The invention relates to long-acting, biologically degradable microparticles based on poly(lactide-co-glycolide) (=PLGA) which contain therapeutically active peptides or their physiologically tolerable salts as the active substance, and to a process for the preparation of such microparticles.

For pharmaceuticals which are enzymatically broken down in the gastrotestinal tract, such as, for example, proteins or peptides, in addition to nasal and dermal administration - often with only a low absorption rate - parenteral pharmaceutical forms are particularly important. Long-term treatment of certain diseases makes it appear desirable to develop parenteral depot pharmaceutical forms which release the pharmaceutical continuously over the course of several weeks. In the literature, in addition to implants (compare, for example EP-B-0,058,481) microcapsules/microspheres are also described for these purposes.

In order to avoid operative removal of these injection forms, biodegradable polymers are used. In addition to polyamides, polyanhydrides, poly(ortho)esters and polyacetals, polyesters are in particular employed. Usually, polyesters based on the monomers lactic acid and glycolic acid or their copolymers are described as suitable polymers.

For the preparation of microcapsules/microspheres, various processes, such as, for example phase separation (EP-B-0,052,510), solvent extraction (EP-B-0,145,240) or spray drying (EP-A-0,315,875 = South African Patent 88/8396, CH 666,406, A5, compare Derwent Ref. No. 88-228036/33) are suitable. Compared to the other processes, spray drying has the advantage that the encapsulation efficiency is as a rule higher than in the other processes mentioned and that no other auxiliaries are needed for encapsulation, which auxiliaries would then lead to possible contamination in the product. It is

disadvantageous that the spray drying has to be carried out at relatively high temperatures, i.e. as a rule far above the boiling point of the respective solvents. If suspensions, emulsions or solutions of copolymers of lactic and glycolic acid (PLGA) are subjected to spray drying under customary conditions, no microparticles are obtained, but thread-like structures which are not suitable for an injectable depot form (EP-A-0,315,875). The microcapsules obtained by spray drying at 59°C described in CH 666,406 A5 do not contain PLGA as the biologically degradable support material but D,L-oligolacoyl-N-(L)-phenylalanine. H.T. Wang et al. (Biomaterials 1990, Volume 11, pages 679-685) describe microparticles which contain albumin and PLGA and are obtained by spray drying at about 37°C.

Surprisingly, it has now been found that PLGA polymer solutions and appropriate aqueous suspensions and emulsions can be sprayed to give microparticles if the spraying temperature is less than 60°C and the spray flow rate is above 500 NL/h. These microparticles are additionally distinguished, compared to microparticles which are prepared by the other processes mentioned, by a lower residual solvent content (organic solvent, water), which is below 1%. The latter is of great importance, in particular in the case of injectables, since high demands are made here on the purity of a pharmaceutical.

The invention therefore relates to microparticles which contain a peptide as the active substance and poly(lactide-co-glycolide) (PLGA) as the support material, which are obtained by spray drying at temperatures below 60°C at a spray flow rate of above 500 NL/h.

(1 NL relates to the conditions at a temperature of 0°C and at a pressure of 1013 hPa and corresponds to 1L).

In the embodiments above and below, microparticles are also understood as meaning microcapsules and microspheres, i.e. both particles in which the active sub-

stance is completely or partially endorsed by polymer, and particles in which the active substance is dispersed in a PLGA matrix.

Peptides are understood as meaning natural and synthetic peptides and also their physiologically tolerable salts.

The invention furthermore relates to a process for the preparation of microparticles which contain a peptide as the active substance and PLGA as the support material, which comprises suspending or dissolving the peptide in a solution of the support material or emulsifying an aqueous solution of the peptide in a solution of the support material and spraying the suspension, solution or emulsion at a temperature below 60°C at a spray flow rate greater than 500 NL/h.

The microparticles according to the invention preferably contain water-soluble peptides. The molecular weight of the peptides is preferably over 800. Particularly suitable peptides are LHRH agonists and antagonists such as, for example buserelin, HOE 013 (Ac-D-Nal-p-Cl-D-Phe-D-Trp-Ser-Tyr-D-Ser(α-L-Rha)-Leu-Arg-Pro-Azagly-NH_2, compare EP-A-0,263,521), nafarelin, triptorelin, leuprorelin and goserelin. Peptides of other types are also suitable such as, for example, TRH, vasopressin, calcitonin, insulin or HOE 427 (= ebiratide, [4-methionine dioxide, 8-D-lysine, 9-phenylamine]-α-MSH-(4-9)-(8-aminooctyl)amide triacetate, compare EP-A-0,179,332).

The biologically degradable support material is PLGA. The release of active substance from the microparticles is not only affected by the physicochemical properties of the active substance but also by the properties of the polymer or of the polymer mixtures such as molecular weight, molar composition and sequence of the lactic acid and glycolic acid units in the polymer. The support material may comprise, for example, a mixture of 50:50

PLGA and 40:60 PLGA. The higher the molecular weight or the inherent viscosity, the longer the release of active substance lasts. The inherent viscosity (20°C, chloroform, 0.1%) is preferably 0.1 to 0.8 dl/g. The molar ratio of lactide to glycolide units is in the range, for example, from 85:15 to 40:60; preferably 50:50 poly(d,l-lactide-co-glycolide) is used which has a viscosity of 0.1 to 0.7 dl/g, in particular of 0.1 to 0.5 dl/g.

Depending on the properties of the polymer, the microparticles prepared by the process according to the invention release the active substance over a period of preferably 2 weeks to 3 months. The degree of loading also has an influence, even if minor, on the duration of active compound release. In the process according to the invention, microparticles having a degree of peptide loading of less than 20%, preferably of less than 12%, are preferably obtained.

In the microparticles prepared by the process according to the invention, the particle size, which also affects the release, is less than 200 μm, preferably less than 100 μm. In particular, the particle size is less than 50 μm.

The microparticles are prepared by spraying of a solution, emulsion or suspension of the active substance in the polymer solution. Solvents which can be employed, for example, are chloroform, methylene chloride, DMF, acetone, ethyl acetate, glacial acetic acid and water or mixtures thereof. Preferably, methylene chloride, glacial acetic acid and water are used.

In the process according to the invention, the peptide is preferably dissolved in water and added to the solution of PLGA in, for example, methylene chloride. The emulsion formed in this way is preferably sprayed. Spraying can also be carried out after addition of, for example,

methanol, a solution then being formed.

The spraying temperature is preferably 50° to 30°C, in particular 40° to 30°C. The spray flow rate must not be too low, as otherwise thread formation can occur. It is preferably about 800 NL/h, as better drying properties are associated with a higher spray flow rate.

In the case of the use of a laboratory sprayer (for example Büchi Mini Spray Dryer 190), the pressure of the spray medium, for example air or nitrogen, should be in the range 3-8 bar. The pump capacity of the sprayer must be suited to the conditions (3-20 ml/min). By appropriate adjustment of the aspirator, for example 18 scale divisions, it is ensured that all the air or nitrogen in the spray medium is removed.

The PLGA polymer should be dissolved in as little solvent as possible, polymers with a high content of lactide requiring a larger amount of solvent. In principle, the concentration of the polymer in the solution, emulsion or suspension to be sprayed should be selected such that spraying is possible under the selected conditions. Preferably, the PLGA concentration in methylene chloride is less than 15% by weight, calculated relative to methylene chloride.

The invention is illustrated by the following examples.

The viscosity given in the examples for PLGA is the inherent viscosity of a 0.1% strength solution in chloroform at 20°C determined by generally customary methods.

The spray parameters a) aspirator and b) pump capacity are adjusted in all examples to 18 (for aspirator) or 6 (for pump capacity) scale divisions.

Example 1: Spray medium - glacial acetic acid

a) 0.064 g of buserelin acetate is dissolved in 2.0 g of water. The aqueous solution is mixed with a solution of 1.936 g of 50:50 PLGA (IV = 0.4 dl/g) in 40 g of glacial acetic acid and sprayed in a laboratory spray dryer. The spray parameters are adjusted as follows:

1. Inlet temperature 50°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 1.2 g = 60% of theory.

Active substance content
(degree of loading): 3%
Residual solvent content:
glacial acetic acid 0.8%

b) In the same manner, microparticles are prepared using 0.192 g of buserelin acetate and 1.808 g of 50:50 PLGA (IV = 0.4 dl/g). Yield: 1.2 g = 60% of theory.
Active substance content: 9%
Residual solvent content:
Glacial acetic acid: 0.8%

For 20 mg of microparticles, the in vitro release rates were determined as follows:
20 mg of microparticles are introduced into 2.0 ml of a pH 7.4 phosphate buffer solution and eluted for 24 h at +37°C. After separation of the eluate from the microparticles, the eluate is tested for buserelin content using an HPLC method. The remaining microparticles are once more added to 2.0 ml of phosphate buffer and again eluted for 24 h at 37°C. This process is continued analogously over 56 days.

The amounts of buserelin acetate determined from the eluates are compiled in extract form in the following table:

	Active Substance Content 3%	Active Substance Content 9%
Day 2	5.8 µg	23.2 µg
Day 9	< 0.7 µg	57.7 µg
Day 16	13.4 µg	51.9 µg
Day 23	20.3 µg	16.7 µg
Day 30	13.2 µg	8.4 µg
Day 37	4.1 µg	3.4 µg
Day 44	2.5 µg	1.5 µg
Day 51	0.7 µg	0.7 µg
Day 56	0.7 µg	0.7 µg

Example 2: Spray medium - chloroform

a) 0.108 g of buserelin acetate is dissolved in 2.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 1.892 g of 50:50 PLGA (IV = 0.4 dl/g) in 92 g of chloroform and sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 0.9 g = 45% of theory.
Active substance content: 5%

b) In the same manner, microparticles are prepared using 0.152 g of buserelin acetate and 1.848 g of 50:50 PLGA (IV = 0.4 dl/g). Yield: 1 g = 50% of theory.
Active substance content: 7%

For 20 mg of microparticles, the in vitro release rates were determined analogously to Example 1 over 35 days:
The amounts of buserelin acetate determined from the eluates are compiled in extract form in the following

table:

	Active Substance Content 5%	Active Substance Content 7%
Day 2	44.7 µg	56.2 µg
Day 9	7.3 µg	10.1 µg
Day 16	30.3 µg	37.8 µg
Day 23	30.0 µg	38.6 µg
Day 30	10.9 µg	13.6 µg
Day 35	11.5 µg	14.5 µg

Example 3: Spray medium - methylene chloride/water/-methanol

a) 0.128 g of buserelin acetate is dissolved in 2.0 g of water. The aqueous solution is mixed with a solution of 1.872 g of 50:50 PLGA (IV = 0.4 dl/g) in 46 g of methylene chloride. 9 g of methanol are added to the mixture. A clear solution is formed which is sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 1.2 g = 60% of theory.
Active substance content: 6%
Residual solvent content: Water 0.9%
Methylene chloride 0.06%
Methanol < 0.01%

For 20 mg of microparticles, the in vitro release rates were determined analogously to Example 1 over 28 days:

The amounts of buserelin acetate determined from the eluates are compiled in extract form in the following

table:

	Active Substance Content 6%
Day 2	93.7 µg
Day 9	5.1 µg
Day 16	7.4 µg
Day 23	12.1 µg
Day 28	12.1 µg

Example 4: Spray medium - methylene chloride/water/-methanol

a) 0.084 g of buserelin acetate is dissolved in 4.0 g of water. The aqueous solution is mixed with a solution of 1.916 g of 50:50 PLGA (IV = 0.42 dl/g) in 65 ml of methylene chloride. 25 g of methanol are added to the mixture. A clear solution is formed which is sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 0.8 g = 40% of theory.
Active substance content: 4%

b) In the same manner, microparticles are prepared using 0.168 g of buserelin acetate and 1.832 g of 50:50 PLGA (IV = 0.42 dl/g). Yield: 0.8 g = 40% of theory.
Active substance content: 8%

For 20 mg of microparticles, the in vitro release rates were determined as follows:
20 mg of microparticles are introduced into 2.0 ml of a pH 7.4 phosphate buffer solution and eluted for 24 h at +37°C. After separation of the eluate from the micro-

particles, the eluate is tested for buserelin content using a radioimmunoassay (RIA). The remaining microparticles are added to 2.0 ml of phosphate buffer once again and again eluted for 24 h at 37°C.

This process is analogously continued over 63 days. The amounts of buserelin acetate determined from the eluates are compiled in extract form in the following table:

	Active Substance Content 4%	Active Substance Content 8%
Day 2	129.3 µg	280.1 µg
Day 9	1.7 µg	4.7 µg
Day 16	1.9 µg	5.7 µg
Day 23	6.3 µg	3.0 µg
Day 30	6.4 µg	8.1 µg
day 37	3.1 µg	5.6 µg
Day 44	1.6 µg	3.9 µg
Day 51	3.1 µg	2.4 µg
Day 58	0.7 µg	1.4 µg
Day 63	0.7 µg	1.4 µg

Example 5: Spray medium - chloroform/water/dimethylformamide

a) 0.13 g of buserelin acetate is dissolved in 2.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 1.87 g of 50:50 PLGA (IV = 0.4 dl/g) in 41.4 g of chloroform and 4.6 g of dimethylformamide and sprayed in a laboratory spray dryer.

The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/g

The microparticles are obtained as a white, pourable powder. Yield: 1.2 g = 60% of theory.

Active substance content: 6%

For 20 mg of microparticles the in vitro release rates were determined analogously to Example 4 over 63 days:

The amounts of buserelin acetate determined from the eluates are compiled in extract form in the following table:

	Active substance content 6%
Day 2	32.1 µg
Day 9	16.8 µg
Day 16	49.1 µg
Day 23	39.1 µg
Day 30	17.5 µg
Day 37	5.7 µg
Day 44	2.5 µg
Day 51	2.5 µg
Day 58	1.4 µg
Day 63	1.4 µg

Example 6: Spray medium - methylene chloride

a) 0.085 g of buserelin acetate is dissolved in 2.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 1.915 g of 50:50 PLGA (IV = 0.4 dl/g) in 46 g of methylene chloride and sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 0.6 g = 30% of theory.
Active substance content: 4%

b) In the same manner, microparticles are prepared using 0.128 g of buserelin acetate and 1.872 g of 50:50 PLGA (IV = 0.4 dl/g). Yield: 0.7 g = 35% of theory.
Active substance content: 6%

c) and also using 0.170 g of buserelin acetate and 1.872 g of 50:50 PLGA (IV = 0.4 dl/g).
Yield: 0.4 g = 20% of theory.
Active substance content: 8%

For 20 mg of the microparticles, the in vitro release rates were determined analogously to Example 4 over 63 days:

The amounts of buserelin acetate determined from the eluates are compiled in extract form in the following table:

	Active substance content 4%	Active substance content 6%	Active substance content 8%
Day 2	39.4 µg	49.8 µg	88.0 µg
Day 9	1.1 µg	2.7 µg	4.7 µg
Day 16	5.9 µg	34.3 µg	21.7 µg
Day 23	17.1 µg	51.5 µg	28.0 µg
Day 30	4.2 µg	18.4 µg	3.2 µg
Day 37	1.5 µg	5.8 µg	2.2 µg
Day 44	1.7 µg	2.5 µg	2.8 µg
Day 51	0.4 µg	0.8 µg	2.4 µg
Day 58	0.3 µg	0.7 µg	2.5 µg
Day 63	0.3 µg	0.3 µg	2.5 µg

Example 7: Spray medium - methylene chloride/water

0.032 g of buserelin acetate is dissolved in 1.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 0.968 g of 85:15 PLGA in 23 g of methylene chloride and sprayed in a

laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 40°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 0.7 g = 70% of theory.
Active substance content: 3%

Example 8: Spray medium - methylene chloride/water

0.064 g of buserelin acetate is dissolved in 2.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 1.936 g of 50:50 PLGA (IV = 0.7 dl/g) in 92 g of methylene chloride and sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 40°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder, yield: 0.9 g = 45% of theory.
Active substance content: 3%

Release of active substance determined analogously to Example 1

	Active substance content 3%
Day 2	48.1 µg
Day 23	3.9 µg
Day 37	7.1 µg
Day 44	3.9 µg
Day 51	1.0 µg

Example 9: Spray medium - methylene chloride/water

0.106 g of buserelin acetate is dissolved in 1.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 0.894 g of 50:50 PLGA (IV = 0.1 dl/g) in 23 g of methylene chloride and sprayed in a laboratory spray dryer.

The spray parameters are adjusted as follows:

1. Inlet temperature 40°C
2. Spray flow rate 800 NL/g

The microparticles are obtained as a white, pourable powder. Yield: 0.3 g = 30% of theory.
Active substance content: 10%

Release of the active substance determined analogously to Example 1

	Active substance content
	10%
Day 2	32.7 µg
day 9	19.6 µg
Day 16	20.6 µg
Day 23	15.4 µg
Day 28	15.4 µg

Example 10: Spray medium - methylene chloride/water

0.13 g of buserelin acetate is dissolved in 2.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 1.87 g of 75:25 PLGA (IV = 0.5 dl/g) in 46 g of methylene chloride and sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 1.2 g = 60% of theory.

Active substance content: 6%

Example 11: Spray medium - methylene chloride/water

0.128 g of buserelin acetate is dissolved in 2.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 1.872 g of 75:25 PLGA (IV = 0.8 dl/g) in 138 g of methylene chloride and sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature: 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 0.9 g = 45% of theory.
Active substance content: 6%

Example 12: Spray medium - methylene chloride/water

0.13 g of buserelin acetate is dissolved in 2.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 1.87 g of 47:53 PLGA (IV = 0.3 dl/g) in 46 g of methylene chloride and sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder, yield: 1.3 g = 65% of theory.
Active substance content: 6%

Example 13: Spray medium - methylene chloride

0.13 g of buserelin acetate is suspended in a solution of 1.87 g of 50:50 PLGA (IV = 0.4 dl/g) and 46 g of methylene chloride using a rotor-stator homogenizer. The

suspension is sprayed in a laboratory spray dryer. The spray parameters are adjusted as follows:

1. Inlet temperature 40°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 0.5 g = 25% of theory.
Active substance content: 6%

Example 14: Spray medium - methylene chloride/water

a) 0.2 g of Hoe 427 is dissolved in 1.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 3.8 g of 50:50 PLGA (IV = 0.4 dl/g) in 92 g of methylene chloride and sprayed in a laboratory spray dryer.
The spray parameters are adjusted as follows:

1. Inlet temperature 40°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 1.9 g = 48% of theory.
Active substance content: 5%

b) In the same manner, microparticles are prepared using 0.6 g of Hoe 427 and 3.4 g of 50:50 PLGA (IV = 0.4 dl/g), Yield: 2.8 g = 70% of theory.
Active substance content: 15%

c) and also using 0.4 g of Hoe 427 and 3.6 g of 50:50 PLGA (IV = 0.4 dl/g).
Yield: 2 g = 50% of theory.
Active substance content: 10%

Example 15: Spray medium - methylene chloride/water

0.08 g of Hoe 013 is dissolved in 1.0 g of water. Using a rotor-stator homogenizer, the aqueous solution is emulsified in a solution of 0.92 g of 50:50 PLGA (IV = 0.4 dl/g) in 23 g of methylene chloride and sprayed in a laboratory spray dryer.

The spray parameters are adjusted as follows:

1. Inlet temperature 30°C
2. Spray flow rate 800 NL/h

The microparticles are obtained as a white, pourable powder. Yield: 0.5 g = 50% of theory.
Active substance content: 6%

Release of active substance determined analogously to Example 1

	Active substance content
	6%
Day 2	13.9 µg
Day 9	5.9 µg
Day 16	3.1 µg
Day 23	3.7 µg
Day 28	2.2 µg

Patent Claims

1. A process for the preparation of long-acting biodegradable microparticles which contain a therapeutically active peptide as the active substance and poly(lactide-co-glycolide)(PLGA) as the support material, which comprises suspending or dissolving the peptide in a solution of the support material or emulsifying an aqueous solution of the peptide in a solution of the support material and spraying the suspension, solution or emulsion at a temperature below 60°C at a spray flow rate greater than 500 L/h (500 NL/h).

2. The process as claimed in claim 1, wherein the peptide is a water-soluble peptide.

3. The process as claimed in claim 1, wherein one or more of the following conditions are kept to:
   a) the peptide is an LHRH agonist or LHRH antagonist
   b) the support material is 50:50 PLGA
   c) the inherent viscosity of the PLGA is 0.1 to 0.5 dl/g (20°C, chloroform, 0.1%)
   d) the solvent is chloroform, methylene chloride, glacial acetic acid, methanol, dimethylformamide or water or a mixture of at least 2 of the solvents mentioned and
   e) the spray flow rate is about 800 L/h (800 NL/h).

4. The process as claimed in claim 1, wherein one or more of the following conditions are kept to:
   a) the peptide is buserelin acetate or HOE 013
   b) the support material is 50:50 PLGA
   c) the inherent viscosity of the PLGA is 0.1 to 0.5 dl/g (20°C, chloroform, 0.1%)
   d) the solvent is methylene chloride, glacial acetic acid or water, or a mixture of 2 of the solvents mentioned and
   e) the spray flow rate is about 800 L/h (= 800 NL/h).

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