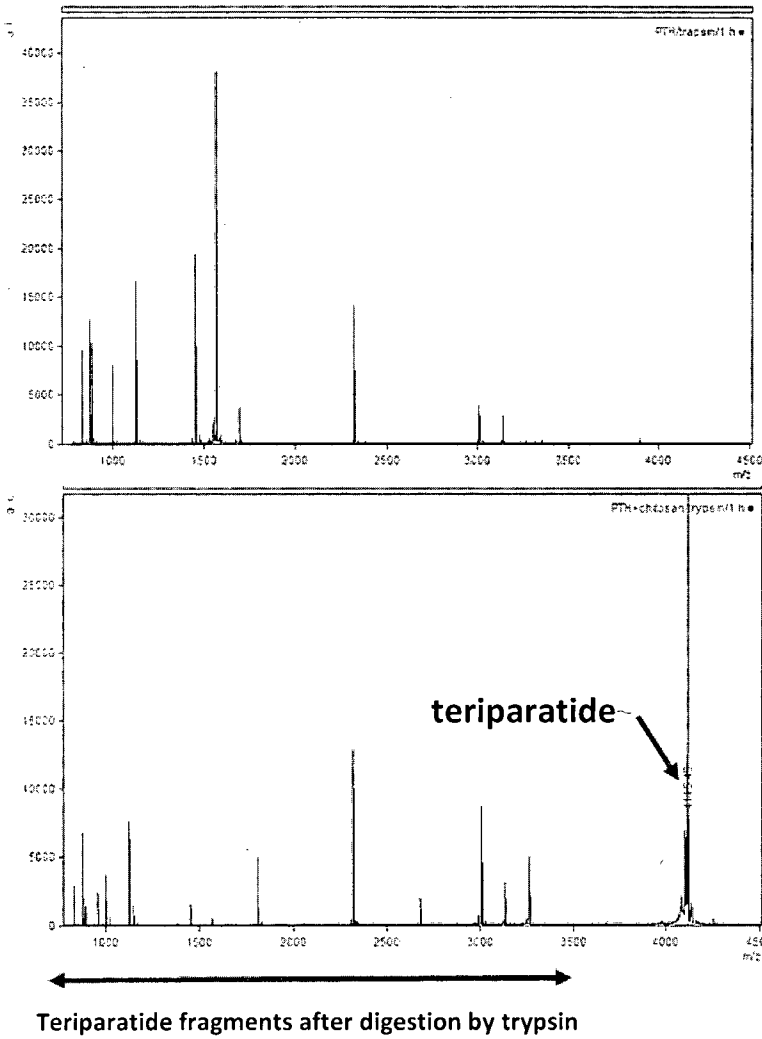


Fig. 10

7/7

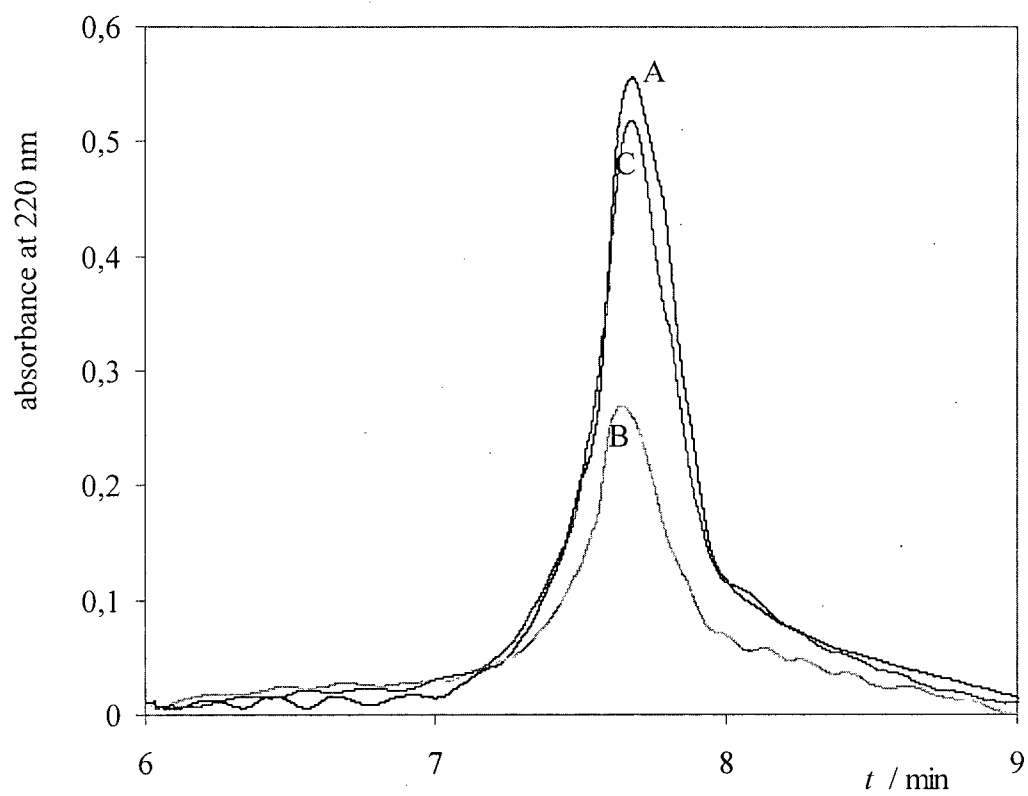


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2012/000025

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/08 A61K47/36 A61K9/00 A61K38/29 A61K47/48
A61K9/14 A61K9/16

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

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

EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/024141 A2 (APOGEE TECHNOLOGY INC [US]; UNIV AKRON [US]; RENEKER DARRELL H [US]; S) 28 February 2008 (2008-02-28) page 38, line 5 - page 41, line 5 page 81, line 2 - page 82, line 1 claims 1-11	1-10
X	LEE CHEN HSEN; WANG YNG JIIN; KUO SHYH MING; CHANG SHWU JEN: "Microencapsulation of parathyroid tissue with photosensitive poly(L-lysine) and short chain alginate-co-MPEG", ARTIFICIAL ORGANS, vol. 28, no. 6, 1 June 2004 (2004-06-01), pages 537-542, XP008154107, abstract page 538, left-hand column, line 17 - page 540, right-hand column, line 8	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

31 July 2012

Date of mailing of the international search report

07/08/2012

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INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2012/000025

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2012/075375 A1 (LANCO BIOSCIENCES INC [US]; DEASEY ANTHONY P [US]; FRANKHAM PATRICK [C] 7 June 2012 (2012-06-07) paragraph [0059] - paragraph [0063] -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/CZ2012/000025

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008024141 A2	28-02-2008	AU 2007288442 A1	28-02-2008
		CA 2650197 A1	28-02-2008
		EP 2015803 A2	21-01-2009
		WO 2008024141 A2	28-02-2008

WO 2012075375 A1	07-06-2012	NONE	
