ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 45 (1998) 49–56

European Journal of Pharmaceutics and Biopharmaceutics

Research article

Development of new chitosan–cellulose multicore microparticles for controlled drug delivery

C. Remuñán-López, M.L. Lorenzo-Lamosa, J.L. Vila-Jato, M.J. Alonso *

University of Santiago de Compostela, Santiago de Compostela, Spain

Received 6 January 1997; accepted 6 May 1997

Abstract

Chitosan (CS) is a very interesting biomaterial for drug delivery; however its use in oral administration is restricted by its fast dissolution in the stomach and limited capacity for controlling the release of drugs. To address this limitation, a new microparticulate CS controlled release system, consisting of hydrophilic CS microcores entrapped in a hydrophobic cellulosic polymer, such as cellulose acetate butyrate (CAB) or ethyl cellulose (EC) was proposed. These microparticles were obtained with different types of CS and various core/coat ratios, with the particle size in all cases being smaller that 70 μm. Using sodium diclofenac (SD) and fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) as model compounds, the properties of these new microparticles for the entrapment and controlled release of drugs and proteins were investigated. Results showed that the entrapment efficiency of SD was very high irrespective of the processing conditions. Furthermore, for both model compounds (SD and FITC-BSA) it was possible to modulate the in vitro release of the encapsulated molecules by changing the core properties (CS salt, M_w, core/coat ratio) or the coating polymer. The microparticles were stable at low pH and thus, suitable for oral delivery without requiring any harmful cross-linkage treatment. © 1998 Elsevier Science B.V.

Keywords: Chitosan; Microencapsulation; Protein delivery; Controlled release; Oral administration; Anti-inflammatory drugs; Cellulose acetate butyrate; Ethyl cellulose

1. Introduction

The use of natural polymers in dosage form design has received considerable attention, especially from the viewpoint of safety. Among these polymers, chitosan (CS), the *N*-deacetylated product of the polysaccharide chitin, is gaining increasing importance in the pharmaceutical field owing to its good biocompatibility, nontoxicity and biodegradability [1,2]. In the early 1980s, CS was proposed as a useful excipient for either sustaining the release of water-soluble drugs [3] or enhancing the bioavailability of poorly water-soluble compounds [4]. More recently, it has also been shown that CS is mucoadhesive [5,6] and enhances the penetration of macromolecules across the intestinal [7] and nasal [6] barriers. These properties have opened promising prospects for the use of this polymer in the oral and nasal administration of proteins and peptides. Furthermore, CS has been presented as a useful polymer for colon-specific drug delivery because of its specific biodegradation by the colonic bacteria [8].

From a technological viewpoint, CS has unique properties which makes it an excellent material for microencapsulation. Due to its hydrophilic and cationic character, CS has the ability to gel upon contact with counter-anions [9,10]. CS has also been demonstrated to possess very good film forming properties [11]. Nev-

* Corresponding author. Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Campus Sur, 15706 Santiago de Compostela, Spain. Tel.: +34 81 594627; fax: +34 81 547148/594595; e-mail: ffmjalon@usc.es

PII S0939-6411(97)00122-7

ertheless, despite the great potential of CS for drug encapsulation, only a few articles on this specific area have been published. Four main approaches have been proposed for the preparation of CS microparticles: (i) ionotropic gelation with an opposite charged polyelectrolyte, such as sodium tripolyphosphate or alginate [9]; (ii) simple or complex coacervation [12,13]; (iii) spray-drying [14] and (iv) solvent evaporation [15]. Independent of the particularities of microparticles produced by these techniques, a common restriction to all of them is their limited capacity for controlling the release of the encapsulated compound and the necessity of a further covalent cross-linking process in order to avoid their rapid dissolution in the gastric cavity. This is due to the free amino groups in the CS molecule which become ionized in acidic media leading to the almost immediate dissolution of the polymer. Chemical crosslinking with aldehydes has been used to overcome this problem [12,13,15]. However, this approach is not adequate for the encapsulation of proteins, peptides and other molecules with amino groups which can also undergo a covalent cross-linkage. Moreover, the toxicity of the aldehydes will significantly limit the exploitation of these cross-linked microcapsules.

In this article we propose a very simple single-step manufacturing process for the preparation of a new multiparticulated CS-controlled release system made of CS microcores entrapped in cellulosic microparticles. When designing this system, we tried to avoid the use of a covalent crosslinking agent as well as to improve the controlled release properties of CS. Two model compounds were selected to evaluate the potential of these new microparticles as oral drug delivery systems: the protein fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) and the anti-inflammatory drug sodium diclofenac (SD). It was expected that these two types of compounds could benefit from the proposed system. It was assumed that both the cellulosic polymer coating and the CS would protect the protein entrapped in the CS cores as it passes through the acidic and enzymatic environment of the stomach, allowing release of the protein during the intestinal transit. The special advantage of the CS particles for anti-inflammatory drugs relies on their ability to weaken the gastro-intestinal side-effects caused by these drugs. This ability is not only related to the prolonged release but also to the well-known antacid and anti-ulcer effects of CS [16,17].

2. Materials and methods

2.1. Materials

The following chemicals were obtained from commercial suppliers and used as received: chitosan glutamate (supplier's specification: viscosity of 1% w/w aqueous solution at 25°C was 20–200 cps for Sea cure G210, high M_w grade), chitosan (supplier's specification: viscosities of 1% w/w aqueous solutions in 1% v/v acetic acid at 25°C were <20 and 20–200 cps and degree of deacetylation was $>80\%$ for Sea cure 123, low M_w grade; and Sea cure 223, high M_w grade) (Pronova, Drammer, Norway); cellulose acetate butyrate (CAB-381-20), cellulose acetate propionate (CAP-482-20) (Eastman, Zug, Switzerland); ethyl cellulose (EC) (Ethocel 10, Dow, Zug, Switzerland); fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA), sodium diclofenac (SD), polyvinyl alcohol (PVA) ($M_w = 30\,000$–$70\,000$) (Sigma, St. Louis, MO); polysorbate 80 (Tween® 80), monohydrated citric acid, acetic acid glacial (Merk, Vorquímica, Vigo, Spain); methylene chloride, ethyl acetate and methanol (Romil, Teknokroma, Barcelona, Spain). Milli-Q (Milli-Q Plus, Millipore Iberica, Madrid, Spain) water was used throughout.

2.2. Preparation of microencapsulated CS microcores

The CS-containing microparticles were prepared by the $W_1/O/W_2$ solvent evaporation technique previously described and conveniently modified [18]. Polysaccharide solutions were prepared by dissolving CS glutamate (5% w/w) in distilled water and CS (1, 3 and 5% w/w) in a diluted acidic solution (acetic acid, citric acid; 0.5 M) at room temperature. The CS solution (400 mg) was dispersed (Branson sonifier® 250, USA) into a solution of the cellulosic polymer (CAB, CAP, EC; 20 mg) in methylene chloride (2 ml) by sonication (sonication time = 10 s, output = 4), and then emulsified in a PVA aqueous solution (100 ml, 1% w/v PVA) under moderate stirring. The system was maintained under agitation (1300 rpm) for 2 h to allow the evaporation of the solvent. Finally, the microparticles were collected, rinsed with water and freeze-dried (-30°C, 24 h; Labcomcon apparatus, Labconco, Kansas City, MI). The microencapsulation process was monitored by optical microscopy (magnification × 1000; Olympus BH-2, Tokyo, Japan).

The active compound (2.5% w/w based on the total amount of polymer) was dissolved (BSA-FITC) or dispersed (SD) in the CS solution, and the process was carried out as previously indicated. Since SD is not soluble in the CS solution, it was first dissolved in methanol (40 μl) and then added to the CS solution, thereby achieving a homogeneous dispersion of the drug.

2.3. Morphological characterization of microparticles

The examination of the surface properties of the microparticles was performed by scanning electron mi-

croscopy (SEM). The particles were freeze-dried, coated with gold palladium to achieve a film of 20 nm thickness (Sputter coater, Balzers SCD 004, Liechtenstein) and observed with a scanning electron microscope (SEM, JSM-6400, Tokyo, Japan).

Particle size analysis was determined on a suspension of microparticles in 0.9% NaCl solution. The samples were analyzed by a Counter Coulter® Multisizer II system (Coulter, Luton, England).

2.4. Determination of the encapsulation efficiency

The drug content in the microparticles was calculated from the difference between the total amount of drug and the amount of drug in the external aqueous phase. The concentration of SD was determined by UV (276 nm) spectroscopy (Schimadzu RF 5001 PC, Kyoto, Japan).

The encapsulation efficiency was calculated from the following expression:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Total amount of drug}} \times 100$$

The concentration of FITC-BSA can be determined by visible spectroscopy ($\lambda = 494$ nm). However, the FITC-BSA encapsulation efficiency could not be determined due to the extraction difficulties of the FITC-BSA from the microparticles.

2.5. Evaluation of SD and FITC-BSA release from the microparticles

In vitro release studies were performed by incubating the microparticles (2 mg for SD and 5 mg for FITC-BSA) in 1 ml isotonic pH 7.4 phosphate buffered saline containing 0.02% w/v polysorbate 80, at 37°C in a horizontal shaker (Heidolf promax 2020, Germany) ($n \geq 3$). At desired times, the microparticles dispersions were centrifuged (0.5, 1.5, 4, 8 and 24 h) (Sigma, model 2-15, Madrid, Spain) for 5 min at 2500 g, and the supernatants filtered (0.2 μm NY filters) (Lida, Kenosha, WI) and assayed for drug release by measuring its absorbance at 276 nm (SD) or 494 nm (FITC-BSA).

3. Results and discussion

The development of drug delivery multicore microparticles, made of a combination of polymers, is receiving increasing attention in the field of microencapsulation. These systems offer significant advantages over the classical one-polymer based microcapsules: (i) by selecting the appropriate core/coating polymer combination it is possible to achieve the encapsulation of hydrophilic and hydrophobic drugs simultaneously; (ii) the active ingredient can be conveniently isolated and protected in the microcores; and (iii) the core material provides the coating polymer with an additional element for controlling the release. In the present study we describe a new drug delivery microparticulate system which consists of microcores made of CS, a hydrophilic swellable polymer, microencapsulated in a water insoluble cellulosic coat. The selection of CS was based on its interesting biopharmaceutical properties in addition to the drawbacks of the CS microparticles developed until now, previously described (introduction). Therefore, it was the aim of this work to present a new approach for the preparation of CS microparticles suitable for oral administration and to evaluate their potential for the encapsulation and controlled release of hydrophobic drugs as well as hydrophilic macromolecules.

3.1. Development of the microencapsulated CS cores

The manufacturing process described in this paper is a single-step procedure based on the $W_1/O/W_2$ emulsion–solvent evaporation technique [18]. To date, this technique has been preferably used for the encapsulation of hydrophilic compounds, mainly antigens and peptides/proteins [19,20]. Therefore, in order to obtain microencapsulated CS microcores, this technique had to be modified accordingly: (i) the inner aqueous phase (W_1) is a very viscous and concentrated solution of CS in which the active ingredient can not only be dissolved, but also dispersed; (ii) the organic phase (O) is a relatively diluted solution of a polymer, the organic solvent and the polymer, both being inert with respect to the CS.

The first part of this work was focused on the search for a hydrophobic polymer and the corresponding organic solvent which would allow the encapsulation of CS. As indicated in Table 1, three cellulose derivatives,

Table 1
Processing variables in the preparation of the CS–cellulose multicore microparticles

Processing variables	Investigated variables
Type of coating polymer	CAB, CAP, EC
Type of organic solvent	MC, EA[a]
Cs concentration	1, 3, 5% w/w
Core/coat ratio	1/5, 1/1.7, 1/1
CS type of salt	Acetate, citrate, glutamate
Cs M_w (viscosity grade)	Low M_w, high M_w

[a] Microparticles were not formed.
CS, chitosan; CAB, cellulose acetate butyrate; CAP, cellulose acetate propionate; EC, ethyl cellulose; MC, methylene chloride; EA, ethyl acetate.

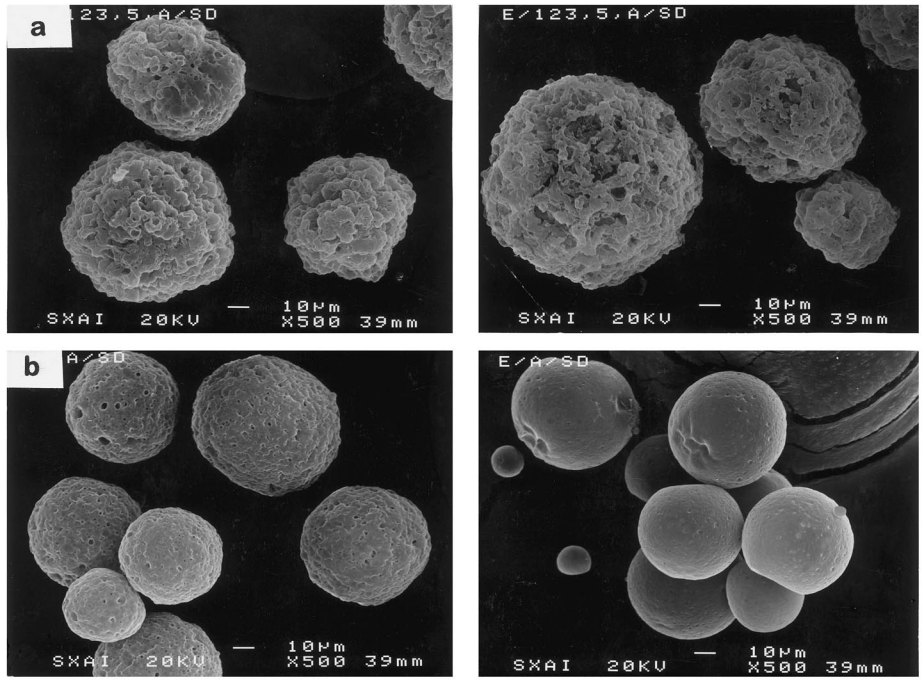

Fig. 1. Scanning electron micrographs of core/coat microparticles and the corresponding control formulations prepared using cellulose acetate butyrate (CAB) and ethylcellulose (EC) as coating polymers. Key: (a) CS/CAB microparticles, (b) control CAB microparticles; (c) CS/EC microparticles and (d) control EC microparticles (core/coat ratio = 1/1; CS = low M_w chitosan acetate).

cellulose acetate butyrate (CAB), cellulose acetate propionate (CAP) and ethyl cellulose (EC), were checked for their ability to coat the CS gels. Two organic solvents typically used in the double emulsion technique, methylene chloride (MC) and ethyl acetate (EA), were selected for the dissolution of the coating polymer. Microscopical (optical microscopy) examination of the samples indicated that, when the organic solvent was MC, the encapsulation of the CS gels occurred properly independent of the coating polymer. In contrast, when EA was used to dissolve the coating polymer, filaments and aggregates of the polymer were obtained but not particles. This could be explained by the rapid diffusion of EA to the inner (W_1) and external (W_2) aqueous phases; a situation that led to the destabilization of the system. Microparticles were obtained for the three polymers tested, however based on the similar properties of CAB and CAP, only EC and CAB were selected as coating polymers for further experiments. In a second step, the feasibility of producing microparticles with different types of CS (M_w and type of salt) and several theoretical core-to-coat (core/coat) ratios was investigated. From these experiments it is interesting to note the following features: (1) It was possible to encapsulate CS gels of various concentrations (1, 3, 5% w/w) leading to the formation of particles with different core/coat ratio (1/5, 1/1.7, 1/1); (2) The encapsulation of CS was achieved independent of the type of acid used to dissolve the CS, and hence the type of salt formed (citrate, acetate), and of the CS M_w grade (low M_w, high M_w).

All the dried CS–cellulosic microparticles prepared using this procedure were quite spherical, free-flowing and non-aggregated. The size of the microparticles varied between 50 and 70 μm and it was not affected by the processing conditions. On the contrary, the appearance of the microparticles clearly varied with the polymer coat and the concentration of CS. Scanning electron micrographs (SEM) shown in Fig. 1 reveal that the control CAB–microparticles, made without CS, are more porous than the corresponding EC-formulations. In addition, for both coating polymers, the entrapment of CS rendered a more irregular and rough surface to the microparticles. The trend observed was that the higher the CS concentration, and hence, the higher the theoretical core/coat ratio, the higher the surface roughness and the higher the irregularity of the microparticles. This observation suggests that these new microparticles consist of CS microcores entrapped in a cellulosic matrix.

3.2. Microencapsulation of sodium diclofenac and FITC–BSA

Two model compounds were selected in order to investigate the suitability of the microencapsulated CS gel cores for the encapsulation and controlled release of bioactive compounds: SD ($M_w = 318$) and FITC–BSA ($M_w = 69\,000$). The effect of the foregoing investigated variables on the encapsulation efficiency and drug/protein release was evaluated.

The encapsulation efficiencies of SD within various microparticles consisting of different CS/CAB ratios, and the control formulation (no CS), are displayed in Fig. 2. It is important to state that the SD entrapment was very high (close to 100%) irrespective of the amount of CS used in the preparation (the amount of CAB was maintained constant). Furthermore, it was found that the encapsulation efficiency was excellent independent of the CS M_w, CS salt and the type of coating polymer (EC vs. CAB).

These results provided evidence for the feasibility of this technique—for the encapsulation of compounds which were not soluble but dispersed in the CS gels. Afterwards, it was important to assess the acceptability of this approach for the encapsulation of hydrophilic macromolecules. As indicated above, the double emulsion technique has been extensively used for the encapsulation of proteins and antigens within PLGA microspheres; however, to our knowledge, there is no report describing the entrapment of proteins within CS particles coated with a cellulosic polymer. In this study, FITC–BSA was selected as a model of hydrophilic macromolecule and dissolved within the CS gels previously to their microencapsulation. Even though it was not possible to determine with accuracy the protein encapsulation efficiency, the micrograph, shown in Fig. 3, demonstrates that cores containing CS and FITC–BSA are entrapped within a CAB microparticle. Furthermore, preliminary SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) studies of FITC–BSA released from microparticles indicated that FITC–BSA did not suffer either aggregation or fragmentation during the preparation process (results not shown).

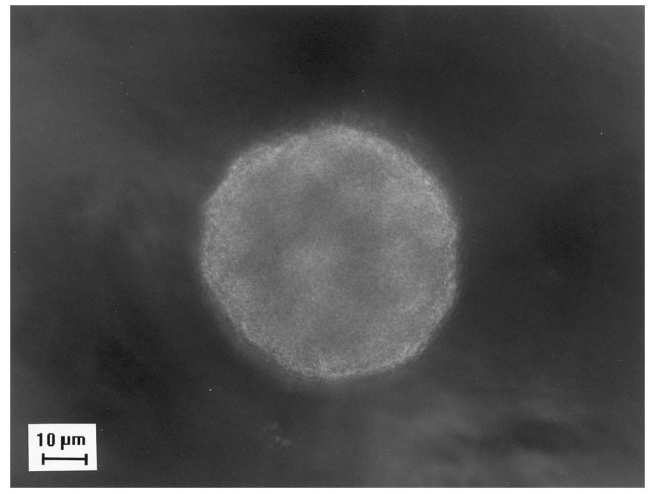

Fig. 3. Optical micrograph of a FITC–BSA loaded CS/CAB microparticle (core/coat = 1/1; CS = low M_w chitosan acetate).

3.3. In vitro release of SD and FITC–BSA

Results in Fig. 4 show the influence of the core/coat ratio on the in vitro release of SD; the higher the amount of core material, the faster the in vitro release rate of SD. This core/coat dependence of the drug release behavior could be logically explained by the hydrophilicity of CS. It was expected that, following their incubation in a buffer medium, the CS core particles entrapped in the cellulosic polymer would absorb water, swell and, consequently, create hydrophilic pathways that should facilitate the release of the drug.

In addition, results in Fig. 5 indicate that the type of acid used to dissolve the CS influence the drug release from CS/CAB micropaticles differently. The CS citrate led to a slower drug release than CS acetate. The explanation to this distinct behavior was found in the differences in the solubilities and swelling/gelling capac-

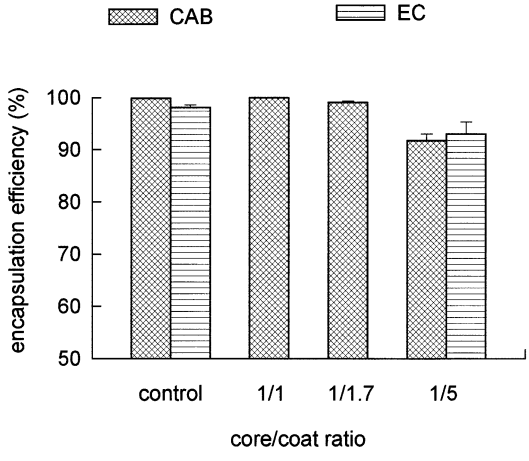

Fig. 2. Effect of the core/coat ratio on the encapsulation efficiency of sodium diclofenac within CS/CAB and CS/EC microparticles (CS = low M_w chitosan acetate) (data shown are the mean ± S.D., $n \geq 3$).

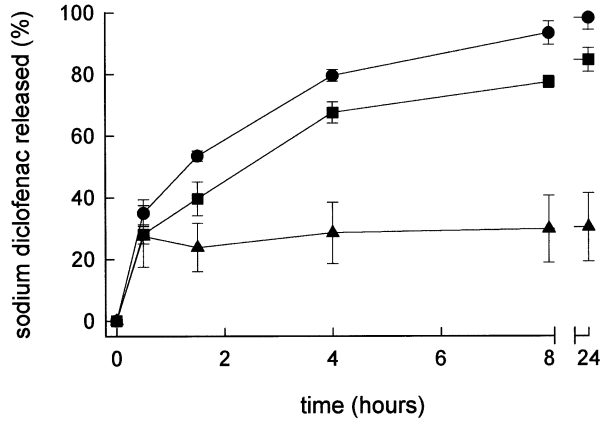

Fig. 4. Effect of the core/coat ratio on the in vitro release of sodium diclofenac from CS/CAB microparticles: (●), 1/1; (■), 1/1.7 and (▲), 1/5 (CS = low M_w chitosan acetate) (data shown are the mean ± S.D., $n \geq 3$).

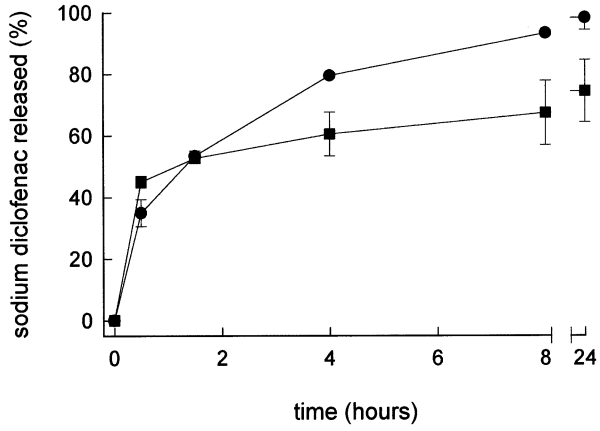

Fig. 5. Effect of the type of chitosan salt on the in vitro release of sodium diclofenac from CS/CAB microparticles: (●), CS acetate and (■), CS citrate (core/coat ratio = 1/1; CS = low M_w chitosan) (data shown are the mean ± S.D., $n \geq 3$).

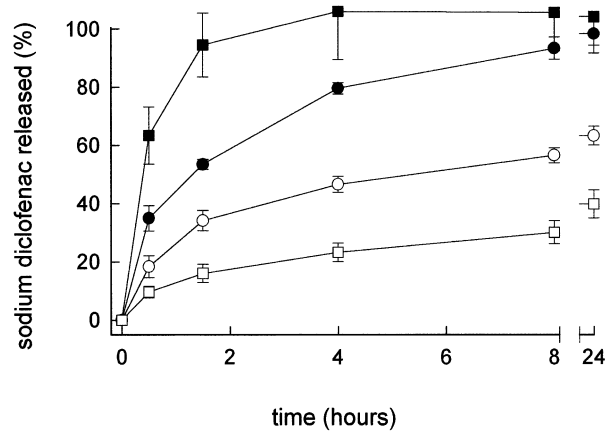

Fig. 6. Effect of the coating polymer on the in vitro release of sodium diclofenac from core/coat microparticles and the corresponding control formulations: (■), CS/EC microparticles; (□), control EC microparticles; (●), CS/CAB microparticles and (○), control CAB microparticles (core/coat ratio = 1/1; CS = low M_w chitosan acetate) (data shown are the mean ± S.D., $n \geq 3$).

ities of the various CS salts [21–23]. In the present study, it was interesting to observe that CS acetate films, prepared by a solvent evaporation method, swelled slowly to a high extent, thus allowing the continuous release of the drug; whereas CS citrate films swelled very rapidly and then formed a very viscous gel-like barrier which hindered the release of the drug. These results agree with those previously reported by Nigalaye et al. [24], who studied the retarding effect of CS–citric acid complexes.

This data permitted us to conclude that, by adequately selecting the CS salt and the core/coat ratio, it was possible to control the release of the encapsulated drug. Other than the factors related to CS, it was expected that a key factor affecting the drug release would be the nature of the coating polymer. The release profiles corresponding to two formulations consisting of low M_w CS acetate microcores coated with CAB or EC, and the corresponding control formulations (no CS) are displayed in Fig. 6. The different release behavior as a function of the coating polymer, could be explained, in part, by the microparticles surface morphology. As shown in Fig. 1, control CAB microparticles were more porous than control EC microparticles, thus, it was logical to conclude that they released the encapsulated drug faster. A drastically different pattern was, however, observed for the CS-containing microparticles: CS/EC microparticles release much faster than CS/CAB. This behavior can be solely attributed to the different efficiency of the coating by the two polymers: EC coats the CS cores less than CAB, thus releasing the encapsulated drug more quickly. In both formulations, the presence of CS creates hydrophilic pathways which would explain the faster release when compared to the corresponding controls.

Once it was demonstrated that the drug release rate could be easily modulated by simply adjusting some formulation variables, the usefulness of this system for controlling the release of the entrapped FITC-BSA was investigated on the CAB formulations prepared with low and high M_w CS and various types of CS salts (acetate, citrate, glutamate) (CS/CAB ratio = 1/1). Release profiles in Fig. 7 indicate that, by selecting the CS M_w and acid used to prepare the gels, it was possible to modulate the protein release rate. As in the case of SD, the distinct behavior of the formulations agrees well with the differences in the swelling/gelling capacities of the different CS cores. In this sense it was found that films made of CS glutamate dissolved much faster than those made of CS acetate and CS citrate. An additional interesting observation from the profiles shown in Fig.

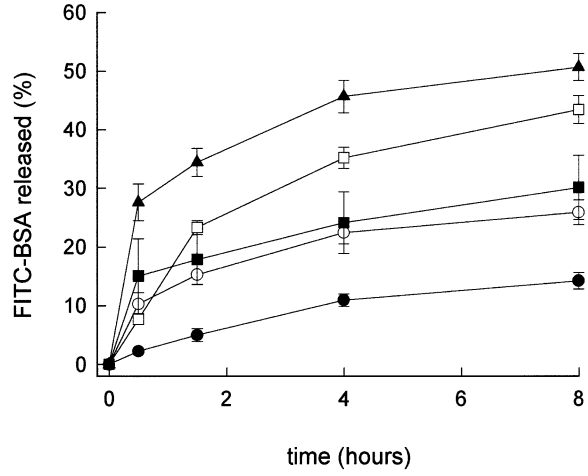

Fig. 7. Effect of the type of salt and M_w of chitosan (CS) on the in vitro release of FITC–BSA from CS/CAB microparticles: (○), low M_w CS acetate; (□), low M_w CS citrate; (●), high M_w CS acetate; (■), high M_w CS citrate and (▲), high M_w CS glutamate (core/coat ratio = 1/1) (data shown are the mean ± S.D., $n \geq 3$).

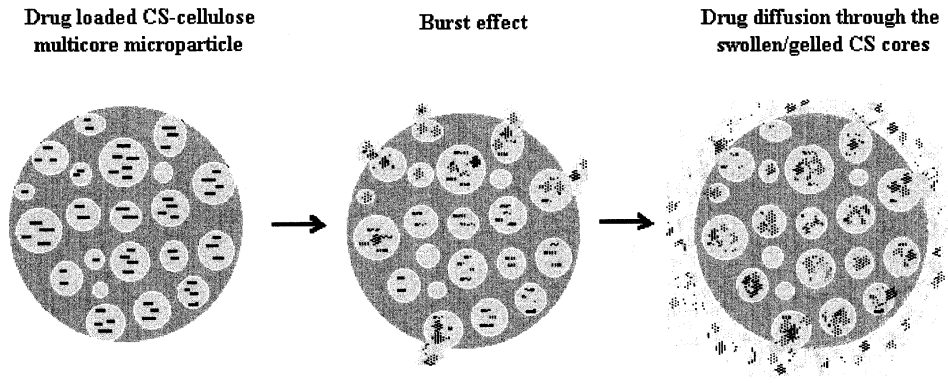

Fig. 8. Theoretical cross-section structure and release mechanisms of drug-containing core/coat microparticles.

7 is that the protein release rate from microparticles prepared with the high M_w CS was slower than from the formulations containing the low M_w CS. This behavior was predictable based on the relationship between M_w and viscosity [25]. Increasing the viscosity of the polymer retarded the diffusion of the drug through the gel into the release medium.

3.4. Microparticles structure and release mechanism

Fig. 8 illustrates the probable drug/protein release mechanisms from the multi-core CS–cellulosic coat systems. The drug release would involve the following processes: (i) water penetration into the microparticles, (ii) CS swelling/gelling and dissolution of the active compound, and (iii) diffusion of the active compound through the CS hydrogels. Therefore, the drug release rate would be controlled by the extent and rate of water absorption/swelling of the CS included within the microparticles, and the rate of diffusion of drug out of the gel, which in turn, will be dependent on the type and amount of CS entrapped. As the CS content in the microparticles increases, the diffusion of the drug/protein out of the microparticles increases as well. The presence of non-totally coated CS cores on the surface of the microparticles would explain the burst effect achieved with a few formulations.

Other factors, such as protein/CS interactions and disintegration of the microparticles did not seem to have a key role in the process. On the other hand, the protein (isoelectric pH = 6) was positively charged, therefore its electrostatic interaction with CS was minimized. The microparticles morphology (as assessed by SEM) did not change significantly following incubation, either in an acidic or neutral medium, further supporting the suitability of these particles for oral administration.

Acknowledgements

This work was supported by grants from the Spanish Commission of Sciences and Technology (CICYT-SAF 94-0579) and XUNTA de Galicia (XUGA 20304A96).

References

[1] J. Knapczyk, L. Krówczynski, J. Krzek, M. Brzeski, E. Nurnberg, D. Schenk, H. Struszcyk, Requirements of chitosan for pharmaceutical and biomedical applications, in: G. Skåk-Bræk, T. Anthonsen, P. Sandford (Eds.), Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications, Elsevier, London, 1989, pp. 657–663.

[2] S. Hirano, H. Seino, I. Akiyama, I. Nonaka, Chitosan: a biocompatible material for oral and intravenous administrations, in: C.G. Gebelein, R.L. Dunn (Eds.), Progress in Biomedical Polymers, Plenum, New York, 1990, pp. 283–289.

[3] W.M. Hou, S. Miyazaki, M. Takada, T. Komai, Sustained release of indomethacin from chitosan granules, Chem. Pharm. Bull. 33 (1985) 3986–3992.

[4] Y. Sawayanagi, N. Nambu, T. Nagai, Enhancement of dissolution properties of griseofulvin from ground mixtures with chitin or chitosan, Chem. Pharm. Bull 30 (1982) 4464–4467.

[5] C.M. Lehr, J.A. Bouwstra, E.H. Schacht, H.E. Junginger, In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers, Int. J. Pharm. 78 (1992) 43–48.

[6] L. Illum, N.F. Farraj, S.S. Davis, Chitosan as a novel nasal delivery system for peptide drugs, Pharm. Res. 11 (1994) 1186–1189.

[7] G. Borchard, H.L. Lueßen, G.A. de Boer, J. Coos Verhoef, C.M. Lehr, H.E. Junginger, The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption. III: Effects of chitosan–glutamate and carbomer on epithelial tight junctions in vitro, J. Control. Release 39 (1996) 131–138.

[8] H. Tozaki, T. Fujita, A. Yamamoto, S. Muranishi, T. Sugiyama, A. Terabe, T. Matsumoto, T. Suzuki, Chitosan capsules for colon specific drug delivery: improvement of insulin absorption from the rat colon, Proc. Int. Symp. Control. Release Bioact. Mater. 23 (1996) 551–552.

[9] R. Bodmeier, K.H. Oh, Y. Pramar, Preparation and evaluation of drug-containing chitosan beads, Drug Dev. Ind. Pharm. 15 (1989) 1475–1494.

[10] P. Calvo, C. Remuñán-López, J.L. Vila-Jato, M.J. Alonso, Novel hydrophilic chitosan–polyethylene oxide nanoparticles as protein carriers, J. Appl. Polym. Sci. 63 (1997) 125–132.

[11] C. Remuñán-López, R. Bodmeier, Mechanical and water vapor transmission properties of polysaccharide films, Drug Dev. Ind. Pharm. 22 (1996) 1201–1209.
[12] A. Berthold, K. Cremer, J. Kreuter, Influence of crosslinking on the acid stability and physicochemical properties of chitosan microspheres, S.T.P. Pharma 6 (1996) 358–364.
[13] C. Remuñan-López, M.J. Alonso, T. Calleja, J.L. Vila-Jato, R. Bodmeier, Development of new chitosan–gelatin microcapsules produced by a complex coacervation process, Proc. Int. Symp. Control. Release Bioact. Mater. 22 (1995) 430–431.
[14] I. Genta, F. Pavanetto, B. Conti, P. Giunchedi, U. Conte, Improvement of dexamethasone dissolution rate from spray-dried chitosan microspheres, S.T.P. Pharma 5 (1995) 202–207.
[15] J. Akbuga, G. Durmaz, Preparation and evaluation of cross-linked chitosan microspheres containing furosemide, Int. J. Pharm. 111 (1994) 217–222.
[16] J. Karlsen, Excipient properties of chitosan, Manufacturing Chemist, June (1991) 18–19.
[17] I.W. Hillyard, J. Doczi, P.B. Kiernan, Antacid and antiulcer properties of the polysaccharide chitosan, Proc. Soc. Exp. Biol. Med. 115 (1964) 1108–1112.
[18] R. Alex, R. Bodmeier, Encapsulation of water-soluble drugs by a modified solvent evaporation method. I. Effect of process and formulation variables on drug encapsulation, J. Microencapsulation 7 (1990) 347–355.
[19] M.J. Alonso, S. Cohen, T.G. Park, R.J. Gupta, G.R. Siber, R. Langer, Determinants of release rate of tetanus vaccine from polyester microspheres, Pharm. Res. 10 (1993) 945–953.
[20] J. Herrmann, R. Bodmeier, Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on W/*O*/W–multiple emulsions, Int. J. Pharm. 126 (1995) 129–138.
[21] C.A. Kienzle-Sterzer, D. Rodríguez-Sánchez, C. Rha, Mechanical Properties of chitosan films: effect of solvent acid, Makromol. Chem. 183 (1982) 1353–1359.
[22] J. Hosokawa, M. Nishiyama, K. Yoshihara, T. Kubo, A. Terabe, Reaction between chitosan and cellulose on biodegradable composite film formation, Ind. Eng. Chem. Res. 30 (1991) 788–792.
[23] R.H. Chen, J.H. Lin, M.H. Yang, Relationships between the chain flexibilities of chitosan molecules and the physical properties of their casted films, Carbohydr. Polym. 24 (1994) 41–46.
[24] A.G. Nigalaye, P. Adusumilli, S. Bolton, Investigation of prolonged drug release from matrix formulations of chitosan, Drug Dev. Ind. Pharm. 16 (1990) 449–467.
[25] P.S. Adusumilli, S.M. Bolton, Evaluation of chitosan citrate complexes as matrices for controlled release formulations using a 3^2 full factorial design, Drug Dev. Ind. Pharm. 17 (1991) 1931–1945.