Pharmaceutical Research, Vol. 15, No. 9, 1998

Review

Chitosan and Its Use as a Pharmaceutical Excipient

Lisbeth Illum[1,2]

Received March 17, 1998; accepted May 26, 1998

Chitosan has been investigated as an excipient in the pharmaceutical industry, to be used in direct tablet compression, as a tablet disintegrant, for the production of controlled release solid dosage forms or for the improvement of drug dissolution. Chitosan has, compared to traditional excipients, been shown to have superior characteristics and especially flexibility in its use. Furthermore, chitosan has been used for production of controlled release implant systems for delivery of hormones over extended periods of time. Lately, the transmucosal absorption promoting characteristics of chitosan has been exploited especially for nasal and oral delivery of polar drugs to include peptides and proteins and for vaccine delivery. These properties, together with the very safe toxicity profile, makes chitosan an exciting and promising excipient for the pharmaceutical industry for present and future applications.

KEY WORDS: chitosan; pharmaceutical excipient; drug dissolution; controlled release implant systems; promotion of the transmucosal absorption.

INTRODUCTION

Chitosan is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine and can be derived by partial deacetylation of chitin from crustacean shells (Figure 1). It is also naturally present in some microorganisms and fungi such as yeast. The term chitosan is used to describe a series of chitosan polymers with different molecular weights (50 kDa–2000 kDa), viscosity (1% chitosan in 1% acetic acid, <2000 mPaS), and degree of deacetylation (40%–98%). Chitosan is insoluble at neutral and alkaline pH values but forms salts with inorganic and organic acids such as glutamic acid, hydrochloric acid, lactic acid and acetic acid. Upon dissolution, the amine groups of the polymer are protonated and the resultant soluble polysaccharide is positively charged. The most commonly used forms of chitosan salts are chitosan glutamate and chitosan chloride.

Chitosan salts are soluble in water, the solubility being dependent on the degree of deacetylation (and thereby the pKa value of the chitosan) and the pH. Chitosans with a relative low degree of deacetylation (40%) have been found to be soluble up to pH 9, whereas chitosans with a degree of deacetylation of about 85% have been found to be soluble only up to a pH of 6.5. The solubility of chitosans is also greatly influenced by the addition of salt to the solution. The higher the ionic strength the lower the solubility. The viscosity of a chitosan solution increases in viscosity with increase in chitosan concentration and decrease in temperature. The viscosity also increases with increasing degree of deacetylation. This is due to the different conformations of the molecule for high and low deacetylated chitosan. At a high degree of deacetylation, where the molecule is highly charged, chitosan has an extended conformation with a more flexible chain, whereas at a lower degree of deacetylation the chitosan molecule adopts a more rod-like shape or coiled shape due to a lower charge (1).

GENERAL INDUSTRIAL USE OF CHITOSAN

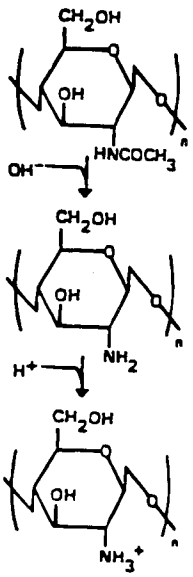
Due to the fact that chitosan has a large molecular weight, exhibits a positive charge, and demonstrates film forming ability and gelation characteristics, the material has been extensively used in the industry, foremost as a flocculant in the clarification of waste water in Japan (2), as a chelating agent for harmful metals for the detoxification of hazardous waste (3), for the clarification of beverages, such as fruit juices and beers (4) and for agricultural purposes such as a fungicide in the protection of crops and the coating of apples. It is also a constituent of many food products, particularly in Japan (5). In addition chitosan has been exploited in the cosmetic industry, the dental industry, for hair care products and for ophthalmic applications, such as for contact lens coatings or as the contact lense material itself (6–9).

In recent years chitosan has been introduced as a material in the nutritional supplement market, especially as a weight loss aid and cholesterol lowering agent. The mechanism behind this apparent effect of chitosan has been suggested to be its effect on the lipid transport mechanism in the gut, wherein free fatty acids (released from consumed fat), cholesterol, bile salts and other components form mixed micelles that comprise an essential step in the fat absorption process. Apparently the positively charged chitosan can bind to free fatty acids and bile salt components and hence disrupt lipid absorption (10).

[1] DanBioSyst UK Ltd, Albert Einstein Centre, Highfields Science Park, Nottingham NG7 2TN, UK.

[2] To whom correspondence should be addressed. (e-mail: enquiry@danbiosyst.co.uk)

0724-8741/98/0900-1326$15.00/0



CHITIN
- Natural structural component of crustacean shells
- Most common natural polymer apart from cellulose
- Biodegradable, non-toxic

CHITOSAN - POLYAMINE
- High molecular weight
- Soluble in dilute acids < pH=6.5
- Polyamine
- Biodegradable, non-toxic

CHITOSAN - CATIONIC
- High positive charge
- Strong film forming properties
- Compatible with cations
- Forms complexes with anions
- Biodegradable, non-toxic

Fig. 1. Chitin and chitosan.

THE USE OF CHITOSAN IN THE PHARMACEUTICAL INDUSTRY

So far as is known chitosan is (or has been) used only in one registered pharmaceutical product; a wound healing product (Tegasorb™) marketed by 3M. However, several other important applications of chitosan as an excipient in pharmaceutical products are now being investigated. These are listed in Table 1. Some of these applications will be discussed in detail below.

Solid Dosage Forms

Tablets

Sawayanagi et al. (11) reported that chitosan had excellent properties as an excipient for direct compression of tablets where the addition of 50% chitosan resulted in rapid disintegration. The degree of deacetylation determined the extent of moisture absorption (11). Chitosan, if added to tablets in a concentration higher than 5%, was superior to corn starch and microcrystalline cellulose as a disintegrant. The efficiency was dependent on chitosan crystallinity, degree of deacetylation, molecular weight and particle size (12). Upadrashta et al. (13) found chitosan to be an excellent tablet binder as compared to other excipients with the rank order correlation for binder efficiency hydroxypropyl methylcellulose > chitosan > methylcellulose > sodium carboxymethyl cellulose.

Table 1. Chitosan as a Pharmaceutical Excipient

Conventional formulations
Direct compression tablets
Controlled release matrix tablets
Wet granulation
Gels
Films
Emulsions
Wetting agent
Coating agent
Microspheres and microcapsules
Novel applications
Bioadhesion
Transmucosal drug transport
Vaccine delivery
DNA delivery

Table 2. Nasal Absorption of Peptide Drugs with Chitosan Powder Delivery Systems

Drug	Bioavalability (F%)
Calcitonin	28%
Goserelin	35%
PTH	39%[a]
Insulin	30%

[a] Rel. to IV injection.

Controlled Release Dosage Forms

Chitosan has been extensively examined in the pharmaceutical industry for its potential in the development of controlled release drug delivery systems. This is due to its unique polymeric cationic character and its gel and film forming properties. Such systems should allow the control of the rate of drug administration and prolong the duration of the therapeutic effect as well as perhaps the targeting of the drug to specific sites. Numerous systems have been described in the literature to include microgranulation systems, sustained release matrices, erodible matrices and controlled release gel systems.

Nagai et al. (14) used chitosan and chitosan derivatives, in combination with other excipients, for the production of tablets with controlled release properties. The rate of release of drug from these tablets was found, to some degree, to be directly related to the amount and type of chitosan used and that a zero order release profile could be so obtained. Most gel-forming polymers provide gels at high pH, hence the potential for chitosan to be used for controlled release in the intestine is obvious. Nigalaye et al. (15) produced theophylline sustained release tablets comprising a hydrocolloidal matrix system containing chitosan, carbomer-934P and citric acid. It was found that when chitosan was used in a concentration of more than 50% of the tablet weight an insoluble non-erosion type matrix was formed; whereas for concentrations less than 33% a fast releasing matrix system was obtained. Chitosan, at a concentration less than 10%, acted as a disintegrant. These results were supported by work by Miyazaki et al. (16), who found that the addition of sodium alginate to the tablet preparation gave the tablets an extended release property. Similar results had been found previously by Kawashima et al. (17), who suggested that citric acid can gel the chitosan and thereby impart the sustained release properties. The carbomer reduces the disintegration property of the chitosan. Akbuga (18) studied the influence of physical-chemical characteristics of drugs on their release characteristics from chitosan maleate matrix tablets and found that the drug solubility, degree of ionisation and the molecular weight of the drug were special factors of importance.

A controlled release system has been produced by film coating theophylline granules with a polyelectrolyte complex

of chitosan and sodium tripolyphosphate (19). The rate of release of drug could be controlled by pH. At low pH values the reduced charge of the anionic tripolyphosphate reduced the electrostatic interaction in the complex and the network in the film loosened.

Gels

Miyazaki et al. (20) investigated the suitability of dried chitosan gels as vehicles for the sustained release of the poorly soluble drugs such as indomethacin and papaverine hydrochloride. The drugs were dispersed in the gel and showed zero order release; with 40% of the indomethacin released into pH 7.4 buffer at 24 hours and 100% papaverine hydrochloride into 0.1N hydrochloric acid in the same time period. Kristl et al. (21) confirmed these results for the release of lidocaine (and its salt) from chitosan hydrocolloids and gels. It was found that the degree of deacetylation and the chitosan content, were important for the observed release properties. The release profiles of the gels followed almost zero order kinetics. Knapczyk (22) prepared gels from 93% and 66% deacetylated chitosans with lactic acid and found that gels prepared from the chitosan, with the highest degree of deacetylation, were more stable in combination with drugs than those prepared with the less deacetylated chitosan.

Enhanced Dissolution

The dissolution of poorly soluble drugs is an important factor for drug absorption. It has been found that grinding of chitosan with poorly soluble drugs, such as griseofulvin or prednisolone, enhances their dissolution properties (23). For low solubility acidic drugs, such as indomethacin, it is possible to exploit the gel forming reaction between the positively charged amino sugar groups of chitosan and the negatively charged drug to increase the solubility of the drug and to control the release. Hou et al. (24) found that granules, formed from chitosan and indomethacin, released the drug faster at pH 7.5 after exposure to acid stomach pH, than if the granules had not been exposed to the low pH. It was thought that this was due to the chitosan swelling and gel formation at this low pH. In comparison, if the granules were crosslinked with glutaraldehyde the swelling and gel forming properties diminished and a sustained release was seen at intestinal pH.

Bioadhesion

The bioadhesive property and the rate of release of a model drug from oral tablets comprising chitosan and sodium hyaluronate has been investigated by Takayama et al. (25). It was found that tablets produced from chitosan alone, were less mucoadhesive than when produced from sodium hyaluronate alone or when using the two polymers as a complex. The release rate of drug was highly dependent upon the weight fraction of chitosan in the tablet, with a constant release rate being obtained between 10 and 60% of chitosan and a rapid increase of release for higher fractions.

Colonic Delivery

Recently, chitosan, in the form of capsules, has been used for the specific delivery of insulin to the colon (26). The chitosan capsules were coated with the enteric coating (hydroxypropyl methylcellulose phthalate) and contained, apart from insulin, various additional absorption enhancers and enzyme inhibitors. It was found that the capsules specifically disintegrated in the colonic region. It was suggested that this disintegration was due to either the lower pH in the ascending colon as compared to the terminal ileum or to the presence of bacterial enzymes which can degrade the chitosan.

Microspheres and Microcapsules

Chitosan microspheres have been widely investigated for use as controlled release delivery systems either for implantation or for oral delivery. Generally, such microspheres are produced, either by an emulsification-crosslinking process or by use of complexation between oppositely charged macromolecules.

Nishioka et al. (27) produced glutaraldehyde crosslinked chitosan microspheres that contained cisplatin. The drug loading efficiency increased markedly with an increasing chitosan and chitin content and the sustained release effect was enhanced with an increase in the chitosan content from 1% to 5% and a chitin content from 0% to 1.5%. Similar microspheres were prepared by Jameela and Jayakrishnan (28) containing mitoxantrone. Drug release was found to be effectively controlled by the degree of crosslinking, with only about 25% of the drug released over 36 days from microspheres with a high degree of crosslinking. The microspheres were not biodegraded in the muscles of rats. Akbuga and Durmaz (29) prepared microspheres containing furosemide from a w/o emulsion system. The microsphere properties were affected by the preparation variables ie type and concentration of chitosan, drug concentration, crosslinking process etc. Microspheres crosslinked by tripolyphosphate have been shown to provide encapsulation of the polypeptide salmon calcitonin and a slow release over 27 days (30). Mi et al. (31) produced chitosan microspheres by two methods involving interfacial acetylation and spray hardening (31). The microspheres contained oxytetracycline and were prepared with chitosan of 70kDa, 700kDa and 2000kDa molecular weight, respectively. The results showed that the higher the molecular weight of the chitosan the more sustained the rate of release of the drug. Similar chitosan microspheres encapsulating insulin were prepared by Aideh et al. (32). The microspheres were crosslinked interfacially by ascorbyl palmitate. The release rate of the drug was dependent on the amount of chitosan in the microspheres and lasted for up to 80 hours. Recently, chitosan microspheres, crosslinked with the polyanion sodium tripolyphosphate and with incorporated polyethyleneoxide-polypropyleneoxide copolymers were suggested for use as carriers of proteins and vaccine for oral delivery. The release of antigen was slow with only 20% of tetanus toxoid being released after 18 days (33). Chitosan microspheres have also been prepared by an emulsification-ionotropic gelation method which basically consists in raising the pH of an emulsion system to render the chitosan insoluble (34).

Oppositely charged polyelectrolytes will interact rapidly in solution to form, normally an insoluble precipitate. This principle has been investigated for the production of chitosan microspheres, hence avoiding the use of crosslinking agents. Polk et al. (35) reacted chitosan with sodium alginate in the presence of calcium chloride and formed microcapsules with

a polyelectrolyte complex membrane. The rate of release of albumin from these microcapsules was dependent on the alginate concentration and the chitosan molecular weight with an increase in these two factors decreasing the rate of release of albumin. A similar principle was used by Remunan-Lopez and Bodmeier (36) who prepared microspheres from chitosan-gelatin coacervates. Recently, Liu et al. (37) prepared porous microspheres by gelling chitosan with sodium alginate followed by freeze-drying. The drug interleukin-2 was incorporated into the preformed microspheres by diffusion from an external aqueous solution of the drug. It was found that the drug was released from the microspheres in a sustained manner and that the drug triggered the induction of cytotoxic T lymphocytes (CTL) more efficiently than free drug due to the slow release of the cytokine.

Wound Healing Products

A scientific basis for the efficacy of chitosan in the promotion of wound healing was first reported in 1978 (38). Chitosan acetate films, which were tough and protective had the advantages of good oxygen permeability, high water absorptivity and slow enzymatic (lysozyme) degradation, thereby avoiding the need for repeated application (9). Malette et al. (39) showed that treating various dog tissues with chitosan solution resulted in the inhibition of fibroplasia with enhanced tissue regeneration. For veterinary wound healing significant progress has been made and the company Sunfive Inc (Japan) has now developed and marketed a chitosan-cotton (Chitopak TM C) and a chitosan suspension (Cbitofine TM S). As mentioned above the 3M company has marketed Tegasorb™ a wound healing product for human use containing chitosan as an excipient.

Absorption Promotion

Illum et al. (40) were the first to show that chitosan is able to promote the transmucosal absorption of small polar molecules and peptide and protein drugs. In the sheep model, they found that the addition of chitosan to a nasal formulation of insulin caused the plasma glucose levels to fall to 43% of base level as compared to 83% of base level without the addition of chitosan. Concurrently, the plasma insulin levels increased from 34 mIU/l to 191 mIU/l and the AUC increased seven fold. Similar results have also been obtained for other small molecular weight drugs that are polar in nature such as morphine and antimigraine drugs and peptides such as calcitonin, desmopressin, goserelin, parathyroid hormone releasing hormone and leuprolide (Table 2). Studies in human volunteers have confirmed the results in sheep (41).

Chitosan can be used as a simple solution formulation with a concentration of 0.5 to 1.0%. Alternatively, the chitosan can be spray dried or formulated into chitosan microspheres. Such powder formulations are superior in providing improved enhancement of drug transport across the membrane as compared to chitosan solutions. Bioavailabilities in the order of 20–40% were obtained with chitosan powders and microspheres in the sheep model for the peptides goserelin, leuprolide and parathyroid hormone. Clinical trials are underway using these systems to confirm the results obtained in the animal model.

In line with the nasal absorption studies Rentel et al. (42) reported that chitosan was able to also enhance the transmucosal absorption of the peptide 9-desglycinamide-8-arginine vasopressin after administration as a solution to intestinal loops of rats. In later studies it was shown that the effect of chitosan on the penetration of mannitol in Caco-2 cells was dependent on the degree of deacetylation in combination with the molecular weight of chitosan (43,44). Recently, it was also reported that a chitosan solution was able to increase fifty fold the intestinal absorption of buserelin (45). Similar absorption enhancing effect has also been demonstrated for the chitosan derivative, N-trimethyl chitosan chloride which was shown to be more soluble and hence easier to formulate into solid oral dosage forms than chitosan itself (45). Studies, with solid dosage forms containing chitosan were less successful due to the slow dissolution of chitosan in powder form (45). Similar results have been obtained by our own group in the rat and pig model for peptides such as insulin and calcitonin (internal communication).

Recently, it has also been demonstrated that chitosan (most likely due to its absorption promoting abilities) is able to act as a material for improving the immunological response of vaccines given via transmucosal routes such as the nasal route. Our group has demonstrated that chitosan in itself does not create a humoral immune response when given either nasally or by injection (Illum et al., unpublished results). Gill et al. (46) have shown that a pertussis vaccine, comprising the antigens filamentus hemaglutinin (FHA) and pertussis toxoid (PT) or comprising the FHA alone, when given nasally in combination with chitosan gave similar plasma IgG levels to an intraperitoneal injection of the antigen and very high secretory IgA levels in nasal washes. This can be compared to no detectable IgA levels in nasal washes for the injection formulation and much lower levels for the nasal formulation without chitosan. An equivalent picture was found for the secretory IgA levels in the lung lavage. A similar highly enhanced immune response was found for influenza vaccine given nasally with chitosan as compared to a subcutaneous injection (47).

The mechanism of action of chitosan in improving transport of drugs across mucosal membranes has been extensively studied by different research groups including our own and is thought to be a combination of bioadhesion and a transient opening of the tight junctions in the cell membrane to allow polar drugs to pass through (41). It has clearly been demonstrated by gamma scintigraphy, that chitosan is mucoadhesive with a nasal clearance times in the order of 25, 40 and 80 minutes for a control solution, a chitosan solution and a chitosan powder, respectively, after application to the nasal cavity of human volunteers (41). It has further been shown in a pulse-chase study that the absorption promoting effect of chitosan on the mucosal membrane is transient, with the effect declining between 30 to 45 minutes after application for a solution formulation (41). The effect of chitosan on a model cell membrane has demonstrated conclusively that chitosan, most likely due to its positive charge, is able to interact with the opening mechanism of the tight junctions as seen by a decrease in ZO-1 proteins and the change in the cytoskeleton protein F-Actin from a filamentous to a globular structure (48,44,49).

REGULATORY AND TOXICOLOGICAL STATUS OF CHITOSAN

Presently chitosan is approved as a food additive in Japan, Italy and Finland. An application for inclusion in the European Pharmacopoeia is being considered and a Drug Master File has

Table 3. Evaluation of Chitosan Toxicity

Caco-2 cells—release of LDH
Frog palate model—mucociliary clearance
Mouse model—immunogenecity
Rat model—nasal histology
Rat model—transport of chitosan across membrane
Rat perfusion model—release of protein, 5'ND, LDH
Guinea pig model—cilia beat frequency, 28 days exposure
Rabbit model—10 day subacute toxicity (solution formulation)
Rabbit model—14 day subacute toxicity (powder formulations)
Human excised turbinates—mucociliary clearance
Human volunteers—mucociliary clearance - saccharine test
Human volunteers—nasal histology on biopsies
Human volunteers—tolerance studies

been filed in the USA. A range of toxicity tests has been performed on chitosan to include tests for effects on cilia beat frequency in guinea pigs after 28 days application (50), effect on mucociliary clearance rates on the frog palate and human nasal tissue (51,52) and effect on nasal membranes in rats (53) (Table 3). In all cases the toxicity was negligible. A ten day subacute toxicity study in rabbits showed neither macroscopic nor microscopic effects on organs or tissues. The mucociliary clearance rate in man, measured by a saccharine clearance test was found to be unaffected after daily nasal application of chitosan (52). The oral toxicity of chitosan has been reported to be 16 g/kg body weight (LD50) (54).

CONCLUSIONS

This review has given an overview of the use of chitosan as an excipient in the pharmaceutical industry, such as for direct tablet compression, as a tablet disintegrant, for the production of controlled release solid dosage forms or for the improvement of drug dissolution. It is evident that when chitosan is compared to traditional excipients for use in certain purposes it appears to have superior characteristics and especially flexibility. Furthermore, chitosan has been found to be of use in production of microspheres and microcapsules which are being investigated for use as controlled release implant systems for delivery of hormones over extended periods of time. Lately, the dramatic property of chitosan, in terms of its transmucosal absorption promoting characteristics has been exploited especially for nasal and oral delivery of polar drugs to include peptides and proteins and for vaccine delivery. These properties, together with the very safe toxicity profile, makes chitosan an exciting and promising excipient for the pharmaceutical industry for present and future applications.

REFERENCES

1. N. Errington, S. E. Harding, K. M. Vårum, and L. Illum. Hydrodynamic characterisation of chitosan varying in molecular weight and degree of acetylation. *Int. J. Biol. Macromol.* **15**:1123–117 (1993).
2. P. A. Sandford and G P Hutchings. Chitosan—A natural cationic biopolymer. In "Industrial Polysaccharides: Genetics Engineering, Structure/Properties Relations and Applications". M. Yalpani (Ed.), Elsevier Science B.V., Amsterdam, pp 363–376 (1987).
3. T. Mitani, C. Nakalima, I. E. Sungkano, and H. Ishii. Effects of ionic strength on the adsorption of heavy metals by swollen chitosan beads. *J. Environ. Sci. Health Part. A. Environ. Sci. Eng. Toxic.* **30**:669–674 (1995).
4. A. G. Imeri and D. Knorr. Effects of chitosan on yield and compositional data of carrot and apple juice. *J. Food Sci.* **53**:1707–1710 (1988).
5. P. Stossel and J. L Leuba. Effect of chitosan, chitin and some aminosugars on growth of various soilborne phytopathogenic fungi. *Phytopathology and Zoology* **111**:82–90 (1984).
6. R. A. A. Muzzarelli. Amphoteric derivatives of chitosan and their biological significance. In "Chitin and Chitosan Sources. Chemistry, Biochemistry, Physical Properties and Applications", G. Skajk-Braek, T. Antonsen, P. Sanford (eds), Elsevier Applied Sciences, London, 1989.
7. P. Gross, E. Konrad, and H. Mager. Patent application DE PS 262714, (1976).
8. J. Dutkiewicz, L. Judkiewicz, A. Papiewski, M. Kucharska, and R. Ciszewski. Some uses of krill chitosan as biomaterial. In "Chitin and Chitosan. Chemistry, Biochemistry, Physical Properties and Applications". G. Skjak-Braek, T. Anthonsen, P. Sandford (eds) Elsevier Applied Sciences, London (1989).
9. G. G. Allan, L. C. Altman, R. E. Bensinger, D. K. Ghosh, Y. Hirabayashi, A. N. Neogi, and S. Neogi. Biomedical application of chitin and chitosan. In "Chitin, Chitosan and Related Enzymes, J. P. Zikakis (ed), Academic Press, Inc., (1984).
10. M. Sugano, T. Fujikawa, Y. Hiratsuji, K. Nakashima, N. Fukuda, and Y. Hasegawa. A novel use of chitosan as a hypocholesterolemic agent in rats. *Am. J. Clin. Nutr.* **33**:787–793 (1980).
11. Y. Sawayanagi, N. Nambu, and T. Nagai. Directly compressed tablets containing chitin or chitosan in addition to lactose or potato starch. *Chem. Pharm. Bull.* **30**:2935–2940 (1982).
12. G. C. Ritthidej, P. Cbomto, S. Pummangura, and P. Menasveta. Chitin and chitosan as disintegrants in paracetamol tablets. *Drug Devel. Ind. Pharm.* **20**:2109–2134 (1994).
13. S. M. Upadrashta, P. R. Katikaneni, and N. O. Nuessle. Chitosan as a tablet binder. *Drug Devel. Ind. Pharm.* **18**:1701–1708 (1992).
14. T. Nagai, Y. Sawayanagi, and N. Nambu. Application of chitin and chitosan to pharmaceutical preparations. In "Chitin, Chitosan and Related Enzymes, J. P. Zikakis (ed), Academic Press, Inc., pp 21–40 (1984).
15. A. G. Nigalaye, P. Adusumilli, and S. Bolton. Investigation of prolonged drug release from matrix formulations of chitosan. *Drug. Devel. Ind. Pharm.* **16**:449–467 (1990).
16. T. Miyazaki, T. Komuro, C. Yomota, and S. Okada. Usage of chitosan as a pharmaceutical material: effectiveness as an additional additives of sodium alginate. *Eisei Shikenjo Hokoku* **108**:95–97 (1990).
17. Y. Kawashima, T. Handa, A. Kasai, H. Takenaka, and S. Y. Lin. The effects of thickness and hardness of the coating film on the drug release rate of theophylline granules coated with chitosan-sodium tripolyphosphate complex. *Chem. Pharm. Bull.* **33**:2469–2474 (1985).
18. J. Akbuga. The effect of physicochemical properties of a drug on its release from chitosan malate tablets. *Int. J. Pharm.* **100**:257–261 (1993).
19. Y. Kawashima, S. Y. Lin, A. Kasai, T. Handa, and H. Takenaka. Preparation of a prolonged release tablet of aspirin with chitosan. *Chem. Pharm. Bull.* **33**:2107–2113 (1985).
20. S. Miyazaki, K. Ishii, and T. Nadai. The use of chitin and chitosan as drug carriers. *Chem. Pharm. Bull.* **29**:3067–3069 (1981).
21. J. Kristl, J. Smid-Korbar, E. Strue, M. Schara, and H. Rupprecht. Hydrocolloids and gels of chitosan as drug carriers. *Int. J. Pharm.* **99**:13–19 (1993).
22. J. Knapczyk. Chitosan hydrogel as a base for semisolid drug forms. *Int. J. Pharm.* **93**:233–237 (1993).
23. Y. Sawayanagi, N. Nambu, and T. Nagai. Enhancement of dissolution properties of griseofulvin from ground mixtures with chitin and chitosan. *Chem. Pharm. Bull.* **30**:4464–4467 (1982).
24. W.-M. Hou, S. Miyazaki, M. Takada, and T. Komai. Sustained release of indomethacin from chitosan granules. *Chem. Pharm. Bull.* **33**:3986–3992 (1985).
25. K. Takayama, M. Hirata, Y. Machida, T. Masada, T. Sannan, and T. Nagai. Effect of interpolymer complex formation on bioadhesive property and drug release phenomenon of compressed tablets

consisting of chitosan and sodium hyaluronate. *Chem. Pharm. Bull.* **38**:1993–1997 (1990).
26. H. Tozaki, J. Komoike, C. Tada, T. Maruyama, A. Terabe, T. Suzuki, A. Yamamoto, and S. Muranishi. Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. *J. Pharm. Sci.* **86**:1016–1021 (1997).
27. Y. Nishioka, S. Kyotani, M. Okamura, M. Miyazaki, K. Okazaki, S. Ohnishi, Y. Yamamoto, and K. Ito. Release characteristics of cisplatin chitosan microspheres and effect of containing chitin. *Chem. Pharm. Bull.* **38**:2871–2873 (1990).
28. S. R. Jameela and A. Jayakrishnan. Glutaraldehyde crosslinked chitosan microspheres as a long acting biodegradable drug delivery vehicle: studies on the in vitro release of mitoxantrone and in vivo degradation of microspheres in rat muscle. *Biomaterials* **16**:769–775 (1995).
29. J. Akbuga and G. Durmaz. Preparation and evaluation of crosslinked chitosan microspheres containing furosemide. *Int. J. Pharm.* **111**:217–222 (1994).
30. Z. Aydin and J. Akbuga. Chitosan beads for the delivery of salmon calcitonin: preparation and release characteristics. *Int. J. Pharm.* **131**:101–103 (1996).
31. F.-L. Mi, T.-B. Wong, and S.-S. Shyu. Sustained-release of oxytetracycline from chitosan microspheres prepared by interfacial acylation and spray hardening methods. *J. Microencapsulation* **14**:577–591 (1997).
32. K. Aiedeh, E. Gianasi, I. Orienti, and V. Zecchi. Chitosan microcapsules as controlled release systems for insulin. *J. Microencapsulation* **14**:567–576 (1997).
33. P. Calvo, C. Remunan-Lopez, J. L. Vila-Jato, and M. J. Alonso. Chitosan and chitosan/ethylene oxide propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. *Pharm. Res.* **14**:1431–1436 (1997).
34. L. Y. Lim, L. S. C. Wan, and P. Y. Thai. Chitosan microspheres prepared by emulsification and ionotropic gelation. *Drug Devel. Ind. Pharm.* **23**:981–985 (1997).
35. A. Polk, B. Amsden, K. De Yao, T. Peng, and F. A. Goosen. Controlled release of albumin from chitosan-alginate microcapsules. *J. Pharm. Sci.* **83**:178–185 (1994).
36. C. Remunan-Lopez and R. Bodmeier. Effect of formulation and process variables on the formation of chitosan-gelatin coacervates. *Int. J. Pharm.* **135**:63–72 (1996).
37. L.-S. Liu, S.-Q. Liu, S. Y. Ng, M. Froix, T. Ohno, and J. Heller. Controlled release of interleukin-2 for tumour immunotherapy using alginate/chitosan porous microspheres. *J. Control. Rel.* **43**:65–74 (1997).
38. L. L. Balassa and J. F. Prudden. Application of chitin and chitosan in wound-healing acceleration. in "Proc. 1st Int. Conf. Chitin/Chitosan", R. A. A. Muzzarelli and E. R. Pariser (eds), MIT Press, Cambridge, MA, USA (1978).
39. W. G. Malette, J. Quigley, and E. D. Adickes. Chitosan effect in vascular surgery, tissue culture and tissue regeneration. In "Chitin in Nature and Technology", R. Muzzarelli, C. Jeuniaux, and G. W. Gooday (eds), Plenum Press, NY (1986).
40. L. Illum, N. F. Farraj, and S. S. Davis. Chitosan as a novel nasal delivery system for peptide drugs. *Pharm. Res.* **11**:1186–1189 (1994).
41. L. Illum. The nasal route for delivery of polypeptides. In "Peptide and Protein Drug Delivery", S. Frøkjær, L. Christrup, and P. Krogsgaard-Larsen (eds.), Munksgaard, Copenhagen (1998).
42. C.-O. Rentel, C.-M. Lehr, J. A. Bouwstra, H. L. Luessen, and H. E. Junginger. Enhanced peptide absorption by the mucoadhesive polymers polycarbophil and chitosan. *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.* **20**:446–447 (1993).
43. N. G. M. Schipper, K. M. Vårum, and P. Artursson. Chitosan as absorption enhancers for poorly absorbed drugs 1: Influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelial Caco-2 cells. *Pharm. Res.* **13**:1668–1692 (1996).
44. N. G. M. Schipper, S. Olsson, J. A. Hoogstraate, A. G. deBoer, K. M. Vårum, and P. Artursson. Chitosan as absorption enhancers for poorly absorbed drugs 2: Mechanism of absorption enhancement. *Pharm. Res.* **14**:923–929 (1997).
45. H. L. Luessen. "Multifunctional polymers for peroral peptide drug absorption", Labor Vincit, Leiden (1996).
46. I. Jabbal-Gill, A. N. Fisher, R. Rappuoli, S. S. Davis, and L. Illum. Stimulation in mice of mucosal and systemic antibody responses against Bordetella pertussis filamentous haemagglutinin and recombinant pertussis toxin after nasal administration with chitosan. *Vaccine* (in press).
47. J. Makin, A. Bacon, M. Roberts, P. J. Sizer, I. Jabbal-Gill, M. Hinchcliffe, L. Illum, and S. Chatfield. Carbohydrate biopolymers enhance antibody response to mucosally delivered vaccine antigens (submitted for publication).
48. P. Artursson, T. Lindmark, S. S. Davis, and L. Illum. Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2). *Pharm. Res.* **11**:1358–1361 (1994).
49. V. Dodane, M. A. Khan, and J. R. Merwin. Effect of chitosan on epithelial permeability and structure. (submitted for publication).
50. T. Aspden, L. Illum, and Ø. Skaugrud. The effect of chronic nasal application of chitosan solution on cilia beat frequency in guinea pigs. *Int. J. Pharm.* **153**:137–146 (1997).
51. T. Aspden, J. Adler, S. S. Davis, Ø. Skaugrud, and L. Illum. Chitosan as a nasal delivery system: Evaluation of the effect of chitosan on mucociliary clearance rate in the frog palate model. *Int. J. Pharm.* **122**:69–78 (1995).
52. T. J. Aspden, J. D. T. Mason, N. Jones, J. Lowe, Ø. Skaugrud, and L. Illum. Chitosan as a nasal delivery system: The effect of chitosan on in vitro and in vivo mucociliary transport rates. *J. Pharm. Sci.* **86**:509–513 (1997).
53. T. Aspden, L. Illum, and Ø. Skaugrud. Chitosan as a nasal delivery system: Evaluation of insulin absorption ednhancement and effect on nasal membrane integrety using rat models. *Eur. J. Pharm. Sci.* **4**:23–31 (1996).
54. K. Arai, T. Kinumaki, and T. Fujita. Toxicity of chitosan. *Bull. Tokai Reg. Fish Lab.* **43**:89–94 (1968).