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Development of chitosan sponges for buccal administration of insulin

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Abstract

This paper describes the development of a new highly porous, flexible device for buccal peptide administration by a very simple and mild casting/freeze-drying procedure. It consists of a mucoadhesive chitosan layer containing the peptide drug and an impermeable protective layer made of ethylcellulose. This structure was expected to provide unidirectional drug release to the mucosa and avoid loss of drug due to washout with saliva. Insulin release was modulated by varying some formulation variables (chitosan salt type and M_w, chitosan solution pH, insulin dose). The main factor affecting insulin release was its diffusion across the matrix, this being related to the water uptake/swelling and dissolution properties of chitosan and the viscosity of the gel formed upon hydration. In addition, an electrostatic interaction could occur between chitosan amino groups and the insulin carboxylic groups. Preliminary mucoadhesion studies showed that the affinity of chitosan sponges to mucin surfaces was related to the swelling and solubility properties of the different salts of chitosan.

Keywords: Bilayered drug delivery device; Buccal peptide administration; Chitosan; Insulin; Mucoadhesive sponge

1. Introduction

The successful exploitation of the new generation of peptides and proteins as therapeutic agents clearly depends on the availability of non-invasive transmucosal (nasal, pulmonary, buccal, peroral) drug delivery systems. These systems must be able to protect the associated macromolecule against enzymatic degradation and promote its passage across the epithelial barriers. The buccal route is a subject of growing interest for systemic delivery of high molecular weight (M_w) drugs because of its numerous advantages, including the bypass of the hepatic first-pass metabolism and gastrointestinal degradation, typically observed following oral administration. Additionally, it has rich blood supply, good accessibility for self-medication, patient compliance and safeness, since the drug device can be removed whenever desired (Hoogstraate & Wertz, 1998; Merkle, Anders, Wermerskirchen, Raehs, & Wolany, 1991). In contrast, the main limitation of the buccal route is the necessity of using penetration enhancers due to the low permeability of the buccal mucosa to high M_w drugs. In practice, drug absorption enhancement is generally accompanied by mucosal damage. Nevertheless, this local irritation is followed by the rapid cellular recovery inherent to the buccal epithelium (Merkle & Wolany, 1992).

An ideal buccal systemic drug delivery system requires intimate contact with the buccal mucosa in order to maintain its position in the mouth for a desired period of time; this can obviously be achieved by using mucoadhesive polymers. Furthermore, the device itself or its components should promote the permeation of the macromolecule across the mucosa, and protect it from environmental degradation (Veuillez, Kalia, Jacques, Deshusses, & Buri, 2001).

Among the mucoadhesive polymers, which are being explored as absorption enhancers across the mucosal

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surfaces, chitosan (CS) is gaining increasing importance due to its good biocompatibility, biodegradability and its favorable toxicological properties (Hirano, Seino, Akiyama, & Nonaka, 1990). CS is degraded by lyzozyme enzyme, that is highly present in the human body tissues and secretions (Muzzarelli, 1997; Varum, Myhr, Hjerde, & Smidsrod, 1997) and its degradation is dependent on the polymer degree of deacetylation (the higher the deacetylation degree, the lower is the degradation) and on the *N*-acetylglucosamine groups distribution along the CS chains (Tomihata & Ikada, 1997). Furthermore, it has also been demonstrated in mice that CS and its degradation products are quickly eliminated by the kidney following intraperitoneal administration to mice, thus overcoming accumulation in the body (Onishi & Machida, 1999). The evidence of the ability of CS to enhance the penetration of macromolecules across several mucosal barriers has been shown not only for CS solutions (Artursson, Lindmark, Davis, & Illum, 1994) but also for CS nanoparticles (Fernández- Urrusuno, Calvo, Remuñán-López, Vila-Jato, & Alonso, 1999). Furthermore, the permeability enhancing effect of CS was recently shown in an *in vitro* model of the human buccal epithelium (Portero, Remuñán-López, & Nielsen, 2002) and in porcine buccal mucosa (Senel et al., 2000). From a technological point of view, it is important to note that CS has excellent film-forming properties as well as a potential for controlling the release of drugs (Remuñán-López & Bodmeier, 1996a, 1996b). For all these reasons, CS has been widely proposed for increasing adhesion of drug delivery systems to oral mucosa and enhancing drug penetration across it (Senel et al., 2002). In spite of this, only a few studies have so far been performed on its usefulness as a vehicle for buccal peptide/protein administration (Langoth, Kalbe, & Bernkop-Schnürch, 2005, 2006; Portero et al., 2002; Senel et al., 2000).

We have previously reported the preparation of mucoadhesive bilayered tablets that are adequate for buccal administration of low M_w drugs (Remuñán-López, Portero, Vila-Jato, & Alonso, 1998b). However, further experiments aimed at investigating its potential application for high M_w drugs, confirmed a limited diffusivity of the macromolecules through these compact matrixes, thus presenting inadequate release patterns (Remuñán-López, Lorenzo-Lamosa, Vila-Jato, & Alonso, 1998a).

The use of CS-based porous matrixes has been reported for different applications, such as for periodontal bone regeneration (Park et al., 2000), matrices for culturing pancreatic islets (Cui, Kim, Imamura, Ion, & Inoue, 2001) or wound dressing structures, in which antibiotics and analgesics could be included (Foda, El-laithy, & Tadros, 2004; Mi, Shyu, Wu, Shyong, & Huang, 2001). However, there are no reports concerning the use of the CS sponges as peptide/protein carriers.

In this article, we describe the development of a porous mucoadhesive bilayered device based on CS intended for buccal systemic delivery of high M_w drugs. Insulin was chosen as a model of peptide drug.

2. Materials and methods

2.1. Materials

The following chemicals were obtained from commercial suppliers and used as received: chitosan (CS) (medium M_w, 150 kDa; deacetylation degree = 87%) and CS glutamate (medium M_w, 150 kDa and high M_w, 350 kDa; deacetylation degree >80%) (*Pronova Lab., Norway*); bovine insulin, dibutylphthalate (*Sigma Chemical Co., Spain*); ethylcellulose (Ethocel 10, standard premium) (*Dow Chemical, USA*); tartaric acid (*Probus, Spain*); citric acid (*Vorquímica, Spain*); hydrochloric acid (*Carlo Elba, France*) and Micro BCA (*Pierce, Rockford Illinois, USA*). Ultrapure water (MilliQ Plus, *Millipore Ibérica, Spain*) was used throughout. All other reagents were of analytical grade and used without further purification.

2.2. Preparation of the mucoadhesive buccal bilayered devices

Bilayered sponges (diameter = 12 mm, thickness = 6 mm) were prepared by a very simple casting/freeze-drying technique. Briefly, 1 g of a 2% w/w polymer solution in water (CS glutamate) or in tartaric or citric acid 3% w/v (CS base), with pH varying from 2 to 6 (pH was adjusted by adding HCl or NaOH) was mixed with insulin (5%, 8.75% and 12.5% w/w based on CS) which had previously been dissolved in a minimal amount of HCl 0.01 M (200 μl HCl/mg insulin). The resulting mixture was poured into a cylindrical mold of adequate size, frozen at −20 °C and freeze-dried (*Labconco freeze-drier, Labconco Co., USA*) to eliminate the solvent, obtaining the CS/insulin mucoadhesive layer. Then, an ethylcellulose solution in acetone containing the plasticizer dibutylphthalate (30% w/w based on polymer) was cast onto it and the solvent evaporated at room temperature obtaining the backing film on the sponge. Sponges were stored in a dessicator at 2–8 °C until used. The preparation method of the devices is illustrated in Fig. 1.

2.3. Viscosity measurements

CS glutamate was dissolved in Milli-Q water with pH varying from 2 to 6, and CS base in tartaric or citric acid 3% w/v by mechanical agitation, and centrifuged (*Sigma 2-15, Sigma, Spain*) at 6000*g* for 15 min in order to remove insoluble impurities. The total polymer concentration was fixed at 2% w/w in all samples and their viscosities were determined at 25 °C using a Cannon-Fenske viscosimeter (*Afora, Spain*). All samples were analyzed nine times.

2.4. Turbidity measurements

CS glutamate solutions (5 g, 1% w/w) at pH 2, 4 and 6 were mixed with 2.5 mg of insulin previously dissolved in

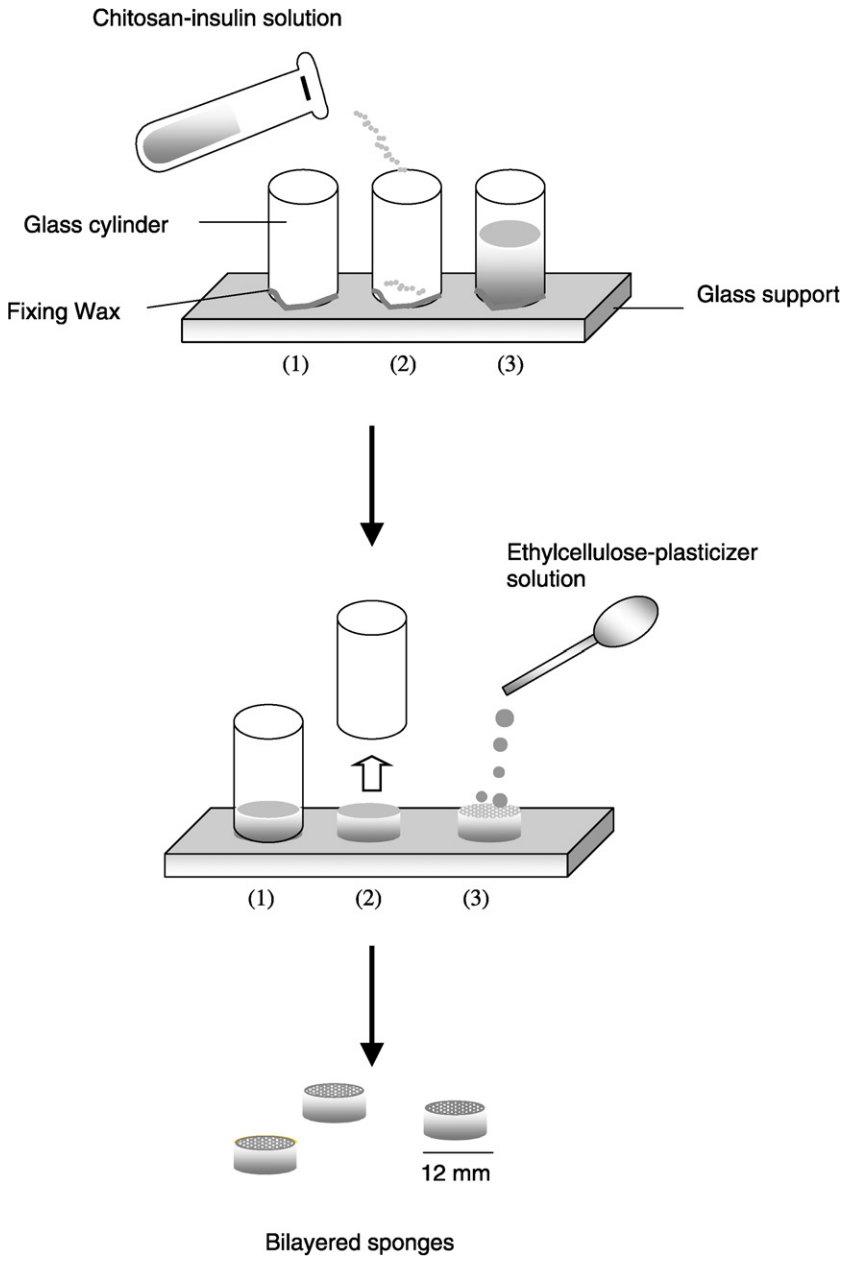

Fig. 1. Preparation method of bilayered chitosan/ethylcellulose sponges.

250 μl of HCl 0.01 M. The mixtures were shaken vigorously and left for 15 min before measuring their turbidities (absorbance at 420 nm) (*Shimadzu RF 5001 PC, Tokyo, Japan*). The turbidities of CS solutions without insulin and insulin solutions without the polymer at the same pH values were also recorded as controls. Afterwards, the samples were centrifuged at 30,000*g* for 40 min. (*Beckman Avanti™30 centrifuge, Beckman, Spain*), the insoluble precipitates were separated and the turbidities and pH of the supernatants were determined. The pellet was resuspended in Milli-Q water and then recentrifuged. The washed precipitates were finally freeze-dried and weighted (*HM-202, AND instruments, Oxford, UK*). Analysis of six replicates was conducted.

2.5. Scanning electron microscopy (SEM)

The surface morphology and cross-sections of the devices were examined by SEM (*JSM-640, Tokyo, Japan*). They were coated under an argon atmosphere with gold–palladium (*Sputter coater, Balzers SCD 004, Liechtenstein*) to achieve a film of 20 nm thickness and then observed with a scanning electron microscope (*SEM., JSM-6400, Tokyo, Japan*).

2.6. Determination of insulin content

Insulin content was determined following incubation of the devices in tubes containing 0.5 M HCl for 6 h under

magnetic stirring at room temperature. The supernatants were filtered (*Durapore Millex®-HV, low protein binding, Millipore, Spain*) and the extracted insulin was spectrophotometrically assayed at 277 nm (*Shimadzu RF 5001 PC, Tokyo, Japan*). Blank sponges were also incubated and the supernatants filtered and used as blanks in order to avoid CS interference at this wavelength. All samples were analyzed in triplicate.

2.7. In vitro release studies

The *in vitro* drug release studies were performed at 23 °C using *Franz-Chien* type vertical diffusion cells (*Vidrafoc, Spain*) whose receptor sides were filled with 5.6 ml of phosphate buffer pH 7.4 and maintained under magnetic stirring ($n = 4$–6). Low protein adsorption membranes (*Durapore® Membrane filters 0,45 μm HV, Millipore, Spain*) were placed between the two cells as supports of the delivery devices, and these were situated on top of them on the donor sides. The release studies of insulin were purposely conducted at 23 °C rather than at 37 °C to minimize the insulin self-aggregation tendency (Sluzky, Klibanov, & Langer, 1992). Samples were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered and insulin released assayed by Micro-BCA protein assay at 562 nm. The pH of the release medium as well as the pH inside the polymer matrix after the release process were recorded (*Crison, micropH 2001, Spain*).

2.8. Swelling studies

The water uptake capacity of sponges was determined gravimetrically. Previously weighed CS sponges were incubated in phosphate buffer pH 7.4 at 23 °C, as described before in the in vitro release studies section. After 6 h, the devices were withdrawn, blotted with paper to eliminate excess water and immediately weighed at room temperature on an electronic balance (*HM-202, AND instruments, Oxford, UK*). Then, the sponges were freeze-dried and the weight of the dry devices recorded.

The water uptake was calculated from the relative weight gain according to the following equation:

$$\text{Weight change(mg/mg device)} = (W_6 - W_0)/W_0$$

where W_0 is the initial weight of each sponge and W_6 is the weight of the swollen device after 6 h. Analysis of 3 replicates was conducted.

The percentage of remaining weight of each sponge after 6 h, which is an indication of its solubility, was calculated from:

$$\text{Remaining weight}(\%) = (W_{6F} \times 100)/W_0$$

where W_{6F} is the weight of the sponge after 6 h incubation in the release medium and further freeze-drying and W_0 is the initial weight of each sponge. Analysis of 3 replicates was conducted.

2.9. Preliminary mucoadhesion studies

Mucoadhesive capacity of the sponges was evaluated by applying them to mucin films which were previously obtained by casting aqueous mucin dispersions on Petri dishes and further solvent evaporation at 40 °C. The system was maintained in desiccators at 100% relative humidity at room temperature (20–25 °C) for different time periods. Afterwards, sponges were manually removed from the mucin films and the detachment forces were qualitatively estimated.

2.10. Statistical evaluation

All data are expressed as the mean ± standard deviation (SD). Statistical differences were investigated by a one-way analysis of variance (ANOVA) with the Pairwise Multiple Comparisson Procedures (Student-Newman-Keuls method) for multiple comparisons (SigmaStat program; Jandel Scientific, Version 3.0). Differences were considered to be significant at a level of $P < 0.05$.

3. Results and discussion

The main goal of this work was to develop a mucoadhesive device adequate for buccal peptide administration, insulin being used as model peptide. We designed a bilayered system that consists of a mucoadhesive CS layer containing the peptide drug and an impermeable protective layer made of ethylcellulose and the plasticizer dibutylphthalate (Fig. 2).

The hypothesis behind the design of this system is that it will increase peptide permeation by releasing it at the absorption site for a prolonged period of time owing to the mucoadhesive properties of CS. Indeed, we have recently found that CS glutamate is able to increase the permeability of large hydrophilic compounds across an *in vitro* model of the buccal mucosa (the TR146 cell culture model) at minimally harmful CS concentrations (Portero et al., 2002). Additionally, the bilayered structure is expected to provide unidirectional peptide delivery to the absorptive mucosa and avoid loss of drug with saliva. With this

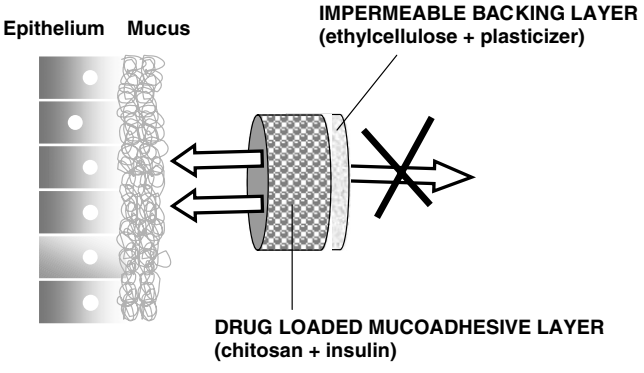

Fig. 2. Structure of the chitosan/ethylcellulose bilayered devices.

idea in mind, we first evaluated the utility of the previously developed bilayered tablets (Remuñán-López et al., 1998b) for insulin formulation and further release. We have already reported that these bilayered tablets have mucoadhesive properties and deliver adequately low M_w drugs in a unidirectional way. However, insulin *in vitro* release from the devices was found to be extremely slow, only 5% of the peptide being delivered after 10 h of study. This poor release behavior was attributed to the limited diffusivity of the high M_w molecules through compact matrixes (Merkle et al., 1991; Remuñán-López et al., 1998a). Therefore, we designed a very porous system, that we have called sponge, and compared the *in vitro* properties of both systems. The sponges were obtained by a very simple and mild casting/freeze-drying technique, in which no heat or vigorous agitation is involved. Flexible and easily manageable CS sponges could be formed without plasticizers. This is very advantageous bearing in mind that the addition of plasticizers would increase the hygroscopicity of the system thereby compromising the stability of the peptide. A critical point while developing new peptide formulations is to assess the stability of the peptide following its incorporation into the device. With this purpose in mind, we extracted the insulin from the sponges with HCl and found that it was possible to recover the total amount (90–100%) of insulin incorporated into the system.

Preliminary studies indicated that insulin release from sponges was significantly faster than from tablets. The noticeable differences found in their release behaviors could be explained by the markedly different internal structure of both devices. SEM pictures revealed that the sponge has a very porous structure (Fig. 3a), which strongly contrasts with the compact non-porous surface of the tablet. When placed in the aqueous medium, the sponge rapidly absorbs water, facilitating insulin diffusion and release. In contrast, the tablet erodes gradually, leading to a slow peptide delivery. Taking into account that the buccal epithelium behaves like a barrier to drug permeation, the porous system was selected as a more appropriate drug delivery system for macromolecules and was further investigated. Furthermore, it is important to mention that the CS (hydrophilic) was easily covered by EC (hydrophobic), and that a perfect binding between the mucoadhesive and the backing layer was achieved as is clearly demonstrated by SEM of sponges cross-sections (Fig. 3b).

In an attempt to control insulin release from the sponges, we investigated a number of variables in the formulation process. The results show that the *in vitro* release properties of the sponges can be easily controlled by simply modifying the formulation variables, such as the pH of the polymer solution prior to freeze-drying (pH 2, 4, 6), the M_w (medium M_w, 150 kDa, and high M_w, 300 kDa), the type of CS salt (glutamate, tartrate and citrate) and the insulin content (1, 1.75, 2.5 mg). The profiles depicted in Fig. 4 indicate that insulin release from the sponges obtained using the pH 2 CS solution was significantly faster

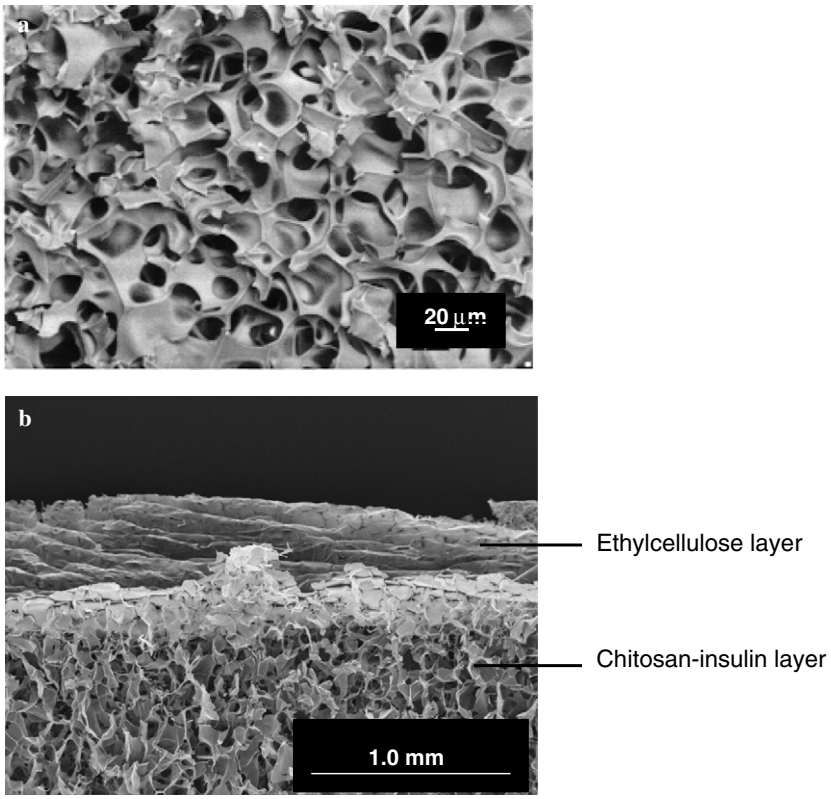

Fig. 3. Scanning electron micrographs of (a) the chitosan surface of a sponge and (b) a cross-section of a bilayered chitosan/ethylcellulose device.

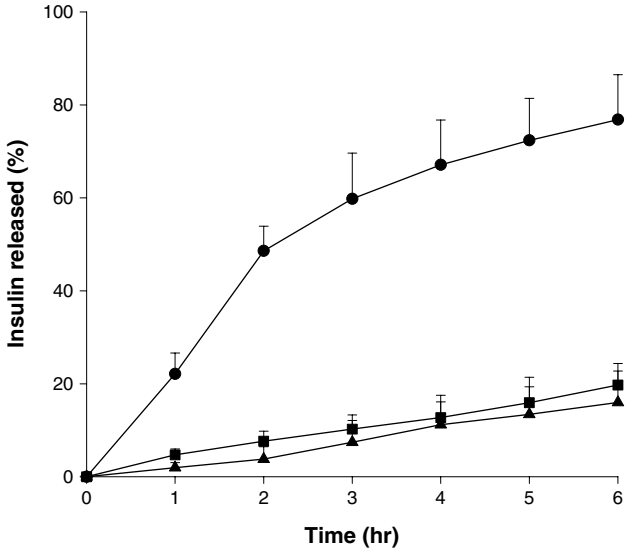

Fig. 4. Effect of the pH of the chitosan-insulin mixture prior to the freeze-drying process on insulin release from the sponges in pH 7.4 phosphate buffer (medium M_w chitosan glutamate; 1 mg insulin): (●) pH 2, (■) pH 4 and (▲) pH 6 (mean ± SD; $n \geqslant 4$).

($P < 0.05$) than from those made using pH 4 and pH 6 solutions. It is important to note that there were no important differences either in the pH of the release medium or in the local internal pH of the CS glutamate sponges made from CS solutions at the different pH values (Table 1). This unexpected behavior could be related to the high volatility of the acid (HCl) used to adjust the pH values of the different CS glutamate solutions, which could have been eliminated during the freeze-drying process. Consequently, the differences in drug release could not be explained by the variations in CS and insulin solubility as a function of the pH inside or outside the sponges.

On the other hand, the viscosity data corresponding to CS glutamate solutions at the different pH values, also depicted in Table 1, show (for the same M_w sponges) a significant ($P < 0.05$) increase of the CS viscosity as the pH was increased from 2 to 6. This increase was less pronounced for pH 6 compared to pH 4 due to the precipitation process suffered by CS at the higher pH. In all cases, this increase in the CS viscosity, related to the pH value, could explain the slower insulin release found for sponges obtained from pH 4 and 6 solutions.

However, it is our hypothesis that the effect of the CS solution pH prior to freeze-drying on drug release could be mainly related to the establishment of electrostatic interactions between peptide and polymer. It is interesting to note that, in the preformulation experiments, we observed that, when insulin was added to the CS solutions at pH 4 and 6, the mixture became cloudy, whereas this phenomenon was not apparent for CS solutions at pH 2. Furthermore, the higher the pH value, the more intense was the turbidity of the CS-insulin mixture. These observations suggest that an electrostatic interaction could occur between insulin and CS upon mixing. In fact, CS is positively charged across the pH range used in this work, whereas the charge on the insulin molecules is pH dependent; insulin has a net positive charge at a pH below its isoelectric point – which is approximately 5.3 – and it has a net negative charge at any pH above it. Therefore, at pH 2 where insulin has a predominantly positive charge, ionic interactions should not be expected. Oppositely, at pH 6, the carboxylic groups of insulin will be ionized (negatively charged) and consequently its ionic interaction with the positively charged amine groups of CS will be favored.

This information was confirmed by measuring the turbidity (absorbance at 420 nm) of CS solutions, insulin solutions and CS-insulin solutions mixtures at those pH values (2, 4 and 6). Results in Fig. 5 show that the turbidity of CS-insulin mixtures gradually increases when raising the pH values of the solution from 2 to 6. In addition, turbidity measurements gave us information about the solubility of both CS and insulin. The absorbance at 420 nm for CS solutions at pH 2 and 4 was similar, but a decrease in turbidity at pH 6 was observed. This behavior can be explained by the fact that, at pH 6, the CS solution begins to precipitate, which leads to a decrease in turbidity. On the contrary, at pH 6, the insulin solution becomes milky, but sedimentation was not appreciable and, consequently, a higher absorbance at 420 nm was found, probably as a result of the increase in pH that could bring insulin to its isoelectric precipitation zone (pH: 4.5–6.5) (Brange & Langkær, 1993). It must be pointed out that the influence

Table 1
Effect of the chitosan type of salt, molecular weight and pH of the polymer solution before freeze-drying on the weight of sponges, viscosity of polymer solutions, and pH inside and outside sponges, swelling capacity and sponge weight remaining after the drug release study (mean ± SD)

Chitosan salt	pH	M_w (kDa)	Sponge weight (mg)[a]	Viscosity (cSt)[b]	pH of release medium[a]	pH of swollen sponge[a]	Swelling (mg water/mg dry device)[a]	Sponge weight remaining (%)[a]
Glutamate	2	350	23.5 (1.16)	260.41 (15.31)	ND	ND	ND	ND
	2	150	23.80 (2.30)	12.22 (0.05)	7.24 (0.04)	6.45 (0.01)	13.26 (0.26)	20.7 (2.27)
	4	150	24.87 (1.56)	20.33 (0.20)	7.29 (0.02)	6.66 (0.01)	14.66 (3.25)	44.50 (8.05)
	6	150	23.52 (0.53)	15.23 (3.39)	7.37 (0.00)	6.40 (0.02)	6.40 (0.49)	62.57 (6.53)
Citrate	2	150	67.92 (3.42)	238.99 (7.53)	7.22 (0.02)	3.08 (0.05)	11.27 (3.61)	35.15 (11.10)
Tartrate	2	150	65.78 (4.21)	205.62 (3.47)	7.18 (0.03)	2.97 (0.08)	10.91 (4.09)	21.10 (5.51)

ND: not determined.
[a] $n = 3$–6.
[b] $n = 9$.

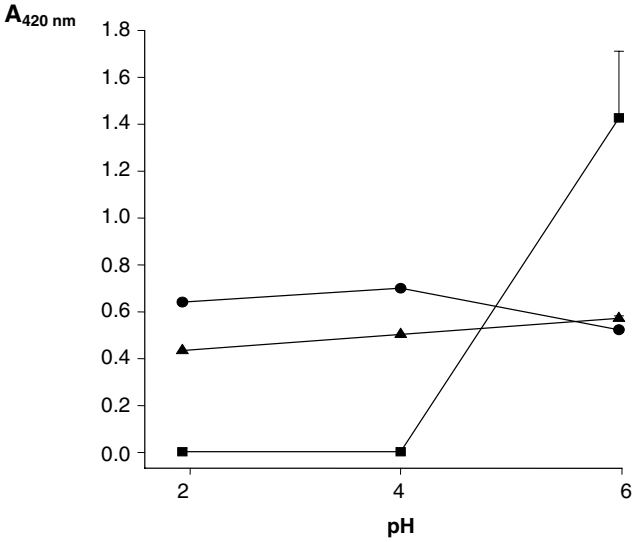

Fig. 5. Effect of the pH of the (●) chitosan solutions, (■) insulin solutions and (▲) chitosan-insulin mixtures on their absorbance values at 420 nm (mean ± SD; $n \geqslant 4$).

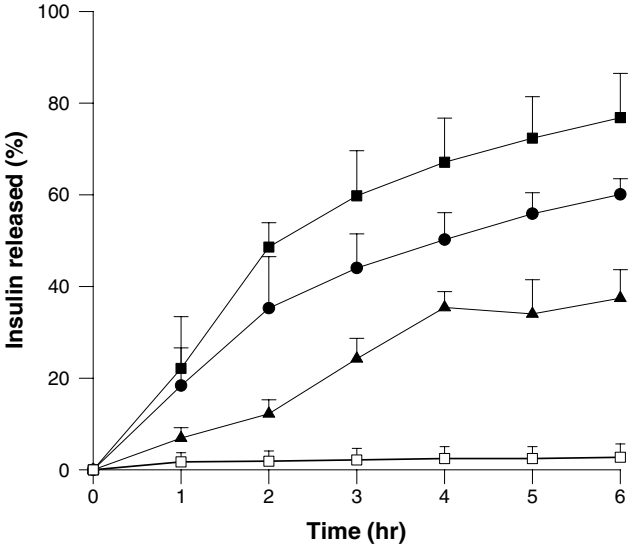

Fig. 6. Effect of the chitosan type of salt and M_w on insulin release from the chitosan sponges in pH 7.4 phosphate buffer (pH 2; 1 mg insulin): (■) medium M_w chitosan glutamate, (●) medium M_w chitosan tartrate, (▲) medium M_w chitosan citrate and (□) high M_w chitosan glutamate (mean ± SD; $n \geqslant 4$).

of pH on turbidity of the CS-insulin mixtures was less pronounced than for each species, individually. This could indicate a stabilizing effect of the polymer, as a result of a probable interaction, against the precipitation/aggregation tendencies of the insulin observed at the higher pH in the absence of CS.

Indeed, there was a clear effect of the initial pH of the CS solution on the percentage of the remaining weight (insoluble fraction of the sponge) of the different devices following incubation in the release medium, which was found to be significantly ($P < 0.05$) higher when the initial pH value was increased (Table 1). In fact, it was observed that after 6 h of incubation in the aqueous release medium, the percentage of remaining weight (insoluble fraction of the sponge) of sponges prepared at pH 2 was around 21% compared to the 45% and 63% observed for the sponges made from pH 4 and 6, respectively. Additionally, the initial pH of the CS-insulin mixtures affected the swelling capacity of the sponges. In this respect, the most noticeable reduction on the swelling properties of CS was found for CS glutamate sponges made at pH 6. It has been previously reported that the type of CS salt has an influence on the solubility and swelling/gelling properties of CS (Chen, Lin, & Yang, 1994; Kienzle-Sterzer, Rodríguez-Sánchez, & Rha, 1982). In fact, we found not only that the swelling properties of the sponges made from the different salts of CS at pH 2 could be ranked: CS glutamate > CS citrate > CS tartrate, but also that for the same CS M_w and initial pH of the CS solution, the viscosity of the polymer solution was a function of the acid used as a solvent (Table 1). Therefore, a strategy to control the peptide delivery from the CS matrixes consists of changing the type of acid used to dissolve the polymer prior to the freeze-drying step in the sponge preparation process. As expected and judging from the results in Fig. 6, the type of acid used to dissolve CS influenced insulin release from the sponges, the release rate ranking being: CS glutamate > CS tartrate > CS citrate. This behavior concurs with the observation that CS glutamate sponges were more soluble than those made of tartrate or citric acid, since less remaining weight was recovered. Furthermore, it was noticeable that CS citrate and tartrate sponges swelled rapidly leading to a very viscous gel-like barrier which hindered the release of the drug. The citrate CS sponges, which were the less soluble ones, exhibited slower peptide release.

Indeed, it was found that upon exposure of the sponges to the release medium, a local variable pH was created inside the sponges, this being dependent on the type of acid used as a solvent of CS, as it is shown in Table 1. This could be a factor to take into account due to the well-known pH-dependent solubility of both CS and insulin (Brange & Langkær, 1993; Rinaudo & Domard, 1989). However, in our opinion, besides the previous arguments, the key factor affecting the insulin release from sponges of different type of salts of CS is the viscosity of the CS gels resulting after hydration of the different CS salts, as it can be seen in Table 1. Note that citric and tartaric acids led to near a 20-fold increase in viscosity, in comparison to the glutamic salt of CS, a fact that greatly influences peptide diffusion through the CS gel. These results agree with those previously reported by Remuñán-López et al. (1998a) and Nigalaye, Adusumilli, and Bolton (1990) who found that CS citrate retarded the release rate of drugs from both microparticles and tablets due to its high viscosity and gel forming capacity. On the other hand, the same type of explanation could be applied to analyze the great influence of CS M_w on insulin release from sponges. As

expected, the lower the CS M_w and, hence, CS viscosity, the faster the peptide diffusion through the swollen polymer. A similar effect has already been described for bovine serum albumin release from a microparticulate system (Remuñán-López et al., 1998a) which was also predictable based on the relationship between M_w and viscosity (Adusumilli & Bolton, 1991).

With respect to the influence of the insulin dose incorporated in the device on the in vitro release results, Fig. 7 shows that low-dose insulin sponges release a significantly higher ($P < 0.05$) percentage of their content than high-dose devices. This could be due to the fact that insulin is closely bound to CS and, as a consequence, the total amount of peptide released is not strictly dependent on the total amount of peptide included in the device, as would be the case if the peptide particles were only physically dispersed in the polymer matrices. In addition, the increase in insulin dose results in a subsequent decrease in the amount of CS on the total device weight. This could lead to limited water penetration into the CS matrix, and thus, to a decrease in the percentage of insulin released. All these in vitro release studies led us to conclude that the sponges designed are versatile in terms of their release properties and that this behavior can be modulated by adequately selecting the formulation parameters.

It is well known that the mucoadhesive properties of CS are mediated by the ionic interaction between its positive amino groups and the negatively charged sialic acid in mucus, which covers the moist surface of the buccal epithelium (Lehr, Bouwstra, Schacht, & Junginger, 1992). For mucoadhesion to occur, an intimate polymer–mucosa contact must take place as a result of a good wetting of the sponge surface with saliva. Afterwards, interpenetration of the chains of the mucoadhesive with those of the mucus must occur. In a preliminary mucoadhesion study, we determined the affinity of the different sponges to the surface of a mucin film. We observed that (data not shown) for sponges made from CS solutions at pH 2, the CS citrate sponges adhered more strongly to the mucin than the tartrate and glutamate ones, the latter being found to overhydrate, loosing its structure. This could be explained by differences in viscosity and swelling properties of the various sponges as we have demonstrated before. For the glutamate salt sponges, the higher the pH, the greater the mucoadhesion. This was also related to the major integrity of sponges made from pH 4 and 6 solutions as was noted before. Unfortunately, it was not possible to quantify the force necessary to separate the mucoadhesive device and the mucin substratum by standardized methods because the polymer–mucin adhesion forces were stronger than those existing within the polymer matrix, hence leading to sponge fracture and, a part of the sponge (CS layer) remaining adhered to the mucin membrane. However, it is evident from this preliminary study that the mucoadhesive capacity of CS sponges to the mucin surface is great and that the intensity of the adhesion is mainly affected by the swelling capacity of the device, as previously demonstrated (Needleman & Smales, 1995; Remuñán-López et al., 1998b). In addition to the permeabilising effect of CS on buccal mucosa (Portero et al., 2002), the demonstrated capacity of these CS sponges to adhere to a mucosal surface and to control insulin delivery by changing several formulation variables highlights the potential of the developed bilayered sponges for buccal peptide delivery.

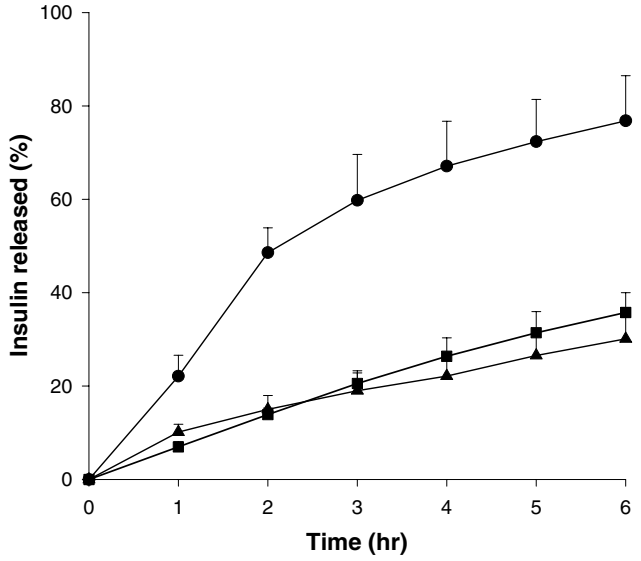

Fig. 7. Effect of drug content on insulin release from the chitosan sponges in pH 7.4 phosphate buffer (medium M_w chitosan glutamate, pH 2): (●) 1 mg, (▲) 1.75 mg and (■) 2.5 mg (mean ± SD; $n \geqslant 4$).

In conclusion, we have designed a bilayered mucoadhesive porous device based on CS, which allows the efficient unidirectional delivery of insulin. When placed in contact with a mucin membrane, the sponge remains strongly adhered to it. The water uptake/swelling and dissolution properties and consequently, the mucoadhesive properties as well as insulin release from the sponges can be modulated by selecting the M_w, type of CS salt and pH of the CS-insulin mixtures prior to freeze-drying. The system developed offers exciting opportunities for the buccal administration of peptide and protein drugs.

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