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Advances and potential applications of chitosan derivatives as mucoadhesive biomaterials in modern drug delivery

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Abstract

Pharmaceutical technologists have been working extensively on various mucoadhesive polymeric systems to create an intimate and prolonged contact at the site of administration. Chitosan is one of the most promising polymers because of its non-toxic, polycationic biocompatible, biodegradable nature, and particularly due to its mucoadhesive and permeation enhancing properties. Due to its potential importance in controlled drug delivery applications, pharmaceutical scientists have exploited this mucoadhesive polymer. However, chitosan suffers from limited solubility at physiological pH and causes presystemic metabolism of drugs in intestinal and gastric fluids in the presence of proteolytic enzymes. These inherent drawbacks of chitosan have been overcome by forming derivatives such as carboxylated, various conjugates, thiolated, and acylated chitosan, thus providing a platform for sustained release formulations at a controlled rate, prolonged residence time, improved patient compliance by reducing dosing frequency, enhanced bioavailability and a significant improvement in therapeutic efficacy. We have explored the potential benefits of these improved chitosan derivatives in modern drug delivery.

Introduction

Extensive work has been done by scientists using mucoadhesive polymers to localize dosage forms in specific regions within the body with a controlled release rate leading to enhanced drug bioavailability and prolonged residence time (Ponchel & Duchene 1992; Lee et al 2000; Edsman & Hagerstom 2005). In the recent years, among the various naturally occurring polymers, chitosan has attracted attention as a potential absorption enhancer and a mucoadhesive agent. The term chitosan, coined in 1894, denotes a copolymer of glucosamine and N-acetyl glucosamine (Hopper-Seyeler 1894; Muzzarelli 1977; Kas 1997; Singla & Chawla 2001; Kato et al 2003). It is derived by alkaline deacetylation of chitin, which is isolated from hard shells of marine living animals (fishes, crustaceans) or synthesized by natural organisms (Sinha et al 2004).

Its favourable characteristics like non-toxicity, polycationic nature, biocompatibility, biodegradability, and permeation enhancing properties render it one of the most promising polymers for various pharmaceutical applications (Chandy & Sharma 1991; Bernkop-Schnurch & Kast 2001; Illum et al 2001; Sinha et al 2004).

Chitosan is a mucoadhesive polymer at physiological pH values due to the presence of OH and NH_2 groups that can give rise to hydrogen bonding. These properties are considered essential for mucoadhesion, causing prolonged residence time of drug in the gastrointestinal tract and thereby improving drug absorption and bioavailability (Leußen et al 1997; He et al 1998; Sinha & Kumaria 2001). These applications of chitosan have been utilized in controlled drug delivery systems (Fiebrig et al 1995; Takeuchi et al 1996; Gavini et al 2005; Shahiwala & Misra 2006).

However, this cationic polymer's inherent drawbacks of insolubility in physiological media and no inhibitory action against proteolytic enzymes present in the gastric and intestinal fluids result in presystemic metabolism and poor bioavailability of drugs, such as polypeptides given non-invasively. This in turn limits its pharmaceutical applications (Bernkop-Schnurch & Kast 2001; Thanou et al 2001).

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Considerable research has been directed towards examining various strategies for eliminating these drawbacks. A promising strategy in this regard is derivatization i.e. synthesizing various derivatives of chitosan such as carboxylated, thiolated, acylated chitosan and conjugates. Chemical modification of chitosan by N-substitution of primary groups of chitosan with moieties having carboxy alkyls have resulted in controlled release formulations with prolonged residence time, improved patient compliance and also enhanced drug absorption at neutral and alkaline pH (Thanou et al 2001; Van der Lubben et al 2001).

Further modification of chitosan at a second position by immobilization of the protease inhibitors like antipain, chymostatin etc. and complexing agents such as EDTA have resulted in chitosan conjugates. These conjugates not only act as enzyme inhibitors, but also display superior swelling properties in neutral and alkaline aqueous media, improved mucoadhesion and controlled release of polymer embedded drugs (Bernkop-Schnurch & Kast 2001).

Another class of chitosan derivatives i.e. thiolated chitosan, have been synthesized by reaction between the primary amino groups of the polycation with coupling reagents. With their increased permeation, mucoadhesion and cohesiveness, these thiomers are also promising tools for of per oral peptide delivery (Bernkop-Schnurch et al 2004b).

Acylated chitosan, such as palmitoyl glycol chitosan, has been shown to exhibit better mucoadhesion and prolonged release and thereby serves as an improved drug carrier (Martin et al 2002, 2003).

Cyclodextrin (CD)-chitosan has displayed characteristics of a potential excipient for a controlled drug delivery system. It has mucoadhesive properties and some inclusion properties from cyclodextrin (Venter et al 2006). Another modification of chitosan, poly (acrylic acid) (PAA)/chitosan polymer complex has also been synthesized. It too exhibits mucoadhesive properties (Ahn et al 2001).

This review explores the potential benefits of these improved chitosan derivatives and their contribution towards pharmaceutical technology and modern drug design. Recent investigations carried out using these novel derivatives have been reported also. Table 1 shows the various chitosan derivatives with mucoadhesive properties and their potential applications.

Quaternized chitosan

N-Trimethyl chitosan (TMC)

A partially quaternized and well soluble derivative of chitosan, TMC was synthesized by reductive methylation of chitosan with indomethane for a duration of 60 min in the presence of a strong base at 60°C. The quaternized polymer was dissolved in water causing the counterion (I^-) to be exchanged with Cl^+, followed by addition of HCl in methanol (Figure 1) (Domard et al 1986; Kotze et al 1997).

TMC possesses permeation-enhancing properties in neutral and basic environments, unlike chitosan. This results from the presence of methyl-substituted primary amine along with the absence of hydrogen bonds between the amine and hydroxyl groups of chitosan (Thanou et al 2001). This positively-charged polymer is reported to have mucoadhesive properties, a key element for the effective absorption enhancement of drugs by prolonging the residence time at the mucosal surfaces. It was observed that there was a decrease in mucoadhesivity with an increase in the degree of quaternization of TMC. This was because of conformational changes in the original polymeric chain structure resulting in decreased flexibility of TMC, thereby influencing the polymer interpenetration into the mucus. However, TMC possessing a 22–48% degree of quaternization retained mucoadhesivity sufficient for manufacture of mucoadhesive dosage forms (Kotze et al 1998; Snyman et al 2003; Van der Merwe et al 2004).

The cytotoxicity of the quaternized polymer was evaluated wherein absence of tissue damaging effects were observed. Thus TMC is a safe polymer for use as an absorption enhancer along with mucoadhesive properties for transmucosal drug delivery (Thanou et al 1999).

Carboxylated chitosan

Carboxymethyl chitosan (CMCh)

Polyampholytic (zwitterionic) polymer CMCh having a carboxyl moiety at the primary amino group of chitosan has been synthesized (Figure 2). The synthesis involved addition of glyoxylic acid to aqueous acetic acid solution of chitosan (0.7%v/v). After stirring, 1 M NaOH was added to adjust the pH to 4.5. The imine formed was reduced in the presence of sodium borohydride. The polymer was precipitated with excess of ethanol and then filtered (Muzzarelli et al 1982; Thanou et al 2001; Colo et al 2004).

CMCh is compatible with anionic macromolecules whereas chitosan and TMC form complexes with polyanions and precipitate out of the solution. It is a potent enhancer of intestinal low molecular weight heparin (LMWH) absorption in rats. The absence of cytotoxicity has confirmed the safety of CMCh polymers (Thanou et al 2001; Van der Lubben et al 2001).

Ocular delivery of ofloxacin via ophthalmic solutions containing polymer N-carboxymethyl chitosan (CMCh) and chitosan hydrochloride (Ch-HCl) was studied for non-invasive treatment of endophthalmic infections. Chitosan has excellent ocular compatibility (Felt et al 1999). The addition of chitosan, especially CMCh, to ofloxacin eye drops for improving transcorneal antibiotic was assessed. Chitosan precipitated after instillation at physiological pH of the tear fluid. Polycationic derivative of chitosan TMC showed permeation enhancing effect. However, CMCh, a polyanion at pH 7.4 failed to enhance the transcorneal penetration rate. It resulted in an increased residence time at a concentration higher than the MIC_{90} at the absorption site, and thereby enhanced the bioavailability of ofloxacin in the aqueous humour. This was due to a viscosity increasing effect, ability to bind the drug and its mucoadhesive properties of the polymer with

Table 1 Various mucoadhesive chitosan derivatives with potential advantages

S. no.	Derivatives	Advantages	Drug	Drug delivery system	References
1	*Quaternised chitosan*				
	N-Trimethyl chitosan	Good mucoadhesion Controlled release	(No specific drug candidate reported)	Oral	Thanou et al (2001), Kotze et al (1998), Snyman et al (2003)
2	*Carboxylated chitosan*				
	N-Carboxymethyl chitosan	Enhanced bioavailability Prolonged pre-corneal residence time Better patient compliance Good mucoadhesion	Ofloxacin	Ocular	Colo et al (2004)
3	*Conjugates*				
	A. Enzyme inhibitors				
	Chitosan–antipain conjugates	Protection from enzymatic degradation A controlled release over a period of 6 h Good mucoadhesion	Insulin	Oral	Bernkop-Schnurch et al (1997)
	B. Complexing agents				
	Chitosan–conjugates EDTA	Excellent mucoadhesion Protection from enzymatic degradation	(No specific drug candidate reported)	Oral	Bernkop-Schnurch & Krajicek (1998)
		Increased stability	Insulin	Oral	Bernkop-Schnurch & Pasta (1998)
	C. Combination of complexing and enzyme inhibitors				
	Chitosan–EDTA–BBI conjugates	Stronger mucoadhesion Protection from enzymatic degradation	(No specific drug candidate reported)	Oral	Bernkop-Schnurch & Pasta (1998), Bernkop-Schnurch & Scerbe-Saiko (1998)
4	*Thiolated chitosan*				
	Chitosan–TGA conjugates	Enhanced mucoadhesion Prolong the residence time Controlled release	Clotrimazole	Vaginal	Kast & Bernkop-Schnurch (2001), Kast et al (2002)
	Chitosan–cysteine conjugates	Enhanced mucoadhesion	—	—	Leitner et al (2003)
	Chitosan–4-TBA conjugates	Enhanced mucoadhesion Improved bioavailability Possesses in-situ gelling properties at physiological pH	Insulin	Oral	Martin et al (2002a), Bernkop-Schnurch et al (2003, 2004a)
		Inhibition of enzymatic degradation	Cefadroxil, glutathione, insulin	Oral	Krauland et al (2004), Roldo et al (2004)
5	*Acylated chitosan*				
	Palmitoyl glycol chitosan	Controlled release Good mucoadhesion	FITC-dextran (MW 4400)	Buccal	Martin et al (2002b)
		Sustained release	Denbufylline	Buccal	Martin et al (2003)
6	*Miscellaneous*				
	Chitosan glyceryl monooleate	Sustained drug delivery and targeting Enhanced mucoadhesion	Lidocaine HCl, ketoprofen, dexamethasone	Oral/ parenteral	Ganguly & Dash (2004)
	Methyl pyrrolidinone chitosan	Good mucoadhesion Enhanced permeation	Aciclovir	Buccal, vaginal	Sandri et al (2004)
	Cyclodextrin (CD)-chitosan	Good mucoadhesion Controlled release	—	Oral	Venter et al (2006)
	PAA/chitosan polymer complex	Good mucoadhesion	—		Ahn et al (2001)

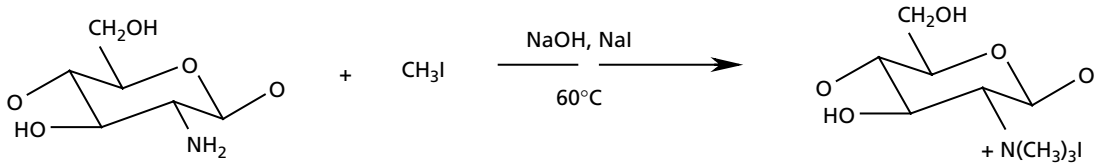

Figure 1 Synthesis of N-trimethyl chitosan chloride (TMC). (Reprinted from Thanou et al (2001), with permission from Elsevier Science Ltd.)

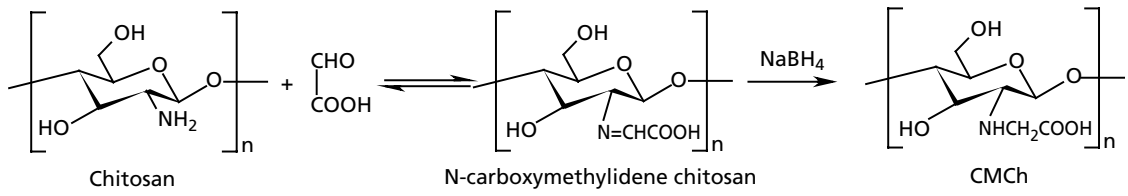

Figure 2 Synthesis of carboxy methyl chitosan. (Reprinted from Thanou et al (2001), with permission from Elsevier Science Ltd.)

respect to control vehicle (commercial eye drops). A zero-order ofloxacin absorption was showing a time-constant effective antibiotic level in the aqueous humour, thus concluding that CMch had the potential to bind to cationic drugs, prolong the precorneal residence of the antibiotic at effective antimicrobial concentrations and thus cause a reduction in installation frequency (Colo et al 2004; Felt et al 2001). No cytotoxicity has been reported with CMCh (Thanou et al 2001).

Conjugates

Chitosan does not inhibit the action of proteolytic enzymes present in the gastric and intestinal fluids and those present locally on the mucosal tissue, causing presystemic metabolism of drugs such as polypeptides given non-invasively, which in turn results in their poor bioavailability (Bernkop-Schnurch & Kast 2001).

The use of modified chitosan as an enzyme inhibitor allowed drug release of polypeptides to be carefully tailored against secreted proteases (trypsin, chymotrypsin elastase, carboxypeptidase A and B) and membrane bound peptidases (endopeptidase, aminopeptidase and carboxypeptidase). Covalent attachment of enzyme inhibitors and/or complexing agents at the primary amino group of the polymer was observed to be the key factor required to achieve the desired result. Covalent immobilization of enzyme inhibitors to the polymer was performed because if these inhibitors were co-administered they were strongly diluted in the intestinal fluid, and thus large amounts of these auxiliary agents were required, which would ultimately cause systemic toxicity.

Thus by the use of immobilized protease inhibitors and complexing agents, chemically-modified chitosan could be developed into an optimal drug delivery system, with improved bioavailability of non-invasively administered peptide drugs (Bernkop-Schnurch & Pasta 1998).

Enzyme inhibitors

The enzymatic barriers found mainly in gastric and intestinal fluids (secreted proteases) and on the surface of various mucosal tissues (membrane bound peptidase) caused presystemic metabolism of polypeptides. The protective effect against the enzymes required only a modified polymer in the delivery system (Bernkop-Schnurch et al 1997). Chitosan conjugates with protease inhibitors like antipain, and Bowman-Birk could inhibit these enzymes through competitive inhibition. These competitive protease inhibitors had at least one carbonic moiety, but not at their active site which reacted with the primary amino group of chitosan, resulting in formation of amide bonds. This transformed carbodiamide into a non-toxic urea derivative formed via a coupling reaction (Bernkop-Schnurch & Kast 2001).

Chitosan–antipain conjugates. Bernkop-Schnurch et al (1997) prepared conjugates by covalently crosslinking the mucoadhesive polymer chitosan to antipain. They observed that insulin tablets prepared using chitosan–antipain conjugates (5% polymer conjugate) shielded the polypeptide from trypsinic degradation more significantly and showed minor proteolysis of tablets as compared with unmodified polymer tablets. The protective effect against trypsin was achieved without affecting the mucoadhesive nature of chitosan and the tablets exhibited a controlled release of the drug over a period of 6 h.

Also, when conjugates with 40:1, 20:1, 10:1 chitosan to inhibitor ratio were taken, it was observed that conjugates containing a higher proportion of inhibitor exhibited a stronger inhibitory effect. However, with increase in covalently-attached inhibitors, mucoadhesion and absorption enhancing properties of the polymer decreased due to reduction in amount of primary amino groups (Bernkop-Schnurch & Scerbe-Saiko 1998).

Complexing agents

Various zinc dependent peptidases like membrane-bound enzymes and proteases and carboxypeptidase A and B exist in the body. Zinc dependent peptidases can be inhibited by the use of complexing agents which deprive the enzyme of Zn^{2+} ion, the essential cation of the enzyme structure.

Various complexing agents such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), and diethylenetriamine pentacetic acid (DTPA) are immobilized on the drug carrier matrices to concentrate the conjugate at the enzyme inhibition site, as the inhibitory effect of these complexing agents is strongly reduced due to dilution in the body fluids.

Chitosan–EDTA conjugates. These conjugates were synthesized by the reaction between amine moieties of chitosan and the carboxyl group of EDTA, which resulted in the formation of amide bonds (Bernkop-Schnurch & Krajicek 1998). The amide bonds caused the immobilization of EDTA on native polymer. Excellent mucoadhesion was observed with chitosan–EDTA conjugates in the ratio 1:20 and 1:40. The enhanced mucoadhesion of the conjugate was attributed to its polyanionic nature in contrast to the polycationic nature of chitosan (Bernkop-Schnurch & Krajicek 1998). The scientists observed that the result was in good accordance with earlier investigations in the literature regarding binding affinity of polymers to mucin surfaces, the results of which had also suggested that a polyanionic polymer was more mucoadhesive and hence preferred over polycationic polymer. It was observed further that the conjugates exhibited a strong inhibitory effect on enzymatic activity of carboxypeptidase A and aminopeptidase. This in turn led to a reduced enzymatic degradation of orally administered proteinaceous drugs (Bernkop-Schnurch & Krajicek 1998). Chitosan–EDTA conjugates were also found to increase the paracellular transport of drugs across biological membranes by affecting the permeability of the tight junctions connecting cells as a consequence of the removal of luminal calcium.

The potential of mucoadhesive chitosan–EDTA conjugate tablets of insulin for oral administration were studied by observing the effects of different methods for drying and ionic crosslinkers on release profile, mucoadhesive strength and cohesiveness of polymers. Formulations embedded with precipitated and air-dried chitosan ionically crosslinked by diamino octane/L-lysine showed better adhesive strength and stability in comparison with lyophilized polymer. Ionic crosslinkers were found to increase the stability, though a significant reduction in bioadhesion of matrix system was observed (Bernkop-Schnurch et al 1998).

Combination of complexing and enzyme inhibitors

Chitosan–EDTA–Bowman-Birk inhibitor (BBI) conjugate. Bioadhesive BBI conjugate containing polypeptide by covalently linking BBI to chitosan–EDTA was prepared and studied. The bioadhesion of polymer–EDTA–BBI conjugates and its inhibitory effects against pancreatic serine proteases and exopeptidases (carboxypeptidase and aminopeptidase N) were compared with unmodified chitosan–EDTA. The bioadhesion of polymer–EDTA–BBI conjugates was found to be stronger as compared with that of unmodified chitosan–EDTA. It also had strong inhibitory activity against serine proteases. The activity was, however, less against elastase. Remarkable protection against exopeptidases due to higher binding attraction of conjugate towards zinc was indicated. It was concluded that polymer–EDTA–BBI conjugates showed good inhibitory action against proteases and were useful as drug carriers (Bernkop-Schnurch & Pasta 1998).

Chitosan–EDTA–protease–inhibitor conjugates

These were formulated for oral delivery of peptides and proteins. In-vivo studies have demonstrated that these conjugates significantly increased the bioavailability of therapeutic peptides. Protection of peptides from degradation by secreted and membrane bound proteases was examined. Due to high binding affinity towards Zn^{2+}, the conjugate inhibited exopeptidases, carboxypeptidase A, B and aminopeptidase N along with serine protease. In-vitro studies showed mucoadhesion of the conjugate to be higher than that of unmodified chitosan. It was thus concluded that polymer–EDTA conjugates were mucoadhesive in nature and could surpass the enzymatic barrier to administer polypeptides orally (Bernkop-Schnurch & Scerbe-Saiko 1998).

Thiolated chitosan

Thiolated chitosan is formed by the immobilization of thiol bearing moieties on the polymeric backbone of chitosan via the formation of amide or amidine groups. Due to the immobilization of thiol groups, various properties of chitosan are improved. The three types of thiolated chitosans synthesized so far include: chitosan–cysteine conjugates, chitosan–thioglycolic acid and chitosan–4-thio-butyl-amidine conjugates.

Thiolated chitosan derivatives have higher and improved mucoadhesion as compared with chitosan. This is because of formation of covalent bonds between thiol groups of the polymer and glycoproteins of the mucus layer (Snyder et al 1983; Bernkop-Schnurch et al 1999). These covalent bonds are stronger than non-covalent bonds, such as ionic interactions of chitosan with anionic substructures of the mucous membrane. By the formation of disulfide bonds with mucus glycoproteins, the mucoadhesive properties of thiomers are up to 130-fold improved compared with the corresponding unmodified polymers. Besides forming disulfide bonds with mucous membrane, the derivative also forms inter- as well as intra-molecular disulfide bonds. Such a cross-linking of the polymer chains results in a higher stability of the drug carrier systems based on thiolated chitosan. The derivative also possesses excellent cohesive property and permeation enhancing effect. The improved permeation of the derivative is also attributed to the immobilization of the thiol groups.

With their increased stability, mucoadhesion, permeation and cohesiveness, these thiomers are promising tools for controlled drug release dosage forms, especially for per oral peptide delivery (Bernkop-Schnurch & Kast 2001; Bernkop-Schnurch et al 2004b).

A controlled drug release with an enhanced mucoadhesion can also be achieved by formulating microparticles based on thiolated chitosan. These microparticles do not disintegrate and are strongly stabilized due to the formation of disulfide bonds within the polymeric network. The microparticles also exhibit improved mucoadhesion due to the immobilization of thiol groups on the chitosan backbone. This is in contrast to chitosan microparticles, which disintegrate rapidly unless combined with multivalent anionic stabilizers such as sodium sulfate, the addition of which in turn reduces the mucoadhesive properties of the polymer.

Besides the above advantages, thiolated chitosan also possesses in-situ gelling properties due to the oxidation of thiol groups at physiological pH values (5–6.8), which results in the formation of inter and intra-molecular disulfide bonds. Thiolated chitosan derivatives therefore seem to be promising excipients for liquid or semi-solid formulations, which should stabilize, once applied on the site of drug delivery. Further, the in-situ gel formation within the pH range 5–6.8 makes the application of thiolated chitosan on vaginal, nasal and ocular mucosa possible.

The potential of thiolated chitosans for the oral administration of hydrophilic macromolecules has also been shown by various in-vivo studies (Bernkop-Schnurch et al 2004c). A pharmacological efficacy of 1% was achieved in rats after oral administration of calcitonin tablets comprising a thiomer. In another study, a pharmacological efficacy of 7% was observed after oral application of tablets comprising of thiomer and pegylated insulin to diabetic mice. Low-molecular-weight heparin embedded in thiolated polycarbophil led to an absolute bioavailability of $\geq 20\%$ after oral administration to rats. In all the above studies, formulations comprising the corresponding unmodified polymer had only a marginal or no effect. These results indicated the potential of thiomers to be used as a promising tool for oral delivery of hydrophilic macromolecular drugs (Bernkop-Schnurch 2004b,c).

Types of thiolated chitosan derivatives

Chitosan–thioglycolic acid conjugates (TGA). A 5–10 fold increase in mucoadhesion in comparison with unmodified chitosan was achieved when TGA was synthesized. Potential application of chitosan–TGA conjugates for developing mucoadhesive systems was investigated. Tensile studies conducted on porcine intestinal mucosa using tablets containing chitosan–TGA conjugates indicated an increased total work of adhesion in comparison with that of unmodified polymer. These thiomers were also observed to prolong the residence time (Kast & Bernkop-Schnurch 2001).

In another study, chitosan–TGA conjugates A and B, containing clotrimazole, were synthesized. The synthesis involved the use of a carbodiimide to covalently link the carboxylic acid moieties of TGA and the primary amino groups of chitosan. Chitosan–TGA conjugate A and chitosan–TGA conjugate B were synthesized by varying the amount of carbodiimide. Conjugate A and conjugate B contained 160 and 280 mM thiol groups per gram of the polymer, respectively. The prepared polymers were characterized by water uptake, disintegration behaviour and bioadhesive properties by using the rotating cylinder method. After the addition of the drug, a 1.6-fold and 100-fold increase in the disintegration time was observed for conjugate A and conjugate B, respectively. Besides providing a controlled release of the drug clotrimazole, the new conjugates also exhibited improved adhesion to the vaginal mucosa. The controlled release of the drug was attributed to the presence of immobilized thiol groups in the conjugates. Based on the results, it was concluded that chitosan–TGA conjugates were promising excipients for the delivery of clotrimazole in the treatment of vaginal infections (Kast et al 2002).

Chitosan–cysteine conjugates (CC). Various studies have shown that cysteine conjugates show mucoadhesive properties (Bernkop-Schnurch et al 2003; Leitner et al 2003). The uptake of fluorescence-labelled bacitracin was improved 1.6-fold when 0.5% of the conjugate was used instead of unmodified chitosan (Bernkop-Schnurch et al 2004b). The conjugate has also been reported to enhance the permeation of hydrophilic compounds and drugs based on the results of in-vitro techniques. The addition of glutathione (GSH), a permeation mediator, further improved this effect. This association is termed thiomer/GSH system, which represents a promising new generation of oral permeation enhancing delivery systems for hydrophilic macromolecules due to their high efficacy and minimal toxicological risks (Cano-Cebrián et al 2005).

Chitosan–glutathione (GSH) conjugates. Chitosan–GSH was synthesized by the covalent attachment of glutathione to the native chitosan by the formation of an amide bond. The reaction was mediated by carbodiimide and N-hydroxysuccinimide. The obtained conjugate consisted of 256.5 μmol immobilized free thiol groups and 397.9 mmol disulphide bonds per gram polymer. Disintegration tests and oxidation experiments were conducted to evaluate the cohesive property and stability of the resulting conjugate, respectively. The permeation enhancing effect of the novel conjugate was evaluated in Ussing chambers by using rhodamine as a model compound. Matrix tablets prepared using the novel conjugate and the model compound were quite stable due to the formation of disulphide bonds within the polymer. A 9.9-fold increase in the total work of adhesion of the conjugate was observed in comparison with unmodified chitosan based on tensile studies. The conjugate exhibited 9.9-fold increase and a 55-fold increase in the total work of adhesion and adhesion time respectively as compared with unmodified chitosan. The enhanced mucoadhesion, cohesion and improved permeation of the novel thiolated chitosan derivatives showed their potential as promising

polymers in controlled drug delivery (Kafedjiiski et al 2005).

Chitosan–4-thiobutyl amide conjugates (TBA). Chitosan–TBA was synthesized by reaction of chitosan with iminothiolactons such as 2-iminothiolane thiol, which resulted in immobilization of the thiol on the polymer. This resulted in an improved cationic nature of chitosan and enhanced viscosity up to 500 fold in the presence of air, thus providing enhanced controlled release on ocular delivery (Martin 2002).

Chitosan–TBA conjugate exhibited improved mucoadhesion and in-situ gelling properties (Martin et al 2002; Bernkop-Schnurch et al 2003). The enhanced mucoadhesion of chitosan–TBA conjugate was due to improved ionic interactions between the additional cationic amidine substructure of the conjugate and anionic substructures within the mucus layer. A 140-fold increase in force of adhesion was observed in comparison with unmodified chitosan. Tensile studies with conjugates of low, medium and high molecular mass (150, 400 and 600 kDa) further indicated that thiolated chitosan with medium molecular mass had the highest mucoadhesion.

A pseudo zero-order release profile of salmon calcitonin over the first 8 h was observed in artificial intestinal fluid from matrix tablets based on chitosan–TBA conjugate. The tablets showed continuous swelling and maintained good cohesiveness during the experiment. The active agent was released from the formulation via a controlled diffusion process (Bernkop-Schnurch et al 2004b).

To improve the therapeutic efficacy of clotrimazole, an antimycotic drug in the treatment of vaginal infections, matrix tablets containing chitosan–TBA conjugate were formulated. The thiolated chitosan tablets were stable over a period of 6 h and no disintegration was observed. The release of the drug from the tablets followed zero-order kinetics. The novel thiolated chitosan conjugate was able to significantly retard the drug release from the tablets and served as a promising tool in mucoadhesive drug delivery systems (Bernkop-Schnurch et al 2003).

Roldo et al (2004) studied the influence of degree of modification and cationic polymer chain length of the thiolated chitosan–TBA on mucoadhesive properties and swelling nature. Results indicated that by increasing covalently attached thiol groups, a remarkable increase of total work of adhesion for medium molecular mass (MMM) chitosan–TBA conjugate was obtained as compared with unmodified chitosan. Polymer chain was found to influence the mucoadhesion of polymer. A fourfold increase in adhesion was indicated for MMM polymer when compared with a derivative of lower mass. Use of MMM polymer thus represented a promising strategy for developing delivery systems with improved mucoadhesion.

Similar results were obtained when chitosan–TGA was used with model drugs cefadroxil and glutathione. Tensile studies and rotating cylinder method were used to study mucoadhesion of chitosan–TBA at pH 3, 5 and 7. At lower pH, thiolated chitosan was observed to have higher mucoadhesion. Augmented permeation was observed in chitosan–TBA/glutathione in comparison with unmodified chitosan. A sustained release of cefadroxil and glutathione was observed for several hours by the use of this derivative, thus improving the bioavailability of poorly absorbed drugs from the gastrointestinal tract (Bernkop-Schnurch et al 2004b).

To exploit chitosan as matrices for oral controlled release, chitosan–TBA insulin tablets were prepared. The preparation involved direct compression of chitosan–TBA insulin, glutathione and two inhibitor conjugates. Immobilization of thiol groups resulted in a 60-fold improvement in mucoadhesion. Chitosan enzyme inhibitor conjugates were added so as to avoid enzymatic degradation of the peptide drug in the gastrointestinal tract. Pharmacological efficacy of the tablets was tested in non-diabetic rats based on the reduction of glucose levels by oral matrix tablets vs intravenous injection. The study was found to present a promising method for oral delivery of insulin (Krauland et al 2004).

In another study, the permeation enhancing effect of chitosan–TBA in comparison with the permeation enhancing effect of unmodified chitosan was shown. The uptake of the cationic marker rhodamine 123 was 3-fold higher in the presence of thiolated chitosan as compared with unmodified chitosan. The mechanism responsible for this improved permeation has been attributed to the inhibition of protein tyrosine phosphatase, a enzyme involved in the opening and closing of the tight junctions of the membrane (Bernkop-Schnurch et al 2004a).

In-vivo studies were conducted to study the effectiveness of chitosan–TBA conjugate for the oral administration of hydrophilic macromolecules. Different drug carrier matrices, comprising chitosan–TBA conjugate as substantial polymer excipient and containing equal amounts of salmon calcitonin were developed. To avoid an enzymatic degradation of the peptide drug in the gastrointestinal tract, chitosan–enzyme-inhibitor conjugates were added. All compounds were homogenously mixed and directly compressed into tablets. The different tablets were given orally to rats and the plasma calcium level was monitored as a function of time. In-vivo studies revealed no statistically significant reduction of the plasma calcium level caused by salmon calcitonin, which was orally given in solution. It was further observed that no significant effect was observed after oral administration of tablets comprising the peptide drug and unmodified chitosan. Among all formulations, stomach-targeted tablets containing chitosan–TBA conjugate were most effective in reducing the plasma calcium level in rats. These tablets led to a decrease of the plasma calcium level of more than10% for at least 12 h. A faster and more reproducible onset of action was also obtained by the use of these conjugates (Bernkop-Schnurch et al 2004c).

The structures of thiomers and conjugates are given in Figure 3 (Kast & Bernkop-Schnurch 2001).

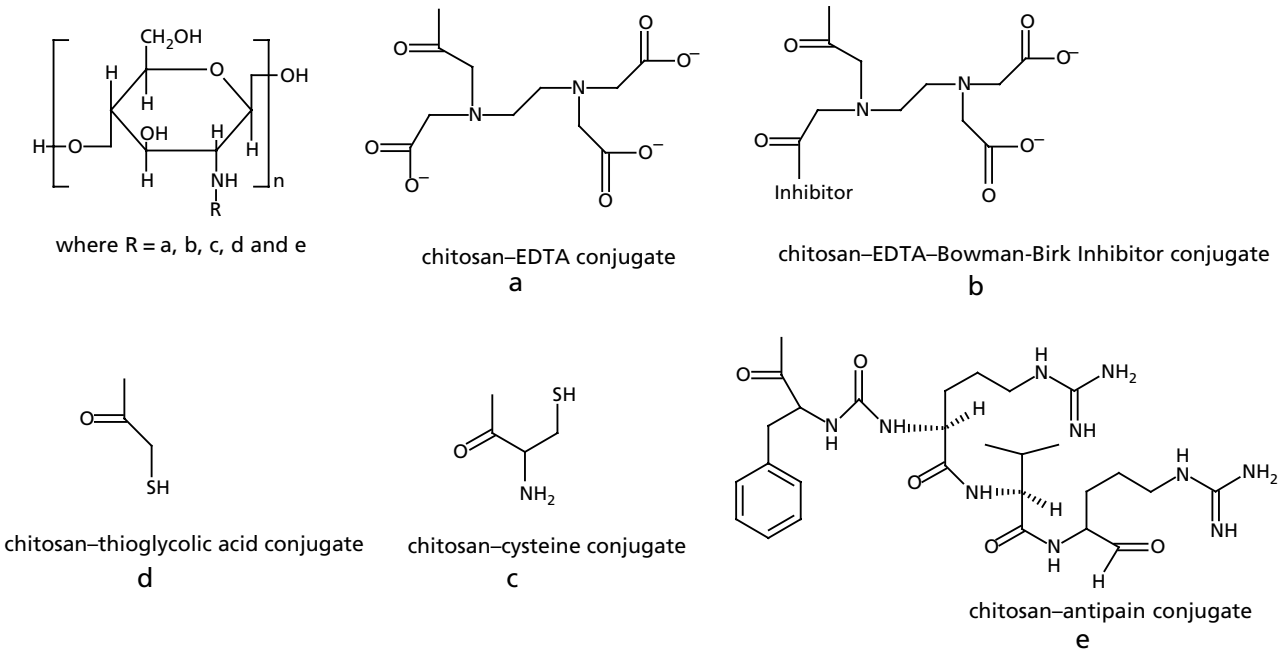

Figure 3 Chemical structures of chitosan derivatives displaying properties mucoadhesive properties. (Reprinted from Bernkop-Schnurch & Kast (2001), with permission from Elsevier Science Ltd.).

Acylated chitosan

Palmitoyl glycol chitosan (GCP)

Martin et al (2002) synthesized GCP with varying degrees of hydrophobicity (GCP21 > GCP11 > GCP12). Glycol chitosan (GC) was dissolved in water (ratio of the reactants varied), to which sodium bicarbonate and absolute ethanol were added. An ethanolic solution of palmitic acid *N*-hydroxysuccinimide (PNS) was added drop-wise to the alkaline solution of GC for 1 h (Figure 4). Acetone was added to the reaction mixture after stirring and evaporated under reduced pressure. The polymer dispersion was finally freeze dried after extraction with diethyl ether and subjected to exhaustive dialysis against water. The physically crosslinked, erodible and porous hydrogel prepared from GCP, was loaded with model macromolecule fluorescein isothiocyanate dextran (FITC)-dextran (MW 4400). The drug release was dependent on the hydrophobicity of the gel polymer control.

The GCP gel formulations were found to be less bioadhesive than carbomer (carbopol), hypromellose, which served as polycontrol. The more hydrophobic gels (GCP11 and GCP12) had stronger bioadhesive strength than GCP21. GCP11 specifically showed better bioadhesion. The cohesiveness of individual components of the gel to the mucosa depends on the degree of dehydration of the mucosal surface (which is the driving force of mucoadhesion), presence of hydrogen bonds between the surfaces of gel and mucosa, and to hydrophobic interactions between the gel surface and the mucosal surface (Lehr

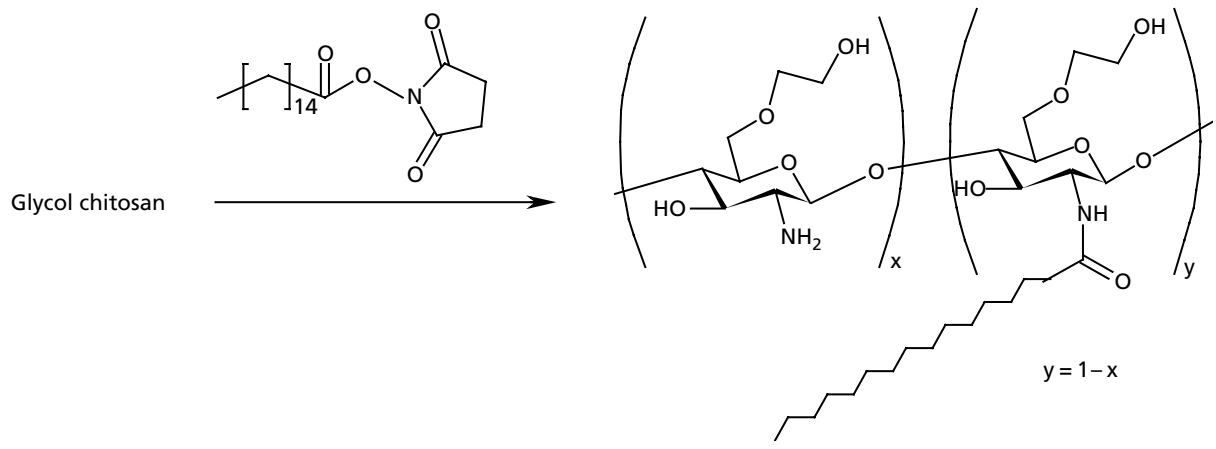

Figure 4 Synthesis of palmitoyl glycol chitosan. (Reproduced From Martin et al (2002), with permission From Elsevier Science Ltd.).

et al 1992; Jacques et al 1997). GCP11 has superior mucoadhesion because of its fast hydrating and minimally eroding properties in comparison with GCP21, which is a fast hydrating and fast eroding gel. Dehydration of the mucus by GCP11 gels thus retains their structure while the dehydration of the mucus by GCP21 gels was accompanied by loss of gel structure. The addition of amphiphilic derivatives Gelucire 50/13 (a mixture of polyethylene glycol triglyceride chitosan amphiphiles) in GCP12 gels showed improved bioadhesion because of this increased hydrophobicity between the gel formulation and the mucosal surface along with reduced gel erosion and hydration. Thus, this bioadhesive hydrogel with high levels of the model macromolecule FITC-dextran adhered to the buccal mucosa providing intimate contact, improved drug absorption and optimum residence time and releasing the drug in a controlled manner into the systemic circulation (Needleman & Smales 1995; Martin et al 2002).

Further, Martin et al (2003) synthesized non-covalently crosslinked GCP for sustained release of denbufylline via the buccal route. This communication also involved various GCP with decreasing hydrophobicity (GCP12 > GCP 11 > GCP 21) in presence of the drug (that is extensively metabolized via the oral route) and a penetration enhancer, sodium glycodeoxycholate (GDC). The hydrophilic parts of the gel GC and GDC caused gel hydration, important for gel mucoadhesion while the hydrophobic parts of the gel–palmitoyl units and denbufylline eroded the gel preventing its solubilization. Drug release from hydrogels was usually as a result of gel hydration (Needleman & Smales 1995). In the case of these hydrophobic drugs, which were bound to hydrophobic gels by non-polar interactions, the level of gel hydrophobicity was used to control the release. Hence a reduced level of hydration gave rise to slow release of the drug (Lowe et al 1999). In-vivo studies were conducted in the rabbit model to evaluate the absorption of drug from tablets prepared using GCP12, denbufylline, and GDC (20:12:5) with Carbopol 974NF, denbufylline, and GDC (20:12:5)(60:36:4) tablets used as controls. The Carbopol 974NF formulations were significantly more mucoadhesive than all GCP formulations. There was an increase in mucoadhesion with GDC (4.5%, w/w) and a higher level of denbufylline (36%, w/w), whereas addition of denbufylline (18.4%, w/w) into GCP12 gels did not alter the mucoadhesion. The inclusion of GDC within denbufylline gels increased hydration, which led to mucoadhesion. Porcine buccal mucosa was used as the model mucosal layer, where the gel absorbed moisture from the mucosal surface and thereby produced good mucoadhesion, followed by dehydration of the mucosal layer, interpenetration of the polymer into the mucosal layer and formation of hydrogen bonding between the mucosal layer and the polymer chains. Denbufylline with GCP12 formulation showed a sustained release for at least 5 h, whereas no sustained release was achieved with control Carbopol 974NF tablets. The results concluded that GCP formulations produced a sustained release for buccal delivery for up to 5 h, although mucoadhesion was less than with the control tablets (Martin et al 2003).

Miscellaneous

Chitosan-glyceryl monooleate (cGMO)

Synthesis of cGMO involved incorporation of GMO (2 and 3% w/v) in solutions (1, 2, 3, 4, and 5%, w/v) of chitosan in 1 M acetic acid. After sonication this solution was added to Sorensen's phosphate buffer forming in-situ gels (Ganguly & Dash 2004).

A novel in-situ delivery system was developed using acidic solutions of chitosan and glyceryl monooleate (GMO). Viscous gels of chitosan were formed when subjected to alkaline pH by losing its charge. This property has been used for sustained drug delivery (Sawayanagi et al 1982), and GMO formed viscous gels known as the cubic phase depending on the amount of water present in the matrix. This GMO has also been used to sustain the delivery of various water-soluble and water-insoluble drugs (Wyatt & Dorschel 1992). Enhanced mucoadhesion (3–7 fold) of chitosan-glyceryl monooleate (cGMO) gel was observed when 3% (w/v) GMO was added to chitosan. The probable mechanism of bioadhesion of GMO involved dehydration of the mucosa (Nielsen et al 1998). Hence a synergistic increase in mucoadhesion of chitosan by GMO was observed along with a prolonged gastrointestinal residence time, thus, utilizing enhanced mucoadhesion for drug targeting for mucin via oral or the parenteral route. This in-situ gel formation by cGMO was employed for controlled delivery of the drugs lidocaine HCl, ketoprofen and dexamethasone. In-vitro release of the drugs from this gel was very quick. Incorporation of 0.2% (v/v) glutaraldehyde (50%, v/v) as a crosslinker could retard drug release. Release of the drug from the gel followed a diffusion-controlled mechanism by incorporation of drug-loaded microspheres. Thus in-situ gel of cGMO has promising physicochemical properties to develop mucoadhesive drug delivery systems (Ganguly & Dash 2004).

5-Methyl-pyrrolidinone chitosan (MPC)

5-Methyl-pyrrolidinone chitosan (MPC) was evaluated for its mucoadhesive properties and characterized as buccal and vaginal penetration enhancers. MPC was synthesized by partially substituting with 5-methyl-pyrrolidinone, a skin penetration enhancer, at the amino groups of glucosamine units (Figure 5) (Muzzarelli et al 1993). Other chitosan derivatives used were two low-molecular weight chitosans (DC1 and DC2) and a partially re-acetylated chitosan (RC). DC1 and DC2 were obtained by partial enzymatic depolarization of high molecular grades, RC was a partially re-acetylated chitosan and chitosan HCl (HCS) was used as a reference. A hydrophilic drug, aciclovir, having poor solubility, was used as the model drug. Mucoadhesion measurements were examined in the presence of two biological substrates i.e. cheek buccal mucosa and bovine mucin

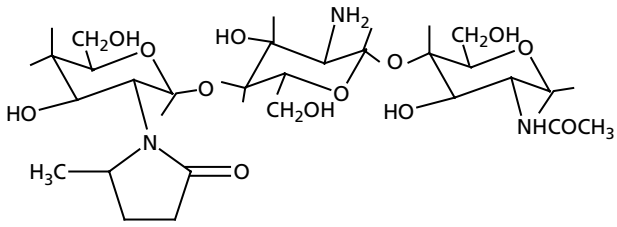

Figure 5 Chemical structure of 5-methyl-pyrrolidinone chitosan. (Reproduced from Sandri et al (2004), with permission from Elsevier Science Ltd.).

mimicking the buccal region; porcine vaginal mucosa and gastric porcine mucin simulating the vaginal environment. Rheological characterization, drug release, drug permeation through porcine cheek epithelium, and penetration measurements into porcine vaginal mucosa were also conducted. Among these derivatives MPC exhibited the best mucoadhesive and penetration enhancement properties in both buccal and vaginal environments. It was observed that biological substrates in the buccal membranes highly influenced the mucoadhesive properties. MPC showed maximum mucoadhesion in the presence of mucin than in the presence of mucosa. Decreased mucoadhesion and lowering of polymer solubility in presence of buccal mucosa was because of its higher buffering capacity in comparison with mucin dispersion. In the vaginal environment derivatives showed a similar trend in the presence of mucosa and mucin, although better results were observed in the presence of mucosa. Better penetration enhancement was observed when MP was attached to chitosan. Thus in both the buccal and vaginal environment MPC showed superior results, thus providing good mucoadhesion (Sandri et al 2004).

Cyclodextrin (CD)-chitosan derivative

CDs are a group of cyclic oligosaccharides with a hydrophilic external and a hydrophobic internal cavity allowing delivery of hydrophobic drugs along with their solubilization and stabilization.

The process of coupling cyclodextrin monoaldehyde to chitosan by reductive amination reaction synthesized CD-chitosan. It involved reaction of chitosan dissolved in aqueous acetic acid with CD monoaldehyde in the presence of sodium cyanoborohydride causing precipitation of CD-chitosan with aqueous sodium hydroxide. The white precipitate was washed with water and ethanol, and when it was dried it gave cyclodextrin-chitosan (Bibby et al 2000; Auzély-Velty & Rinaudo 2001).

Modified chitosan, by grafting with CD, resulted in the synergistic effects of transport properties of cyclodextrin and mucoadhesive properties of the polymeric matrix. The grafted CD-chitosan showed considerable mucoadhesive properties, however lower in comparison with chitosan alone. Conformational changes in CD-chitosan chain caused less availability of positive charges for interaction with the negatively-charged mucus resulting in decreased mucoadhesion. Further, the molecular weight of CD-chitosan, another reason affecting mucoadhesion, might lead to a diminished interpenetration into the mucus layer, following decrease in mucoadhesivity. Thus, a monosubstituted CD-chitosan displayed characteristics of a possible controlled mucoadhesive drug delivery system with some inclusion properties from cyclodextrin (Venter et al 2006).

PAA/chitosan polymer complex

A novel polymer processing mucoadhesive properties for transmucosal drug delivery was prepared based on template polymerization of acrylic acid in the presence of chitosan. The mucoadhesive force of the complex was similar to a commercial product, Carbopol 971P NF. Based on the results of FTIR it was concluded that the polymer complex was formed between poly (acrylic acid) and chitosan through hydrogen bonding. The dissolution rate of the PAA/chitosan polymer complex was dependent on pH and ratio of PAA/chitosan (Ahn et al 2001).

Conclusion

The chemically modified derivatives of chitosan provide intimate contact with the mucosa by attachment of various functional groups, resulting in prolonged residence time and solubility at physiological pH, protective action against enzymatic degradation and control of polycationic, anionic and hydrophobic properties.

This scientific approach has thus offered great potential to contribute towards the development of various mucoadhesive controlled drug delivery systems which provide enhanced bioavailability at a controlled rate, improved therapeutic efficacy, prolonged residence time, improved patient compliance and convenience.

Further studies and development of chitosan derivatives need to be explored for contributing towards human health care.

References

Ahn, J. S., Chio, H. K., Cho, C. S. (2001) A novel mucoadhesive polymer prepared by template polymerization of acrylic acid in the presence of chitosan. *Biomaterials* **22**: 923–928

Auzély-Velty, R., Rinaudo, M. (2001) Chitosan derivatives bearing pendant cyclodextrin cavities: synthesis and inclusion performance. *Macromolecules* **34**: 3574–3580

Bernkop-Schnurch, A., Kast, C. E. (2001) Chemically modified chitosans as enzyme inhibitors. *Adv. Drug Deliv. Rev.* **52**: 127–137

Bernkop-Schnurch, A., Krajicek, M. E. (1998) Mucoadhesive polymers as platforms for peroral peptide delivery and absorption: synthesis and evaluation of different chitosan–EDTA conjugates. *J. Control. Release* **50**: 215–223

Bernkop-Schnurch, A., Pasta, M. (1998) Intestinal peptide and protein delivery: novel bioadhesive drug carrier matrix shielding from enzymatic attach. *J. Pharm. Sci.* **87**: 430–434

Bernkop-Schnurch, A., Scerbe-Saiko, A. (1998) Synthesis and in vitro evaluation of Chitosan–EDTA–protease–inhibitor conjugates which might be useful in oral delivery of peptides and proteins. *Pharm. Res.* **15**: 263–269

Bernkop-Schnurch, A., Bratengeyer, I., Valenta, C. (1997) Development and in vitro evaluation of a drug delivery system protecting from trypsinic degradation. *Int. J. Pharm.* **157**: 17–25

Bernkop-Schnurch, A., Humenberger, C., Valenta, C. (1998) Basic studies on bioadhesive delivery systems for peptide and proteins. *Int. J. Pharm.* **165**: 217–225

Bernkop-Schnurch, A., Schwarz, V., Steininger, S. (1999) Polymers with thiol groups: a new generation of mucoadhesive polymers? *Pharm. Res.* **16**: 876–881

Bernkop-Schnurch, A., Hornof, M., Zoidl, T. (2003) Thiolated polymers – thiomers: modification of chitosan with 2-uninothiolane. *Int. J. Pharm.* **260**: 229–237

Bernkop-Schnurch, A., Guggi, D., Pinter, Y. (2004a) Thiolated chitosan: development and *in vitro* evaluation of a mucoadhesive, permeation enhancing oral drug delivery system. *J. Control. Release* **94**: 177–186

Bernkop-Schnurch, A., Hornof, M., Guggi, D. (2004b) Thiolated chitosan. *Eur. J. Pharm. Sci.* **57**: 9–17

Bernkop-Schnürch, A., Hoffer, M. H., Kafedjiiski, K. (2004c) Thiomers for oral delivery of hydrophilic macromolecular drugs. *Expert Opin. Drug Deliv.* **1**: 187–198

Bibby, D. C., Davies, N. M., Tucker, I. G. (2000) Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems. *Int. J. Pharm.* **197**: 1–11

Cano-Cebrián, M. J., Zornoza, T., Granero, L., Polache, A. (2005) Intestinal absorption enhancement via the paracellular route by fatty acids, chitosans and others: a target for drug delivery. *Curr. Drug Deliv.* **2**: 9–22

Chandy, T., Sharma, C. P. (1991) Biodegradable chitosan matrix for the controlled release of steroids. *Biomater. Artif. Cells Immobilization Biotechnol.* **19**: 745–760

Colo, G. D., Zambito, Y., Burgalsassi, S., Nardini, I., Saettone, M. F. (2004) Effect of chitosan and N-carboxymethyl chitosan on intraocular penetration of topically applied ofloxacin. *Int. J. Pharm.* **273**: 37–44

Domard, A., Rinaudo, M., Terrassin, C. (1986) Now method for the quartenization of chitosan. *Int. J. Biol. Macromol.* **8:** 105–107

Edsman, K., Hägerström, H. (2005) Pharmaceutical applications of mucoadhesion for the non-oral routes. *J. Pharm. Pharmacol.* **57**: 3–22

Felt, O., Furrer, P., Mayer, J. M., Plazonet, B., Buri, P., Gurny, R. (1999) Topical use of chitosan in ophthalmology: tolerance assessment and evaluation of precorneal retention. *Int. J. Pharm.* **180**: 185–193.

Felt, O., Baeyens, V., Buri, P., Gurny, R. (2001) Delivery of antibiotics to the eye using a positively charged polysaccharide as vehicle. *AAPS Pharm. Sci.* **3** (article 34)

Fiebrig, I., Harding, S. E., Rowe, A. J., Hyman, S. C., Davis, S. S. (1995) Transmission electron microscopy on pig gastric mucin and its interaction with chitosan. *Carbohydr. Polym.* **28**: 239–244

Ganguly, S., Dash, A. K. (2004) A novel in situ gel for sustained drug delivery and targeting. *Int. J. Pharm.* **276**: 83–92

Gavini, E., Rassu, G., Sanna, V., Cossu, M. (2005) Mucoadhesive microspheres for nasal administration of an antiemetic drug, metoclopramide: in-vitro/ex-vivo studies. *J. Pharm. Pharmacol.* **57**: 287–294

He, P., Davis, S. S., Illum, L. (1998) In vitro evaluation of the mucoadhesive properties of chitosan microspheres. *Int. J. Pharm.* **166**: 75–88

Hopper-Seyeler (1894) Ueber Chitosan and Zellulose. *Ber. Deut. Chem. Gesell.* **27**: 3329–3331

Illum, L., Jabbal-Gill, I., Hinchcliffe, M., Fisher, A. N., Davis, S. S. (2001) Chitosan as a novel nasal delivery system for vaccines. *Adv. Drug Deliv. Rev.* **51**: 81–96

Jacques, Y., NguyenXuan, T., Ionescu, E., Ravelli, G. P., Buri, P., Baehni, P., Gurny, R. (1997) In vivo evaluation of hydrophilic and hydrophobic mucoadhesive semi-solid formulations containing sucralfate and lidocaine for intraoral use. *Eur. J. Pharm. Biopharm.* **43**: 59–63

Kafedjiisji, K., Foger, F., Werle, M., Bernkop-Schnurch, A. (2005) Synthesis and in vitro evaluation of a novel chitosan–glutathione conjugate. *Pharm. Res.* **22**: 1480–1488.

Kas, H. S. (1997) Chitosan: properties, preparation and application to microparticulate systems. *J. Microencapsul.* **14**: 689–711

Kast, C. E., Bernkop-Schnurch, A. (2001) Thiolated polymers – thiomers: development and *in vitro* evaluation of chitosan thioglycolic acid conjugates. *Biomaterials* **22**: 2345–2352

Kast, C. E., Valenta, C., Leopold, M., Bernkop-Schnurch, A. (2002) Design and *in vitro* evaluation of a novel bioadhesive vaginal drug delivery system for clotrimazole. *J. Control. Release* **81**: 347–354

Kato, Y., Onishi, H., Manchida, Y. (2003) Application of chitin and chitosan derivatives in the pharmaceutical field. *Curr. Pharm. Biotechnol.* **4**: 303–309

Kotze, A. F., Leußen, H. L., Leeuw, B .J., Boer, B. G., Verhoef, J. C., Juninger, H. E. (1997) N-trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: in vitro evaluation in intestinal epithelial cells (Caco-2). *Pharm. Res.* **14**: 1197–1202.

Kotze, A. F., Lueßen, H. L., de Boer, A. G., Verhoef, J. C., Junginger, H. E. (1998) Chitosan for enhanced intestinal permeability: prospects for derivatives soluble in neutral and basic environments. *Eur. J. Pharm. Sci.* **7**: 145–151

Krauland, A. H., Guggi, D., Bernkop-Schnurch, A. (2004) Oral insulin delivery: the potential of thiolated chitosan insulin tablets on non diabetic rats. *J. Control. Release* **95**: 547–555

Lee, J. W., Park, J. H., Robinson, J. R. (2000) Bioadhesive-based dosage forms: the next generation. *J. Pharm. Sci.* **89**: 850–866

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

Leitner, V. M., Marchitz, M. K., Bernkop-Schnurch, A. (2003) Mucoadhesive and cohesive properties of poly (acrylic acid)–cysteine conjugates with regard to their molecular mass. *Eur. J. Pharm. Sci.* **18**: 89–96

Leußen, H. L., de Leeuw, B. J., Langemeyer, W. M. E., de Boer, A. G., Verhoef, J. C., Junginger, H. E. (1997) Mucoadhesive polymers in peroral peptide drug delivery. IV. Polycarbophil and chitosan are potent enhancers of peptide transport across intestinal mucosae in vitro. *J. Control. Release* **45**: 15–23

Lowe, T. L., Tenhu, H., Tylli, H. (1999) Effect of hydrophobicity of a drug on its release from hydrogels with different topological structures. *J. Appl. Polym. Sci.* **73**: 1031–1039

Martin, P. (2002) Chitosan-thio-amide conjugates and their cosmetic as well as pharmaceutics use. EP Patent PCT/EP02/06250/

Martin, L., Wilson, C. G., Koosha, F., Tetley, L., Gray, A. I., Senel, S., Uchegbu, I. F. (2002) The release of model macromolecules may be controlled by the hydrophobicity of palmitoyl glycol chitosan hydrogels. *J. Control. Release* **80**: 87–100

Martin, L., Wilson, C. G., Koosha, F., Uchegbu, I. F. (2003) Sustained buccal delivery of the hydrophobic drug denbufylline using physical cross-linked palmitoyl glycol chitosan hydrogels. *Eur. J. Pharm. Biopharm.* **55**: 35–45

Muzzarelli, R. A. A. (1977) *Chitin*. Pergamon Press, New York, pp 1–37

Muzzarelli, R. A. A., Tanfani, F., Emmanueli, M., Mariotti, S. (1982) N-(Carboxymethylidene)-chitosans and N-(carboxymethyl)-chitosans: novel chelating polyampholytes obtained from chitosan glyoxylate. *Carbohydr. Res.* **107**: 199–214

Muzzarelli, R. A. A., Ilari, P., Tomasetti, M. (1993) Preparation and characteristic properties of 5-methyl-pyrrolidine chitosan. *Carbohydr. Polym.* **20**: 99–105.

Needleman, I. G., Smales, F. C. (1995) In-vitro assessment of bioadhesion for periodontal and buccal drug-delivery. *Biomaterials* **16**: 617–624

Nielsen, L. S., Schubert, L., Hansen, J. (1998) Bioadhesive drug delivery systems I. Characterization of mucoadhesive properties of systems based on glyceryl mono-oleate and glyceryl monolinooleate. *Eur. J. Pharm. Sci.* **6**: 231–239

Ponchel, G., Duchene, D. (1992) Principle and investigation of the bioadhesion mechanism of solid dosage form. *Biomaterials* **13**: 709–714

Roldo, M., Hornof, H., Caliceti, P., Bernkop-Schnurch, A. (2004) Mucoadhesive thiolated chitosan as platforms for oral controlled drug delivery: synthesis and in vitro evaluation. *Eur J. Pharm. Biopharm.* **57**: 115–121

Sandri, G., Rossi, S., Ferrari, F., Bonferoni, M. C., Muzzarelli, C., Caramella, C. (2004) Assessment of chitosan derivatives as buccal and vaginal penetration enhancers. *Eur. J. Pharm. Sci.* **21**: 351–359

Sawayanagi, Y., Nambu, N., Nagai, T. (1982) Use of chitosan for sustained-release preparations of water-soluble drugs. *Chem. Pharm. Bull.* **30**: 4213–4215

Shahiwala, A., Misra, A. (2006) Preliminary investigation of the nasal delivery of liposomal leuprorelin acetate for contraception in rats. *J. Pharm. Pharmacol.* **58**: 19–26

Singla, A. K., Chawla, M. (2001) Chitosan: some pharmaceutical and biological aspects—an update. *J. Pharm. Pharmacol.* **53**: 1047–1067

Sinha, V. R., Kumaria, R. (2001) Polysaccharides in colon-specific drug delivery. *Int. J. Pharm.* **224**: 19–38

Sinha, V. R., Singla, A. K., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., Dhawan, S. (2004) Chitosan microspheres as a potential carrier for drugs. *Int. J. Pharm.* **274**: 1–33

Snyman, D., Hamman, H., Kotze, A. F. (2003) Evaluation of the mucoadhesive properties of N-trimethyl chitosan chloride. *Drug Dev. Ind. Pharm.* **29**: 61–69

Snyder, G. H., Reddy, M. K., Cennerazzo, M. J., Field, D. (1983) Use of local electrostatic environments of cysteines to enhance formation of a desired species in a reversible disulfide exchange reaction. *Biochim. Biophys. Acta.* **749**: 219–226

Takeuchi, H., Yamamoto, H., Niwa, T., Hino, T., Kawashima, Y. (1996) Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. *Pharm. Res.* **13**: 896–900

Thanou, M., Verhoef, J. C., Romeijn, S. G., Nagelkerke, J. F., Merkus, F. W. H. M., Junginger, H. E. (1999) Effects of N-trimethyl chitosan chloride, a novel absorption enhancer, on Caco-2 intestinal epithelia and the ciliary beat frequency of chicken embryo trachea. *Int. J. Pharm.* **185**: 73–82

Thanou, M., Verhoef, J. C., Junginger, H. E. (2001) Oral drug absorption enhancement by chitosan and its derivatives. *Adv. Drug Deliv. Rev.* **52**: 117–126

Van der Lubben, I. M., Verhoef, J. C., Borchard, G., Junginger, H. F. (2001) Chitosan and its derivatives in mucosal drug and vaccine delivery. *Eur. J. Pharm. Sci.* **14**: 201–207

Van der Merwe, S. M., Verhoef, J. C., Uerheijden, J. H., Kotze, A. F., Tuninger, H. E. (2004) Trimethylated chitosan as polymeric absorption enhancer for improved per oral delivery of peptide drugs. *Euro. J. Pharm. Biopharm.* **58**: 225–235.

Venter, J. P., Kotzé, A. F., Auzély-Velty, R., Rinaudo, M. (2006) Synthesis and evaluation of the mucoadhesivity of a CD-chitosan derivative. *Int. J. Pharm.* **313**: 36–42

Wyatt, D. M., Dorschel, D. (1992) A cubic-phase delivery system composed of glyceryl monooleate and water for sustained release of water-soluble drugs. *Pharm. Technol.* **10**: 116–130