Expert Opinion

Nanoparticle strategies for the oral delivery of insulin

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Since its discovery, insulin remains the major treatment for Type 1 diabetes and many Type 2 diabetic patients, with insulin being administered parenterally. The oral route of insulin delivery, being the most comfortable, would also be the most physiologically advantageous in taking advantage of the portal–hepatic route of absorption. However, insulin is less absorbed by the intestinal mucosa and is rapidly degraded enzymatically in the gastrointestinal tract. Polymeric biodegradable and biocompatible nanoparticles have been developed. These nanoparticles protect insulin against degradation and facilitate the uptake of insulin (associated or not associated to the nanoparticles) through a paracellular or a transcellular pathway. In this review, the physicochemical characteristics of polymer composition, *in vitro* release kinetics and the biological effects of insulin-loaded nanoparticles in experimental diabetes and healthy animals are discussed.

Keywords: diabetes, insulin, intestinal uptake, nanoparticles, nanospheres, oral delivery

Expert Opin. Drug Deliv. (2008) 5(1):45-68

1. Introduction

Insulin is a 51 amino acid peptide that was discovered in 1921 – 1922 by Banting and Best, together with Macleod and Collip at the University of Toronto [1]. This natural hormone is produced by the pancreatic β cells and controls the level of sugar in the blood by facilitating the uptake of glucose, especially in the liver, muscle and adipose tissue. Thus, insulin is considered as the essential treatment for diabetes mellitus – a metabolic disorder due to the autoimmune destruction of β cells, leading to a strong decrease in insulin (Type 1 diabetes). Insulin controls the homeostasis of glucose by binding to its receptors on target cells, a process which is disturbed in Type 2 diabetes, leading to a dysfunction of insulin and an insulin resistance. Thus, in Type 2 diabetes, which appears later in life, the pancreas is still able to produce insulin – at least at an early stage of diabetes – but insulin is not fully active. Regardless the type of diabetes, the consequence is an increase in blood glucose level, which is the cause of diabetic complications such as neuropathy, nephropathy, blindness, cardiac failure, stroke and amputations [2].

Diabetes is a general health problem in the world. The global number of diabetes patients has increased from 30 million in 1985 to 194 million in 2003, and is expected to grow to 333 millions by 2025 [3]. Diabetes is generally controlled with the administration of oral medication (Type 2 diabetes) or by the use of insulin injections (Type 1 and more often Type 2 diabetes). The present practice is the use of one or more doses of insulin, injected subcutaneously. It can be considered that during a lifetime (75 years) a Type 1 diabetic patient receives nearly 80,000 injections. Despite advances in devices for the injection of insulin (insulin pens and pumps), several approaches for alternative routes of insulin administration have been developed, such as buccal, nasal, oral, rectal, pulmonary, ocular, transdermal, vaginal and intrauterine [4-6]. However, the oral route seems

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to have been the dream since the discovery of insulin. Indeed, between 1922 and 1980, > 125 reports were published on this subject [7]. The last 20 years have added > 160 new articles. In fact, oral insulin administration seems to be the most convenient and physiologically advantageous. Indeed, insulin absorbed by the intestinal epithelium reaches the liver through the portal vein and can directly inhibit hepatic glucose output [6]. The effect of insulin on the liver is essential in the maintenance of glucose homeostasis. The parenteral administration of insulin never mimics the natural secretion of insulin by the pancreatic islets of Langerhans, as it is first delivered to peripheral tissues [6]. However, the absorption of insulin by the intestinal epithelium is much reduced (< 0.5% under physiological conditions) due to the large size and hydrophilicity of the molecule. In addition, insulin is strongly degraded by proteolytic enzymes in the gastrointestinal tract (GIT) – the same enzymes that degrade dietary peptides and proteins. In order to protect insulin from biodegradation and to improve its intestinal absorption, insulin has been associated to antiproteases [8-11] and hydrogels [12], or combined with absorption enhancers such as cyclodextrins [13], bile salts or surfactants [14,15]. Others have associated insulin with oligoarginine – a cell-penetrating peptide [16] or chemically modified molecule of insulin [17-22]. A more interesting strategy has been to associate insulin with a drug delivery system. Liposomes were the first to be developed (since 1976) [23]. However, the glycemic responses to peroral insulin liposomes were extremely variable [5]. Thus, another strategy was to encapsulate insulin in polymeric nanoparticulate systems that are able to protect insulin from proteolytic enzymes and facilitate its transport through the GIT to its target organs (especially the liver, muscle and fat). Initially developed for parenteral use, in the form of filled polymeric structures, nanoparticles have been shown to improve the efficiency of drugs, and to minimise their unwanted toxic effects. These carriers must be biocompatible, stable in suspension, and should be degraded under physiological conditions.

A number of review articles on insulin formulation for alternative routes of delivery have already been published [4-6,24-33]. Therefore, the aim of this review is to focus on the specific features and applications of polymeric nanoparticulate systems designed to deliver insulin orally, excluding liposomes, microcapsules, beads and chemical modifications of the molecule.

2. The gastrointestinal barrier to oral insulin nanoparticles

2.1 The physical barrier

The GIT is a major organ that possesses a very highly absorptive surface – the small intestine – representing a surface of 250 m^2 in an adult human [6]. Indeed, the initial function of the GIT is to digest and absorb nutrients, water and vitamins from food. However, it is considered as a barrier for the entry of pathogens, toxins and undigested macromolecules. Thus, before reaching the bloodstream, insulin nanoparticles encounter mainly three organs: the stomach, the small intestine (subdivided in three parts: duodenum, jejunum and ileum) and the colon.

The epithelium that lines the small intestine is mainly composed of two types of cells: columnar epithelial cells called enterocytes, which are involved in the absorption of nutrients, and goblets cells that secrete mucus. Finally, a few endocrine cells are dispersed throughout the mucosa. This epithelium also lines the crypts that form the germinal area of the villi involved in the renewal, water, ion, exocrine and endocrine secretions. This epithelium is supported by the lamina propria and muscularis mucosa.

At their apical part, the plasma membrane of enterocytes form numerous folds called microvilli. The resulting brush border increases the absorptive area of the intestine by approximately two orders of magnitude [32]. It is lined by a glycocalix – a sulfated mucopolysaccharide sheet. Together with mucus, a viscous fluid composed of highly glycosylated proteins (mucins) secreted by goblet cells, enzymes, electrolytes and water hinder the absorption of proteins and peptides.

The small intestine has another particularity. Lymphoid nodules, individually or aggregated into Peyer's patches, are dispersed through the intestinal epithelium. They are more abundant in the ileum than in the jejunum. The follicle-associated epithelium is composed of enterocytes, a few goblet cells and M cells. These latter structures attracted the attention of scientists for a long time because they are involved in the immunological responses of the mucosa. M cells are highly specialised cells that differ from their neighbouring enterocytes by the absence of the classical brush border and a thinner mucous gel layer at the apical side. M cells transport macromolecules, particles and organisms from the gut by endocytosis at the apical part of the cell to the basolateral hollow cavity, where particles are released by exocytosis [6,26,28,29,34]. This space contains a few macrophages and lymphocytes that can interact with transported antigens, leading to the first step of a mucosal immune response. Although the number of Peyer's patches varies between species, it is generally accepted that their number decreases with age [34].

The physical integrity of the intestinal epithelium results from the presence of tight junctions between the cells. The junctional complexes (zona occludens, zona adherens and macula adherens) interconnect epithelial cells on their apical side, preventing the uptake of macromolecules and pathogens by a paracellular pathway. Considered recently as a dynamic semipermeable diffusion barrier between the cells, they open under physiological conditions at the apical part of the villi, leading to the desquamation of mature absorptive cells into the intestinal lumen. Indeed, during the renewal of epithelial cells, which occurs for 2 to 4 days, undifferentiated cells from the crypt of Lieberkühn migrate during their differentiation toward the tip of the villus, where they desquamate [34].

Thus, intercellular spaces will be larger and can allow the paracellular uptake of nanoparticles. This process, called 'persoption', allows the uptake of 5 – 150 µm particles and only occurs at the most apical part of the villi [35]. The assembly of tight junctions, rendering the epithelium impermeable to macromolecules, can be influenced by a wide range of signalling pathways. Surfactants and chitosan have been shown to transiently open the intercellular tight junctions, thus increasing the permeability of the epithelium by the activation of protein kinase C-dependent signal transduction pathways which affect tight junction integrity [36].

2.2 The enzymatic barrier

In addition to the physical barrier, insulin nanoparticles have to face another obstacle that is probably more important: the enzymatic barrier. In the stomach they encounter pepsin, and in the duodenum they are in presence of trypsin, chymotrypsin and carboxypeptidases secreted in the intestinal lumen from the pancreas. Trypsin is the most efficient enzyme for the degradation of insulin. However, other enzymes located in the brush border membrane, such as peptidases, also contribute to insulin degradation. In addition, there is also a specific cytosolic enzyme called insulin-degrading enzyme, which completely denaturates insulin inside enterocytes [37].

2.3 The stability of insulin

Insulin, a peptide of ~ 5.8 kDa, is composed of two peptide chains referred to as the A chain and B chain. A and B chains are linked together by two disulfide bonds, and an intramolecular disulfide bond formed within the A chain (**Figure 1**). The isoelectric point of insulin is 5.3 – 5.4 [38]. Although the amino acid sequence of insulin varies among species, certain segments of the molecule are highly conserved, including the positions of the three disulfide bonds, both ends of the A chain and the C-terminal residues of the B chain. These similarities in the amino acid sequence of insulin lead to a three-dimensional conformation of insulin that is very similar among species, and insulin from one animal is very likely biologically active in other species. Insulin molecules have a tendency to form dimers in solution due to hydrogen bonding between the C-terminals of B chains. Additionally, in the presence of zinc ions, insulin dimers associate into hexamers. However, at high concentrations, insulin dimers are able to form hexamers in the absence of zinc ions. These interactions have important clinical ramifications. Monomers and dimers readily diffuse into blood, whereas hexamers diffuse poorly. Hence, absorption of insulin preparations containing a high proportion of hexamers is delayed and somewhat slow. This phenomenon, among others, has stimulated the development of a number of recombinant insulin analogs.

The biological activity of insulin is directly related to its internal structure. Thus, any disorganisation of its structure leads to the inactivation of the protein. Most proteins including insulin are stable at neutral pH. In the GIT, insulin is subjected to great pH variations, passing from an acidic medium in the stomach to the neutral pH in the duodenum. Thus, the degradation of insulin begins in the stomach by pepsin and continues in the duodenum by the combined action of pancreatic enzymes (trypsin, chymotrypsin, elastase and carboxypeptidases). Finally, insulin is degraded by peptidases present in the brush border membrane and insulin degrading enzyme present in the cytoplasm of absorptive cells [37]. This enzyme should be able to degrade 92% of insulin present in the cytoplasm.

3. Nanoparticle formulation and physicochemical properties

Nanoparticles are solid colloidal drug carriers ranging from 10 to 1000 nm in size. They are composed of natural, semisynthetic or synthetic polymers that may or may not be biodegradable [39]. Nanoparticles are a collective name for nanospheres and nanocapsules (illustrated in **Figure 2**). Nanocapsules form a vesicular structure with an inner liquid core surrounded by a polymeric wall. Nanospheres are full polymeric structures. Insulin may be adsorbed at the surface of the polymer or entrapped in the polymer (**Figure 2**). However, in the case of nanocapsules, insulin is usually inside the inner core of the nanoparticulate system [40].

3.1 Polymers used in nanoparticle manufacturing

Since the pioneering work by Damgé *et al.* in 1988 [41], who reported a sustained reduction of glycemia that lasted from 1 – 3 weeks in diabetic rats treated orally with insulin nanocapsules, a number of researchers have developed nanoparticle-based oral delivery systems for insulin. The main properties of these systems are the biocompatibility, biodegradability and ability to prolong drug release behaviour. The nature of the polymers used influences the size of the nanoparticles and the profile of insulin release (**Table 1**). A number of techniques have been described to produce nanoparticles [28,39,42,43]. However, the choice of the method to prepare insulin nanoparticles depends on both the polymer and the solubility characteristics of insulin associated with nanoparticles. In addition, insulin stability during nanoencapsulation and subsequent release is essential for retaining biological activity. In particular, insulin is a peptide very sensitive to thermal and shear stress [44]. Thus, the method to prepare insulin nanoparticles must be adapted.

The polymeric materials used to prepare insulin nanoparticles include synthetic polymers such as poly(alkylcyanoacrylate) [41,45-51], poly(methacrylate) and acrylic acid based copolymers [52-56]. Polyesters alone and in combination with other polymers have also been used: poly(lactic acids) (PLA) [57,58], poly(lactic-co-glycolic acids) (PLGA) [59-63], poly(ε-caprolactone) (PCL) [64,65].

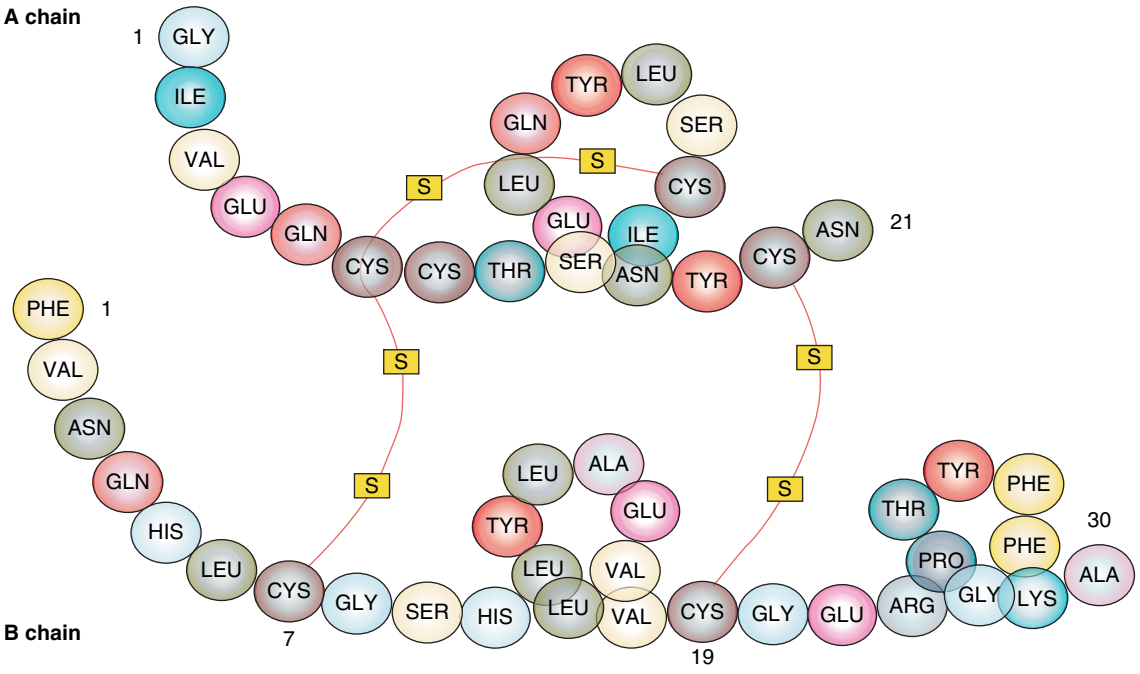

Figure 1. An insulin molecule formed by two chains (A and B) linked with disulfide bonds.

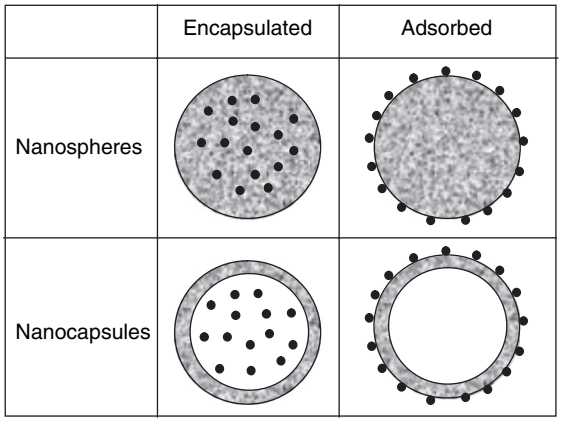

Figure 2. A schematic representation of insulin nanoparticles.
Insulin visualised by black dots can be encapsulated or adsorbed at the surface of the nanoparticle.

More recently, nanoparticulate systems composed of natural polymers have been developed. These include particles based on chitosan [25,27,55,66-76] or alginate [77] or alginate/chitosan [78,79] or alginate/chitosan/poly(ethylene glycol) (PEG) [80]. Solid lipid insulin nanoparticles [81-84], insulin–phospholipids nanoparticles [22], vitamin B_{12}–nanosphere conjugate systems [85], calcium pectinate nanoparticles [86] and calcium phosphate–PEG–insulin–casein (CAPIC) particles [87] have also been developed.

3.2 Formulation principles

Many methods have been developed in order to prepare insulin nanoparticles (**Table 1**); these methods can be classified into two main categories according to whether the formulation requires a polymerisation reaction [40,41,45-51,88-93] or is achieved directly from a macromolecule or preformed polymer [59,61-63,66,70-72,75,77,78,86,94-96].

The method of choice depends on the goals, such as a high encapsulation efficiency, a small resultant particle size, the safety of the compound, a low shear stress, the integrity of insulin, a high oral bioavailability, an easiness of scale-up and fewer time-consuming techniques.

In fact, a polymerisation reaction through an emulsion procedure is one of the fastest methods for preparing nanoparticles, and is readily scalable [43]. The method is classified into two categories, based on the use of an organic phase or, more commonly, the use of an aqueous continuous phase. The polymerisation process can be initiated by different mechanisms. Poly(alkylcyanoacrylate) insulin-loaded nanocapsules have been prepared by interfacial polymerisation [40,41,45,49,50,88-91,97]. An advantage of this technique is high-efficiency of drug encapsulation, but the main disadvantage is the use of organic solvents required for the external phase. With few exceptions, most of the monomers suitable for a polymerisation process in an aqueous phase lead to slowly biodegradable or non-biodegradable polymers [43]. In addition, residual molecules in the polymerisation medium (monomer, oligomer, surfactant) can be toxic, requiring meticulous purification of the

Table 1. Physico-chemical characteristics of insulin nanoparticles.

Particulate system	Method of preparation	Size (nm)	Zeta potential (mV)	Incorporation efficiency (%)	Cumulative release (%)	Ref.
PIBCA	Emulsion polymerization	< 500	n.d.	65 – 95	n.d.	[86]
PECA	Emulsion polymerization	< 500	n.d.	87	n.d.	[104]
PIBCA	Emulsion polymerization	245	n.d.	72	An initial burst release from the surface (20%) followed by a slower diffusional release	[87]
PIBCA	Anionic polymerization	85 – 182	n.d.	53 – 59%	n.d.	[48]
Polymethacrylic acid–chitosan–polyether (polyethylene glycol–polypropylene glycol copolymer)	Free radical polymerization	500 – 800	n.d.	> 85	Less than 20% insulin released at pH 1.2 after 150 min and complete insulin release after 350 min at pH 7.4	[52]
Chitosan and methacrylates derivatives	Free radical polymerization	150 – 280	+17.2 to +29.4	19.6 – 100%	Initial burst release followed by a slowly sustained release for more than 24 h	[66]
PLGA	Nanoprecipitation	104.7 – 169.5	n.d.	6.8 – 12.1	n.d.	[56]
SLN	Emulsion/solvent evaporation	50.2 – 64.5	-13 to -46.27	17.8 – 67.9	n.d.	[77]
WGA–modified SLN	Emulsion/solvent evaporation	75.3	-13.11	40.2	n.d.	[77]
PLGA	Emulsion/solvent evaporation	216.2 – 1145.8	n.d.	8.5 – 74.8	Variable depending formulation	[59]
PLGA–insulin phospholipid complex	Reverse micelle–solvent evaporation	200	-12.6 to -22.6	> 90	Initial burst and subsequent delayed release in both pH 6.8 and pH 1.2 dissolution mediums	[20]
Poly-ε-caprolactone and Eudragit RS	Emulsion/solvent evaporation	358	+41.8	96	n.d.	[62]
PLGA and PLGA modified	Emulsion/solvent diffusion	150 – 169	n.d.	n.d.	Insulin release at pH 1.2 around 19.7 to 50.5% and then 65.6 and 61.6%	[60]
Poly(L-malic acid) and chitosan	Salting-out	100 – 250		53 – 81	Rapid release of insulin followed by a slowly declining release rate at 8 h	[65]
Polyethylenimine dextran	Polyelectrolytes complexation	250	Around +30	80 – 90	Burst release within 5 min around 65 – 100%	[89]
Chitosan/TPP	Ionotropic gelation	250 – 400	+27.3 to +40.71	> 80	Initial burst release from the surface (20%) followed by a slower dissociation mechanism	[71]

PECA: Poly(ethylcyanoacrylate); PEG: Polyethylene glycol; PIBCA: Poly(isobutylcyanoacrylate); SLN: Solid lipid nanoparticles; TPP: Tripolyphosphate; WGA: Wheat germ agglutinin-*N*-glutaryl phosphatidylethanolmamine.

Table 1. Physico-chemical characteristics of insulin nanoparticles (continued).

Particulate system	Method of preparation	Size (nm)	Zeta potential (mV)	Incorporation efficiency (%)	Cumulative release (%)	Ref.
Pectinate	Ionotropic gelation	< 1000	Large interval of negative zeta potential	32.8 – 93.3	Up to 75% of the associated insulin was released into the simulated gastric fluid over a 4-h period whereas 50% of the associated insulin was released into the intestinal fluid within the first hour, with insignificant amounts of insulin released over the next 5 h	[80]
Chitosan/TPP	Ionotropic gelation	237 – 253	n.d.	2 – 85	F5.3np showed a burst effect within the first 30 min, followed by little release (20%) while F6.1np showed a burst effect within the first 30 min (63.4 – 95% depending pH)	[63]
Chitosan/dextran sulfate	Complexation	500	-15	> 70%	pH dependent release of insulin in simulated gastric and intestinal fluids	[70]
Alginate and chitosan	Ionotropic pre-gelation	748	-5.6	72.8	At gastric pH, around 40% of the insulin was immediately released while an additional 20% was released when pH was 6.8	[74]
Poly(γ-glutamic acid) and chitosan	Ionic gelation	110 – 150	-23.7 to +36.8	56.8	20% within the first hour. At pH 6.6, there was only a minimal amount of insulin released (10%). In contrast, at pH 7.4, fairly fast release of insulin	[90]
Chitosan and Eudragit	Complex coacervation	200	-30	> 70%	Less than 10% of FITC-insulin released from the nanoparticles in simulated gastric fluid after 6 h, but in the simulated intestinal fluid, almost 90% of FITC-insulin was released	[93]
PLA–PEG	Supercritical CO_2	400 – 600	n.d.	> 90	< 3% of the loaded insulin was released in 80 h	[54]
PLA–PEG	Supercritical CO_2	< 1000	n.d.	65 – 95	Different insulin release profiles with formulations containing different amounts of PEG	[94]
Gold	Adsorption	< 50	n.d.	86 – 95%	n.d.	[95]

PECA: Poly(ethylcyanoacrylate); PEG: Polyethylene glycol; PIBCA: Poly(isobutylcyanoacrylate); SLN: Solid lipid nanoparticles; TPP: Tripolyphosphate; WGA: Wheat germ agglutinin-*N*-glutaryl phosphatidylethanolmamine.

colloidal material. To avoid these limitations, methods using preformed polymers instead of monomers have been proposed [43].

Generally, nanoparticles composed of synthetic polymers are prepared by dispersion of preformed polymers [29]. There are several techniques to produce insulin nanoparticles from preformed polymers, such as nanoprecipitation, emulsification/solvent evaporation, emulsification/solvent diffusion, salting-out, desolvation, complexation, emulsification/polymer gelation and so on (**Table 1**).

The nanoprecipitation method has been employed in one study to encapsulate insulin in PLGA nanoparticles [59]. This technique involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant. The obtained PLGA particles were < 200 nm in size, but the encapsulation efficiency was low [59].

The emulsification/solvent evaporation technique has also been applied to produce insulin nanoparticles based on polyesters (PLGA and poly-ε-caprolactone) [22,61-65]. Emulsification/solvent evaporation involves two steps: the first step requires emulsification of the polymer solution into an aqueous phase. A polymer organic solution containing the dissolved drug is dispersed into nanodroplets, using a dispersing agent and high-energy homogenisation, in a non-solvent or suspension medium. Then polymer precipitates in the form of nanospheres in which the drug is finely dispersed in the polymer matrix network. This technique, producing small nanoparticles, has also been applied to produce solid lipid nanoparticles (SLN) with insulin [81,82].

The emulsion/solvent diffusion technique involves forming an oil-in-water emulsion with a partially water-soluble solvent, containing polymers and an aqueous solution containing a stabiliser. The addition of a large volume of water causes the diffusion of the partially water-soluble solvent into the external phase, and then the formation of nanoparticles [29]. In particular, the emulsion solvent diffusion method is widely used because of its relative simplicity. However, this conventional method sometimes results in lower encapsulation efficiency for water-soluble agents, and the initial burst (early rapid release). Insulin-loaded PLGA with and without hypromellose phthalate nanoparticles have been produced by this technique [63].

Another technique to produce insulin nanoparticles is the salting-out technique. This is based on the separation of a water-miscible solvent from aqueous solution via a salting-out effect. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride and magnesium acetate, or non-electrolytes such as sucrose) and a colloidal stabiliser. This oil-in-water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres [43]. Insulin nanoaggregates have been prepared by this technique and then encapsulated with two oppositely charged polyelectrolytes: poly(L-malic acid) and chitosan [68].

Another technique used to encapsulate insulin into polymeric nanoparticles is based on desolvation process. This technique was the first technique applied to produce insulin-loaded nanospheres. Nanoparticles of ~ 200 nm have been prepared from a neutral insulin solution by desolvation of the peptide, followed by crosslinking with glutaraldehyde. Typically, the soluble insulin was first desolvated by hydrochloric acid and then slightly resolvated by sodium hydroxide [98].

However, the most recent advances in nanotechnology applied to insulin are towards safer, simpler and easier scale-up methods, using naturally occurring polymers such as alginate and chitosan. Insulin-loaded alginate–dextran nanospheres have been prepared by emulsion dispersion followed by triggered polymer in situ gelation [77]. The alginate/crosslinking agent mixture containing encapsulant is dispersed into an oil phase (vegetable or mineral oil) under agitation. Once the emulsion is formed, gelation is initiated by pH adjustment with an oil-soluble acid. Following polymer gelation, the resulting emulsion is mixed gently and nanospheres are partitioned from the oil dispersion into an aqueous phase. This is followed by the application of a high centrifugal force and washing to remove residual oil.

There are other ongoing investigations to improve the oral bioavailability of insulin using chitosan [25,55,66,74,75,94,96]. Generally, nanospheres are prepared by ionotropic gelation of chitosan with tripolyphosphate anions [25,75]. Other techniques have been applied to produce chitosan nanoparticles, such as free radical polymerisation [69], simple ionic gelation [96] and complexation [70,71].

Apart from alginate and chitosan, other natural materials can be used as matrix encapsulation polymers, such as pectin. Insulin has also been encapsulated in pectin nanoparticles by a ionotropic gelation technique with calcium ions [86].

Other techniques have been applied to produce nanoparticles, such as complex coacervation [99], supercritical techniques [57,100] or simple adsorption of the drug to recently formed nanoparticles [101].

The size of nanoparticles prepared with these techniques, the zeta potential and the incorporation efficiency of insulin are represented in **Table 1**.

3.3 Formulation parameters influencing nanoparticle properties

Many different types of nanoparticulates have been investigated for oral insulin delivery, as previously described. In these studies, several properties have been identified as important for the uptake of nanoparticles, including the size, surface charge and surface hydrophobicity [102]. The size of particles is considered to be a major parameter in

determining the efficiency of particulate drug delivery systems [102]. In general, smaller nanoparticles are absorbed to a greater degree than larger particles, as particle uptake by intestinal cells is size dependent [29,102]. Smaller particles are distributed more easily to distant sites, and remain detectable for longer periods of time [102]. Key parameters modulating nanoparticle size during the formulation process have been extensively studied, such as the effect of processing parameters on the size of insulin-loaded poly(isobutylcyanoacrylate) nanoparticles, the stirring rate of the organic phase, the flow rate of injection of the organic phase into the aqueous phase, the order of addition of insulin to the organic phase, the type of insulin, the pH, and the insulin monomer source [47,50]. The effect of the type of encapsulation method, the volume of the internal phase and insulin loading on particle size were investigated in insulin-loaded PLGA nanoparticles [62]. Other parameters have been studied in different nanoparticulate systems, such as polymer ratio [22,47,57,64,96,100], drying process [95], range of pH [64,66,94], composition and pH of the buffer [59], polymer molecular weight [100], surfactant concentration [51,75], filtration cycles [66], chemical characteristics of the polymer [86], crosslinking agent concentration [74], presence of coating polymer [63] and oil–water phase ratio. All factors were shown to have some influence on nanoparticle size.

Apart from particle size, nanoparticle surface properties seem to influence the uptake by intestinal epithelia [26]. The surface of nanoparticles can be modified either by coating or by grafting a molecule that changes nanoparticle surface characteristics. Nanoparticle zeta potential plays an important role in the interactions with intestinal mucosa. In fact, the mucin network along the GIT contains sialic acid and L-fucose residues. As the pK_a of sialic acid is 2.6, it is completely ionised at a physiological pH of 7.4 [64]. Together with the presence of sulfate residues, the mucin network is known to carry a considerable negative charge at physiological pH. Thus, positively charged nanoparticles are likely to interact with mucin glycoprotein and facilitate the intestinal absorption of insulin, but the affinity of charged colloidal carriers to intestinal tissues is the subject of much discussion [26]. In general, nanoparticles of chitosan have demonstrated a positive surface charge [66,69,75,96], but pectin [86] or poly(isobutylcyanoacrylate) or poly-ε-caprolactone nanoparticles have a negative surface charge [64]. Zeta potential can be modulated by the incorporation of additional material, and is not only involved in the mucoadhesive interactions of nanoparticles with intestinal mucosa, but it also may influence particle stability and the occurrence of agglomerates.

Additionally, the uptake of nanoparticles prepared from hydrophobic polymers seems to be higher than from particles with more hydrophilic surfaces [26]. Hydrophobicity is greatly influenced by polymer composition [22].

Finally, the integrity of encapsulated insulin has been verified [78,95], which obviously has a crucial impact on its bioavailability and pharmacological efficiency [77]. Parameters improving the encapsulation efficiency of insulin into polymeric nanoparticles have been investigated, such as the pH of the aqueous-phase insulin solution [50,86], the origin of the insulin monomer [50], insulin concentration [62], volume of the aqueous phase [64], surfactant concentration [75], polymer molecular weight [57,100] and pH of the buffer [59,94].

3.4 Modification of the nanoparticle surface to improve transport across the intestinal mucosa

It is fully accepted that particle surface properties are of outmost importance for their uptake by intestinal epithelial cells [26,29]. Consequently, many strategies have been developed to improve the intestinal absorption of insulin-loaded nanoparticles, either by the modification of nanoparticle surface properties, or by coupling a targeting molecule at their surface.

The modification of nanoparticle surface properties can be achieved either by a coating process with a hydrophilic stabilising agent, incorporating stabilising agents in the core matrix, using bioadhesive polymers or surfactants or by incorporating biodegradable copolymers containing an hydrophilic moiety in the formulation. These modifications mainly change zeta potential and nanoparticles hydrophobicity. Thus, they influence the colloidal stability of the formulation, mucoadhesion properties and protein adsorption at their surface, and consequently the oral absorption of the insulin-loaded nanoparticles.

PEG has been employed as a coating/matrix material in insulin nanoparticulate delivery for its stabilising properties [55,57,74,100]. It has been reported that PEG chains form a steric barrier at the surface of nanoparticles, which stabilises the complex [6] and prevents opsonisation or interactions with macrophages. Due to these interesting properties, PEGylated insulin nanoparticulate formulations have been developed, as this process is known to improve protein stability towards proteases, reduce immunogenicity and antigenicity and prolong the presence of protein in the blood [25,100].

Due to its mucoadhesive properties, chitosan has been one of the most employed polymers to coat nanoparticles [73,96] or as core material [55,66,75,94]. Others have also been applied as mucoadhesive polymers, such as Eudragit [65] or poly(methacrylic acid) [55,56].

Targeting strategies to improve the interaction of nanoparticles with absorptive cells and M cells of Peyer's patches can be classified into those using specific binding to ligands or receptors, and those based on nonspecific/adsorptive interactions, such as size, surface charge and hydrophobicity.

The first strategy consists of grafting a ligand at the nanoparticle surface to specifically target receptors expressed on enterocytes or M cells. Certain glycoproteins (lectins) selectively bind to this type of surface structure by specific, receptor-mediated mechanisms. Lectins have been chosen

for their relatively good resistance to acidic pH and enzymatic degradation, and due to the presence of binding sites in the GIT [6]. They are involved in many cell recognition and adhesion processes that significantly increase the transport of nanoparticles across the intestinal mucosa, by efficiently increasing interactions with mucus and/or the surface of epithelial cells and by promoting particle translocation [29,81,82]. An example of the application of lectin in the oral insulin delivery field is that of SLNs with wheat germ agglutinin-*N*-glutaryl-phosphatidylethanolamine. This modification led to an increase in insulin oral bioavailability [82]. Vitamin B-12 absorption from the gut under physiological conditions occurs via receptor-mediated endocytosis in the ileum. The ability to increase the oral availability of insulin has been observed by the covalent coupling of vitamin B-12 to dextran nanoparticles [85]. Another type of targeting molecule that has been tested to improve insulin oral bioavailability is the transferrin molecule [6]. Transferrin is a single-chain protein with a molecular weight of a ~ 80 kDa. Serum transferrin is involved in the uptake of iron by the cells and tissues. This molecule was first tested in *in vitro* studies and then in diabetic rats. Insulin–transferrin conjugates, when administered orally to diabetic rats, have been shown to cause slow, but prolonged hypoglycemic effect [17].

3.5 The *in vitro* release of insulin

The nanoencapsulation process can improve insulin intestinal absorption by protecting insulin from the harmful gastric environment, by preventing enzymatic attack and by delivering insulin in the small intestinal region, where it has a chance to act. However, some nanoparticulate systems lead to rapid insulin release from polymeric matrix and, thus, insulin is susceptible to enzyme attack before the peptide drug reaches the target action site. The key for success, in terms of insulin-controlled release, will be an absence of insulin release in the stomach, followed by controlled release during its intestinal passage. **Table 1** summarises the *in vitro* analysed liberation profiles of insulin from nanoparticles in simulated gastrointestinal fluids. Most nanoparticulate systems lead to an initial burst of insulin release in the stomach (10 – 20%) within the first few hours [22,55,56,63,96], followed by a fast and generally complete insulin release when nanoparticles reach a high pH region in the intestine. High percentages of insulin release in gastric pH have also been obtained [22,86]. An exception to this insulin release profile has been observed with chitosan–dextran and alginate–dextran nanospheres, with the clear absence of insulin release during its gastric passage and rapid and complete release when nanospheres were transferred to intestinal fluid [71,77]. Cheng and Lim [86] prepared calcium pectinate nanoparticles and observed that up to 75% of the associated insulin was released from those nanoparticles into the simulated gastric fluid over a 4-h period. There was an initial burst effect in the first hour, followed by a low level of insulin release thereafter. Similarly, ~ 50% of the associated insulin was released into the intestinal fluid within the first hour, with insignificant amounts of insulin released over the following 5 h [86]. However, there are a few studies where there is a lack of information on the release profile of insulin, as only one pH was studied and, thus, gastrointestinal passage cannot be simulated [69,95]. In addition, there are some studies that have investigated a range of pH values [75]. With regards to the release mechanism, some studies have claimed a constant insulin diffusion mechanism from the nanoparticle matrix [69,100]. Others have described an initial burst release of insulin from the surfaces of the particles, followed by a slow diffusion process from the swollen polymer matrices [54]. Furthermore, some studies have shown different mechanisms, such as dissociation [75] or an initial burst release of insulin located at the particles' surfaces, followed by a slow erosion process of the polymer matrices [93]. In fact, it is obvious that the polymer composition influences insulin release. For example, the release profile of polypeptides or proteins from a lipophilic polymer such as PLGA is divided in three phases [62]. In the first phase, protein located next to the surface of the polymeric matrix is released, and during second phase the molecules diffuse through a network of newly generated water-filled pores, and finally protein diffusion from PLGA nanoparticles is modulated by the degradation of the polymer. Moreover, the nanoparticle preparation method may also influence the insulin release profile. Insulin-loaded nanoparticles prepared by a double emulsion method have shown only one release phase, which was attributed to particle agglomeration. Several strategies have been applied to modulate insulin release, such as the addition of a surfactant [62], a pH modulating agent [62], different types of polymers such as Eudragit® (Röhm Pharma GmbH) [54], hypromellose phthalate [63] or PEG [57].

4. The mechanism of absorption enhancement of insulin by nanoparticles

As already reported above [41], insulin associated to polymeric nanoparticles remains biologically active after oral administration in diabetic or healthy animals. In contrast, free insulin has no significant effect. Thus, the question has been of how do these particles act? Do they protect insulin in the acidic medium of the stomach, do they protect insulin against proteolytic enzymes in the small intestine, or do they facilitate insulin uptake by the intestinal mucosa and by which mechanism?

4.1 The stabilisation of insulin against proteolytic enzymes

The protection of insulin against proteolytic enzymes was first investigated *in vitro* by the incubation of insulin-loaded poly(alkylcyanoacrylate) nanocapsules or nanospheres in the presence of gastric (pepsin) and pancreatic enzymes (trypsin, chymotrypsin, pancreatin) [45,47,90]. It was demonstrated that

nanoparticles preserved at least 75% of insulin, but free insulin in solution was largely degraded. However, in the presence of pancreatin and bile extract, the protection was lower [90]. Using vitamin B_{12}–dextran nanoparticle conjugates, a 65 – 83% protection of insulin against proteolytic enzymes was reported, and the protective ability was ranked in the order pepsin > trypsin > chymotrypsin [85]. A protective effect of insulin against trypsin and pepsin was also noted for lectin-modified SLNs, but this effect was greater when these nanoparticles were modified with wheat germ agglutinin-*N*-glutaryl-phosphatidylethanolamine [82]. However, lower inhibitory effects against trypsin were observed with polymethacrylic acid–chitosan–PEG nanoparticles and β-cyclodextrin–insulin complex encapsulated in polymethacrylic acid–chitosan–polyether (PEG–polypropylene glycol copolymer [PMCP]) nanoparticles [55]. Chitosan–cyclodextrin nanoparticles also preserved the stability of insulin in simulated intestinal fluid [72]. However, PEG stabilises the molecule of insulin, thus increasing the plasma half-life and resistance to proteolytic degradation [38].

4.2 Mucoadhesion of insulin-loaded nanoparticles

The mucoadhesive properties of nanoparticulate polymers has been employed to improve the bioavailability of the peptide [55,65,66,71,75,79,80,99]. The adhesive ability is an important factor in prolonging retention in the GIT and promoting penetration into the mucus layer. Mucoadhesiveness can be explained by the interaction of the electropositive nanoparticles and the electronegative mucus layer, which covers the intestinal epithelium. Indeed, sialic acid residues of mucus are negatively charged at physiological pH. This may increase the residence time of insulin nanoparticles next to the absorption surface of the GIT and create a drug gradient concentration towards the blood. This has been confirmed macroscopically and histologically after the labelling of insulin with fluorescein isothiocyanate (FITC). Indeed, as previously reported [65], electro-positively charged insulin nanoparticles composed of poly(ε-caprolactone)/Eudragit RS strongly adhered to the luminal part of the intestinal epithelium after administration in an *in situ* isolated intestinal loop in the rat (**Figure 3D**). These nanoparticles also reduced glycemia for a prolonged period of time. Mucoadhesiveness has also been described for poly(alkylcyanoacrylate) nanocapsules [45,97,103]. Observed by a scanning (SEM) or a transmission electron microscope, these nanoparticles were found in close contact with the mucus layer over the brush border membranes. Other polymers, such as chitosan, also play a mucoadhesive role, due to their electropositive charges [71,104]. Sajeesh and Sharma [55] showed that > 84% of nanoparticles composed of PMCP were retained on the mucosal surface of an *ex vivo* rat small intestinal segment flushed with saline to remove luminal contents. Finally, using insulin-loaded alginate–dextran nanoparticles shelled with chitosan-albumin-PEG, the present authors have also observed a strong adherence of FITC-insulin-labelled nanoparticles after oral administration to diabetic rats [80].

Thus, due to the mucoadhesive properties of nanoparticulate polymers, nanoparticles remain in close contact with the apical membrane of epithelial cells. They will then either be absorbed by a paracellular or transcellular route. Another possibility is the release of insulin in the vicinity of the apical cell membrane followed by its local absorption.

4.3 Paracellular transport

Paracellular transport is possible only after opening the tight junctions that maintain the integrity of the intestinal epithelium. This transport more often occurs at the tip of the villi, where absorptive cell desquamation permanently occurs [45,103]. Indeed, poly(isobutylcyanoacrylate) nanocapsules have been observed in the intercellular spaces between absorptive cells (**Figure 3B**) [45,103]. They were also recovered in the lumen of capillaries, being in close contact with endothelial cells and red cells [45,103]. These SEM observations [45,103] were confirmed later by transmission electron microscopy after labelling insulin with colloidal gold particles [97]. All these results confirm the paracellular uptake of nanocapsules of < 150 nm in diameter through the intestinal mucosa.

The paracellular uptake of insulin nanoparticles can be improved by cationic polymers such as chitosan or poly(acrylate), which reversibly open the tight junctions between enterocytes, allowing the transport of macromolecules [30,104]. Chitosan is thought to act via the electrostatic interaction between positively charged chitosan in solution and the negatively charged components of cell membranes. It has been shown that chitosan decreases the trans-epithelial electrical resistance between cells of a Caco-2 monolayer (derived from a colonic adenocarcinoma) – a well accepted *in vitro* model that mimics the epithelial barrier [104]. Chitosan acts through tight junction disruption, as indicated by the translocation of the tight junction proteins ZO-1 and occludin from the plasma membrane [36]. Finally, it has been demonstrated that chitosan activates protein kinase C-dependent signal transduction pathways, which affect tight junction integrity [36]. The opening of tight junctions results in the enhancement of absorption of insulin and a reduction in blood glucose levels after the oral administration of insulin-loaded chitosan nanoparticles in the rat [66]. Ma *et al.* [66] conjugated FITC to chitosan and confirmed the presence of the labelling agent at different stages of intestinal villi 3 h after oral administration to diabetic rats. The present authors' work has also reported marked labelling on the epithelium of intestinal villi, after administration in an *in situ* isolated intestinal loop in the rat of FITC-labelled insulin-loaded alginate–dextran nanoparticles encapsulated with albumin–chitosan–PEG [80]. It is interesting to note that the surface charge of nanoparticles is an important factor for the disruption of tight junctions characterised

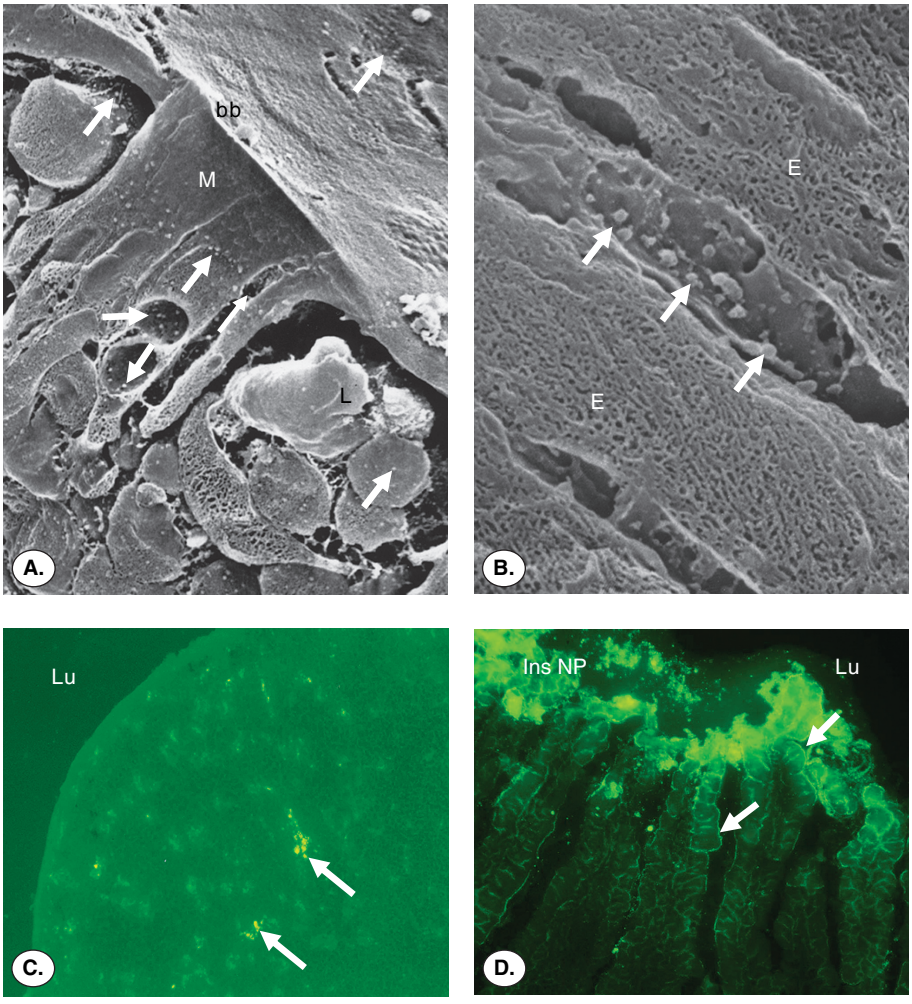

Figure 3. The transport of nanoparticles across the intestinal mucosa. Nanoparticles in the follicle associated epithelium (**A**, **C**) and in villi of a non-follicular epithelium (**B, D**). **A** and **B**: scanning electron microscopy of polyalkylcyanoacrylate nanocapsules indicated by arrows in intercellular spaces between enterocytes (E) (**B**), at the luminal side of the apical membrane of M cells (M), in the cytoplasm of M cells and in contact with lymphoid cells (L) situated in interdigitated spaces under M cells (**A**). Nanocapsules were identified by their content in iodine analysed by X-ray analysis. **C** and **D**: FITC-labelled insulin-loaded poly(ε-polycaprolactone)/Eudragit RS nanospheres in the dome of a Peyer's patch (arrows, **C**) and in the non-follicular intestinal epithelium (**D**). Note the marked labelling of insulin-labelled nanospheres in the intestinal lumen, in close contact with brush border membranes (arrows, **D**) and in intercellular spaces (**D**). Magnifications: **A**: ×2500; **B**: ×10,000; **C**: ×800; **D**: ×200.

Figure 3C : Reprinted from Damgé C, Maincent P, Ubrich N. Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. J Control Rel 2007;117(2):163-70, with permission from Elsevier.

bb: Brush border membrane; Lu: Intestinal lumen.

in Caco-2 cells by trans-epithelial electrical resistance reduction. Indeed, positively charged nanoparticles encapsulated with chitosan have been found to transiently open tight junctions, but negatively charged nanoparticles of poly(γ-glutamic acid) had no effect [96].

4.4 Transcellular transport

As previously reported [65,97], insulin-loaded nanoparticles should be degraded in the intestinal lumen in order to release their insulin. However, this degradation depends on the polymer used. A protective role of the mucus against poly(alkylcyanoacrylate) degradation has been reported *in vitro*, resulting in the presence of insulin-loaded nanocapsules in the vicinity of brush border membranes from enterocytes located at the apical part of the villi [97]. In contrast, nanocapsules appeared to be degraded in the lumen of the crypts, where mucus was reduced and isolated gold labelled insulin colloidal particles could be released [97]. It is quite possible that other polymers behave differently when in contact with the mucus layer. Due to their muco-adhesive properties, poly(ε-caprolactone)/Eudragit RS or alginate-chitosan-based nanoparticles should achieve close

contact with the brush border membrane of enterocytes (**Figure 3D**), releasing insulin at their proximity [65,79,80]. Thus, as reported by Bendayan *et al.* [9,10], insulin should be transported within the cytoplasm of enterocytes by transcytosis. Indeed, in the presence of antiproteases (aprotinin) and a surfactant agent (sodium cholate), gold-labelled insulin particles detected in the lumen of the intestinal tract, were absorbed through the endosomal compartment of epithelial cells rather than passing between cells. Internalisation occurred through invaginations of the luminal plasma membrane and vesicular structures of the endosomal compartment. In 5 – 10 min, insulin was transferred to the basolateral membrane and released into the interstitial space to reach the circulation. Insulin receptors present along the intestinal epithelium [105,106] should play a role in insulin uptake via receptor-mediated transport (RME) [107], and allow the transport of 60- to 500-nm particles [26,106]. RME could explain the presence of insulin-labelled gold particles in enterocytes after oral administration of insulin-loaded poly(isobutylcyanoacrylate) nanocapsules in the rat [97]. However, insulin should also be degraded inside the cytoplasm of absorptive cells by the specific insulin-degrading enzyme [37]. As a consequence, the exact amount of insulin arriving in the blood compartment through transcytosis remains unknown. Nevertheless, Bendayan *et al.* [9] found a significant reduction of blood glucose after the introduction of insulin (associated to antiproteases and surfactants) in the intestinal lumen of normal and diabetic rats.

A more classical insulin nanoparticle uptake pathway concerns translocation through M cells of the follicle-associated epithelium. It has been known for a long time that these cells are able to take up small and large particles, up to 15 μm [35]. However, small particles were taken up to a greater extent than larger particles. This uptake can be nonspecific or specific, using the properties of the polymers to bind to specific transporters such as lectins or transferrins, as described above. In addition, nanoparticles characterised by SEM were found in the cytoplasm of M cells, then in the intercellular spaces containing phagocytic cells, including lymphocytes and macrophages (**Figure 3A**) [48]. However, according to Alphandary *et al.* [97], poly(isobutylcyanoacrylate) nanocapsules should be degraded because free insulin-labelled gold particles were found under the M cells, in the intercellular pockets, and also in the underlining capillaries. Thus, there is a possibility that free insulin and insulin-loaded particulates can escape the lymphatics and eventually reach the bloodstream.

More recently, using insulin labelled with FITC, Sarmento *et al.* [79] with alginate-chitosan nanoparticles and Damgé *et al.* [65] with poly(ε-caprolactone)/Eudragit RS nanoparticles, found marked labelling in the dome of Peyer's patches after the oral administration of insulin nanoparticles, as illustrated in **Figure 3C**. However, this labelling was the most intense when alginate–chitosan insulin-loaded nanospheres were coated with albumin and PEG [80]. Thus, it seems that the presence of PEG on the surface of nanoparticles should increase their uptake by Peyer's patches.

Finally, insulin-loaded nanoparticles or free insulin have been shown to penetrate into blood capillaries through the fenestrations of their basement membrane, which generally have diameters from 500 nm to 5 μm [108]. Indeed, gold-labelled nanoparticles, as well as colloidal gold particles representing free insulin, were observed inside intravillus blood capillaries [97].

Thus, different mechanisms coexist to allow insulin-loaded nanoparticles to be taken up by the intestinal epithelium. These depend on the nature of the polymer forming the nanoparticle or the coating material.

It is generally accepted that hydrophobic nanoparticles should be taken up more extensively by the intestinal epithelium than hydrophilic nanoparticles [29]. Indeed, aminated nanoparticles have been shown to be more efficiently transported through follicle-associated epithelium-like cells than carboxylated nanoparticles, suggesting an influence of nanoparticle hydrophobicity and surface functional groups on cellular uptake [29]. In fact, hydrophobic nanoparticles seem to have a higher affinity for M cells than for absorptive cells [26]. Indeed, they poorly penetrate into the mucus, which is dense over epithelial cells [102]. Thus, not only the surface charge, but also the hydrophobic/hydrophilic balance, are important factors in the way nanoparticles are taken up through the intestinal epithelium [26,29,102].

The different mechanisms of absorption of insulin-loaded nanoparticles and free insulin, which may be released in the intestinal lumen in close contact with the brush border membrane, are illustrated in **Figure 4**.

5. Nanoparticles as oral delivery systems for insulin

The biological effects of insulin nanoparticles in diabetic and healthy animals are illustrated in **Table 2**.

5.1 Poly(alkylcyanoacrylate) nanoparticles

In a previous work resulting from the present authors collaboration with Couvreur *et al.* [41], it was shown for the first time that insulin-loaded poly(isobutylcyanoacrylate) (PIBCA) nanocapsules given orally to fasted streptozotocin-induced diabetic rats were able to reduce the blood glucose level from 300 mg/dl to 125 mg/dl. Thus, glycemia was nearly normalised. The duration of this effect was dose dependent [41,45]: 20 days with a single administration of 50 IU/kg encapsulated insulin, 9 days with 25 IU/kg and 6 days with 12.5 IU/kg. This effect was less obvious in fed animals. In contrast, free insulin was without any effect on blood glucose level – a result in agreement with the physiological absorption of insulin, which never exceeds 0.5% [6]. Thus, the encapsulation of insulin in polymeric nanocapsules allowed the preservation of the biological

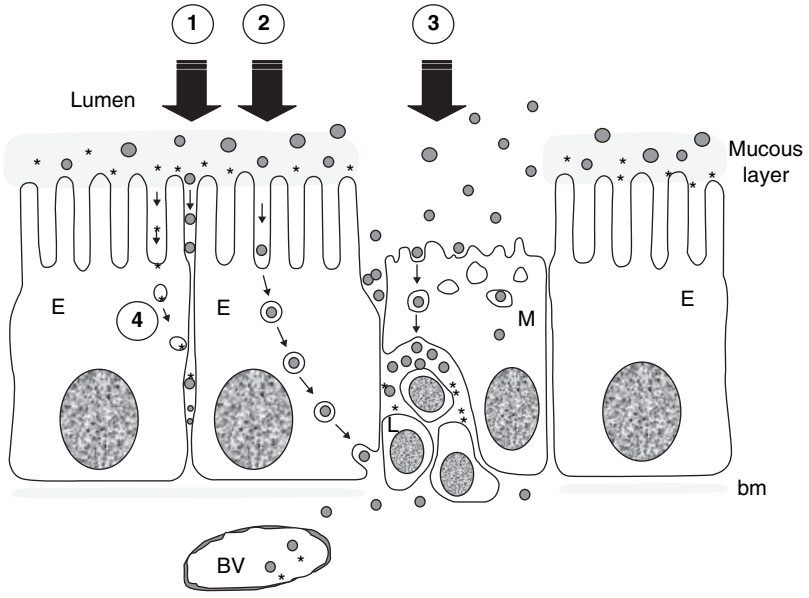

Figure 4. Shematic representation of insulin-loaded nanoparticles transport across the intestinal epithelium. 1. Paracellular transport. **2.** Transcytosis by enterocytes. **3.** Transcellular transport by M cells; **4.** Receptor-mediated transport of free insulin.
*Free insulin.
bm: Basement membrane; BV: Blood vessel; E: Enterocyte; M: M cell.

activity of insulin in diabetic rats. It was also demonstrated that the intensity of the reduction in blood glucose level was dependent on the site of administration along the GIT, the ileum being the most potent site of absorption [89]. Later, these results were confirmed in dogs [46]. Indeed, a single oral administration of 100 IU/kg insulin-loaded PIBCA nanocapsules to normal dogs reduced the peak of glycemia and insulinemia induced by an intravenous administration of glucose, with a maximal effect at day 9 post-administration. In addition, in alloxan-induced diabetic dogs, these insulin nanocapsules, given orally, reduced postprandial glycemia, glycosuria and blood glucagon and somatostatin levels – parameters which rose as a consequence of diabetes [46]. Finally, Sai *et al.* [109] showed that feeding 100 IU/kg insulin nanocapsules to non-obese diabetic mice – a genetically autoimmune Type 1 diabetic model – reduced the incidence of diabetes and the severity of lymphocytic inflammation of endogenous islets.

The nanocapsules responsible for such therapeutic effects consisted of polymeric colloidal particles of < 300 nm in diameter, made of an oily core surrounded by a polymeric wall. Poloxamer 188 was used as a surfactant during formulation. Further, the investigators loaded full polymeric nanospheres with insulin and analysed their biological effects after oral administration to fasted streptozotocin-induced diabetic rats [47]. A sustained reduction of blood glucose level from the second hour, up to 10 – 13 days, was observed when nanospheres were dispersed in an oily medium containing surfactant agents (poloxamer 188 and deoxycholic acid), but they were without any effect after suspension in water. This indicates that oil and surfactant agents play a considerable role in the protection of insulin against proteolytic enzymes and in its absorption by the GIT. These findings are in agreement with those of others. Radwan and Aboul-Enein [110] reported that orally administered insulin-loaded poly(ethylcyanoacrylate) nanospheres retained its biological activity up to 12 days in 50% of diabetic rats in the presence of glycyrrhizic acid and capric acid as absorption enhancers. However, Radwan [92] only found a blood glucose reduction that lasted from 2 h up to > 8 h after the oral administration of 75 IU/kg PIBCA nanospheres, in the presence of surfactants, to diabetic rats. Meisha *et al.* [51] found, after the oral administration of PIBCA nanospheres to diabetic rats, blood glucose lowering from the second hour, which extended over 40 h. In addition, Cournarie *et al.* [49] only detected a significant, but rapid, increase in plasma insulin levels (30 min to 1 h post-administration) after an oral administration of insulin-loaded PIBCA nanocapsules to diabetic rats, without decreasing glycemia. According to these authors, insulin-resistance developed by their diabetic rat model could explain these results.

5.2 Polymethacrylic acid based nanoparticles

Although several articles have described the preparation and *in vitro* characterisation of insulin nanoparticles based on methacrylic acid copolymers, only one work has investigated the *in vivo* effects of methacrylic acid and

Table 2. Oral administration of insulin encapsulated in nanoparticles.

Polymer	Size (nm)	Type of NPs	Species	Observations	Biological and pharmacological availability	Ref.
PIBCA	220	NC	Diabetic rats	12.5 – 50 IU/kg 50% decreased glycemia from 2 days up to 21 days, depending on insulin concentration	n.d.	[38,42]
PIBCA	297	NC	Diabetic rats	100 IU/kg Marked and prolonged reduction of BG from 2 days to 18 days depending on the site of administration: ileum > stomach > duodenum and jejunum > colon	n.d.	[83]
PIBCA	280	NC	Normal and diabetic dogs	100 IU/kg Sustained decrease of glycemia and improved response to i.v. GTT and metabolic parameters glycosuria, glucagon and somatostatin levels up to 6 – 8 days	n.d.	[43]
PIBCA	280	NC	Non obese diabetic mice	100 IU/kg Reduced incidence of diabetes and inflammation of islets of Langerhans	n.d.	[103]
PIBCA	Not indicated	NC	Diabetic rats	50 IU/kg High variability in the concentration of insulin crossing the intestinal barrier (peak at 1 h) and absence of modification of glycemia	n.d.	[46]
PIBCA with surfactants and Miglyol	145	NS	Diabetic rats	100 IU/kg 50% reduction of BG from 2 h up to 10 – 13 days	n.d.	[44]
PECA with surfactants	< 500	NS	Diabetic rats	BG level reduction up to 12 days in 50% of rats with NS + glycyrrhizic acid or capric acid	PA: 0.5 – 1% depending on the surfactant	[104]
PIBCA/pluronic acid	85	NS	Diabetic rats	100 IU/kg Lowering BG level from 2 h, extended over 40 h	n.d.	[48]
PIBCA with surfactants	< 500	NS	Diabetic rats	75 IU/kg BG reduction from 2 h to >8 h	PA: 37.6%	[86]
P(MAA-g-PEG) P(AA-g-PEG)	200 nm at pH 2.0 2 µm at pH 6.0	NS	Normal and diabetic rats	50 IU/kg Low hypoglycemic effect but > 6 h post-administration	n.d.	[50]
PLA-F127-PLA	10 – 100	Vesicles	Diabetic mice	50 and 100 IU/kg Sustained reduction of BG with max effect (-75%) from 4.5 – 24 h. Same effect with 50 and 100 IU/kg	n.d.	[55]

BA: Relative bioavailability; BG: Blood glucose concentration; CAPIC: Calcium-phosphate-PEG-insulin-casein; CS-DMAEMC: Chitosan *N*-dimethylaminoethyl methacrylate hydrochloride; CS-MMA: Chitosan monomer methyl methacrylate; CS-N-trimethylAEMC: Chitosan *N*-trimethylaminoethyl methacrylate hydrochloride; F 5.3 np and F 6.1 np: Chitosan nanoparticles prepared at pH 5.3 and 6.1, respectively; NPs: Nanoparticles; P(AA-g-PEG): Poly(acrylic acid grafted with PEG); P(MAA-g-PEG): Poly(methacrylic acid) grafted with PEG; PA: Pharmacological availability; PECA: Poly(ethylcyanoacrylate); PEG: Polyethylene glycol; PIBCA: Poly(isobutylcyanoacrylate); PLA: Poly(lactic acid); PLGA/FAO: PLGA with fumaric anhydride oligomer and iron oxide additives; PLGA: Poly(lactide-co-glycolide) acid; SLNs: Solid lipid NPs; WGA-SLNs: Lectin-modified solid lipid NPs.

Table 2. Oral administration of insulin encapsulated in nanoparticles (continued).

Polymer	Size (nm)	Type of NPs	Species	Observations	Biological and pharmacological availability	Ref.
PLGA	160 – 170	NS	Normal rats	Strong hypoglycemic effect after adm. the ileal loop	n.d.	[56]
PLGA	261 – 1145	NS	Diabetic rats	Prolonged reduction of BG	n.d.	[59]
PLGA	149.6	NS	Diabetic rats	10 IU/kg Sustained hypoglycemic effect from 4 h with a max effect (-52%) at 10 h	PA: 10.3%	[58]
PLGA	150	NS	Diabetic rats	20 IU/kg Prolonged reduction in BG from 4 h to 24 h. Max decrease (-30%) at 16 h	BA: 3.68%	[60]
PLGA–Chitosan		NS	Diabetic rats	Sustained hypoglycemic effect from 14 – 16 h	+15.4% versus non coated PLGA NP	[72]
PLGA–Hp55	169	NS	Diabetic rats	20 IU/kg Prolonged reduction in BG from 4 h to 24 h. Max decrease (-65%) at 16 h	BA: 6.27%	[60]
PLGA–insulin phospholipids complex	200	NS	Diabetic rats	20 IU/kg 57.4% reduction in BG from 12 h to 24 h	BA: 7.7%	[20]
PLGA/FAO	< 1 µm	NS	Normal rats	64 IU/kg. Sustained reduction in BG	PA 11.4% vs. intraperitoneal injection	[57]
Poly(ε-caprolactone)/Eudragit RS	358	NS	Diabetic rats	50 – 100 IU/kg Dose dependent sustained reduction in BG with max effect (-40%) between 4 – 8 h	BA: 13.21%	[62]
Chitosan	250 – 400	NS	Diabetic rats	21 IU/kg Prolonged hypoglycemia over 15 h	BA: 14.9%	[71]
Chitosan: F 5.3 np F 6.1 np	269 339	NS	Diabetic rats	50 and 100 IU/kg Marked and sustained lowering of glycemia from 2 h to 24 h	No increase of plasma insulin	[63]
Chitosan–dextran sulfate	500	NS	Diabetic rats	50 and 100 IU/kg Sustained reduction (-22 and -27%) in BG with max effect after 14 h	PA: 5.6% with 50 IU/kg and 3.4% with 100 IU/kg	[71]
Chitosan–poly(glutamic acid)	110 – 150	NS	Diabetic rats	15 and 30 IU/kg Dose-dependent decrease in blood glucose for 2 – 10 h post-adm.	n.d.	[90]

BA: Relative bioavailability; BG: Blood glucose concentration; CAPIC: Calcium-phosphate-PEG-insulin-casein; CS-DMAEMC: Chitosan *N*-dimethylaminoethyl methacrylate hydrochloride; CS-MMA: Chitosan monomer methyl methacrylate; CS-N-trimethylAEMC: Chitosan *N*-trimethylaminoethyl methacrylate hydrochloride; F 5.3 np and F 6.1 np: Chitosan nanoparticles prepared at pH 5.3 and 6.1, respectively; NPs: Nanoparticles; P(AA-g-PEG): Poly(acrylic acid grafted with PEG); P(MAA-g-PEG): Poly(methacrylic acid) grafted with PEG; PA: Pharmacological availability; PECA: Poly(ethylcyanoacrylate); PEG: Polyethylene glycol; PIBCA: Poly(isobutylcyanoacrylate); PLA: Poly(lactic acid); PLGA/FAO: PLGA with fumaric anhydride oligomer and iron oxide additives; PLGA: Poly(lactide-co-glycolide) acid; SLNs: Solid lipid NPs; WGA-SLNs: Lectin-modified solid lipid NPs.

Table 2. Oral administration of insulin encapsulated in nanoparticles (continued).

Polymer	Size (nm)	Type of NPs	Species	Observations	Biological and pharmacological availability	Ref.
Chitosan–mehtacrylate: CS-MMA CS-DMAEMC CS-N-trimethylAEMC	150 – 280	NS	Normal rats	100 IU/kg Hypoglycaemic effect depending on the copolymer. -30 to -40% with CTM-CS for 6 – 10 h post-adm	n.d.	[66]
Alginate–dextran shelled with chitosan	750	NS	Diabetic rats	50 and 100 IU/kg 40% reduction of blood glucose sustained for over 18 h	PA: 6.8% with 50 IU/kg; 3.4% with 100 IU/kg	[74]
Alginate–dextran shelled with chitosan–albumin–PEG	< 600 nm	NS	Diabetic rats	25, 50 and 100 IU/kg Reduction of blood glucose up to 48 h with a max. at 12 h (-73%)	PA: 22% with 50 IU/kg	[75]
Lectin–modified solid lipid nanoparticles	50 – 75	NS	Normal rats	50 IU/kg Sustained reduction in blood glucose SLNs: max at 1 h WGA-SLNs: 3 – 6 h post-adm	BA: 6.08% for SLNs; 7.11% for WGA-SLNs	[77]
Gold NPs	35	NS	Diabetic rats	50 IU/kg Blood glucose reduction max (50 – 55%) after 3 h	n.d.	[95]
Chitosan–reduced gold NPs	10 – 50	NS	Diabetic rats	50 IU/kg Sustained reduction in BG with a max (-30%) at 2 h post-adm	n.d.	[69]
CAPIC	600	NS	Diabetic mice	100 IU/kg -80% BG reduction from 1 – 12 h in fasted mice -50% BG reduction from 1 – 5 h in fed mice	n.d.	[81]

BA: Relative bioavailability; BG: Blood glucose concentration; CAPIC: Calcium-phosphate-PEG-insulin-casein; CS-DMAEMC: Chitosan *N*-dimethylaminoethyl methacrylate hydrochloride; CS-MMA: Chitosan monomer methyl methacrylate; CS-N-trimethylAEMC: Chitosan *N*-trimethylaminoethyl methacrylate hydrochloride; F 5.3 np and F 6.1 np: Chitosan nanoparticles prepared at pH 5.3 and 6.1, respectively; NPs: Nanoparticles; P(AA-g-PEG): Poly(acrylic acid grafted with PEG); P(MAA-g-PEG): Poly(methacrylic acid) grafted with PEG; PA: Pharmacological availability; PECA: Poly(ethylcyanoacrylate); PEG: Polyethylene glycol; PIBCA: Poly(isobutylcyanoacrylate); PLA: Poly(lactic acid); PLGA/FAO: PLGA with fumaric anhydride oligomer and iron oxide additives; PLGA: Poly(lactide-co-glycolide) acid; SLNs: Solid lipid NPs; WGA-SLNs: Lectin-modified solid lipid NPs.

acrylic acid grafted with PEG nanoparticles in healthy and diabetic rats [53]. The obtained insulin nanoparticles (diameter of either 200 nm at pH 2.0 or 2 µm at a pH of ~ 6.0) reduced serum glucose levels for at least 6 h after an oral administration of 50 IU/kg.

5.3 PLA and PLGA nanoparticles

5.3.1 *PLA nanoparticles*

Vesicular insulin nanoparticles formed by PLA-*b*-pluronic-*b*-PLA (PLA-F127-PLA) block copolymers, 10 – 100 nm in diameter, were orally administered to alloxan-induced diabetic mice at a concentration of 50 IU/kg [58]. They reduced blood glucose level by 71% with a maximal effect 4.5 h after administration; this effect lasted at least up to 25 h. However, increasing the concentration of insulin to 100 IU/kg did not improve this hypoglycemic effect.

5.3.2 *PLGA nanoparticles*

PLGA insulin nanoparticles prepared by different groups have been investigated in healthy and diabetic rats after oral administration. These nanoparticles were generally < 200 nm, except those of Carino *et al.* [60], which had a larger diameter, although < 1 µm. After intragastric administration in diabetic rats, at 10 IU/kg insulin, they reduced glycemia from 4 h post-administration, with a maximal effect (-52%) after 10 h [75]. A similar prolonged reduction in blood glucose was also observed by Kumar *et al.* [62] and Cui *et al.* [63] in diabetic rats, and Barichello *et al.* [59], who injected insulin nanoparticles in the ileal loop from non-diabetic rats. However, the relative bioavailability was low (3.68% compared with a subcutaneous injection of insulin) [63]. Pan *et al.* [76] coated PLGA nanoparticles with chitosan, increasing the relative pharmaceutical availability by 15.4% versus uncoated nanoparticles. One of the strategies used was to improve the liposolubility of insulin with soybean phosphatidylcholine, leading to the formation of PLGA nanoparticles loaded with a insulin–lipid complex [22] or to prevent the release of insulin in the stomach using a pH-sensitive cellulose copolymer (HPMCP-55), leading to the formation of PLGA-Hp55 nanoparticles [63]. These modifications improved the relative bioavailabilities of insulin to 7.7 and 6.27%, respectively. Both formulations (20 IU/kg) worked for up to 24 h, but the maximal decrease of blood glucose was more obvious with PLGA-Hp55 than with PLGA insulin–lipid complex nanoparticles. Finally, Carino *et al.* [60] prepared PLGA nanoparticles with fumaric anhydride oligomer and iron oxide additives (FAO/PLGA). When administered orally to normal rats at the dose of 64 IU/kg, they reduced glycemia between 4 and 6 h and completely blocked increased glycemia in response to a subcutaneous glucose tolerance test. This formulation has a 11.4% efficacy by comparison to an intraperitoneally delivered insulin.

5.4 Poly(ε-caprolactone) nanoparticles

Nanoparticles composed of a biodegradable polymer poly(ε-caprolactone) and a non-degradable biocompatible mucoadhesive polymer (Eudragit RS) with polymers ratio 50/50 were prepared by Damgé *et al.* [65]. These nanoparticles reduced glycemia for a prolonged period (from 4 h up to 24 h) with a maximal effect after 6 – 8 h (-40%). This effect was dose dependent (**Figure** 5). In addition, insulin nanoparticles improved the glucose response to an oral glucose tolerance test. The relative bioavailability was 13.21% versus a subcutaneous administration of insulin.

5.5 Chitosan-based nanoparticles

Chitosan has been proposed for the manufacturing of nanoparticles because this natural polysaccharide increases the permeability of proteins and peptides across epithelial cells. Thus, chitosan nanoparticles have been extensively applied to the nasal and pulmonary delivery of insulin [25,30]. However, these alternative routes of administration of insulin will not be discussed in the present review.

For the oral delivery of insulin, chitosan has been used as a unique polymer or a copolymer, or as coating material for polymeric nanoparticles.

Ma *et al.* [66] prepared insulin-loaded chitosan nanoparticles at pH 5.3 and 6.1. When administered orally to diabetic rats, the former formulation (100 IU/kg) induced a marked and sustained lowering of serum blood glucose levels from 10 h post-administration, for up to 24 h. The second formulation elicited, at the same concentration, a faster onset of action, producing a significant reduction of glycemia as early as 2 h. However, despite such actions on glycemia, there was no increase in plasma insulin levels.

Sarmento *et al.* [71] prepared insulin-loaded dextran sulfate/chitosan nanoparticles. After oral administration in diabetic rats, they lowered serum glucose levels by 22 and 27%, respectively, with 50 and 100 IU/kg doses of encapsulated insulin, with a maximal effect after 14 h.

Lin *et al.* [96] developed insulin-loaded chitosan–poly(γ-glutamic acid) nanoparticles. When administered orally to diabetic rats, they also decreased blood glucose level in a dose-dependent manner (15 or 30 IU/kg) from 2 to 10 h post-administration.

Chitosan has also been associated to methacrylate derivatives [69], creating a chitosan-monomer methyl methacrylate or chitosan-*N*-dimethylaminoethyl methacrylate hydrochloride or chitosan-*N*-trimethylaminoethyl methacrylate chloride nanoparticles. When administered orally to healthy rats, they reduced glycemia from 6 to 10 h post-administration, with chitosan-*N*-trimethylaminoethyl methacrylate chloride nanoparticles being the most efficient. Chitosan has also been used as a coating material for insulin-loaded PLGA [76] and alginate [79,80] nanoparticles.

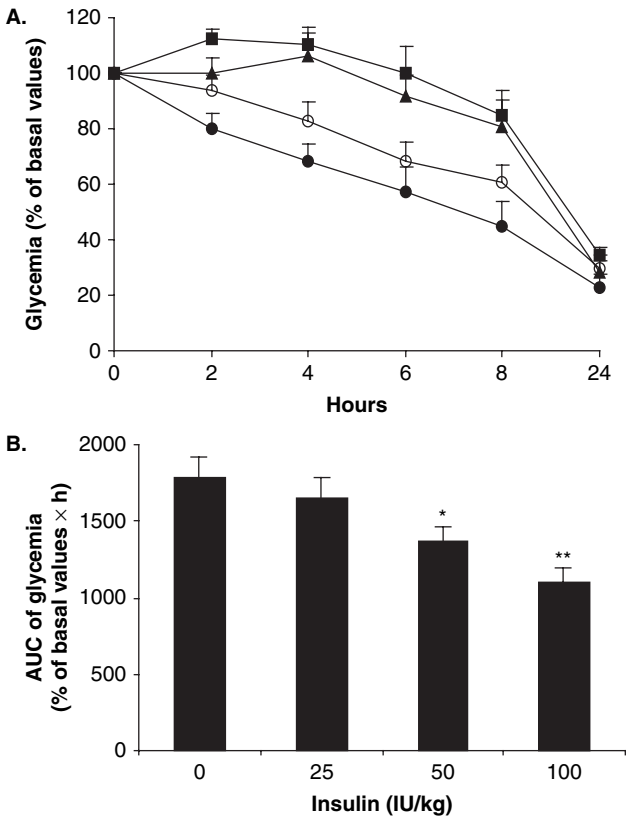

Figure 5. A. Blood glucose levels after a single oral administration of insulin-loaded poly(ε-caprolactone)/Eudragit RS nanoparticles at the concentrations of 25 IU/kg (triangles, n = 11), 50 IU/kg (empty circles, n = 11), 100 IU/kg (full circles, n = 8) or empty nanoparticles for controls (squares, n = 22) in fasted diabetic rats. Results are expressed as means ± S.E.M. Mean basal values at T_0 were: 249 ± 18 mg/dl. **B.** Areas under the curves according to the oral tested doses. Comparisons calculated against the control group on the corresponding areas under the curves for 0 to 24 h.

*$p < 0.05$.

**$p < 0.01$.

Reprinted from Damgé C, Maincent P, Ubrich N. Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. J Control Rel 2007;117(2):163-70, with permission from Elsevier.

5.6 Alginate-based nanoparticles

Alginate was initially used for cell encapsulation, in particular for the transplantation of islets of Langerhans. More recently it was applied to deliver insulin orally [77,79,80]. Chitosan has been used as a coating material in order to preserve insulin release at low pH, namely in the stomach and for its mucoadhesive properties. When administered orally to diabetic rats, they lowered basal serum glucose levels by ~ 40% with 50 and 100 IU/kg doses, sustaining hypoglycemia for > 18 h. The pharmacological availability was 6.8 and 3.4%, respectively [79].

Recently, dextran–alginate nanoparticles were coated with chitosan, PEG 4000 and albumin [80]. When administered orally to diabetic rats, they induced a marked reduction in the blood glucose level from 4 h up to 48 h. The most obvious effect was noted after 12 h (-73% of basal glycemia values), and was dose dependent. The pharmacological availability of insulin was 22% for the 50 IU/kg dose. Thus, PEG and albumin coating has been shown to improve the biological efficacy of insulin-loaded alginate nanoparticles coated with chitosan alone [79].

5.7 Solid lipid nanoparticles

Insulin-loaded SLNs, 65 nm in diameter, have been modified with wheat germ agglutinin-*N*-glutaryl-phosphatidylethanolamine (WGA-SLNs) in order to stabilise them [82]. WGA was chosen for its relatively good resistance to acidic pH and enzymatic degradation. After oral administration to healthy rats, insulin-loaded SLNs (50 IU/kg) reduced the blood glucose level rapidly, with the maximal effect being noted after 1 h. Insulin-loaded WGA-SLNs exerted a less intense effect, but more was prolonged (up to 6 h) at the same dose. Thus, the relative bioavailabilities of these formulations were 4.99 and 7.11%, respectively, in comparison with a subcutaneous injection.

Nevertheless, cetyl palmitate-based SLNs containing insulin, 350 nm in size, administered orally to diabetic rats, have shown a considerable hypoglycemic effect during 24 h [84].

5.8 Insulin-loaded vitamin B_{12}-coupled dextran nanoparticles

In order to enhance nanoparticle translocation across the GIT, dextran nanoparticles have been coupled with vitamin B_{12} as a specific targeting ligand [85]. The insulin-loaded conjugates (20 IU/kg), when administered orally to fed diabetic rats, reduced glycemia by 70 – 75% within 5 h, with basal levels being attained in 8 – 10 h. Interestingly, this effect was followed by a prolonged second phase of blood level reduction, which lasted up to 54 h. The coupling of vitamin B_{12} to dextran nanospheres improved the pharmacological bioavailability of insulin.

5.9 Calcium phosphate–PEG–insulin–casein nanoparticles

Calcium phosphate–PEG–insulin–casein (CAPIC) nanoparticles composed of a hydrophobic core (calcium phosphate–PEG–insulin) surrounded by a hydrophilic layer (mostly casein), which may protect insulin from the harsh acidic environment of the stomach [87], have been created, with a size of 600 nm. In this formulation, PEG increased the loading capacity of insulin from 10 to 60%. When administered orally to fasted non-obese diabetic mice, CAPIC nanoparticles (100 IU/kg) reduced the blood glucose level by 80% after 1 h, with this effect being maintained at least up to 12 h. When administered at the same dose to fed mice, the maximal reduction of blood glucose level observed after 3 h was still ~ -50% of the initial value, and lasted for up to 5 h. Although the authors did not report the relative bioavailability of insulin in

CAPIC nanoparticles, it seems to be an interesting formulation, working fast (as early as 1 h post-administration) and for a sustained period (at least 12 h in fasted mice and 5 h in fed mice).

5.10 Insulin-loaded gold nanoparticles

Insulin-loaded gold nanoparticles have been prepared with sodium borohydride [101] or chitosan [73] as reducing agents. These insulin-loaded nanoparticles, 10 – 50 nm in diameter, were administered orally to diabetic rats at a dose of 50 IU/kg. They reduced the blood glucose level by 30% after 2 h for the second formulation, and 18% after 3 h for the first formulation. Thus, it seems that chitosan is not only acting as a reducing agent in the synthesis of gold nanoparticles, but also promotes the penetration and uptake of insulin across the mucosa; this was confirmed by increased serum gold levels and insulin 2 h post-administration [73].

6. Conclusion

The bioavailability of insulin delivered orally is physiologically very low (0.5%) because insulin, a 51 amino acid peptide, is less absorbed and degraded in the GIT. Several strategies have been employed to improve the bioavailability insulin. Among them, the association to a polymeric drug carrier, such as nanoparticles of < 1 µm in diameter (nanocapsules or nanospheres), is a promising approach. When administered orally to diabetic or healthy animals, they generally induce a sustained reduction in blood glucose level. The intensity of the effect and duration has depended on the physicochemical characteristics of the polymer, copolymers and added substances used in their preparation. In a similar way, the size, efficiency of insulin encapsulation, zeta potential and *in vitro* release profile of insulin also depend on the composition of the nanoparticles. Finally, it has been shown that nanoparticles can protect insulin against degradation from enzymes involved in digestion. Permeation enhancers improve the absorption of insulin, whether associated to nanoparticles or not, by a transcellular pathway (by endocytosis through M cells or absorptive cells) or a paracellular route (by opening the tight junctions between adjacent cells). Finally, by increasing the mucoadhesive properties of nanoparticulate polymers, it has been possible to improve the residence time of insulin in contact with the intestinal mucosa, leading to a sustained absorption of the peptide. Insulin may be internalised by enterocytes, reaching the basolateral spaces. More specific targeting has resulted from receptor-mediated targeting using receptors on the epithelial cells (lectins, transferring, vitamin B_{12}) or to change the physicochemical properties of insulin (insulin–phospholipid complex) associated to nanoparticles. In addition, insulin-loaded nanoparticles can be transported directly to the liver through the portal vein [47]. Indeed, after double labelling of the polymer and insulin, the present authors have observed a marked uptake of insulin-loaded nanoparticles in different organs, and especially in the liver, after oral administration of poly(isobutylcyanoacrylate) nanospheres in the rat [47]. Once in this organ, which is implicated in the regulation of glycemia, nanoparticles should be taken up by the Kuppfer cells, which could act as a reservoir, progressively releasing insulin as a function of the polymer degradation, leading to a sustained reduction in glycemia [111].

7. Expert opinion

In recent years, a number of potential oral insulin formulations have been developed and investigated for their biological activity in experimental animals – generally rats and mice – that are often diabetic, but also healthy. However, to the best of our knowledge, no nanoparticulate system containing insulin has been investigated in humans. The formulations under clinical development concern Generex's buccal insulin mouth spray, Emisphere's SNAC-insulin capsule formulation, and Nobex's hexyl-PEG-modified peroral insulin [18,20,112-114]. Generex oral insulin spray (Oralin™) uses the formation of microfine mixed micelles made from the combination of absorption enhancers, which encapsulate and protect insulin molecules. After buccal and oropharyngual absorption, insulin molecules rapidly appear in the bloodstream, within 10 min of application. The insulin concentration peak has been demonstrated to be 40 – 60 min [112]. Oralin has been successfully applied in Type 1 and Type 2 diabetic patients, resulting in appropriate control of glycemia when compared with subcutaneous injections [113]. Emisphere have developed insulin tablets combining insulin with the delivery agent SNAC (sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate) [114]. When given orally to healthy subjects, this formulation lowered the blood glucose level and suppressed the endogenous secretion of insulin, suggesting that it is absorbed under a biologically form. SNAC-insulin is also fast acting insulin; the peak of plasma insulin appeared 30 min after oral delivery [114]. Nobex's insulin technology includes the modification of insulin by the covalent attachment of one or more low-molecular-weight amphiphilic oligomers (carriers) [18]. The absorption of encapsulated hexyl-insulin monoconjugate-2 (HIM2) across the GIT has been demonstrated in healthy and Type 2 diabetic subjects [18]. This covalent modification of insulin increases its solubility and improves stability against proteolytic enzymes. Finally, it has been demonstrated that HIM2 given orally to healthy subjects is equivalent to short-acting lispro-insulin given subcutaneously, and reduces postprandial glycemia in Type 2 diabetics [19,20].

Thus, it appears that all formulations in clinical trials are short-acting insulins. Their effect is quite similar to inhaled insulin, which exerts good efficacy in Type 1 and Type 2 diabetic patients [4], but presently available devices are cumbersome and the route of administration is systemic.

Both alternative insulin formulations require 10- to 100-times more insulin than the subcutaneous route, but are better accepted by the patients than the classical injections through an insulin pen. Formulations developed with nanoparticulate technology, as described above, generally lead to a sustained release of insulin lasting for several hours (up to 24 h or more). Thus, these oral formulations should replace a long-acting insulin formulation given subcutaneously. A long-acting 'bed time' insulin is generally first proposed in Type 2 diabetic patients, in combination with oral antidiabetic agents when these are no loner effective. Oral insulin nanoparticles should also be proposed for Type 1 diabetics in combination with pulmonary delivered insulin, or with the short-acting oral formulations developed by the above companies.

Oral insulin presents a number of advantages when compared with subcutaneous insulin. Primarily, it is the only way of administering insulin in away that physiologically reproduces the pancreatic response to food intake. Indeed, after absorption through the gut, insulin arrives directly to the portal vein. Then the hormone suppresses hepatic glucose production, leading to blood glucose reduction. As we have already demonstrated, nanoparticles are taken up by the liver [92], where they are degraded, progressively delivering insulin. Another advantage of the oral route comes from the liver itself. Only ~ 20% of insulin injected subcutaneously is available to the liver, requiring high amounts of insulin. Excessive peripheral hyperinsulinemia is, in part, responsible for insulin resistance in Type 2 diabetes. Thus, the oral administration of insulin has clinical importance because the liver is exposed to higher concentrations of insulin, compared with the systemic circulation. Thus, in theory, oral insulin should require less insulin per dose to moderate hepatic glucose production – the primary effect of insulin. Furthermore, the amount of insulin that reaches the systemic circulation should be smaller and the peak shorter-lived than with subcutaneous insulin, thus reducing unwanted hypoglycaemia.

Now the question remains why insulin nanoparticles have not been transferred to clinical investigation though the number of experimental studies described above. We have to prove that these systems, which should be administered daily to patients, are safe, non-toxic, stable, biocompatible, biodegradable and elicit reproducible biological effects. A good understanding of the mechanisms of absorption of polymers and surface-modified polymers should allow the development of nanoparticulate systems with better bioavailability than existing systems. Indeed, formulations have been investigated in a Type 1 diabetes experimental model (streptozotocin or alloxan-induced diabetic rats or mice) that need lower amounts of insulin than Type 2 diabetic models characterised by insulin resistance. Insulin nanoparticles should be preferentially applied to Type 2 diabetes. This application needs the development of insulin nanoparticles with higher bioavailabilities, and their investigation in experimental models of Type 2 diabetes that closely match humans – although the appropriateness of these models is a further matter of debate.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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