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Paper A(Ch)

Chemistry

This paper comprises:

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Letter from the applicant

Nano BioTech&No.Logic
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Oficina de D. Arturo Barea. Patentes
Alcalá 42
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Dear Mr Barea,

[001] We are a small university spin-off company in the field of biopharmaceuticals. We are working on strategies for the administration of insulin for the treatment of diabetes by the oral route. Novel and promising biodegradable nanoparticles based on our research are now ready for clinical tests. We kindly ask you to file a European patent application on our behalf covering all aspects of our technology.

[002] As we are currently engaged in attracting funding for the development of our nanoparticles, we kindly ask you to draft the application so that a patent can be granted by the EPO without delay. Please note that due to financial reasons we would like to avoid payment of claims fees.

[003] We enclose with this letter all necessary information for the preparation of the patent application. You will presumably need to consider the attached document D1 for drafting the application. We also enclose a short article (D2) from the *Journal of Controlled Release* which we published one year ago and will give you more insight about the field of the invention.

[004] The vast majority of peptide biopharmaceuticals are administered by intravenous or subcutaneous injection. These routes of administration are often inconvenient and painful, particularly when multiple daily injections are required.

[005] Oral administration of peptide biopharmaceuticals would overcome these drawbacks.

[006] The term “peptide biopharmaceutical” refers to therapeutically active peptides comprising 70 or fewer amino acids.

[007] The term “oral administration” refers to administration by swallowing. The absorption of the therapeutically active peptides into the bloodstream circulation usually occurs through the intestinal mucosa.

[008] Oral administration of peptide biopharmaceuticals is challenging since the therapeutically active peptides are subject to degradation and/or destabilisation in the acidic conditions of the stomach. Another obstacle is the permeability of the intestinal mucosa to peptides, which determines the extent of intestinal absorption and the efficiency of therapeutic action.

[009] Sophisticated strategies are therefore required to preserve the therapeutically active peptides in the stomach and to enhance intestinal absorption.

[010] A well-known approach is the encapsulation in polymeric nanoparticles. However, this strategy may not be suitable for oral administration of therapeutically active peptides due to the difficulty of encapsulation with acceptable drug loading.

[011] The term “drug loading” refers to the amount of therapeutically active peptide encapsulated or entrapped in the polymeric nanoparticle.

[012] Our aim was to develop a technology for effective oral administration of peptide biopharmaceuticals, in particular metabolic peptides, which at present require injections.

[013] The term “metabolic peptide” refers to any metabolism regulating hormone such as amylin, calcitonin, glucagon, insulin, or secretin. We have specifically focused on insulin. The term “insulin” refers to insulin or any synthetic analogue of insulin.

[014] Our technology comprises: 1) biodegradable nanoparticles based on polyalkyl cyanoacrylate (PACA) comprising a metabolic peptide encapsulated or entrapped therein, 2) methods for producing the PACA nanoparticles and 3) pharmaceutical dosage forms for oral administration comprising the PACA nanoparticles.

[015] The term “polyalkyl cyanoacrylate (PACA)” as used herein refers to biodegradable homopolymers of C_2-C_{12} alkyl 2-cyanoacrylate monomers, more specifically biodegradable homopolymers of C_2-C_8 alkyl 2-cyanoacrylate monomers including, but not limited to, ethyl 2-cyanoacrylate, n-butyl 2-cyanoacrylate, isobutyl 2-cyanoacrylate, n-hexyl 2-cyanoacrylate, isohexyl 2-cyanoacrylate, and 2-octyl 2-cyanoacrylate.

[016] The biodegradable PACA nanoparticles described herein have a hydrodynamic diameter between 10 nm and 500 nm as measured by dynamic light scattering. Nanoparticles suitable for intestinal absorption must have a hydrodynamic diameter of 300 nm or less. Preferably a population of biodegradable PACA nanoparticles is provided wherein at least 90% of the nanoparticles have a hydrodynamic diameter between 100 nm and 300 nm.

[017] Upon oral administration, the integrity of the biodegradable PACA nanoparticles is preserved during passage through the stomach until reaching the small intestine. In the small intestine, the PACA nanoparticles are absorbed and the biodegradable polymer slowly degrades, releasing the therapeutically active peptide in a controlled fashion.

[018] The rates of polymer degradation and release of the therapeutically active peptide depend primarily on the alkyl chain length of the alkyl 2-cyanoacrylate monomers. The polymers of short chain alkyl 2-cyanoacrylate monomers, such as C_6 alkyl or lower, have suitable degradation rates and are advantageously used. In this regard, the preferred alkyl 2-cyanoacrylates are ethyl 2-cyanoacrylate and n-butyl 2-cyanoacrylate.

[019] According to our technology, a therapeutically active peptide, specifically a metabolic peptide such as insulin, is encapsulated or entrapped within the biodegradable PACA nanoparticles during the polymerisation process, i.e. the peptide is dissolved or dispersed in the polymerisation medium and then the polymerisation reaction is carried out.

[020] Any polymerisation method known in the art, such as interfacial polymerisation or anionic polymerisation, is suitable.

[021] Preferably, the biodegradable PACA nanoparticles are prepared by anionic polymerisation comprising the steps of:

a) dissolving a therapeutically effective amount of peptide in an acidic aqueous solution;
b) mixing the aqueous solution with an oil and a nonionic surfactant and stirring to form a water-in-oil nanoemulsion;
c) dissolving a C_2-C_{12} alkyl 2-cyanoacrylate monomer in an organic solvent;
d) slowly adding the organic solution of the monomer from step c) to the nanoemulsion from step b) under continuous stirring thereby spontaneously initiating polymerisation;

e) allowing polymerisation to progress and the organic solvent to evaporate, thereby producing PACA nanoparticles;

f) separating the nanoparticles from the nanoemulsion and purifying them.

[022] The organic solvent is typically chloroform. Any acid can be used to generate the acidic aqueous solution of step a). The oil is typically ethyl oleate. The nonionic surfactant is typically sorbitan monolaurate or polyvinyl alcohol.

[023] The C_2-C_{12} alkyl 2-cyanoacrylate monomer and the therapeutically active peptide are typically in a weight ratio of 2:1.

[024] The PACA nanoparticles have a drug loading of 10% to 30% by weight of therapeutically active peptide relative to the total weight of the nanoparticles, preferably 15% to 30% by weight. A drug loading below 10% by weight of therapeutically active peptide relative to the total weight of the nanoparticles results in poor encapsulation efficiency and is insufficient to provide a significant pharmacological effect.

[025] A pharmaceutically acceptable stabiliser such as dextran, chitosan, fucoidan, pectin, amylase or amylopectin can also be incorporated into the nanoparticles. In that case, the stabiliser is added to the therapeutically active peptide in step a), typically in an amount of 0.5 to 1% relative to the weight of therapeutically active peptide.

[026] The pH of the acidic aqueous solution used in step a) is typically in the range 1 to 6. In a preferred embodiment, the pH of the aqueous solution in step a) is 2 or less.

[027] We have successfully developed this method for the preparation of insulin-loaded PACA nanoparticles. In preliminary tests shown in the enclosed examples, we have orally administered insulin-loaded biodegradable PACA nanoparticles to rats. We have observed that the PACA nanoparticles release therapeutically effective amounts of insulin over a prolonged period after oral administration. This is demonstrated by a significant lowering of blood glucose levels (see figure 1).

[028] Further, we have unexpectedly observed that, at a pH of 2 or less, insulin forms a non-covalent complex with PACA during the polymerisation reaction. The complex is then entrapped within the biodegradable PACA nanoparticles. However, at a pH greater than 2, no complex is formed and insulin is incorporated into the PACA nanoparticles as free insulin.

[029] We believe that this is due to the physicochemical properties of insulin and to the mechanism of anionic polymerisation. Insulin has an isoelectric point of 5.3. At a pH below 5.3, it is capable of being positively charged. At a pH of 2 or less, insulin has a strong positive net charge and therefore, it can form a non-covalent complex with the negatively charged PACA chains during the polymerisation reaction.

[030] PACA nanoparticles entrapping insulin in the form of a non-covalent complex have the advantage of releasing insulin over a prolonged period after oral administration. Further, we assume that insulin in the form of a non-covalent complex has an enhanced intestinal absorption. This results in therapeutically effective insulin concentrations in the blood for longer periods which can satisfy physiological insulin needs and lower blood glucose levels (see figure 1).

[031] However, we also observed that at a pH of 2 or less the encapsulation of insulin during the polymerisation reaction results in PACA nanoparticles having irregular, unstable surfaces comprising crystalline material. The physical instability is associated with poor encapsulation efficiency and insufficient insulin loading.

[032] To avoid this, it is essential to incorporate a pharmaceutically acceptable stabiliser into PACA nanoparticles prepared at a pH of 2 or less. For this purpose, dextran is used. Dextran provides the necessary stabilisation resulting in formation of nanoparticles with acceptable morphology. Chitosan and pectin are also used for this purpose with the same good results. However, efficient nanoparticle stabilisation also depends on the alkyl chain length of the alkyl 2-cyanoacrylate monomers forming the PACA.

[033] The biodegradable PACA nanoparticles described herein can be incorporated into oral dosage forms such as capsules or tablets. A coating can be applied to the oral dosage forms for additional protection while passing through the stomach.

[034] The biodegradable PACA nanoparticles and the pharmaceutical oral dosage forms can be used to treat a wide variety of metabolic diseases, such as disorders associated with elevated blood glucose levels. Insulin-loaded PACA nanoparticles can be used to provide insulin to diabetes patients, in particular patients with type 2 diabetes.

[035] The technology described herein is further illustrated by the following examples:

Example 1

[036] Biodegradable PACA nanoparticles comprising insulin were prepared by anionic polymerisation. Ethyl 2-cyanoacrylate (ECA), n-butyl 2-cyanoacrylate (BCA), n-hexyl 2-cyanoacrylate (HCA) and 2-octyl 2-cyanoacrylate (OCA) were used as monomers.

[037] 0.25 g of insulin was dissolved in 5 ml of water having a pH of 5. The aqueous solution was mixed with ethyl oleate and sorbitan monolaurate at a weight ratio 1:4:5. A water-in-oil nanoemulsion was formed by stirring at 700 rpm at 4°C. 0.5 g of the monomer was dissolved in 2 ml of chloroform and slowly added under continuous stirring to the nanoemulsion. The mixture was stirred for 3 hours and left to stand overnight. The nanoparticles were separated by centrifugation, washed with absolute ethanol, and purified by filtration. Nanoparticle surface morphology was monitored by Scanning Electron Microscopy (SEM). Nanoparticles were spherical, with smooth surfaces, and free of crystalline material. Particle size was estimated by dynamic light scattering. 90% of the nanoparticles were in the hydrodynamic diameter range of 120 nm to 280 nm. Insulin loading was determined by methods known in the art.

Table 1

Composition	Monomer	Insulin loading (weight%)
A	ECA	22.1
B	BCA	22.7
C	HCA	21.8
D	OCA	21.5

[038] The results show the formation of insulin-loaded PACA nanoparticles with high insulin loading which was not significantly influenced by the choice of the monomer.

Example 2

[039] Insulin-loaded nanoparticles were prepared using BCA by the method outlined in example 1. Dextran 10 or Dextran 20 (molecular weight 10,000 and 20,000, respectively) was incorporated into the nanoparticles as stabiliser. Dextran in amounts of 0.5% or 1% (relative to the weight of insulin) was added to the aqueous solution of insulin before mixing with the oil and the surfactant.

Table 2

Composition	Dextran	Concentration (weight%)	Insulin loading (weight%)
E	Dextran 10	0.5	22.6
F	Dextran 10	1	23.1
G	Dextran 20	0.5	23.2
H	Dextran 20	1	22.8

[040] No significant difference in insulin loading was observed when either the molecular weight or concentration of dextran was varied.

Example 3

[041] Insulin-loaded PACA nanoparticles were prepared from various monomers by the method outlined in example 1, except that the aqueous solution of insulin was adjusted to pH 2 with hydrochloric acid and optionally supplemented with Dextran 10 as stabiliser. Insulin loading and nanoparticle surface morphology were analysed.

Table 3

Composition	Monomer	Dextran 10 (weight%)	Surface morphology (by SEM)	Insulin loading (weight%)
I	ECA	-	irregular, crystalline	3.9*
J	ECA	0.5	smooth, no cryst. material	17.1
K	BCA	-	irregular, crystalline	4.2*
L	BCA	0.5	smooth, no cryst. material	18.9
M	HCA	-	irregular, crystalline	3.8*
N	HCA	0.5	smooth, no cryst. material	17.6
O	OCA	-	irregular, crystalline	5.6*
P	OCA	0.5	irregular, crystalline	5.4*

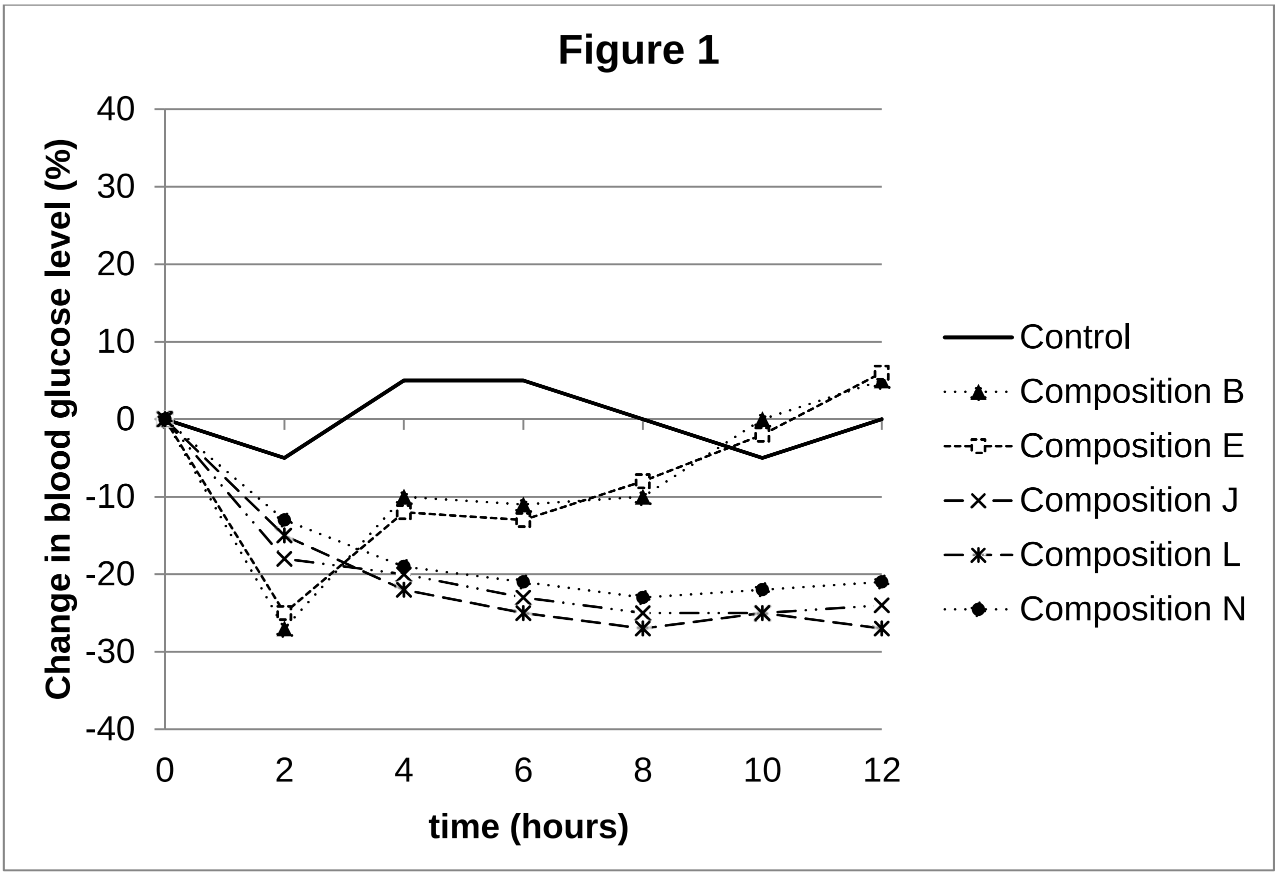
* Insulin loading was difficult to measure due to nanoparticle instability

[042] Nanoparticles with acceptable stability, morphology and insulin loading could not be obtained using OCA, even when higher concentrations of dextran or other stabilisers were used.

Example 4

[043] An *in vivo* study was carried out using Wistar rats. Insulin-loaded PACA nanoparticles were suspended in aqueous saline solution. Rats were given a single dose of PACA nanoparticles (20 mg/kg body weight) by oral administration. The control group was given the saline solution only. Blood samples were taken before administration (reference), and at defined time periods (2, 4, 6, 8, 10 and 12 hours) after administration, and glucose level was determined by commercially available tests. The results are expressed in figure 1 as percentage change in glucose level (%) from reference. They demonstrate that a significant reduction in blood glucose levels can be maintained over a prolonged time period after oral administration of insulin-loaded PACA nanoparticles.

Figure 1: Percentage change in blood glucose level (%) vs. time (hours) after oral administration



Document D1
WO 91/00XXX

POLYMERIC NANOPARTICLES FOR ORAL ADMINISTRATION OF INSULIN

Technical field

[001] This invention relates to polyisobutyl cyanoacrylate nanoparticles as drug carrier for oral administration of insulin.

Background

[002] Since the discovery of insulin by Banting and Best in 1922 attempts have been made to find the best route of administration of insulin in the treatment of diabetes. Oral administration of insulin would be a desirable approach. It requires that insulin is protected from degradation in the stomach.

[003] Polyisobutyl cyanoacrylate, first used as tissue glue in surgery, can be a suitable drug carrier. The rate of biodegradation of polyalkyl cyanoacrylates (PACA) depends on the length of the alkyl chain. Polyisobutyl cyanoacrylate has the great advantage of being both biocompatible and showing an excellent biodegradation rate.

Disclosure of the invention

[004] The object of the invention is to provide polymeric nanoparticles based on polyisobutyl cyanoacrylate which can entrap and transport insulin for intestinal absorption to treat metabolic diseases such as diabetes, specifically type 2 diabetes, by oral administration.

[005] The nanoparticles are prepared by interfacial polymerisation using isobutyl 2-cyanoacrylate. Insulin is added to a lipophilic phase which contains fatty acid triglycerides of medium-chain length and isobutyl 2-cyanoacrylate dissolved in absolute ethanol. The lipophilic phase is then added to an aqueous solution at neutral or slightly acidic pH containing a nonionic surfactant, and the nanoparticles are immediately formed on mechanical stirring. The colloidal suspension obtained can be concentrated by evaporation and purified by filtration.

[006] The nanoparticles of the invention may additionally comprise a further substance or additive entrapped therein, for example a pharmaceutically acceptable stabiliser such as chitosan, dextran or pectin. In that case, the stabiliser is added to the lipophilic phase together with the insulin.

[007] The hydrodynamic diameter of the insulin-loaded nanoparticles of the invention measured by dynamic light scattering is such that 95% of the nanoparticles are within the range of 140 nm to 300 nm.

[008] The average drug loading of the nanoparticles is about 25% by weight of insulin relative to the total weight of the nanoparticles. The high insulin loading achieved is a very exciting result since the nanoparticles protect insulin in the stomach and can allow a therapeutically effective absorption in the small intestine.

Claims

1. Biodegradable polyisobutyl cyanoacrylate nanoparticles comprising insulin entrapped therein.

2. The nanoparticles of claim 1 for use in a method of treatment of a metabolic disease such as diabetes, preferably diabetes type 2.

Document D2

Journal of Controlled Release, March 2015

PEPTIDE-POLYMER INTERACTIONS DURING POLYMERISATION OF POLYALKYL CYANOACRYLATE NANOPARTICLES

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[001] Biodegradable polyalkyl cyanoacrylate (PACA) nanoparticles can be of interest as a drug carrier for controlled release of peptide biopharmaceuticals. High entrapment efficiencies can be achieved by anionic polymerisation of alkyl 2-cyanoacrylate. This polymerisation method requires that the bioactive peptide is present in the polymerisation medium. The peptide can thus disturb the polymerisation reaction. Specifically, formation of strong covalent peptide-polymer bonds may occur due to the possibility of various amino acid residues of the peptide bonding to the alkyl 2-cyanoacrylate and blocking the completion of the polymerisation.

[002] Covalent peptide-polymer bonds may cause large amounts of the peptide entrapped in the nanoparticles to remain unreleased. This undesirable effect can impair the bioactivity and therapeutic effectiveness of PACA nanoparticles. In this paper, we investigate the occurrence of covalent peptide-polymer interactions during formation of polyethyl cyanoacrylate and poly-n-butyl cyanoacrylate nanoparticles with the bioactive peptides angiotensin II, insulin, insulin-like growth factor 1 (IGF-1) and secretin.

Experimental

[003] PACA nanoparticles were prepared by anionic polymerisation. 0.1 g of peptide was dissolved in 2.5 ml water at pH 5. A water-in-oil nanoemulsion was formed by mixing the aqueous phase, ethyl oleate and polyvinyl alcohol (weight ratio 1:3.5:5.5). 0.2 g of ethyl 2-cyanoacrylate (ECA) or n-butyl 2-cyanoacrylate (BCA) dissolved in 1 ml chloroform was slowly added to the nanoemulsion under continuous stirring. The mixture was left to stand overnight to complete polymerisation. Nanoparticles were separated by centrifugation. Hydrodynamic diameter measured by dynamic light scattering was such that 90% of the nanoparticles were in the range 120 nm to 280 nm. The average drug loading (by weight of bioactive peptide relative to the total weight of the nanoparticles) was between 21.7% and 22.5%. Covalent peptide-polymer bonds were characterized by mass spectrometry.

Results and discussion

[004] Three of the four bioactive peptides investigated were found to disturb with the polymerisation process.

Bioactive peptide	Covalent peptide-polymer bond		Site of covalent bonding
	ECA	BCA	
Angiotensin II	yes	yes	Histidine
Insulin	no	no	-
IGF-1	yes	yes	Glutamic acid
Secretin	yes	yes	Histidine

[005] Covalent peptide-polymer bonds were identified for angiotensin II, IGF-1 and secretin. Covalent bonding results from reaction of histidine and glutamic acid of the peptides with ECA and BCA. However, insulin did not disturb the polymerisation reaction at pH 5. The three-dimensional folding of insulin under the polymerisation conditions makes the reactive amino acid groups sterically unavailable for covalent interaction.

[006] In order to study the influence of pH on the occurrence of peptide-polymer covalent interactions, the polymerisation reaction was repeated at pH 1.9. Lowering the pH did not prevent the covalent bonding of angiotensin II, IGF-1 and secretin to ECA and BCA. For these peptides, the reactivity of the amino acid groups appears to be independent from the pH.

[007] For insulin at pH 1.9, however, we observed that formation of distinct, well-shaped PACA nanoparticles with both ECA and BCA was impaired. We could only isolate unstable nanoparticles having irregular shape and unacceptably low insulin loading of about 4%.