Expert Opinion

The potential of chitosan for the oral administration of peptides

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Over recent years, a major challenge in drug delivery has been the design of appropriate vehicles for the oral administration of macromolecular drugs (peptides and proteins). Indeed, despite the increasing market value of these complex molecules, their clinical use has been highly limited by their reduced oral bioavailability. Among the different delivery approaches explored so far, those based on the use of the polysaccharide chitosan have opened promising alternatives towards this ambitious goal. This is due to the interesting physicochemical and biopharmaceutical properties of this polymer. This article describes the advances that have been made in the design of chitosan-based systems specially adapted for the oral administration of peptides. These systems include solutions, microspheres, nanoparticles, nanocapsules and liposomes. More specifically, this article discusses the efficacy of the different delivery approaches for improving the absorption of peptides, and analyses the various mechanisms that have been proposed for the understanding of their efficacy.

Keywords: chitosan, delivery systems, oral administration, peptides/proteins

Expert Opin. Drug Deliv. (2005) 2(5):843-854

1. Introduction

Chitosan is a natural polymer synthesised by alkaline deacetylation of chitin, which is the second most abundant polysaccharide in nature after cellulose. This polymer can be found in the exoskeleton of crustacean, insects and some fungi. The main difference in the chemical structure between chitin and chitosan (α [1→4] 2-amino 2-deoxy β-D glucan) is related to the number of acetyl groups (Figure 1). This small difference makes chitin insoluble in water or in the most common organic solvents, whereas chitosan is soluble in acidic solutions.

Chitosan was discovered in 1859 by Rouget [1]; however, most of the reports on its biological and pharmaceutical applications have been published in the last couple of decades. Biomedical and biological applications of chitosan include its use as a dietary supplement for weight loss and as a hypocholesterolaemic [2], antimicrobial [3] and wound healing [4] agent. On the other hand, properties such as good biocompatibility, low toxicity [5,6] and biodegradation by lysozymes [5,7] make chitosan a new promising biomaterial.

From a pharmaceutical perspective, chitosan has also attracted significant attention for a variety of applications and modalities of administration. One area in which interest is growing is related to the use of chitosan as a material for transmucosal drug delivery. This is justified by some interesting characteristics of chitosan, such as bioadhesiveness and absorption-promoting characteristics. These two properties have been exploited for achieving either local or systemic drug delivery; for example, chitosan has become particularly well known because of its ability to increase the systemic absorption of drugs and vaccines administered intranasally [8-13]. A clear proof of its efficacy and acceptability is that there are several formulations in different stages of clinical trials; for example, at this moment, a chitosan-based liquid formulation is

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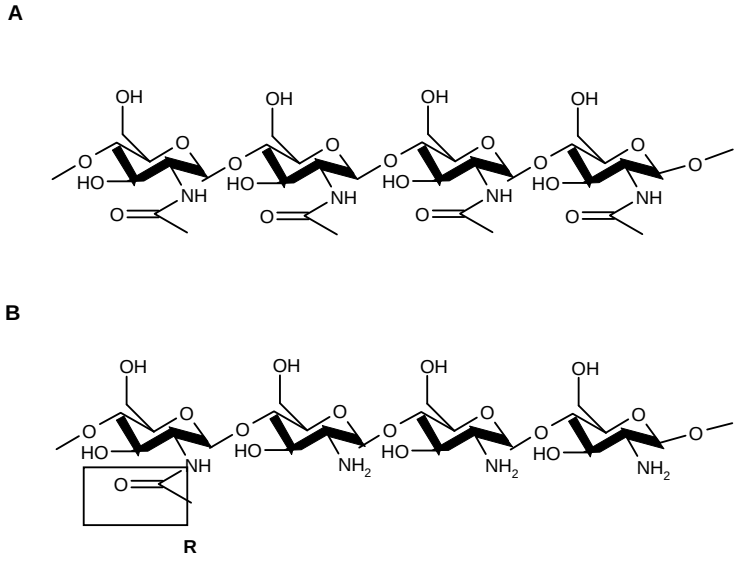

Figure 1. Chemical structures of A. chitin and B. chitosan.
R: *N*-acetyl group (acetylation percentage usually < 50%).

in clinical evaluation for nasal administration of several drugs and vaccines [14,15]. Chitosan has also shown interesting potential as an ocular drug delivery agent [16]. For this specific mode of administration, the interest relies on the ability of chitosan-based systems to enhance the intensity and time of retention of topically applied drugs [17-19]. A wide range of applications were also identified for the use of chitosan in oral drug delivery. A simple one would be its use as a hydrophilic excipient to increase the solubility of poorly soluble drugs [20]. Chitosan can also be presented in the form of microspheres (crosslinked or reacetylated), which have the ability to control the release of therapeutic agents along the intestinal tract [21,22] or to deliver drugs (i.e., antibiotics) locally to the gastric wall [23]. Finally, a number of chitosan derivatives and chitosan-based carriers have been proposed to increase the systemic absorption of peptides by providing the polymer with a better solubility at the absorption intestinal pH or improving mucoadhesive and permeation-enhancing properties [24-26].

There are already several general reviews on the overall potential of chitosan in the field of drug delivery [26-28], as well as specific reviews covering the application of chitosan for nasal [29] and ocular drug delivery [16]; therefore, this article reviews a specific application of chitosan with great market potential: enhancement of the oral absorption of peptides. This is without a doubt one of the greatest challenges confronted by pharmaceutical scientists in the last decade: a challenge that is clearly justified by the increasing number of macromolecular drugs approved and which have to be administered parenterally. Keeping this goal in mind, this article describes first the physicochemical and biopharmaceutical properties of chitosan that are critical for its oral application. Then, the features and efficacy of the different chitosan delivery systems evaluated so far for oral peptide delivery are presented, making the distinction between solutions, microspheres and nanostructures. Finally, the mechanisms suggested to explain the way that these systems are able to enhance the bioavailability of orally administered peptides are analysed.

2. Chitosan properties

Bearing in mind that this review is focused on the use of chitosan as a material for oral peptide delivery, this section discusses the physicochemical and biological properties that may affect this specific application of chitosan. In this sense, it is important to keep in mind that chitosan comprises a series of polymers, which vary in the percentage of *N*-acetyl groups, degree of deacetylation and molecular weight. Both characteristics are critical, not only from a physicochemical point of view but also from a biological perspective.

2.1 Solubility properties

A property that greatly affects the solubility of chitosan is its deacetylation degree. Highly deacetylated (85%) chitosans are readily soluble up to a pH value of 6.5; however, their solubility decreases significantly with the deacetylation degree [30]. Consequently, most studies regarding the pharmaceutical use of chitosan were performed with highly deacetylated chitosan.

Highly deacetylated and purified chitosans are commercially available in a broad range of molecular weights, in the form of

a base and also as a salt. The chitosan base form is soluble in acidic solutions such as hydrochloric, glutamic, acetic and lactic acid solutions, in which the amino groups of chitosan become protonated, leading to a positively charged polymer. Obviously, chitosan salts do not require the use of acids and are readily soluble in water; however, irrespective of the initial form, the solubility of chitosan significantly decreases when the pH is raised to neutral or basic values. In addition to the pH, the ionic strength affects the solubility of chitosan. The higher the ionic strength is, the lower the solubility; in fact, a higher electrolyte concentration results in a salting-out effect that leads to the precipitation of the polymer [31].

This solubility behaviour is important from the perspective of the use of chitosan for oral administration. Indeed, chitosans administered orally in the form of an aqueous solution are expected to precipitate on reaching the intestinal region due to the increase in the pH up to values in the range of 6.5 – 7.5. On the other hand, chitosans administered as powders are supposed to dissolve in the acidic pH of the gastric cavity and, then, precipitate in the intestinal compartment. Consequently, the behaviour of classical solutions and powders justifies the search for optimised presentations of chitosan, either in the form of a chemical derivative that is soluble at the intestinal pH or in the form of a device (nanoparticles/microspheres) that is stable in the physiological conditions of the gastrointestinal tract.

2.2 Penetration enhancement properties

It is well known that chitosan can enhance the permeability of different compounds through the intestinal monolayer. This was very clearly shown using the Caco-2 cell line for a variety of compounds, such as buserelin, inulin, mannitol and horseradish peroxidase [32-36]. The increase in the permeability has been generally related to a decrease in the transepithelial electric resistance (TEER), which was attributed to a partial disruption of the tight junctions. Moreover, the results of these studies show that the increase in the permeability of the monolayer and, hence, on the transport of drugs is dependent on a number of factors, such as the chitosan dose, molecular weight and deacetylation degree [33-35]. More specifically, Schipper *et al.* [34] observed, using mannitol as a model molecule, that chitosans with a low acetylation degree (1 – 15%) show a clear effect on the transport of the molecule, regardless of the molecular weight. In contrast, for highly acetylated chitosan (> 35%), a permeability enhancement of mannitol across the monolayers was only achieved with high molecular-weight chitosans.

Despite the evidence of the penetration-enhancement ability of chitosan, the mechanism of action remains unclear. Studies performed in the early 1990s concluded that the exposure of the cell surface to chitosan solutions induces clear changes in the F-actin distribution [32]. Some years later, it was observed that chitosan induces not only a redistribution of F-actin but also that of the tight junction proteins zona occludens-1 (ZO-1) [33,35]. In addition, it was found that the content of ZO-1 and occludin in the cytoskeleton increase, revealing the ability of chitosan to disrupt the epithelial cell tight junctions involving the translocation of the proteins ZO-1 and occludin from the membrane to the cytoskeleton [35]. Recently, the same authors found that chitosan acts, at least in part, via an activation of protein kinase C (PKC) [37]. Interestingly, the results of the previous studies [33-35] also indicate that the effect of chitosan on the Caco-2 cell monolayer is reversible and, hence, that the opening of the cellular barrier is transient. This specific behaviour makes a great difference in terms of toxicity between chitosan and the classical penetration enhancers, which are known to cause irreversible epithelial damage.

Within the context of this review article, the authors found it important to analyse this mechanism of action of chitosan with regard to its ability to enhance the absorption of peptides; however, the relevance of this mechanism on the efficacy of a specific chitosan-based oral drug delivery system will be dependent on the characteristics of the device and physical state of chitosan.

2.3 Mucoadhesive properties

A limitation of the oral route is the rapid transit, which reduces the chances for the drug to interact with the absorptive epithelium. One of the approaches to deal with this limitation has been based on the use of materials that favour the interaction of the drug with the mucus layer that covers the intestinal epithelium. Chitosan belongs to the category of these so-called mucoadhesive materials. Indeed, due to its positive charge, chitosan is able to interact with the negatively charged mucus components [38,39]. This property has attracted significant attention to the use of chitosan for transmucosal drug delivery and, in particular, for nasal drug delivery; for example, in this case, the chitosan–mucus interaction leads to the formation of a viscous gel that reduces the mucociliary clearance and increases the residence time of the drug in the absorptive mucosa [29]. However, although the advantage of this property is clear for nasal application, its contribution to the potential of chitosan for oral drug delivery is uncertain. This is due to the fact that the mucus covering the intestinal wall undergoes a rapid turnover and, consequently, the mucoadhesion of chitosan does not necessarily imply a more intense and prolonged contact of the coadministered drug with the absorptive epithelium. Therefore, a critical point in the design of a chitosan-based mucoadhesive delivery device would be to achieve, first, a facilitated interaction with the mucus and, second, an adequate diffusion through the mucus layer towards the underlying epithelium. In addition, classical chitosan solutions and powders are expected to precipitate on reaching the intestinal tract, and this uncontrolled precipitation may logically affect the inherent mucoadhesive properties of chitosan.

Irrespective of the importance of the role of mucoadhesion in the efficacy of chitosan-based delivery systems, there are some characteristics of chitosan that were found to affect the

intensity of the mucoadhesion phenomenon. Logically, as expected from the mechanism of mucoadhesion, the mucoadhesive character of chitosan is dependent on its acetylation degree. Indeed, a higher deacetylation degree leads to a more important number of positive charges and, hence, to greater marked adhesiveness [39].

On the other hand, the molecular weight of chitosan was also regarded as a parameter that affects the mucoadhesive properties of chitosan; for example, Kawashima *et al.* [40] observed, using a rat everted intestinal sac, that the mucoadhesion of chitosan increased with the molecular weight. The same conclusion was derived from a rheological study aimed at predicting the mucoadhesive properties of chitosan. The results of this study indicate that the mucoadhesion forces could be modulated by adjusting the chitosan molecular weight and concentration [41].

As in the case of the permeability-enhancing property, it is worthwhile to mention that, despite the evidence of the chitosan inherent properties on its mucoadhesive character, the consequence that these properties may have on the behaviour of chitosan-based delivery devices will be highly dependent on the specific characteristics of the device.

2.4 Chitosan toxicity issues and regulatory status

The safety of chitosan has been investigated, showing its low toxicity and biocompatibility [5,6,42]. It has been reported that the oral dose in rodents to kill 50% (LD_{50}) of the animals treated is > 16 g/kg [42], showing that chitosan is safe following oral administration. On the other hand, results in humans indicate that it is necessary to consume several grams of chitosan a day in order to observe signs of constipation or diarrhoea [43,44]. These amounts are far beyond those needed in pharmaceutical formulations and, consequently, it is accepted that the risk of side effects following oral administration of chitosan formulations is negligible.

With regard to the regulatory aspects, there is a monograph of chitosan hydrochloride in the European Pharmacopoeia (EP1774). In addition, the American Society for Testing and Materials (ASTM) has published guidelines (ASTM F 2103) for the characterisation of chitosans for use in tissueengineered medical products. Finally, according to the information provided by Novamatrix, a drug master file covering the chitosan salts and bases was submitted to the FDA in July 2004.

3. Chitosan-based systems for oral peptide delivery

Recently, a number of chitosan-based formulations (i.e., solutions, microspheres and nanostructures) were developed to improve the oral administration of peptides and proteins. In this section, the performance of these vehicles in terms of their ability to enhance the intestinal absorption as well as the mechanistic details are described. The pharmacological efficacy obtained for the two model peptides, insulin and calcitonin, that have received the greatest attention as candidates for these chitosan-based carriers are summarised in Tables 1 and 2.

3.1 Chitosan solutions

Most of the studies intended to evaluate the ability of chitosan solutions to improve the absorption of drugs across the intestinal epithelium were performed *in vitro*, either in cell culture [45] or in the rat intestinal sac model [46]. The *in vivo* evaluation of the effectiveness of chitosan solutions for oral peptide delivery is limited. This is understandable if the fact we take into account that, as indicated above, chitosan precipitates at the pH of the intestinal tract (~ 6.5 – 7.5). In fact, only one study has been found in the literature reporting the *in vivo* absorption-enhancing effects of chitosan hydrochloride [47]; concretely, the results showed an increase in the bioavailability of a nonapeptide following intraduodenal injection to rats. This positive result was attributed to the inherent capacity of chitosan to open the intercellular junctions.

The problem associated with the low solubility of chitosan at neutral and high pH values could be overcome by chemical modification of the chitosan molecule; for example, the derivative, *N*-trimethyl chitosan, was found to be readily soluble at neutral and basic pH values [48]. Unfortunately, the studies performed in Caco-2 cells showed that the mucoadhesive [49] and permeation-enhancing [48] properties of trimethylated chitosan were not as remarkable as those of the parent molecule. According to the authors [48,49], this phenomenon could be related to a change in the conformation of the trimethylated chitosan that reduces the flexibility of the polymer molecules and, therefore, the interpenetration into the mucus layer. This reduced mucoadhesion could also be due to a decrease in the density of the amino groups available for protonation subsequent to the chemical modification. Nevertheless, despite the reduced mucoadhesion of the chemically modified polymers in the *in vitro* cell line, the *in vivo* studies that were performed in rats or pigs revealed that trimethylated chitosan was significantly more efficient than chitosan hydrochloride at increasing the absorption of the peptide octreotide [50,51]. This greater efficacy was attributed to the more important absorption-enhancing effect of trimethylated chitosan at neutral pH values compared with chitosan hydrochloride [52].

A relatively successful approach has been the chemical modification of chitosan by the introduction of a thiol group [53]. Thiolated chitosans exhibit improved mucoadhesive properties *in vitro* [54] as well as an enhancement in the epithelial drug permeability [55]. Their *in vitro* behaviour correlates well with their ability to increase the absorption of calcitonin or insulin following oral administration to rats [53,56]; however, it should be clarified that chitosan was presented in the form of a solid matrix (1.5-mm minitablets) in these studies.

3.2 Chitosan-based microspheres

A different strategy towards increasing the systemic absorption of peptides administered orally has been designed specifically to deliver drugs in the colonic region. The strategy was proposed

Table 1. Pharmacological efficacy obtained after oral administration of insulin encapsulated in chitosan-based carriers to rats.

Chitosan-based carrier	Dose (IU/kg)	R_{max}* (%)	t_{max}‡ (h)	Duration of effect	Ref.
Chitosan nanoparticles§	7, 14 and 21	60	10	8 – 24 h	[68]
Chitosan nanoparticles§	50 and 100	50	19	13 – 24 h	[69]
Chitosan/ glucomannan nanoparticles	50	50	14	14 – 24 h	[73]
Chitosan-coated liposomes	100	30	3	0.5 – 12 h	[83]

*Maximum pharmacological effect.‡Time of the maximum pharmacological effect. §*In vivo* study performed in diabetic rats.

Table 2. Pharmacological efficacy obtained after oral administration to rats of calcitonin encapsulated in chitosan-based carriers.

Chitosan-based carrier	Dose (IU/kg)	R_{max}* (%)	t_{max}‡ (h)	Duration of effect	Ref.
Enteric-coated chitosan-based microspheres	500§	15	10	8 h - 12 h	[59]
Chitosan nanocapsules	500	30	1	> 24 h	[76]
Chitosan-coated solid nanoparticles	500	30	1	> 24 h	[82]
Chitosan-coated PLGA nanoparticles	125, 250 and 500	25	8	36 h	[40]
Chitosan-coated liposomes	500	22	2	8 h	[84]

*Maximum pharmacological effect. ‡Time of the maximum pharmacological effect. §Expressed by the authors in mg/kg and converted to IU taking the relation 1 mg = 5000 IU.

to take advantage of two critical facts: the limited peptide enzymatic activity compared with that of the small intestine; and the markedly slower rate of colonic transit. Chitosan-based microspheres were chosen as candidate vehicles to achieve this goal due to the specific degradation of chitosan in the colonic microflora [57,58], and due to its mucoadhesive/absorption-enhancing effects. Indeed, if conveniently designed, chitosan-based microspheres can travel intact along the gastrointestinal tract and reach the colonic region. Once in this region, the polymer matrix degrades and releases the peptide that, at this level, is free to cross the colonic mucosa.

The technological approach, adopted to prevent the alteration of chitosan-based microspheres during their gastrointestinal transit, was their entrapment or coating with pH-sensitive polymers (acrylic or cellulosic) [59-61]. Using fluorescent markers (i.e., carboxyfluorescein or the peptide insulin), it was shown that the pH-sensitive microspheres containing chitosan exhibit a pH-dependent release behaviour. More specifically, when exposed to a pH gradient, they provide a negligible release until the colonic pH was reached (~ 6.5 – 7), followed by a continuous and controlled drug release [59-61].

Unfortunately, clear conclusions can not be drawn from the limited number of *in vivo* studies that were intended to evidence the efficacy of these pH-sensitive chitosan systems regarding the efficacy of this approach; for example, in the study performed by Lamprecht *et al.* [59], aimed at evaluating the performance of enteric-coated chitosan-based microspheres containing calcitonin, it was observed that the pharmacological response was not affected by the presence of chitosan. Consequently, the authors concluded that the success of the formulation was due to the enteric coating and also that the role of chitosan was negligible in this type of formulation. These results differ from those observed by Tozaki *et al.* [62], who studied the efficacy of a large chitosan capsule orally administered to rats. The chitosan capsule had an enteric polymer coating and contained insulin in association with an absorption enhancer and enzyme inhibitor. A hypoglycaemic response was noted at 6 h postadministration, when the capsule reached the colon, giving an insulin bioavailability of 5.73%. The authors justified the success of this formulation by the specific disintegration of the capsules in the colonic region and by the absorption-enhancing properties of chitosan. In addition, they found that the positive effect of chitosan could be reinforced by the coadministration of other absorption enhancers.

Therefore, although this colonic delivery approach offers some potential, it is possible that the performance of a pH-sensitive chitosan delivery system will be dependent on the type of peptide as well as the specific composition of the final formulation.

3.3 Chitosan nanostructures

Over the last decade, a number of nanostructures based on chitosan and chitosan derivatives have been proposed for the oral administration of peptides. These nanostructures can be classified into three categories; chitosan-based self-assembling structures, chitosan-based nanoparticles and chitosan-coated nanosystems. Self-assembling nanostructures can be formed using specific chemical derivatives of chitosan. Chitosan-based nanoparticles can be composed of crosslinked chitosan or chitosan in combination with other hydrophilic polymers. Chitosan-coated nanosystems include chitosan-coated solid hydrophobic nanoparticles, liposomes and oily nanodroplets, also called nanocapsules.

3.3.1 *Chitosan-based self-assembling nanostructures*

Recently, a number of authors have reported the chemical modification of chitosan with hydrophobic groups, such as

palmitoyl, or linolenic or deoxycholic acid. The attachment of the hydrophobic moiety enabled the polymer to self-assemble forming a micelle-like nanostructure [63-65]; moreover, these systems have shown a capacity to entrap peptide molecules such as bovine serum albumin [65].

A different type of nanostructure was described by Ohya *et al.* [66] who reported that the grafting of hydrophilic polymers such as polyethyleneglycol (PEG) to the chitosan backbone provides the polymer with the ability to self-aggregate in aqueous media due to the formation of intermolecular hydrogen bridges; moreover, these nanostructures could associate insulin and release it in a controlled fashion depending on the degree of PEGylation of chitosan.

Unfortunately, although these self-assembling nanostructures have been proposed as promising carriers for the delivery of labile molecules like peptides and proteins, so far, there is no evidence of their performance following *in vivo* administration.

3.3.2 Chitosan-based nanoparticles

Chitosan has the ability to gel on contact with specific polyanions. Taking advantage of this specific property, a few years ago, Calvo *et al.* [67] developed nanoparticles solely made of chitosan or chitosan in combination with other hydrophilic polymers, such as PEG. These nanoparticles are formed immediately on mixing the two aqueous phases (one containing chitosan and the other containing sodium tripolyphosphate) through inter- and intramolecular linkages created between phosphate and chitosan amino groups. Besides the advantage of being produced under extremely mild conditions, these nanoparticles have shown a great capacity for the association of peptides and proteins, such as bovine serum albumin, tetanus toxoid and insulin [11,12,67]. The physical appearance of these nanoparticles as viewed by transmission electronic microscopy is shown in Figure 2A and compared with that of chitosan-coated systems, which will be described later (Section 3.3.3).

Recently, these ionically crosslinked nanoparticles were tested for their efficacy as carriers for oral peptide administration [68]. The results obtained following oral administration of insulin-loaded chitosan nanoparticles to diabetic rats revealed their efficacy at improving the hypoglyacemic response of insulin. More specifically, they observed a reduction of the blood glucose levels at 8 h post-administration, and this response was prolonged for ≤ 24 h. In contrast, chitosan solution, used as a control, was wholly inefficient at increasing the intestinal absorption of insulin. This report was coincident with that of Ma *et al.* [69], who evaluated the efficacy of the same type of nanoparticles after oral administration to normal and diabetic rats. They observed that, although no significant hypoglycaemic response was registered in normal rats, a marked hypoglycaemic effect was observed in similarly dosed diabetic rats. The onset of action occurred at 10 h post-administration, and the effect was maintained for a few hours.

With respect to the mechanism by which chitosan nanoparticles are able to increase the hypoglycaemic response of orally administered insulin, the authors of this work [68,69] speculated about several possible effects: the protection of the peptide from degradation in the gastrointestinal tract; and the potential mucoadhesive and absorption-enhancing properties of chitosan. Furthermore, the authors argue that the long-term response could be associated with the uptake of the nanoparticles by the M cells overlaying the Peyer's patches. Despite the advances made in this sense, the mechanism of action of these nanoparticles has not been fully elucidated; for example, using the Caco-2 model cell line, it was observed that some chitosan nanoparticles were able to enter into the cells and that this interaction with the monolayer was more important in the case of the mucus-secreting cells (MTX-E12) [70]. Consequently, from this work it was concluded that the presence of mucus favoured the interaction of the particles with the underlying epithelium. Unfortunately, this interpretation cannot be translated to the *in vivo* situation; however, it could be reasonably accepedt that the nanoparticles may protect the peptide molecules from degradation and facilitate their interaction with the absorptive mucosa.

Recently, Cuña *et al.* [71] produced a different type of nanoparticle made of chitosan in association with the polysaccharide glucomannan by using the same principle of ionic crosslinking. The rationale for the design of this novel colloidal carrier was that glucomannan would improve the stability of nanoparticles in the gastrointestinal fluids and also facilitate the interaction of nanoparticles with mannose receptors present in the epithelial cells [72]. In order to validate this hypothesis, insulin was chosen as a model peptide and tested the efficacy of the carrier following oral administration to normal rats [73]. Interestingly, chitosan–glucomannan nanoparticles were able to elicit a delayed hypoglycaemic response at 14 h post-administration, and this response was maintained for ≥ 10h. The success of chitosan–glucomannan nanoparticles as compared with those made of chitosan could be related to the observed stabilising effect of glucomannan [73]. In fact, recently, the same group observed a similar response for chitosan nanoparticles stabilised with poloxamer (A Vila, MJ Alonso, unpublished data). Although the stabilisation effect of glucomannan has been verified, its ability to improve the uptake of nanoparticles remains to be investigated.

Chitosan nanoparticles were also explored for their efficacy to increase the systemic absorption of hydrophobic peptides such as cyclosporin A [74]. In this study, chitosan nanoparticles were administered orally to beagle dogs, and the currently available cyclosporin A microemulsion (Neoral®) was used as a control. The results indicate that chitosan nanoparticles provided an improved absorption and, hence, a greater bioavailability of cyclosporin A compared with the control microemulsion. The authors of this work understood that this positive behaviour was due to a combination of the mucoadhesion and the ability to open the tight junctions of

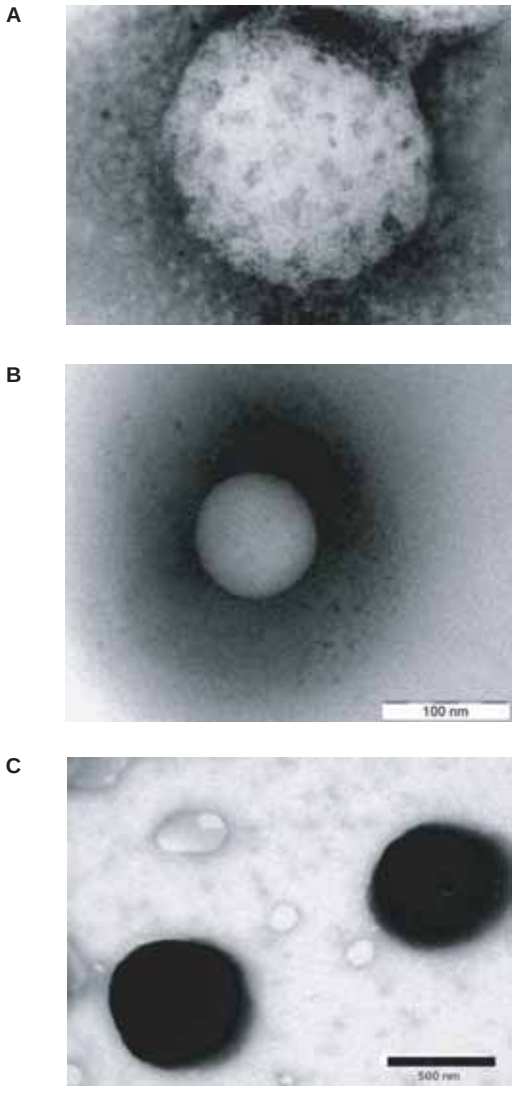

Figure 2. Transmission electron micrographs of A. chitosan nanoparticles; B. chitosan nanocapsules; and C. chitosan-coated tripalmitin nanoparticles. Reprinted from GARCIA-FUENTES M, TORRES D, ALONSO MJ: New surface-modified lipid nanoparticles as delivery vehicles for salmon calcitonin. *Int. J. Pharm.* (2005) **296**(1-2):122-132 [79], Copyright 2005, with permission from Elsevier.

epithelial cells, which are inherent properties of chitosan; however, no mechanistic studies were undertaken in order to verify this proposed mechanism.

3.3.3 Chitosan-coated nanosystems

Chitosan has also been used as a coating material in order to change the surface properties of colloidal drug carriers. Different types of nanosystems, oily nanodroplets, solid nanoparticles and liposomes were selected as cores for the coating process. The purpose of designing these systems was to improve the interaction of the cores with mucosal surfaces. Obviously, the nature of the core is expected to affect the drug encapsulation and release properties of the system.

3.3.3.1 Chitosan nanocapsules

A few years ago, Calvo *et al.* [75] developed chitosan-coated oily nanodroplets. The formation of these nanosystems was possible using the solvent displacement technique with an important modification; the incorporation of chitosan to the aqueous phase. The physical appearance of the coated systems is the one presented in Figure 2B. Recently, the efficacy of chitosan nanocapsules as carriers for oral peptide delivery using salmon calcitonin as a model peptide was investigated [76,77]. The reduction of the serum calcium levels after oral administration of calcitonin-loaded chitosan nanocapsules were monitored and an aqueous solution and a nanoemulsion containing calcitonin were used as controls. Interestingly, as shown in Figure 3, a marked hypocalcaemic response was noted when the peptide was associated to the nanocapsules, whereas no significant effect was observed after administration of the solution or the uncoated nanoemulsion. Therefore, a conclusion from these data was that the chitosan coating was critical for the performance of the formulation. Moreover, the percentage of reduction of the serum calcium levels achieved following administration of chitosan nanocapsules was maintained for $\geq$ 24 h [76]. This pronounced and long-lasting hypocalcaemic effect led us to speculate about the adhesion of the carrier and a sustained release of the associated peptide from the absorptive epithelium towards the bloodstream.

In order to obtain some evidence on the mechanism of action of chitosan nanocapsules Prego *et al.* [76] have performed some studies in the Caco-2 model cell line. Two main conclusions were extracted from these experiments. First, chitosan nanocapsules were able to reduce the TEER of the Caco-2 cell monolayer in a dose-dependent manner; however, the important concentration required to detect a significant change in permeability led to the conclusion that this phenomenon cannot be taken as an explanation for the success of the formulation following *in vivo* administration. Second, confocal experiments performed with rhodamine-labelled chitosan nanocapsules suggest that both, the nanocapsules and the corresponding control emulsions, were able to enter Caco-2 cells monolayer; however, the level of internalisation was very low and similar for both coated and uncoated carriers and, consequently, these results do not justify the *in vivo* success of the chitosan-coated systems. Recently, the same authors tested chitosan nanocapsules using a co-culture of Caco-2 cells and mucus-secreting cells (HT29-M6), and observed the fluorescence signals by confocal microscopy [78]. The images indicated that the level of interaction of chitosan nanocapsules is greatly enhanced by the presence of the mucus-secreting cells. In addition, no transport of the particles across the monolayer was observed [78]. Therefore, overall, these results led to the suggestion that the expected mucoadhesion of the chitosan-coated system could be responsible for the facilitated access of

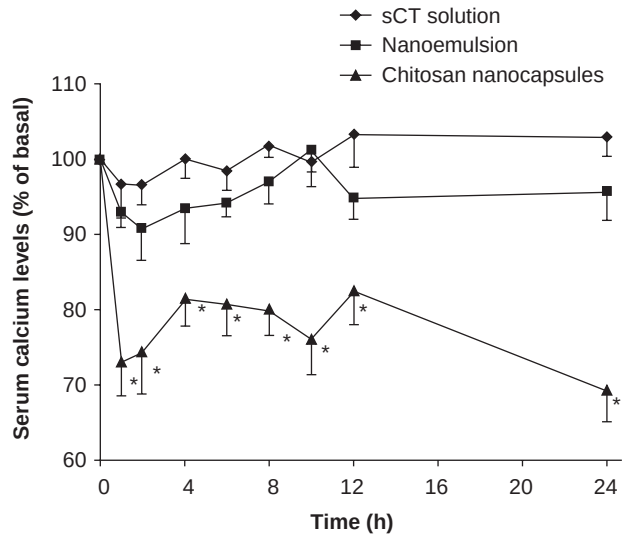

Figure 3. Hypocalcaemic effect after oral administration in rats of chitosan nanocapsules as well as the control nanoemulsion and aqueous solution of salmon calcitonin (mean ± SE; n = 6). Reprinted from PREGO C, GARCIA M, TORRES D, ALONSO MJ: Transmucosal macromolecular drug delivery. *J. Control. Release* (2005) **101**:151-162 [76], Copyright 2005, with permission from Elsevier.

*Statistically significant differences from salmon calcitonin solution ($p < 0.05$). SE: Standard error.

the drug to the underlying epithelium, and, hence, for the pronounced and long-lasting hypocalcaemic effect. An illustration of this mechanism is presented in Figure 4.

3.3.3.2 *Chitosan-coated solid nanoparticles*

In order to investigate the importance of the nature of the lipid core in the performance of chitosan-coated lipid systems, chitosan-coated tripalmitin nanoparticles were produced [79]. First, calcitonin-loaded tripalmitin nanoparticles were prepared by the double emulsion-solvent emulsification method [80] and, then, coated with chitosan by simple incubation in a aqueous chitosan solution (Figure 2C). After oral administration of calcitonin-loaded chitosan-coated tripalmitin nanoparticles to rats, the hypocalcaemic effect was evaluated. A great and long-lasting reduction of the serum calcium levels was obtained [81,82]. This response was similar to the one observed for calcitonin-containing chitosan nanocapsules [76]; however, as in the case of uncoated nanoemulsion, tripalmitin cores were inefficient at reducing the serum calcium levels. An obvious conclusion from these observations was that the chitosan coating was critical for the success of the formulation. As in the case of the chitosan nanocapsules, the interpretation was that the presence of chitosan could facilitate the interaction with the overlying mucus layer, leading to a prolonged site-specific delivery of calcitonin and, thus, an extended pharmacological response.

These results agree with those previously reported for chitosan-coated polylactic acid/glycolic acid (PLGA) nanoparticles [40]. In this study, elcatonin-loaded PLGA nanoparticles were prepared by the emulsion solvent diffusion method. Then, the nanoparticles were isolated and incubated with a chitosan solution to form chitosan-coated PLGA nanoparticles. The effectiveness of chitosan-coated PLGA nanoparticles was assayed in rats, showing a reduction of the serum calcium levels compared with the peptide solution and uncoated nanoparticles. As in the case of the chitosan-coated lipid nanoparticles, this positive *in vivo* behaviour was attributed to the mucoadhesive character of the carrier and its intimate contact with the intestine. This explanation was justified by the observed mucoadhesion of the carrier using the everted intestinal sac model.

3.3.3.3 *Chitosan-coated liposomes*

Liposomes have been considered as candidate vehicles for oral peptide delivery due to their capacity to encapsulate peptides and to protect them from enzymatic degradation. One of the approaches to improve their interaction with the intestinal mucosa and, hence, to increase the absorption of the associated peptide was their coating with mucoadhesive polymers such as chitosan; for example, Takeuchi *et al.* [83,84] prepared multillamelar liposomes coated with chitosan by hydrating the lipid film with an aqueous solution of the polymer. As expected, these chitosan-coated liposomes exhibited a mucoadhesive character whose degree was dependent on the amount of chitosan attached to their surface [83]. The efficacy of the coated liposomes as carriers for oral peptide delivery was tested *in vivo* for two model peptides, insulin and salmon calcitonin [83-85]. The results of these studies indicated that the chitosan-coated liposomes are more effective than the uncoated ones in terms of improving the pharmacological effect of the peptides administered orally to rats. This improved response was related to the mucoadhesive properties of the chitosan-coated liposomes. Moreover, the same authors tested the performance of the chitosan solutions for improving the absorption of calcitonin and observed that the simple presence of chitosan in solution did not help the absorption of this peptide. As a consequence, they concluded that the protection of the peptide in the liposomal core as well as their coating with the mucoadhesive polymer were critical for the success of the formulation.

Overall, the results obtained from chitosan-coated nanostructures underline the efficacy of this type of colloidal carrier. In general, the chitosan coating around the carriers showed a positive effect at improving the pharmacological response of the peptide, which is mainly attributed to the mucoadhesive properties of the polymer. However, the differences in the core material could also influence the level of protection of the associated peptide and its release from the carrier. As a consequence, these differences could have an impact on the intensity and duration of the pharmacological response.

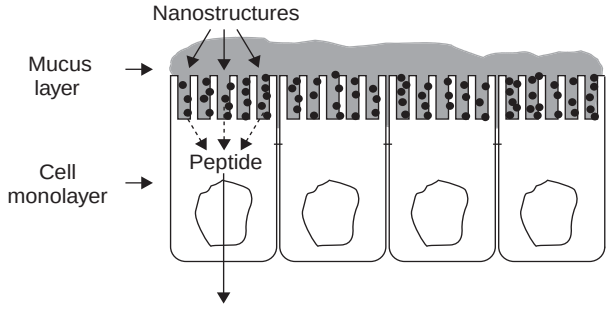

Figure 4. Schematic illustration of the interaction of chitosan-based nanostructures with the intestinal mucosa and the transport of the associated peptide.

4. Conclusion

Different chitosan-based drug delivery systems were revealed as promising peptide carriers. Among them, those based on chemically modified chitosan and also nanoparticulate carriers (nanoparticles and -capsules) have been particularly successful. Moreover, there is clear evidence of the greater performance of nanoparticulate chitosan carriers, compared with the solutions of unmodified chitosan in terms of enhancing the absorption of peptides such as insulin and salmon calcitonin. The explanation to this phenomenon is that the particulate carriers are able not only to increase the peptide absorption due to the mucoadhesive properties of chitosan but also to offer protection from enzymatic degradation. Furthermore, although more work is needed to fully understand the mechanism of action and efficacy of these carriers, it can be concluded that chitosan-based systems have a promising future in oral drug delivery.

5. Expert opinion

At present, only the hydrophobic peptide cyclosporin A can be administered orally in the form of a microemulsion; however, the progress made over recent years towards making feasible the oral administration of peptides offers an optimistic perspective. Indeed, the Eligen® technology (based on using a low molecular-weight delivery agent) has reached Phase II clinical trials for insulin and salmon calcitonin [86,87]. Similarly, hexyl–insulin monoconjugate 2, a modified insulin conjugated to an amphiphilic polymer [88], as well as oral formulations of calcitonin and parathyroid hormone consisting of a combination of enzyme inhibitors, absorption enhancers and enteric coating, have been evaluated in humans [89]. On the other hand, a number of particulate polymer and bioadhesive systems have provided evidence of their effectiveness in large-scale animals [90,91]. Therefore, these delivery-based strategies are opening the way for future great developments, preferably based on nanosystems and polymers. This article presents the value of a more immature but promising strategy based on the use of the bioadhesive polysaccharide chitosan. Chitosan as such (in solution or powder) cannot be used because it is soluble in the gastric fluids but precipitates at the intestinal pH; however, a number of delivery approaches based on chitosan have shown a degree of success in small-scale animals: the use of chemically modified chitosan, which is soluble at the intestinal pH; the use of nanosystems that protect the peptide and facilitate its interaction with the absorptive epithelium; and the design of devices that specifically deliver the peptide together with chitosan in the colonic region. Despite the difficulties in comparing the results of these approaches, the specific characteristics of the nanosystems and documented information about their efficacy lead to the consideration of their special potential for oral peptide administration. More detailed studies about their mechanism of action will help to design the way to proceed for their further optimisation.

At present, there is the proof-of-concept that chitosan-based nanocarriers can enhance the absorption of model peptides such as insulin and salmon calcitonin in small-scale animal models. It could be anticipated that these initial results will stimulate the optimisation of oral peptide formulations based on chitosan delivery nanosystems. Furthermore, this accumulated information is expected to lead to the evaluation of the efficacy of these nanosystems in large-scale animals in fed and fasting conditions. These results, together with those of the efficacy of these nanosystems during storage and also in a solid dosage, will soon give an indication of the potential of these nanosystems for clinical use.

Acknowledgements

The studies reported in this review were performed in our laboratory and have been supported by grants from the Ministry of Sciences and Education of Spain and the 'Xunta de Galicia', Spain.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. ROUGET C: Des substances amylacées dans le tissu des animaux, spécialement les articulés (chitine). *Comp. Rend.* (1859) 48:792-795.
2. SUGANO M, WATANABE S, KISHI A *et al.*: Hypocholesterolemic action of chitosans with different viscosities in rats. *Lipids* (1988) 23:187-191.
3. ALLAN CR, HADWIGER LA: The fungicidal effect of chitosan on fungi of varying cell wall composition. *Exp. Micol.* (1979) 3:285-287.
4. BALASSA LL, PRUDDEN JF: Application of chitin and chitosan in wound healing acceleration. *Proceedings of the First*

International Conference on Chitin/Chitosan. Cambridge, USA (1978): 269-305.

5. HIRANO S, SEINO H, AKIYAMA I, NONAKA I: Biocompatibility of chitosan by oral and intravenous administration. *Polym. Mat. Eng. Sci.* (1988) 59:897-901.
6. HIRANO S, SEINO H, AKIYAMA I, NONAKA I: Chitosan: a biocompatible material for oral and intravenous administrations. In: *Progress in Biomedical Polymers*, CG Gebelein, RL Dunn (Eds.), Plenum Press, New York (1990):283-290.
7. PANGBURN SH, TRESCONY PV, HELLER J: Lysozyme degradation of partially deacetylated chitin, its films and hydrogels. *Biomaterials* (1982) 3:105-108.
8. ILLUM L: Chitosan and its use as a pharmaceutical excipient. *Pharm. Res.* (1998) 15:1326-1331.
9. ILLUM L, JABBAL-GILL I, HINCHCLIFFE M, FISHER AN, DAVIS SS: Chitosan as a novel nasal delivery system for vaccines. *Adv. Drug Deliv. Rev.* (2001) 51:81-96.
10. FERNANDEZ-URRUSUNO R, ROMANI D, CALVO P, VILA-JATO JL, ALONSO MJ: Development of a freeze-dried formulation of insulin-loaded chitosan nanoparticles intended for nasal administration. *S.T.P. Pharma Sci.* (1999) 9:429-436.
11. FERNANDEZ-URRUSUNO R, CALVO P, REMUÑAN-LOPEZ C, VILA-JATO JL, ALONSO MJ: Enhancement of nasal absorption of insulin using chitosan nanoparticles. *Pharm. Res.* (1999) 16:1576-1581.

• Shows the potential of chitosan nanoparticles as nasal peptide carriers.

12. VILA A, SANCHEZ A, TOBIO M, CALVO P, ALONSO MJ: Design of biodegradable particles for protein delivery. *J. Control. Release* (2002) 78:15-24.
13. VILA A, SANCHEZ A, JANES K *et al.*: Low molecular weight chitosan nanoparticles as new carriers for nasal vaccine delivery in mice. *Eur. J. Pharm. Biopharm.* (2004) 57:123-131.
14. ILLUM L, DODANE V, IQBAL K: Chitosan technology to enhance the effectiveness of nasal drug delivery. *Drug Deliv. Technol.* (2002) 2:40-43.
15. DAVIS SS, ILLUM L: Absorption enhancers for nasal drug delivery. *Clin. Pharmacokinet.* (2003) 42:1107-1128.
16. ALONSO MJ, SANCHEZ A: The potential of chitosan in ocular drug delivery. *J. Pharm. Pharmacol.* (2003) 55:1451-1463.
17. DE CAMPOS AM, SANCHEZ A, ALONSO MJ: Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. *Int. J. Pharm.* (2001) 224:159-168.
18. DE CAMPOS AM, SANCHEZ A, GREF R, CALVO P, ALONSO MJ: The effect of a PEG versus a chitosan coating on the interaction of drug colloidal carriers with the ocular mucosa. *Eur. J. Pharm. Sci.* (2003) 20:73-81.
19. DE CAMPOS AM, DIEBOLD Y, CARVALHO ELS, SANCHEZ A, ALONSO MJ: Chitosan nanoparticles as new ocular drug delivery systems: *in vitro* stability, *in vivo* fate, and cellular toxicity. *Pharm. Res.* (2004) 21:803-810.
20. PORTERO A, REMUÑAN-LOPEZ C, VILA-JATO JL: Effect of chitosan and chitosan glutamate enhancing the dissolution properties of the poorly water soluble drug nifedipine. *Int. J. Pharm.* (1998) 175:75-84.
21. AYDIN Z, AKBUGA J: Chitosan beads for the delivery of salmon calcitonin: preparation and release characteristics. *Int. J. Pharm.* (1996) 131:101-103.
22. SINHA VR, SINGLA AK, WADHAWAN R: Chitosan microspheres as a potential carrier for drugs. *Int. J. Pharm.* (2004) 274:1-33.
23. REMUÑAN-LOPEZ C, PORTERO A, LEMOS M *et al.*: Chitosan microspheres for the specific delivery of amoxycillin to the gastric cavity. *S.T.P. Pharma. Sci.* (2000) 10:69-76.
24. BERNKOP-SCHNÜRCH A, HOFFER MH, KAFEDJIISKI: Thiomers for oral delivery of hydrophilic macromolecular drugs. *Expert Opin. Drug Deliv.* (2004) 1:87-98.
25. VAN DER MERWE SM, VERHOEF JC, VERHEIJDEN JH, KOTZE AF, JUNGINGER HE: Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. *Eur. J. Pharm. Biopharm.* (2004) 58:225-235.
26. JANES KA, CALVO P, ALONSO MJ: Polysaccharide colloidal particles as delivery systems for macromolecules. *Adv. Drug Deliv. Rev.* (2001) 47:83-97.

•• Provides a good review of the chitosan-based colloidal carriers for macromolecular drug delivery.

27. RADI HEJAZI, MANSOOR A: Chitosan-based gastrointestinal delivery systems. *J. Control. Release* (2003) 89:151-165.
28. SINGLA AK, CHAWLA M: Chitosan: some pharmaceutical and biological aspects-an update. *J. Pharm. Pharmacol.* (2001) 53:1047-1067.
29. ILLUM L: Nasal drug delivery-possibilities, problems and solutions. *J. Control. Release* (2003) 87:187-198.
30. CHO YW, JANG J, PARK CR, KO SW: Preparation and solubility in acid and water of partially deacetylated chitins. *Biomacromolecules* (2000) 1:609-614.
31. TSAIH ML, CHEN RH: Effects of ionic strength and pH on the diffusion coefficients and conformation of chitosans molecule in solution. *J. Appl. Polym. Sci.* (1999) 73:2041-2050.
32. ARTURSSON P, LINDMARK T, DAVIS SS, ILLUM L: Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2). *Pharm. Res.* (1994) 11:1358-1361.
33. DODANE V, KHAN MA, MERWIN JR: Effect of chitosan on epithelial permeability and structure. *Int. J. Pharm.* (1999) 182:21-32.
34. SCHIPPER NGM, VARUM KM, ARTURSSON P: Chitosans as absorption enhancers for poorly absorbable drugs.1: Influence of the molecular weight and the degree of acetylation on drug transport across human intestinal epithelium (Caco-2) cells. *Pharm. Res.* (1996) 13:1686-1692.
35. SMITH J, WOOD E, DORNISH M: Effect of chitosan on epithelial cell tight junctions. *Pharm. Res.* (2004) 21:43-49.
36. KOTZE AF, DE LEEUW BJ, LUESSEN HL *et al.*: Chitosans for enhanced delivery of therapeutic peptides across the intestinal epithelium: *in vitro* evaluation in Caco-2 cell monolayers. *Int. J. Pharm.* (1997) 159:243-253.
37. SMITH JM, DORNISH M, WORD EJ: Involvement of protein kinase C in chitosan glutamate-mediated tight junction disruption. *Biomaterials* (2005) 26:3269-3276.
38. LEHR C-M, BOUWSTRA JA, SCHACHT EH, JUNGINGER HE: *In vitro* evaluation of mucoadhesive properties of chitosan and some other

natural polymers. *Int. J. Pharm.* (1992) 78:43-48.

39. HE P, DAVIS SS, ILLUM L: *In vitro* evaluation of the mucoadhesive properties of chitosan microspheres. *Int. J. Pharm.* (1998) 166:75-88.

40. KAWASHIMA Y, YAMAMOTO H, TAKEUCHI H, KUNO Y: Mucoadhesive dl-lactide/glycolide copolymer nanospheres coated with chitosan to improve oral delivery of elcatonin. *Pharm. Dev. Technol.* (2000) 5:77-85.

41. CARAMELLA CM, ROSSI S, BONFERONI MC: A rheological approach to explain the mucoadhesive behavior of polymers hydrogels. In*: Encyclopedia of controlled drug delivery (Volume 1)*. Mathiowit E (Ed.), John Wiley & Sons Inc., New York, NY, USA (1999): 25-65.

42. ARAI K, KINUMAKI T, FUJITA T: Toxicity of chitosan. *Bull. Tokai Reg. Fish. Res. Lab.* (1968) 43:89-94.

43. KOIDE SS: Chitin-chitosan – properties, benefits and risks. *Nutr. Res.* (1998) 18:1091-1101.

44. PITTLER MH, ABBOT NC, HARKNESS EF, ERNST E: Randomized, double-blind trial of chitosan for body weight reduction. *Eur. J. Clin. Nutr.* (1999) 53:379-381.

45. ARTURSSON P: Cell cultures as models for intestinal peptide transport. *S.T.P. Pharma Sci.* (1993) 3:5-10.

46. LUESSEN HL, RENTEL CO, KOTZE AF *et al.*: Mucoadhesive polymers in peroral peptide drug delivery. IV. Polycarbophil and chitosan are potent enhancers of peptide transport across intestinal mucosa *in vitro*. *J. Control. Release* (1997) 45:15-23.

47. LUESSEN HL, DE LEEUW BJ, LANGEMEYER MW *et al.*: Mucoadhesive polymers in peroral peptide drug delivery. VI. Carbomer and chitosan improve the intestinal absorption of the peptide drug buserelin *in vivo*. *Pharm. Res.* (1996) 13:1668-1672.

48. KOTZE AF, LUESSEN HL, DE LEEUW BJ *et al.*: Comparison of the effect of different chitosan salts and and N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells (Caco-2). *J. Control. Release* (1998) 51:35–46.

49. SNYMAN D, HAMMAN JH, KOTZE AF: Evaluation of the mucoadhesive properties of *N*-trimethyl chitosan chloride. *Drug Dev. Ind. Pharm.* (2003) 29:59-67.

50. THANOU M, VERHOEF JC, MARBACH P, JUNGINGER HE: Intestinal absorption of octreotide: *N*-trimethyl chitosan chloride (TMC) ameliorates the permeability and absorption properties of the somatostatin analogue *in vitro* and *in vivo*. *J. Pharm. Sci.* (2000) 89:951-957.

51. THANOU M, VERHOEF JC, VERHEIJDEN JHM, JUNGINGER HE: Intestinal absorption of octreotide using trimethyl chitosan chloride: studies in pigs. *Pharm. Res.* (2001) 18:823-828.

52. KOTZE AF, THANOU MM, LUESSEN HL *et al.*: Enhancement of paracellular drug transport with highly quaternized *N*-trimethyl chitosan chloride in neutral environments. *J. Pharm. Sci.* (1999) 88:253-257.

53. GUGGI D, KAST CE, BERNKOP-SCHNÜRCH A: *In vivo* evaluation of an oral calcitonin-delivery system based on a thiolated chitosan carrier matrix. *Pharm. Res.* (2003) 20:1989-1994.

54. BERNKOP-SCHNÜRCH A, GUGGI D, PINTER Y: Thiolated chitosans: development and *in vitro* evaluation of a mucoadhesive, permeation enhancing oral drug delivery system. *J. Control. Release* (2004) 94:177-186.

55. LANGOTH N, GUGGI D, PINTER Y, BERNKOP-SCHNÜRCH A: Thiolated chitosan: *in vitro* evaluation of its permeation enhancing properties. *Proceedings of the 30th Annual Meeting of the Controlled Release Society,* Glasgow, UK (2003):34.

56. KRAULAND HA, GUGGI D, BERNKOP-SCHNÜRCH A: Oral insulin delivery: the potential of thiolated chitosan-insulin tablets on non-diabetic rats. *J. Control. Release* (2004) 95:547-555.

57. YAMAMOTO A, TOZAKI H, OKADA N, FUJITA T: Colon-specific delivery of peptide drugs and anti-inflamatory drugs using chitosan capsules. *S.T.P. Pharma Sci.* (2000) 10:23-34.

58. SINHA VR, KUMRIA R: Microbially triggered drug delivery to the colon. *Eur. J. Pharm. Sci.* (2003) 18:3-18.

59. LAMPRECHT A, YAMAMOTO H, TAKEUCHI H, KAWASHIMA Y: pH-sensitive microsphere delivery increases oral bioavailability of calcitonin. *J. Control. Release* (2004) 98:1-9.

60. TORRES D, RODRIGUEZ M, CUÑA M: Microencapsulated lipid cores for site-specific delivery of corticosteroid and peptide drugs to the colonic region. *S.T.P. Pharma Sci.* (2003) 13:49-56.

61. LORENZO-LAMOSA ML, REMUÑAN-LOPEZ C, VILA-JATO JL, ALONSO MJ: Design of microencapsulated chitosan microspheres for colonic drug delivery. *J. Control. Release* (1998) 52:109-118.

62. TOZAKI H, KOMOIKE J, TADA C *et al.*: Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. *J. Pharm. Sci.* (1997) 86:1016-1021.

63. UCHEGBU IF, SCHATZLEIN AG, TETLEY L *et al.*: Polymeric chitosan-based vesicles for drug delivery. *J. Pharm. Pharmacol.* (1998) 50:453-458.

64. KIM K, KWON S, PARK JH, CHUNG H, JEONG SY, ICK CK: Physicochemical characterizations of self-assembled nanoparticles of glycol chitosan-deoxycholic acid conjugates. *Biomacromolecules.* (2005) 6:1154-1158.

65. LIU C-G, DESAI KGH, CHEN X-G, PARK H-J: Linolenic acid-modified chitosan for formation of self-assembled nanoparticles. *J. Agric. Food Chem.* (2005) 53:437-441.

66. OHYA Y, CAI R, NISHIZAWA H, HARA K, OUCHI T: Preparation of PEG-grafted chitosan nanoparticles as peptide drug carriers. *S.T.P. Pharma Sci.* (2000) 10:77-82.

67. CALVO P, REMUÑAN-LOPEZ C, VILA-JATO JL, ALONSO MJ: Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *J. Appl. Polym. Sci.* (1997) 63:125-132.

68. PAN Y, LI Y, ZHAO H *et al.*: Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin *in vivo*. *Int. J. Pharm.* (2002) 249:139-147.

69. MA Z, LIM TM, LIM LY: Pharmacological activity of peroral chitosan-insulin nanoparticles in diabetic rats. *Pharm. Nanotechnol.* (2005) 293:271-280.

70. BEHRENS I, VILA-PENA AI, ALONSO MJ, KISSEL T: Comparative uptake studies of bioadhesive and non-bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. *Pharm. Res.* (2002) 19:1185-1193.

- Presents a comparison of the mechanisms of interaction of different types of nanoparticles in Caco-2 and mucus-secreting model cell lines.

71. CUÑA M, ALONSO-SANDE M, REMUÑAN-LOPEZ C *et al.*: Development of chitosan/glucomannan nanoparticles as carriers for oral protein administration. *Proceedings of the 29th Annual Meeting of the Controlled Release Society.* Seoul, Korea (2002):136.
72. TOMIZAWA H, ARAMAKI Y, FUJII Y *et al.*: Uptake of phosphatidylserine liposomes by rat Peyer´s patches following intraluminal administration. *Pharm. Res.* (1993) 10:549-552.
73. ALONSO M, TEIJEIRO D, REMUÑAN-LOPEZ C, ALONSO MJ: Chitosan/glucomannan nanoparticles as oral delivery systems for insulin. *2nd Pharmaceutical Sciences World Congress*, Kyoto, Japan (2004): 239.
74. EL-SHABOURI MH: Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. *Int. J. Pharm.* (2002) 249:101-108.
75. CALVO P, REMUÑAN-LOPEZ C, VILA-JATO JL, ALONSO MJ: Development of positively charged colloidal drug carriers: chitosan-coated polyester nanocapsules and submicron-emulsion. *Colloid. Polym. Sci*. (1997) 275:46-53.
76. PREGO C, GARCIA M, TORRES D, ALONSO MJ: Transmucosal macromolecular drug delivery. *J. Control. Release* (2005) 101:151-162.

- Describes the efficacy of chitosan-coated nanostructures as oral carriers for calcitonin.

77. PREGO C, FERNANDEZ-MEGIA E, NOVOA-CARBALLAL R *et al.*: Chitosan and chitosan-PEG nanocapsules: new carriers for improving the oral absorption of calcitonin. *Proceedings of the 30th Annual Meeting of the Controlled Release Society,* Glasgow, UK (2003): 70.
78. PREGO C, FABRE M, TORRES D, ALONSO MJ: Study of the interaction of chitosan nanocapsules with intestinal model cell lines. *30th Annual Meeting of the Controlled Release Society,* Miami, FL, USA (2005)
79. GARCIA-FUENTES M, TORRES D, ALONSO MJ: New surface-modified lipid nanoparticles as delivery vehicles for salmon calcitonin. *Int. J. Pharm.* (2005) 296 (1-2):122-132.
80. GARCIA-FUENTES M, TORRES D, ALONSO MJ: Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. *Colloid. Surf. B: Biointerf.* (2002) 27:159-168.
81. PREGO C, GARCIA-FUENTES M, ALONSO MJ, TORRES D: Chitosan-coated lipid nanoparticles enhance the oral absorption of calcitonin, *Proceedings of the 30th Annual Meeting of the Controlled Release Society,* Glasgow, UK (2003) 308.
82. GARCIA-FUENTES M, PREGO C, TORRES D, ALONSO MJ: A comparative study of the potential of solid triglyceride nanostructures coated with chitosan or poly (ethylene glycol) as carriers for oral calcitonin delivery. *Eur J. Pharm. Sci.*, (2005) 25:133-143.
83. TAKEUCHI H, YAMAMOTO H, NIWA T, KAWASHIMA Y: Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. *Pharm. Res.* (1996) 13:896-901.
84. TAKEUCHI H, MATSUI Y, YAMAMOTO H, KAWASHIMA Y: Mucoadhesive properties of carbopol and chitosan-coated liposomes and their effectiveness in the oral administration of calcitonin to rats. *J. Control. Release* (2003) 86:235-242.
85. WU Z-H, PING Q-N, WEI Y, LAI J-M: Hypoglycemic efficacy of chitosan-coated insulin liposomes after oral administration in mice. *Acta Pharm. Sinica* (2004) 25:966-972.
86. SINGH BN, MAJURU S: Oral delivery of therapeutic macromolecules: a perspective using the Eligen™ technology. *Drug Del. Technol.* (2003) 3:58-62.
87. MAJURU S: Advances in the oral delivery of heparin from solid dosage forms using Emisphere's Eligen® oral drug delivery technology. *Drug Del. Technol.* (2004) 4:84-89.
88. CLEMENT S, DANDONA P, STILL JG, KOSUTIC G: Oral modified insulin (HIM2) in patients with type 1 diabetes mellitus: results from a Phase I/II clinical trial. *Metabolism* (2004) 53:54-58.
89. MEHTA NM: Oral delivery and recombinant production of peptide hormones. Part I: making oral delivery possible. *BioPharm. Int.* (2004) 17:38-43.
90. DAMGE C, HILLAIRE-BUYS D, PUECH R *et al.*: Effects of orally administered insulin nanocapsules in normal and diabetic dogs. *Diabetes, Nutr. Metab.* (1995) 8:3-9.
91. PEPPAS NA, WOOD KM, BLANCHETTE JO: Hydrogels for oral delivery of therapeutic proteins*: Expert Opin. Biol. Ther.* (2004) 4:881-887.

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