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Review Article

Chitosan: some pharmaceutical and biological aspects – an update

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Abstract

Chitosan, a natural polysaccharide, is being widely used as a pharmaceutical excipient. It is obtained by the partial deacetylation of chitin, the second most abundant natural polymer. Chitosan comprises a series of polymers varying in their degree of deacetylation, molecular weight, viscosity, pKa etc. The presence of a number of amino groups permit chitosan to chemically react with anionic systems, thereby resulting in alteration of physicochemical characteristics of such combinations.

Chitosan has found wide applicability in conventional pharmaceutical devices as a potential formulation excipient, some of which include binding, disintegrating and tablet coating properties. The polymer has also been investigated as a potential adjuvant for swellable controlled drug delivery systems. Use of chitosan in novel drug delivery as mucoadhesive, gene and peptide drug administration via the oral route as well as its absorption enhancing effects have been explored by a number of researchers.

Chitosan exhibits myriad biological actions, namely hypocholesterolemic, antimicrobial and wound healing properties. Low toxicity coupled with wide applicability makes it a promising candidate not only for the purpose of drug delivery for a host of drug moieties (anti-inflammatories, peptides etc.) but also as a biologically active agent.

It is the endeavour of the present review to provide an insight into the biological and pharmaceutical profile of chitosan. Various investigations carried out recently are reported, although references to research performed on chitosan prior to the recent reviews have also been included, where appropriate.

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Introduction

Chitin, the second most abundant natural polysaccharide, is a straight homo-polymer composed of B (1,4)-linked GlcNAc (N-acetyl glucosamine) units with a three dimensional α-helical configuration stabilised by intramolecular hydrogen bonding (Kas 1997). Partial deacetylation of chitin results in the production of chitosan, which is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine. It is also naturally present in some micro-organisms and fungi. The term chitosan is used to describe a series of chitosan polymers with different molecular weights (50 kDa to 2000 kDa), viscosity and degree of deacetylation (Illum 1998). The chemical structure of chitin and chitosan is shown in Figure 1.

Manufacture of chitosan

Chitosan is commercially produced in different parts of the world (Japan, North America, Poland, Italy, Russia, Norway and India) on a large scale. The raw

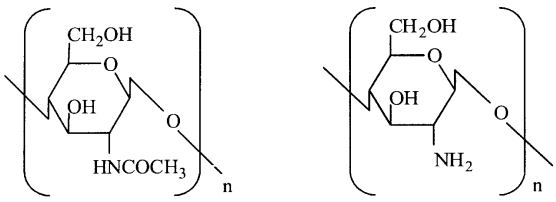

Figure 1 Chemical structure of N-acetyl glucosamine (A) and glucosamine (B).

Table 1 Principle sources of chitin (Felt et al 1998).

Organism		Chitin content (%)
Crustacea	Crab[a]	72.1
	Shrimp[a]	69.1
	Lobster[a]	69.8
Insects	True fly[a]	54.8
	Sulphur butterfly[a]	64.0
Fungi	*Aspergillus niger*[b]	42.0
	Mucor rouxii	44.5

[a]Organic weight of cuticle; [b]dry weight of the cell wall.

material for chitin generally consists of crustacean shells. Even though more species are used, chitin itself never seems to alter in terms of chemical composition. However, adjustments during processing may be needed to make the end product of consistent quality. Generally, shells from crab and shrimps are utilised including those of Dungeness crab (*Cancer magister*), the King crab (*Paralithodes camschatica*) and the Pacific shrimp (*Pandalus borealis*) (Skaugrud 1991). Principle sources of chitin are summarised in Table 1 (Felt et al 1998).

The basic process for the manufacture of chitosan involves the removal of proteins and minerals such as calcium carbonate and calcium phosphate, by treatment with alkali and acid, respectively. Before the treatment, the shells are ground to make them more accessible, and after the completion of the manufacturing procedure chitin is dried so that it can be stored as a stable intermediate for deacetylation to chitosan at a later stage. The process of deacetylation is achieved by treating chitin with a strong solution of sodium hydroxide at an elevated temperature (Skaugrud 1991; Ilango et al 1998). A flow diagram depicting the manufacture of chitosan is given in Figure 2.

Physicochemical properties

The term chitosan describes a series of chitosan polymers with different molecular weight, viscosity and degree of deacetylation (40–98 %). It is a linear polyamine with a number of amino groups that are readily available for chemical reaction and salt formation with acids. Important characteristics of chitosan are its molecular weight, viscosity, degree of deacetylation (Bodek 1994; Ferreira et al 1994a, b), crystallinity index, number of monomeric units (n), water retention value, pKa and energy of hydration (Kas 1997). Chitosan has a high charge density, adheres to negatively charged surfaces and chelates metal ions.

Due to high molecular weight and a linear unbranched structure, chitosan is an excellent viscosity enhancing agent in an acidic environment. It behaves as a pseudoplastic material exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan

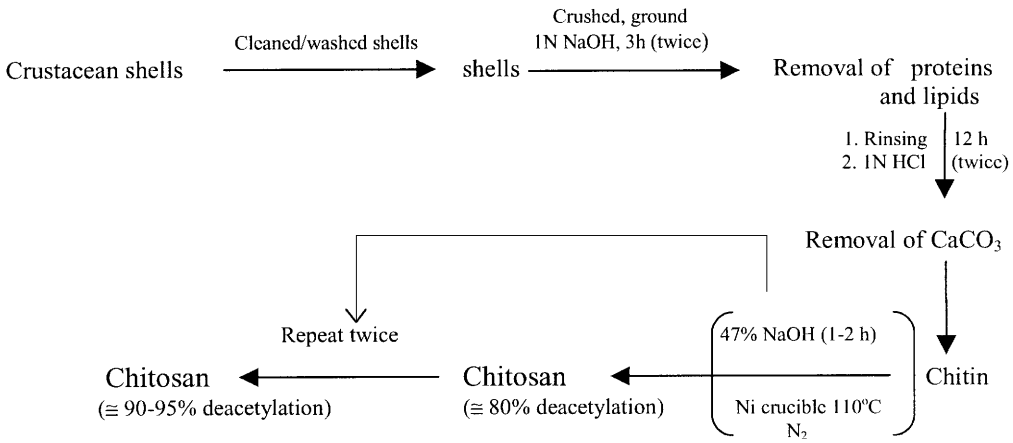

Figure 2 Manufacture of chitosan.

Table 2 Viscosity of 1% chitosan solution (w/v) in different acids (Skaugrud 1991).

Acid	Viscosity (cps)/pH of 1% (w/v) chitosan in various concentrations in acid (v/v)		
	1%	5%	10%
Acetic	260/4.1	260/3.3	260/2.9
Adipic	190/4.1	–	–
Citric	35/3.0	195/2.3	215/2.0
Formic	240/2.6	185/2.0	185/1.7
Lactic	235/3.3	235/2.7	270/2.1
Malic	180/3.3	205/2.3	220/2.1
Malonic	195/2.5	–	–
Oxalic	12/1.8	100/1.1	100/0.8
Tartaric	52/2.8	135/2.0	160/1.7

Table 3 Physicochemical properties of chitosan (Knapczyk et al 1989; Sanford 1990).

Properties		
Physical	Particle size	$< 30\ \mu$m
	Density	1.35–1.40 g/cc
	pH	6.5–7.5
	Solubility	Insoluble in water but soluble in acids
Chemical	Cationic polyamine	
	● High charge density at pH < 6.5	
	● Adheres to negatively charged surfaces	
	● Forms gels with polyanions	
	High molecular weight, linear polyelectrolyte	
	● Viscosity – high to low	
	Chelates certain transitional metals	
	Amiable to chemical modifications	
	● Reactive hydroxyl/amino groups	

solution increases with an increase in chitosan concentration, decrease in temperature and with increasing degree of deacetylation. The viscosity for 1% chitosan (w/v) in various organic acids at different concentrations (Skaugrud 1991) is given in Table 2.

Chitosan is insoluble at an alkaline and neutral pH. The solubility of chitosan in inorganic acids is limited when compared with its solubility in common organic acids. Upon dissolution, amine groups of the polymer become protonated, with a resultant positively charged soluble polysaccharide (RNH_3^+). However, chitosan salts (glutamate, chloride etc.) are soluble in water, the solubility being dependent on the degree of deacetylation. Chitosan with a low degree of deacetylation (40%) has been found (Errington et al 1993) to be soluble up to pH 9.0, whereas chitosan with a degree of deacetylation of about 85% is soluble only up to pH 6.5. Solubility is also greatly influenced by the addition of salt to the solution. The higher the ionic strength, the lower the solubility (Errington et al 1993). This is due to the fact that chitosan in solution exists in an extended conformation due to the repelling effect of each positively charged deacetylated unit on the neighbouring glucosamine unit. Addition of an electrolyte reduces this effect, resulting in a more random coil-like conformation in the molecule. A higher electrolyte concentration thus results in a salting-out effect leading to the precipitation of chitosan from solution (Skaugrud 1991). The physicochemical characteristics of chitosan are summarised in Table 3 (Knapczyk et al 1989; Sanford 1990).

Table 4 Pharmaceutical applications of chitosan (Illum 1998; Paul & Sharma 2000).

Conventional formulation	
Directly compressible vehicle for tablets	Emulsions
CR matrix tablets	Wetting agent
Wet granulation	Coating agent
Gels	Microspheres and microparticles
Films	
Novel applications	
Bioadhesion	Transmucosal drug transport
Vaccine delivery	Gene delivery
Peptide delivery	

Pharmaceutical uses

Chitosan has been investigated for a number of important pharmaceutical applications (Table 4; Illum 1998; Paul & Sharma 2000).

Drug delivery

Recently the use of chitosan in formulation development has increased many fold. Though chitosan exhibits excellent compatibility with organic compounds as cationic dyes and surfactants, starches, quarternary ammonium salts and with most cationic and non-ionic polymers, multivalent anions easily crosslink with chitosan to form gels and precipitates. The cationic nature permits it to form complexes with oppositely charged drug(s)/excipient(s), thereby altering the physicochemical characteristics of the formulation. Reacting chitosan with controlled amounts of multivalent anions result in crosslinking between chitosan molecules. This may be achieved in acidic, neutral or basic environments depending on the method applied. Table 5 lists several counterions for ionotropic gelling of chitosan (Skaugrud 1991).

Table 5 Counterions for ionotropic gelation of chitosan (Skaugrud 1991).

Low molecular weight counterions	High molecular weight counterions	Hydrophobic counterions
Pyrophosphate	Alginate	Octyl sulfate
Tripolyphosphate	κ-carragenan	Lauryl sulfate
Tetrapolyphosphate	Poly-1-hydroxy-1-sulfonate-propene-2	Hexadecyl sulfate
Octapolyphosphate	Polyaldehydro-carbonic acid	Cetylstearyl sulfate
Hexametaphosphate		
$(Fe(CN)_6)^{-4}/(Fe(CN)_6)^{-3}$		

Table 6 Comparison of pharmacokinetic parameters following administration of conventional formulation, commercial sustained release preparation and chitosan granules of indometacin (Miyazaki et al 1988).

Preparation	Drug/chitosan ratio	C_{max}[a] ($\mu g\ mL^{-1}$)	t_{max} (h)	$AUC_{(0-8\,h)}$[a]
Commercial conventional capsule	–	5.76 ± 1.22	0.5–1.5	9.39 ± 1.45
Commercial SR capsule	–	2.59 ± 0.20[c]	1.0–2.0	9.37 ± 0.90
Chitosan granules	2:1	3.62 ± 1.01	1.5–2.0	11.63 ± 1.80
Chitosan granules	1:2	2.23 ± 0.19[b]	3.0–6.0	10.65 ± 1.33

[a]mean $\pm$ s.e.m. of 3 rabbits; [b]significantly different from commercial capsules ($P < 0.05$); [c]$P < 0.10$.
C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}.

Chitosan has been investigated for its possible role in controlling the delivery of active medicaments, which is ascribable to its unique gel forming ability. Chitosan also finds application in fast release dosage forms. The polymer (molecular weight 19.8–400 kDa, degree of deacetylation 74–97%) has been evaluated as a directly compressible vehicle for tablets, but has found limited utility due to lack of good flow properties and compressibility (Sabnis et al 1997).

Influence of excipients (lactose, sodium lauryl sulfate, sodium alginate, hydroxypropyl methyl cellulose, carbopol 934P and citric acid) on drug release from chitosan matrix tablets was investigated using diltiazem hydrochloride as a model water soluble drug (Kristmundsdottir et al 1995). Tablets prepared with chitosan alone exhibited higher friability (1.2%). Addition of carbopol 934P and citric acid resulted in a decrease in friability and an increase in tablet hardness. On the other hand, addition of sodium lauryl sulfate and lactose had an opposite effect.

Upadrashta and co-workers (1992) investigated the potential application of chitosan as a tablet binder. Chitosan, when used at a concentration of 2% tablet weight, resulted in 75% drug dissolution within 5 min. However, an increase in the concentration to 5% of the tablet weight led to only 30% of the drug being dissolved in the same time period. Ilango et al (1997) utilised chitosan as a granulating agent (2% and 4% levels) for sulfamethoxazole tablets, which exhibited sustained release in dogs when compared with the conventional formulation.

Controlled drug delivery. Miyazaki et al (1988) observed the sustaining effect of chitosan on the release of indometacin (water insoluble drug) from granules. A sustained plateau level of indometacin was obtained for drug/chitosan granules (1:2 mixture) when compared with a sharp peak of plasma concentration with conventional commercial capsules (in rabbits). A summary of the results obtained in-vivo, in rabbits, is given in Table 6. Further, the applicability of chitosan (degree of deacetylation 92.7%) as a vehicle for sustained release (SR)-preparation of water soluble drug (propranolol HCl) was examined. Retardation in drug release was observed to be proportional to chitosan content and was attributed to the gel forming ability of chitosan in media of low pH (Sawayanagi et al 1982a).

Miyazaki and co-workers (1990) demonstrated that the addition of alginate sodium to tablet preparations containing chitosan resulted in an extended release property. However, it was observed that an immediate release of the drug was obtained when chitosan was

Table 7 Energies of interaction between chitosan and mucin (Rillosi & Buckton 1995b).

Sample	pH	ΔG_{132}^{LW} (mJ m^{-2})	ΔG_{132}^{AB} (mJ m^{-2})	ΔG_{132}^{TOT}(mJ m^{-2})
Chitosan–mucin	1.2	4.4	−1.5	2.9
	6.4	−1.4	−4.6	−6.0
	7.5	−0.7	0.3	−0.4
Chitosan (hydrated)–mucin	1.2	3.1	−1.0	2.2

used alone and that the addition of alginate sodium, 1000 cps (> 5% level) resulted in an extended release for various drugs. Addition of citric acid to chitosan resulted in gel formation, thereby imparting sustained release properties (Kawashima et al 1985).

Fukuda and Kikuchi (1978) observed that mixing a solution of chitosan with sodium carboxymethyl dextran resulted in the formation of a water insoluble complex which was attributed to the interaction of amino groups (NH_3^+) of chitosan with the carboxylic groups (COO^-) of sodium carboxymethyl dextran. Formation of a polyelectrolyte complex (PEC) between chitosan and pectin has been reported by Macleod et al (1999). It was observed that PEC formation between pectin and chitosan occurred at pH 5.0, when the pectin–chitosan ratio was between 2:1 and 3:1. Further, it was postulated that at this pH, when amino and carboxylic groups were present in both the ionised and the unionised form (NH_3^+, COO^-, NH_2, COOH), an interaction could take place between positively charged chitosan and a negatively charged pectin, resulting in tightening of the PEC network leading to a reduced swelling. Within the PEC, the possibility of intramolecular hydrogen bond formation between -COOH groups of pectin or -NH_2 groups of chitosan and -OH or -$COOCH_3$ groups elsewhere within the network was also not ruled out. Interpolymer complex (IPC) of chitosan with pectin/acacia and its subsequent effect on the release behaviour of chlorpromazine HCl was also investigated by Meshali and Gabr (1993). Binding ratios of the complexes were found to be 1:10 and 1:20 for chitosan/pectin and chitosan/acacia, respectively. A good sustaining effect on the release of chlorpromazine HCl was obtained with a physical mixture of the polymer rather than with the complex. This led the authors to conclude that a better control over the release of the drug could be obtained when the complex was allowed to be formed in-situ during the dissolution process.

An interaction between amino groups of chitosan and carboxyl groups of sodium hyaluronate was reported in an earlier study by Takayama et al (1990). Binding ratio of the complex was affected by changes in pH of the media and ionic bonding force seemed to be a primary binding force responsible for complex formation. The phenomenon of ionic bonding between chitosan (degree of deacetylation > 95%), polyacrylic acid and sodium alginate was further confirmed by Takahashi et al (1990). The rigidity or flexibility of the polymer chains were thought to contribute towards the formation of complexes. Chavasit and Torres (1990) have also reported the formation of a complex between chitosan and polyacrylic acid. Further, the rheological properties and fractal structure of the polyion complex between chitosan and sodium alginate were investigated (Matsumoto et al 1993). Complexation was observed to occur between carboxyl anion of alginate and an amino group of chitosan. The complex developed most markedly at a mixing ratio between 1:1 and 1:2 (molar ratio for chitosan–alginate) at which the dynamic viscoelastic functions of the system manifested a plateau region due to a heterogenous behaviour.

Release of diltiazem HCl from chitosan matrices was retarded by the presence of sodium lauryl sulfate, with the rate of drug release being observed to be decreased with increased surfactant concentration. It was argued that a complex formation between sodium lauryl sulfate and chitosan could change the consistency of the gel layer, thereby leading to a decline in drug release rate (Kristmundsdottir et al 1995).

Mucoadhesive polymers have received considerable attention for controlled drug delivery. The main reason for this interest is that mucoadhesive polymers may fulfil the following desirable features of a controlled drug delivery system (Lueßen et al 1994):

(1) Prolonged residence time at the site of drug absorption;
(2) Increased contact to the absorbing mucosa, resulting in a steep concentration gradient to favour drug absorption; and
(3) Localisation in specified regions to improve and enhance the bioavailability of the drug.

It has been proposed that positive charges on the surface of chitosan could give rise to a strong electrostatic interaction with mucus or a negatively charged mucosal surface (He et al 1999a). In an earlier study, He and coworkers (1998) had proposed a salt bridge effect for the

Table 8 Force of detachment for various chitosan derivatives (Lehr et al 1992).

Polymer	Force of detachment (mN cm^{-2})
Chitosan (Wella 'low viscosity')	3.9 ± 1.2
Chitosan (Wella 'high viscosity')	6.7 ± 0.7
Chitosan	5.7 ± 1.1
Daichitosan H (mw 500000 to 800000)	8.0 ± 5.7
Daichitosan VH (mw 1400000)	9.5 ± 2.4
SeaCure 240 (chitosan-pharmaceutical grade)	4.1 ± 2.9
SeaCure 210^{+} (chitosan glutamate)	9.5 ± 2.5
Chitosan (Sigma)	6.6 ± 3.0

Values are mean ± s.d. of 2–6 observations.

Table 9 Comparison of the adhesive strength of tablets containing 66.7% polymer, 3% insulin, and 30% mannitol (Bernkop-Schnurch et al 1998).

Test tablet	Maximum detachment force (mN)
Precipitated chitosan–EDTA	93.2 ± 15.6
Lyophilised chitosan–EDTA	57.7 ± 9.5
Lyophilised chitosan–EDTA L-lysine (completely neutralised with L-lysine)	51.8 ± 10.9
Lyophilised chitosan–EDTA (completely neutralised with 1,8-diamino octane)	41.6 ± 9.8

Values are mean ± s.d., n = 5.

interaction of positively charged chitosan microspheres with the negatively charged mucus glycoprotein.

Rillosi and Buckton (1995a) investigated surface energies of chitosan (molecular weight 325000; degree of deacetylation 77%) from contact angle and surface tension experiments. Chitosan was observed to exhibit higher adhesivity in intestinal fluids (13599 ± 2800 mg, 5 min contact time) than in an acidic environment (8856 ± 1837 mg, 5 min contact time). Total free energy of interaction (ΔG_{132}^{TOT}) between the three phases (chitosan, mucin and test fluid) and the contributing polar (ΔG_{132}^{AB}) and apolar (ΔG_{132}^{LW}), of chitosan is summarised in Table 7 (Rillosi and Buckton 1995b).

In a novel in-vitro study, Patel et al (1999) evaluated the adsorption and retention of chitosan on the buccal cells obtained from human oral cavity and its subsequent ability to mask the surface glycoconjugates, thereby retarding lectin binding (concanavalin A). Chitosan exhibited a high lectin binding inhibition, thus confirming its mucoadhesive potential. It was able to inhibit over 86% of lectin binding to cells. Mucoadhesive potential of chitosan, as confirmed in this study, corroborated the earlier reported results of Lehr et al (1992), Needleman and Smales (1995) and Henriksen et al (1997). Table 8 lists the force of detachment of chitosan and its various derivatives, as determined by Lehr et al (1992).

In a study by Bernkop-Schnurch et al (1998), influence of different drying methods and of ionic crosslinkers on adhesive strength, cohesiveness, as well as release behaviour from tablets containing chitosan and its conjugate, was investigated. The results for tablets (in 50 mM Tris HCl, pH 8.0 with 0.9% NaCl at 37°C) containing 66.7% polymer, 3% insulin, and 30% mannitol, and using native porcine mucosa as the biological membrane are summarised in Table 9. Reduced adhesivity of the lyophilised polymer was explained on the basis of its amorphous state obtained during the freeze drying technique, leading to a strongly reduced cohesiveness.

Fast release dosage forms. Dissolution of a drug is a prerequisite to drug absorption in the gastrointestinal tract. Chitosan (degree of deacetylation 92.7%) has been associated with enhanced dissolution of griseofulvin from co-ground mixtures and which was observed to be due to a decrease in the crystal size of the drug (Sawayanagi et al 1982b). Theophylline tablets employing chitosan as a hydrocolloid matrix system was investigated by Nigalaye et al (1990) and observed that chitosan at a concentration greater than 50% of the tablet weight resulted in an insoluble non-erosion matrix. However, a fast release of the drug from the formulation was obtained at a chitosan concentration of 33%. It was further observed that at levels < 10%, chitosan acted as a tablet disintegrant. In a related study by Portero et al (1998), chitosan (degree of deacetylation > 80%) was found to result in enhanced dissolution of nifedipine. The effect was observed to be dependent on the polymer–drug mixing ratio, chitosan type and the method adopted to disperse the drug within the polymer. A maximum dissolution was obtained when chitosan to drug ratio was 3:1. The dissolution was highest for the polymer–drug solid dispersion, followed by kneaded mixtures, co-ground mixtures and finally the physical mixtures. The phenomenon of enhanced dissolution was attributed to the decreased drug crystallinity and size and the polymer wetting effect. In a related study, Shiraishi et al (1990) had attributed the enhanced dissolution of poorly water-soluble drugs by low molecular weight chitosan (molecular weight 3800) to its ability to make the drug powder hydrophilic.

Interaction of four kinds of low molecular weight chitosans (varying in molecular weight and degree of deacetylation) with indometacin was investigated by Imai et al (1991). Carboxylic groups of indometacin were observed to be associated with the amino groups of chitosan (molecular weight 3800, degree of deacetylation 66%) through complexation in both aqueous as well as in the solid-state. Dissolution rate of indometacin from the complex was significantly higher as when compared with the drug alone. Though a high dissolution rate was obtained, such complexes could only have very limited utility in the pharmaceutical field due to a very low yield of the complex.

In a novel study (Yao et al 1997), chitosan was reported to protect tablet coating membrane from mechanical damage of the compression force. Co-compression of particles coated with ethylcellulose/HPC and small particle size chitosan prevented the rupture of coating membrane during compression. Plasma concentration time profile of theophylline tablets prepared with chitosan coincided with that of the coated particles, and the compressed tablets disintegrated rapidly, with subsequent redispersion into many discrete particles, following oral administration to dogs.

The influence of the physicochemical characteristics of the drug on the release characteristics from chitosan malate tablets was investigated by Akbuga (1993). Drug solubility, degree of ionisation and molecular weight of the drug was observed to greatly influence the release profile.

Chitosan and peptide delivery. Increasing attention is being paid towards the alternate oral route for the delivery of peptides or peptide based drugs. Although the oral route of peptide delivery offers the greatest ease of application, difficulties still exist in achieving a predictable and reproducible absorption in therapeutic doses without wasting a major fraction of the drug (Lueßen et al 1994).

In a study by Lueßen and co-workers (1997) chitosan glutamate was observed to strongly increase the transport of 9-desglycinamide, 8-arginine vasopressin (DGAVP) across Caco-2 cell monolayers at a concentration level between 0.4–1.0% (w/v). A similarity in transport between two different chitosan glutamate concentrations indicated the maximum transport rate to be achieved at a level of 0.4%. These observations were in accordance with the results obtained with chitosan glutamate at pH values between 4.9 and 6.0, where a plateau level in P_{app} (apparent permeability coefficient) was reached at a polymer concentration of 0.25% and 0.5% w/v (Artursson et al 1994). Results with Seacure210^{+} and Daichitosan VH indicated a comparable improvement in the transport of DGAVP in an in-vitro model of rats. The effect was thought to be mediated through the ability of chitosan derivatives to open the tight junctions (Lueßen et al 1994), and was attributed to the interaction of positively charged amino groups of chitosan with the negatively charged sialic acid groups of the membrane bound glycoproteins (Artursson et al 1994). Similar results were reported in other studies by Lueßen and co-workers (1996) for the peptide drug buserelin, in-vivo. The enhancing effect of 1.5% (w/v) chitosan HCl resulted in the highest absolute bioavailability for intraduodenally administered buserelin in rats (5.1 ± 1.5%) when compared with the control (0.1 ± 0.1%). Chitosan was suggested to improve the mucosal peptide absorption by opening of intercellular junctions with no inhibitory effect being seen towards the proteases. Keeping this in mind, and also to incorporate some protease inhibitory activity to chitosan, Bernkop-Schnurch and Krajicek (1998) synthesized and evaluated various chitosan–EDTA conjugates for their ability to increase peptide absorption due to its capacity to bind bivalent cations, which are essential co-factors for intestinal proteolytic enzymes. Chitosan–EDTA conjugates (1:20 and 1:40) displayed excellent mucoadhesive properties and exhibited a strong inhibitory effect on enzymatic activity of carboxypeptidase A as well as aminopeptidase N. Lowering the percentage of covalently linked EDTA on the polymer resulted in a significant decrease in the adhesive force. This led to the conclusion that chitosan–EDTA conjugates exhibiting the lowest amount of free amino groups appeared to be useful in overcoming the enzymatic barrier for orally administered peptides.

In an earlier study, Bernkop-Schnurch and co-workers (1997) demonstrated the ability of chitosan–EDTA conjugate to bind 2.01 ± 0.12 mM of zinc per gram of the polymer at pH 6.5 ($n = 3 \pm$ s.d.). As zinc is an essential co-factor for aminopeptidase N, enzyme activity (48 mU mL^{-1}) could be completely inhibited under the use of 1% chitosan–EDTA conjugates. Inhibitory effect of chitosan–EDTA conjugate (1%) on the degradation of leu-enkephalin by aminopeptidase N was 2.9-fold higher than that of zinc complexing bacitracin–polyacrylic acid conjugate at the same concentration. Chitosan conjugate exhibited a higher bioadhesion than the unmodified one, was easily hydratable in water and basic aqueous solutions and therefore exhibited quick swelling properties. Further, chitosan–EDTA was covalently linked to Bowman-Birk inhibitor (BBI) (Bernkop-Schnurch & Pasta 1998) and evaluated for its mucoadhesive potential as well as its

Table 10 Inhibitory effect of Ac-YIGSRβAG-chitosan in melanoma B16-BL6 in mice (Hojo et al 1999).

Treatment	Dose (mg)	Tumour colonies
Control	–	218.50 ± 34.02
Ac-YIGSRβA-OH	0.3 (0.4)[a]	153.13 ± 26.74
	0.1 (0.12)[a]	140.97 ± 48.61
Ac-YIGSRβAG	0.3 (0.12)[a]	77.78 ± 24.31
	0.1 (0.04)[a]	170.14 ± 38.89

[a]Peptide content in μmol, results are mean ± s.e.m., n = 5. Melanoma B16-BL6 cells (1×10^5/0.1 mL i.v.) and Ac-YIGSRβAG-chitosan hybrid (1 mg/0.1 mL, i.v.) were administered separately. Control mice received the same amount of melanoma B16-BL6 cells. Lung tumour colonies were counted after 14 days.

ability to inhibit various proteases. The conjugate exhibited an adhesive strength of 54.4 ± 7.7 mN (mean ± s.d., n = 5) whereas an adhesive strength of 21.0 ± 3.8 mN (mean ± s.d., n = 5) was reported for the polymer–BBI complex. A strong inhibitory activity was shown by all polymer–BBI conjugates towards trypsin and chymotrypsin but a relatively lower inhibitory effect towards elastase. Due to the high binding affinity of chitosan–EDTA–BBI for zinc, a strong protective effect was observed towards carboxypeptidase A and aminopeptidase N. Similar results have been reported for chitosan–EDTA complex covalently linked to antipain, chymostatin and elastatinal (Bernkop-Schnurch & Scerbe-Saiko, 1998).

Recently Hojo et al (1999) prepared a hybrid of chitosan (molecular weight 3750) and an antimetastatic laminin related peptide Acetyl-Tyr-Ile-Gly-Ser-Arg-βAla-OH (Ac-YIGSRβA-OH) by a solid phase method, and subsequent reaction with water soluble chitosan. Chitosan amino groups did not react with the peptide using diphenylphosphonyl azide/diisopropylcarbodiimide/1-hydroxybenzotriazole, water soluble carbodiimide/1-hydroxybenzotriazole, phosphazo or 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU). A small spacer (tert-butyloxycarbonyl-Gly) was reacted with chitosan using the TBTU method. Following removal of the protecting groups, Gly-chitosan was coupled with Ac-YIGSRβA-OH by the water soluble carbodiimide method to give Ac-YIGSRβAG-chitosan. It was observed that the inhibitory effect of the peptide–chitosan hybrid on experimental metastasis in mice was not reduced, but actually potentiated (Table 10), suggesting the possible use of chitosan as a drug carrier for peptides. The potentiation of the antimetastatic effect of a peptide by hybrid with a polymer was proposed to be based on slower enzymatic degradation and more stable receptor binding of the hybrid as compared with the parent peptide.

Bugamelli et al (1998) prepared and evaluated chitosan microspheres containing insulin. A constant release of the peptide for a period in excess of 80 h was observed in-vitro.

A recent review by Bernkop-Schnurch (2000) has exhaustively dealt with the applications of chitosan and its derivatives for peroral peptide drug delivery.

Chitosan as an absorption enhancer. Chitosan has recently been reported as an absorption enhancer for hydrophilic drugs across the intestinal and nasal mucosa. Schipper et al (1996) investigated the effects of the chemical composition and molecular weight of chitosan on the intestinal permeability and toxicity, using human intestinal Caco-2 cells as model epithelium and ^{14}C mannitol as a model drug. Chitosans with a low degree of acetylation (1 and 15%) were found to be active absorption enhancers at low and high molecular weight, but a dose dependent toxicity of chitosan was observed. On the other hand, chitosan with a degree of acetylation of 35 and 49% enhanced ^{14}C mannitol absorption at high molecular weight only but exhibited lesser toxicity. Chitosan (degree of acetylation 35%, molecular weight 170 kD; C 35:170) was found to provide an early onset of action, very low toxicity and a flat dose absorption enhancement response relationship. The mechanism underlying the above mentioned effects of chitosan were further investigated at the cellular level by Schipper and co-workers (1997). It was observed that chitosan with a low degree of acetylation (DA, 1%) and molecular weight (31 kD; C1:31) and one with a higher DA (35%) and molecular weight (170 kD) bound tightly to the epithelium. No cellular uptake of chitosan was observed, both were able to increase the apical but not basolateral cell membrane permeability and induced a redistribution of cytoskeletal-F actin and the tight junction protein ZO-1, leading to a higher paracellular permeability of hydrophilic markers which was inhibited by the addition of negatively charged heparin. This led to the conclusion that the absorption enhancing effects of chitosan were mediated through its positive charge. This was further confirmed by Dodane et al (1999) who demonstrated the activity of chitosan on the functions and structure of monolayer epithelial cells. Involvement of tight junctions was visualised by confocal scanning microscopy using tight junctional proteins occludin and ZO-1. An increased transport of compounds was obtained in the acidic medium where the pH was less or in the order of the pKa of chitosan (Yalpani & Hall, 1984).

Table 11 Effect of TMC, chitosan glutamate and chitosan HCl on the permeability of ^{14}C mannitol and PEG 4000 at pH 6.2 (Kotze et al 1998b).

Chitosan salt	Marker	Control	Concentration of chitosan (% w/v)					
			0.25	0.50	1.0	1.5	2.0	2.5
TMC	^{14}C mannitol	0.72 ± 0.08 (1)	8.11 ± 0.21* (11)	9.26 ± 0.35* (13)	14.0 ± 0.4* (19)	7.52 ± 0.86* (17)	12.35 ± 0.43* (19)	15.21 ± 1.37* (21)
($P_{app} \times 10^{-7}$ cm s^{-1})	^{14}C PEG 4000	0.09 ± 0.02 (1)	0.39 ± 0.02* (4)	0.38 ± 0.03* (4)	0.57 ± 0.08* (6)	0.46 ± 0.06* (5)	0.67 ± 0.08* (7)	0.80 ± 0.03* (9)
Chitosan glutamate	^{14}C mannitol	0.72 ± 0.08 (1)	18.25 ± 1.10* (25)	14.17 ± 0.45*,† (29)	20.82 ± 0.30* (29)	18.29 ± 1.53* (25)	nd	nd
($P_{app} \times 10^{-7}$ cm s^{-1})	^{14}C PEG 4000	0.09 ± 0.02 (1)	0.91 ± 0.03* (10)	1.00 ± 0.05* (11)	1.28 ± 0.29* (14)	1.02 ± 0.19* (11)	nd	nd
Chitosan HCl	^{14}C mannitol	0.72 ± 0.08 (1)	24.65 ± 2.13* (34)	23.28 ± 1.00* (32)	25.56 ± 2.95* (36)	26.16 ± 1.86* (36)	nd	nd
($P_{app} \times 10^{-7}$ cm s^{-1})	^{14}C PEG 4000	0.09 ± 0.02 (1)	2.44 ± 0.14* (27)	2.30 ± 0.19* (26)	3.54 ± 0.38*,† (39)	4.36 ± 0.29*,† (48)	nd	nd

Each value represents the mean ± s.d., n = 3; *significantly different from control ($P < 0.05$); †significantly different from all other treatments in the group ($P < 0.05$); nd, not determined due to the insolubility of chitosan salts; value in parentheses, absorption enhancement ratio; P_{app}, apparent permeability coefficient.

This suggested charge density of chitosan to be an important factor for enhancement of mucosal transport (Kotze et al 1996; Schipper et al 1997). Chitosan, being a weak base, loses its charge with subsequent precipitation in neutral and basic environment thus proving to be ineffective as an absorption enhancer, limiting its use in the alkaline pH of the intestine and colon. A similar phenomenon was demonstrated by Kotze et al (1998a). No significant increase in the transport of ^{14}C mannitol at pH 7.4 in the presence of chitosan salts (glutamate and hydrochloride; both degree of deacetylation 83%) was observed. However, at pH 6.2, incubation with the polymers resulted in a marked accumulation of ^{14}C mannitol in the receptor compartment. The cumulative amounts transported up to 4 h after incubation with 0.5% polymers were 14.3 ± 1.0% (chitosan HCl) and 8.9 ± 0.2% (chitosan glutamate) of the total applied dose. At a concentration of 1.5% of the polymer, the cumulative amounts transported were 17.4 ± 1.2% (chitosan HCl) and 11.8 ± 1.1% (chitosan glutamate) of the total dose applied and this represented a 36-fold and 25-fold increase, respectively, in the transport rate compared with the control.

Due to the insolubility of chitosan glutamate and chitosan HCl at the relatively neutral pH of intestine, a partially soluble and quarternised derivative of chitosan, N-trimethyl chitosan chloride, was synthesised and its effects on transepithelial electrical resistance (TEER) and intestinal epithelial permeability were investigated in-vitro. The polymer (at 1.5–2.5% w/v) was observed to cause a pronounced and an immediate reduction (25–85%) in TEER of Caco-2 cells. Large increases in the transport rates of ^{14}C mannitol (37–60 fold), buserelin (28–73 fold) and fluorescein isothiocyanate labelled dextran (molecular weight 4400, 167–373 fold) were demonstrated. The effect was proposed to be mediated by the ability of chitosan to open the tight junctions of the intestinal epithelial cells which was confirmed by confocal laser scanning microscopy (Kotze et al 1997). Further, Kotze and co-workers (1998b) compared the effects of N-trimethyl chitosan chloride (TMC) on the permeability of intestinal epithelial cells using Caco-2 cell monolayer with chitosan HCl and chitosan glutamate, using ^{14}C mannitol and PEG 4000 (Table 11). Chitosan salts were found to reduce the TEER of intestinal cell monolayers in a reversible manner to allow for paracellular transport. The influence of chitosan microspheres on the transport of prednisolone phosphate across the epithelial barrier was investigated by Mooren et al (1998). Studies were performed in polarised human cell line HT-29B6. Binding of prednisolone to chitosan microspheres was observed to drastically enhance the drug permeability when compared with the drug solution ($P = 35.37 \pm 3.21 \times 10^{-6}$ cm s^{-1}, and $8.68 \pm 8.24 \times 10^{-6}$ cm s^{-1} for chitosan microsphere and drug solution, respectively).

As the gastrointestinal tract is covered with a mucosal barrier throughout its length, mannitol permeability was investigated in mucus producing cell line HT29-H. The presence of an intact mucus layer was observed to reduce the absorption enhancement by chitosan. Mucus may, therefore, act as a diffusion barrier for chitosan, suggesting that in the presence of mucus chitosan might not reach the epithelium and may, therefore, exert its absorption enhancing effects to a limited extent only (Schipper et al 1999). These studies could have an important bearing on the use of chitosan as an absorption enhancer.

Fernandez-Urrusuno et al (1999a) investigated the potential of chitosan (molecular weight < 50 000–130 000; degree of deacetylation 70–87 %) nanoparticles as a system for improving the systemic absorption of insulin following nasal instillation. Nanoparticles prepared by ionotropic gelation with tripolyphosphate enhanced the nasal absorption of the peptide to a greater extent than an aqueous solution of chitosan in a conscious rabbit model by monitoring the plasma glucose levels. The amount and molecular weight of chitosan did not have a significant effect on insulin release. In a related study, Fernandez-Urrusuno et al (1999b) assessed the efficacy of freeze-dried insulin-loaded chitosan nanoparticles in lowering the plasma glucose levels following nasal administration. Fresh chitosan nanoparticles displayed a positive charge and a high insulin loading (> 55 %). Upon freeze-drying in the presence of trehalose or sucrose (cryoprotective agents), the nanoparticles were freely reconstituted in water without a significant change in their physicochemical properties and release rate. Further in-vivo evaluation in the rabbit model revealed that insulin associated nanoparticles could reduce the plasma glucose levels to a greater extent than aqueous solution. Consequently, freeze-dried nanoparticles were proposed as useful vehicles for increasing the nasal absorption of insulin.

Colon targeting. Interest has been shown in the application of chitosan to deliver peptides or other drugs directly to the colon. In one study, Tozaki et al (1997) investigated the potential of chitosan for colon specific delivery of insulin. Following oral administration of chitosan capsules containing insulin (20 IU) to rats, it was observed that the capsules reached the large intestine after 6–12 h. The hypoglycemic effect (as determined by monitoring plasma glucose levels) started 6 h post-chitosan capsule administration. Plasma insulin concentration reached a maximum of 326 μU mL^{-1}, at 7 h post-dosing with the bioavailability of insulin being 5.73 %. However, no significant hypoglycemic effect was noted after colonic administration of insulin solution (20 IU). The results were attributed to the improved stability of insulin in the gastrointestinal tract within the chitosan capsules.

The enteral absorption of insulin (measured as a function of blood glucose levels) from chitosan coated liposomes was also investigated by Takeuchi et al (1996) in the rat model. Degree of adhesion of such liposomes to gastrointestinal tract was dependent on the amount of chitosan. Blood glucose levels were significantly decreased following administration of chitosan coated liposomes, with the hypoglycemic effect being maintained for more than 12 h, suggesting possible mucoadhesion of chitosan coated liposomes to the intestinal tract of rats.

In a study by Tozaki et al (1999a), 5-amino salicylic acid (5-ASA) containing chitosan capsules were also observed to be more effective than other 5-ASA formulations for the treatment of 2,4,6-trinitrobenzene sulfonic acid sodium salt induced colitis in rats. Similar results were obtained with the colon specific delivery of R68070, a new thromboxane inhibitor (Tozaki et al 1999b).

Chitosan and gene delivery. Understanding of the molecular basis of disease provides the opportunity to modulate or replace a dysfunctional gene. Vectors are, therefore, being designed to efficiently transfect and target cells in-vivo. In recent years, it has been increasingly recognised that cationic polymers have the potential to complex DNA and, hence, may prove to be useful as non-viral vectors for gene delivery. This is an attractive choice as simple mixing with DNA in-vitro could lead to electrostatically driven self assembly of polymer–DNA complexes into nanoscale polyplexes (Felgner et al 1997). Use of chitosan in gene delivery has several advantages, some of which are listed below (Leong et al 1998):

(1) Conjugation of ligands to the nanospheres, for targeting or stimulating receptor-mediated endocytosis.
(2) Incorporation of lysosomolytic agents to reduce the degradation of DNA in the endosomal and lysosomal compartments.
(3) Co-encapsulation of other bioactive agents for multiple plasmids.
(4) Improvement of bioavailability of DNA due to the protection from serum nuclease degradation by the polymer matrix.
(5) Lyophilisation of nanospheres for storage, without the loss of any activity.

In a study by Erbacher et al (1998), formulation of plasmid DNA with chitosan resulted in a homogeneous population of complexes that were stable and had a diameter of approximately 50–100 nm. These complexes were found to effectively transfect HeLa cells, independently of the presence of serum (10 %) without the need for the presence of an endosomolytic agent. The gene expression was also observed to gradually increase over time (from 24–96 h). At 96 h, chitosan was found to be 10-times more efficient than polyethylene-imine-mediated transfection. Roy et al (1999) described an immunoprophylactic strategy using oral allergen-gene immunisation to moderate peanut antigen induced murine anaphylactic responses. Oral administration of

Table 12 Applications of chitosan.

Polymer(s)	Drug (dosage form)	Summary of the study	Reference
Chitosan	Chlorhexidine gluconate (gels/films for oral mucosal delivery)	Prolonged release of the drug from films Chitosan gels less active than chlorhexidine solution against *Candida albicans* Highest antifungal activity obtained with 2 % chitosan gel containing 0.1 % chlorhexidine Synergistic effect observed between chitosan and chlorhexidine	Senel et al 2000
Chitosan	Lidocaine HCl (beads)	20–35 % of the total theoretical amount of lidocaine entrapped in the beads Release of lidocaine prolonged	Kofuji et al 1999
Chitosan	Cis-platin (microspheres)	Incorporation efficiency of the drug ranged between 28–99 % Initial burst effect observed during dissolution	Akbuga & Bergisadi 1999
Chitosan	Metoclopramide (microspheres)	Spherical microspheres with shallow invaginations obtained Chitosan microspheres exhibited CR (> 8 h) Release rate from microspheres prepared with 20 % formaldehyde was unaffected by pH	Ganza-Gonzalez et al 1999
Chitosan (mw 140–169 kDa)	Cimetidine/famotidine (microspheres)	Prepared by novel w/o/w emulsion spray drying method Severe retardation in drug release attributed to poor wettability of the drug	He et al 1999a
Chitosan	Cimetidine/famotidine/ nizatidine (microspheres)	Prepared by conventional spray drying technique Faster rate of drug release with an accompanying burst effect	He et al 1999b
Chitosan	Chlorpheniramine maleate (microspheres)	Spherical microspheres ranging between 82–420 μm diameter Increasing weight ratio of chitosan coating to chlorpheniramine maleate resinate (1 : 1 to 4 : 1) moderately decreased release profile (up to 33 h)	Huang et al 1999
Chitosan	Ibuprofen (tablets)	Tablets prepared using chitosan paste (1, 3 and 5 %) *In vivo* studies in dogs exhibited SR (sustained release)	Ilango et al 1999
Chitinosans	Salicylic acid (tablets)	Chitinosans (degree of deacetylation < 75 %) significantly affected the crushing strength as well as the release profile of salicylic acid An interaction between amino groups of chitinosan with oppositely charged drug ions confirmed by DSC	Rege et al 1999
Chitosan	Dextran molecular weight 70 000 (beads)	Chitosan treated alginate beads prepared as oral CR system for a macromolecular drug by ionotropic gelation method Addition of chitosan increased the drug loading capacity and at the same time reduced the release of the drug Chitosan treatment suppressed erosion of the beads	Sezer and Akbuga 1999
Chitosan	Amoxicillin and metronidazole (microspheres)	Microspheres developed for stomach specific drug delivery and, hence, could prove very beneficial for increasing local absorption of the antibacterial agents and eradicating *Helicobacter pylori* infection High porosity of drug loaded microspheres led to a complete drug release within 2 h	Shah et al 1999
Chitosan	– (microspheres)	Chitosan microspheres reduced the rate of clearance from nasal cavity ($t_{1/2cl}$ 84 min) as compared with chitosan solution ($t_{1/2cl}$ 41 min) and control ($t_{1/2cl}$ 21 min)	Soane et al 1999
Chitosan	Phenobarbital (microspheres)	Spherical microspheres with a loading efficiency up to 57.2 % Molecular weight and concentration of chitosan affected the performance of the microspheres as did the concentration of the stabilising agent (sorbitan monooleate) Sustained drug release obtained after an initial burst effect A high concentration of sorbitan monooleate increased drug release rate	al-Helw et al 1998
Chitosan/casein	Diltiazem HCl (microspheres)	Interaction between chitosan solution in dilute acetic acid (5 % v/v) and casein solution in 0.5 M NaOH was the basis of microsphere formation Free flowing microspheres obtained, whereas the drug was non-flowable Retardation in drug release observed, which was increased with an increase in casein concentration and stirring time On the other hand, increasing chitosan concentration and using a high initial drug loading exhibited a faster drug release	Bayomi et al 1998
Chitosan	Salbutamol sulfate (beads)	Chitosan beads exhibited a rapid drug release in an acidic pH SR obtained in phosphate buffer (pH 7.4)	el-Fattah et al 1998
Chitosan	Ketoprofen (microspheres)	The drug release was modulated over a period of 48 h Microspheres consisting of high (2 000 000) and low (70 000) molecular weight chitosan (1 : 2 w/w) were the most suitable formulation in controlling drug release	Genta et al 1998
Chitosan	– (microspheres)	Produced by spray drying A strong interaction between microspheres and mucin was detected with > 50 % microspheres being retained in the rat intestine, when compared with the control ethylcellulose microspheres	He et al 1998
Chitosan	Propranolol HCl (microspheres)	Investigated the effect of the addition of magnesium stearate on chitosan microspheres prepared by emulsification coacervation technique Degree of aggregation of chitosan microspheres reduced by the incorporation of magnesium stearate, which also resulted in discrete spherical microspheres with smooth surfaces However, propranolol loaded microspheres had convoluted surface and ill-defined shape Release of the drug was fast, irrespective of magnesium stearate content which, in fact, increased drug loading with increasing concentration	Lim & Wan 1998

Table 12 (*cont.*)

Polymer(s)	Drug (dosage form)	Summary of the study	Reference
Chitosan/ ethylcellulose	Nifedipine and propranolol HCl (tablets/films)	These new devices exhibited promising potential for use in CDDS Tablets produced by *in situ* crosslinking with polycarbophil displayed controlled swelling, release and adequate adhesivity	Remunan-Lopez et al 1998
Chitosan/N,O-carboxymethyl chitosan	Morphine sulfate (sub cutaneous injectable gel)	Gel resulted in significant nociception within 10 min with a maximum being achieved at 60 min Effect sustained for 6 h in rats	Tasker et al 1998
Chitosan/sodium alginate	Interleukin-2 (microspheres)	Drug was released in a sustaining manner and the drug loaded microspheres triggered the induction of cytotoxic T-lymphocytes more efficiently than the pure drug	Liu et al 1997
Chitosan	Oxytetracycline (microspheres)	Prepared with chitosans of various molecular weights (70 000 to 2 000 000) Results indicated a greater sustaining effect for higher molecular weight chitosan	Mi et al 1997
Chitosan	Diclofenac sodium (tablets)	Degree of acetylation and N-deacetylation of chitosan significantly affected drug release at pH 1.2 and 6.8 ($P < 0.0001$) Factors preventing the release of the drug in an acidic environment were insolubility of the drug, formation of a rate limiting chitosan gel barrier and ionic interaction of diclofenac sodium with ionised amino groups of chitosan	Sabnis et al 1997
Chitosan/N,O-carboxymethyl chitosan	Morphine sulfate (injectable gel)	Gel prepared with a combination of the two polymers was easily injectable Prolonged but complete bioavailability from the gel in dogs	Tasker et al 1997
Chitosan	Prednisolone (microspheres)	Drug release found to be dependent on drug polymer ratio	Berthold et al 1996
Chitosan/chondroitin sulfate	Diclofenac sodium (beads)	Complex formation between chitosan and chondroitin sulfate suppressed the disintegration of alginate gel beads Prolongation of preparation time resulted in a decrease in apparent release rate, though it did not markedly affect the release pattern	Murata et al 1996
Chitosan/sodium alginate	Diltiazem HCl (tablets)	Rapid release of drug, which could be modified by changing the mixing ratios of chitosan and sodium alginate Chitosan content in the tablet and/or the viscosity grade of either of the polymers resulted in a reduction in drug release rate Significant improvement in bioavailability after sublingual administration (69.6%) of tablets prepared with a 1:4 chitosan:alginate weight ratio, when compared with oral administration (30.4%)	Miyazaki et al 1995
Chitosan/sodium alginate	Ketoprofen (tablets)	Magnitude of adhesion force comparable to that of Aftach® A decrease in release rate observed with increasing chitosan content in the tablets Plasma concentration curve in rabbits for tablets with 1:4 (chitosan/alginate) demonstrated SR 3 h post-administration	Miyazaki et al 1994
Chitosan/sodium alginate	Theophylline (directly compressible tablets)	SR of the drug was independent of the medium pH Drug release at a lower pH occurred primarily by chitosan dissolution, whereas, at a neutral pH it was controlled by gelation and dissolution of alginate	Yomota et al 1994
Chitosan	Lidocaine HCl (gel)	Degree of deacetylation (approx. 70%) and chitosan content important for release properties Release profile was zero order	Kristl et al 1993
Chitosan	5-fluorouracil (microspheres)	Chitosan found to be a suitable agent for SR	Li et al 1991
Chitosan/sodium hyaluronate	Brilliant blue FCF (tablets)	Strong mucoadhesion observed for a physical mixture of sodium hyaluronate and chitosan The pH of the medium did not affect adhesion of the tablets Release rate greatly decreased for tablets prepared with chitosan and sodium hyaluronate (3:7), which was non-fickian, suggesting the release to be due to diffusion and swelling of the matrix	Takayama et al 1990
Chitosan	Cis-platin (microspheres)	Drug loading efficiency increased with an increase in chitosan concentration with a simultaneous increase in SR effect	Nishioka et al 1990

DNA nanoparticles synthesised by complexing plasmid DNA with chitosan resulted in transduced gene expression in the intestinal epithelium, thus indicating the probable use of chitosan–DNA nanoparticles in murine anaphylactic responses. The successful transfection of HeLa cells with a plasmid that coded for betagalactosidase was achieved by Venkatesh and Smith (1998). A non-ionic interaction between the carboxylate backbone of chitosan and cell surface proteins was thought to probably play an important role in chitosan mediated transfection. On the other hand, Hayatsu et al (1997) observed that a treatment with phosphate buffered saline (pH 7.0, 37°C for 26 h) resulted in the release of only a minute quantity of DNA (0.05%) from chitosan–DNA complex, thereby indicating the formation of a tight complex, possibly through ionic interactions.

The potential of chitosan (molecular weight < 5000 to 10 000; degree of deacetylation 55.3–65.4%) in gene delivery has recently been investigated by Richardson and co-workers (1999). Chitosan molecular mass fractions were observed to readily complex DNA even down to a chitosan:DNA charge ratio of 1:0.1, which also resulted in a significant decrease in the degradation by DNase II with no degradation being apparent at a charge ratio of 1:1. It was further observed that low molecular mass fractions of chitosan were non-toxic against L 132 human embryonic lung cells or CCRF-CEM human lymphoblastic leukaemia cells up to a concentration of 500 μg mL^{-1}, suggesting the use of low molecular weight chitosan in i.v. administration. These results are in contradiction to those of Carreno-Gomez and Duncan (1997), who investigated chitosan with different degrees of deacetylation and molecular weights for cytotoxicity towards B16F10 cells and their ability to lyse rat erythrocytes. Cytotoxicity towards B16F10 cells was observed to be dependent upon the type and concentration of chitosan salt used and the polymer molecular weight. Chitosan HCl was the most toxic having an IC50 (inhibitory concentration 50%) of 0.21 ± 0.04 mg mL^{-1} which was only 4-fold less toxic than poly-L-lysine (IC50 0.05 ± 0.01 mg mL^{-1}). High molecular weight chitosan was the most toxic, the resulting ranking of toxicity being chitosan HCl > chitosan hydroglutamate > glycol chitosan > chitosan hydrolactate. Haemoglobin was released by exposure to all soluble chitosans in a time dependent manner. This was further confirmed by scanning electron microscopy (SEM) which showed changes in B16F10 and red cell morphology.

However, in a study (Quong & Neufeld 1998) involving the immobilisation of DNA within an alginate gel matrix coated with chitosan, almost total hydrolysis of DNA was observed in alginate beads following exposure to nucleases and less than 1% of the total double stranded DNA remained unhydrolysed within chitosan beads corresponding with an increase in DNA residuals. It was further noted that chitosan membranes did not offer sufficient protection against DNase diffusion.

Table 12 summarises varied drugs and polymer combinations using chitosan for the purpose of drug delivery.

Biological properties of chitosan

It is considered very advantageous that chitin and chitosan possess low toxicity, are only slightly allergenic, exert moderate immunostimulating effects and are also metabolised by lysosomes (Chobot et al 1995). In-vivo toxicity studies show chitosan to be inert, non-toxic and biodegradable. The LD50 (lethal dose 50%) of chitosan in mice was determined to be greater than 16 g kg^{-1} (Arai et al 1968). A summary of the biomedical properties of chitosan is given in Table 13 (Sanford et al 1990; Skaugrud 1991).

Table 13 Biomedical applications of chitosan (Sanford et al 1990; Skaugrud 1991).

Biocompatibility	Anticholesterolimic
Natural polymer	Contact lens – (crosslinked to give porous grindable lens material which is non-allergenic)
Biodegradable sutures	
Safe and non-toxic	
Haemostatic	Wound healing – absorbable sutures
Fungistatic	Eye bandages – forms tough protective coating
Spermicidal	Dental – bioadhesive
Anticarcinogen	Orthopaedic – temporary bioengineering material

Hypocholesterolemic effects

Sugano et al (1988) studied the relationship between hypocholesterolemic efficacy and average molecular weight of chitosan, in rats fed on cholesterol-enriched diet. At a 5% dietary level, chitosan almost completely prevented the rise of serum cholesterol level. However, at a 2% level, chitosan with viscosity at both the extremes exerted a comparable cholesterol lowering action. Thus, the hypocholesterolemic action of chitosan was proposed to be independent of its molecular weight.

Effect of various different grades of chitosan on faecal excretion of fat in rats fed on a high fat diet was investigated by Deuchi et al (1995). Chitosan intake resulted in a higher level of fat to be excreted in the corn oil receiving rats than the lard receiving ones, although the effect was strong for both diet groups. A supplement of ascorbic acid to each chitosan diet resulted in a significant depression of fat digestion and absorption in the lumen. It was observed that an increase in the viscosity or the degree of deacetylation of chitosan resulted in a pronounced effect on the apparent digestibility of fats.

The mechanism of inhibition of fat digestion by chitosan was also investigated by Kanauchi et al (1995), who attributed this ability to the dissolution of chitosan in the acidic environment of the stomach with a subsequent change to a gel form capable of entrapping fats

in the intestine. A synergistic effect was observed with sodium ascorbate (AS) and was attributed to:

(1) A viscosity reduction of the polymer combination in the stomach, thus implying that chitosan mixed with a lipid might be better than chitosan alone;
(2) An increase in oil holding capacity of the chitosan gel; and
(3) Chitosan-fat gel being more flexible and less likely to leak the entrapped fat in the intestinal tract.

The fact that orally administered chitosan binds fats in the intestine, blocking absorption, has led various researchers to believe that dietary supplementation with chitosan may be able to inhibit the formation of atherosclerotic plaque. This hypothesis was investigated by Ormrod et al (1998) using the apolipoprotein E-deficient mouse model of atherosclerosis. Mice were fed for 20 weeks on a diet containing 5% chitosan or on a control diet. Blood cholesterol levels were found to be significantly lower in chitosan treated mice and at 20 weeks were 64% of control levels. On comparing the aortic plaque in the two groups, a highly significant inhibition of atherogenesis in both the whole aorta (42%) and the aortic arch (50%) was observed in the chitosan fed mice, suggesting the possible use of chitosan in inhibiting the development of atherosclerosis in individuals with hypercholesterolemia.

LeHoux and Grondin (1993) investigated the effects of chitosan on plasma and liver cholesterol levels, liver weight and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key regulatory enzyme of cholesterogenesis, in rats fed on a sterol diet (1% cholesterol and 0.2% cholic acid). Chitosan at a level of 5% lowered plasma and liver cholesterol levels by 54% and 64%, respectively. High molecular weight chitosan ($>$ 750 kDa) had less hypocholesterolemic potential than a 70 kDa preparation. Incorporation of chitosan (70 kDa, 7.5%) for 3 weeks in a sterol diet completely prevented any decrease in plasma high-density lipoproteins cholesterol, or increase in plasma cholesterol level and liver weight. HMG-CoA reductase activity was 7.7-times more elevated in the sterol chitosan group than in the sterol group. No significant difference was observed in results obtained at 1, 3, or 6 weeks. It was concluded that chitosan at 7.5% was able to adequately maintain cholesterol homeostasis in rats despite a greatly increased intake of cholesterol.

Han et al (1999) assessed the effect of chitosan treatment on the activity of pancreatic lipase in-vitro and on the degree of fat storage induced in mice by oral administration of a high fat diet for 9 weeks. Chitosan prevented the increase in body weight, hyperlipidemia and fatty liver induced by a high fat diet. The antiobesity effects of chitosan in high fat-treated mice was attributed to the inhibition of intestinal absorption of dietary fat with no effect being observed on pancreatic lipase activity. However, in a double blind study (Wuolijoki et al 1999) involving 51 obese females, administration of microcrystalline chitosan (McCh, 3 capsules each 400 mg, twice a day for 8 weeks) did not significantly alter serum total and high density lipoprotein cholesterol ($P > 0.01$) but slightly increased serum triglycerides when compared with placebo ($P = 0.015$–0.060). No reduction in body weight was observed for any treatment group. Similar results were reported by Pittler et al (1999), who did not observe any reduction in body weight in obese subjects following oral treatment with chitosan.

Chitosan was compared with other hypocholesterolemic agents such as cholestyramine and oat gum for its effects on lipid lowering and on intestinal morphology in rats (Jennings et al 1988). Cholestyramine, chitosan and oat gum were able to significantly lower liver cholesterol. Cholestyramine feeding yielded results identical to the non-cholesterol group. On the other hand, chitosan and oat gum moderately lowered liver cholesterol. However, chitosan and cholestyramine significantly lowered serum cholesterol compared with the cellulose group. Histological examination of small and large bowel with morphometry revealed a statistically significant increase in both proximal and distal large bowel mucosal thickness in the cholestyramine fed group. No changes were noted in the proximal large bowel. Neither chitosan nor oat gum produced any mucosal changes other than an increase in the distal small bowel with the oat meal diet. It was concluded that chitosan may have the lipid lowering effects similar to those of cholestyramine without producing any deleterious changes in the intestinal mucosa.

Renal effects

Chitosan has been suggested to be effective in the treatment of patients with renal failure. In a study involving 40 patients with renal failure undergoing haemodialysis treatment, administration of chitosan (1350 mg, twice a day), resulted in the reduction in total serum cholesterol levels (10.4 ± 4.4 to 5.82 ± 2.19 mM) with an increase in serum haemoglobin levels (58.2 ± 12.1 to 68.0 ± 9.0 g L^{-1}), but a significant reduction in urea and creatinine levels in serum were observed after 4 weeks of chitosan administration (Jing et al 1997), suggesting the potential application of the polymer in treating patients with renal failure.

Wound Healing

Okamoto et al (1995) analysed the accelerating effects of open wound healing by chitin and chitosan in dogs. A greater re-epithelialisation was observed in chitin and chitosan treated groups than in the control group, though the difference was found to be insignificant. Similar results were reported by Sall et al (1987), who did not observe any statistically significant difference in corneal wound healing tensile strength between chitosan treated eyes and control in a rabbit model. However, in a survey conducted by Bartone and Adickes (1988), effect of chitosan on wounds of the genitourinary system in dogs was explored with no adverse effects being observed on urogenital wound healing. In all the tissues investigated (kidney, ureter, and penile foreskin), a decrease in fibrosis was seen in wounds treated with chitosan. It was concluded that treatment with chitosan could result in decreased morbidity of urogenital surgery. Chitosan was also found to have a haemostatic effect, which was proposed to be due to an interaction between the cell membrane of erythrocytes and chitosan, and was independent of the classical coagulation cascade (Rao & Sharma 1997). Similar results were observed by Klokkevold et al (1999), who found that the topical application of chitosan to lingual incisions effectively decreased the intraoral bleeding time in a therapeutically anticoagulated (heparinised) rabbit model. Chitosan was able to facilitate lingual haemostasis, possibly through interaction with erythrocytes, linking them together to establish a cellular clot or a haemostatic plug. On the other hand, Kind et al (1990) reported that topical application of chitosan did not improve the control of bleeding in a liver laceration model when compared with pressure alone, in both normal and heparinised rats. Further, histologic results suggested that there might be an increase in inflammatory response by the healing liver following topical application of chitosan. The effect that chitin and chitosan might have on the release of arachidonic acid products was investigated by Usami et al (1998). Supernatants of canine polymorphonuclear cell (PMN) suspensions incubated with chitin and chitosan contained leukotriene B_4, the concentration of which was high enough to induce canine PMN migration in-vitro. The supernatants also contained the same concentration of PGE_2 as that normally found in the peripheral blood of dogs.

An earlier study by Malette et al (1983) had demonstrated the possible use of chitosan as a haemostatic. A study by Hoekstra and co-workers (1998) indicated the use of microcrystalline chitosan (McCh) sealant installation via an arterial sheath at the completion of catheterisation with a subsequent improvement in haemostasis. Results using McCh in 8 heparinised dogs exhibited significant reduction in manual compression time ($P < 0.016$) of the artery, following withdrawal of both the sheath introducer and catheter.

Recently, Ono et al (2000) observed that photocrosslinkable chitosan, to which both azide and lactose moieties were introduced (Az-CH-LA), was prepared as a biological adhesive for soft tissues, and its effectiveness compared with fibrin glue. Az-CH-LA produced a hydrogel within 60 s when irradiated with UV with a binding strength similar to fibrin glue. Az-CH-LA was able to seal leakage from pinholes on isolated small intestine and aorta and from incisions on isolated trachea. No cytotoxicity of the hydrogel was observed and it could prove to be a new tissue adhesive. Further, Stone et al (2000) evaluated chitosan as a dressing material and its effectiveness in healing at split skin graft donor sites, dressed half with chitosan and half with conventional dressing. Chitosan was observed to facilitate rapid wound re-epithelialisation and the regeneration of nerves within a vascular dermis. While the exact mechanism of interaction between chitosan and skin wound was not clearly defined, a gel like fibronectin matrix was shown to form at the chitosan wound interface which might act either as a scaffold for inward epithelial cell migration (Wang & Lou 1989) or facilitate the formation of heparin–chitosan complexes, thereby stabilising and binding to the immobilised heparin (Kratz et al 1997).

Effect on gastric ulcers

Meshali et al (1989) investigated the effects of chitosan on gastric ulceration caused by non-steroidal anti-inflammatory drugs. Chitosan was able to reduce the ulcerogenic activity of oxyphenbutazone and glafenine when administered to albino rats. The mechanism underlying this reduction in ulcerogenic activity of an acidic as well as a basic drug was explained on the basis of the solubility of chitosan in the acidic environment and its subsequent demulcent and protective effect on the stomach mucosa. The ability of chitosan to weaken gastric mucosal injury associated with the administration of diclofenac sodium has also been reported by Acikgoz et al (1995).

Antimicrobial effects

The antibacterial effects of chitosan (degree of deacetylation 69%), sulfonated chitosan (sulfur content 0.63%), and sulfobenzoyl chitosan were investigated by Chen et al (1998). The minimum inhibitory concentration (MIC) of sulfonated chitosan against *Shigella dysenteriae*, *Aeromonas hydrophila*, *Salmonella*

typhimurium and *Bacillus cereus* were found to be lower than that of chitosan. A high sulfur content in sulfoanted chitosan was reported to adversely influence its antibacterial activity. Sulfobenzoyl chitosan at 1000 and 2000 ppm extended the shelf-life of oysters at 5°C by 4 days at the former or by 7 days at least at the latter concentration. Chitosan and sulfobenzoyl chitosan were also able to retard the growth of C*oliforms*, *Pseudomonas*, *Aeromonas* and *Vibrio* species on oysters.

The MIC of a 4% chitosan gel on *Candida albicans* was shown to be 0.1 mg cm^{-3} (Knapczyk 1994). However, Staroniewicz et al (1994) observed the MIC of chitosan to be 0.6 mg cm^{-3} against the same species.

The mechanism underlying the inhibitory activity of shrimp chitosan (98% degree of deacetylation) against *Escherichia coli* was investigated (Tsai & Su 1999). The age of the bacterial culture affected its susceptibility to chitosan, with cells in the late exponential phase being more sensitive. The bactericidal effect was potentiated in an acidic pH and a higher temperature. On the other hand, presence of divalent cations reduced this antibacterial activity. The antibacterial action was proposed to be due to crosslinking between polycations of chitosan and the anions on the bacterial surface, which altered the membrane permeability, thereby resulting in the leakage of glucose and lactate dehydrogenase from *E. coli* cells. Similar results were earlier reported by Valenta et al (1998) for chitosan–EDTA conjugate neutralised with sodium hydroxide. The antibacterial effect was explained by its high binding affinity for Mg^{2+}, which was responsible for the stabilisation of outer membranes of Gram-negative bacteria.

In another study (Tarsi et al 1997) chitosan has been shown to be an interesting candidate capable of preventing the adherence of *Streptococcus mutans* to hydroxyapatite in dental caries. This effect was attributed to the ability of chitosan to stimulate ordered regeneration of oral soft tissues, prevention of the deleterious effects of organic acids and bactericidal effects. The desorptive effect of chitosan was weaker when *Streptococcus mutans* had adhered to the saliva coated hydroxyapatite in the presence of sucrose. These results indicated the potential of the presence of minor amounts of chitosan in toothpastes, mouthrinses or chewing gums to impair the colonisation of the tooth surface.

Miscellaneous effects

Antigenotoxic activity of chitosan and chitin were investigated utilising the sister chromatid exchange assay (Ohe 1996). The antigenotoxic activity of chitin and chitosan for mitomycin C were 87 and 0% and for adriamycin were 47 and 78%, respectively, thereby indicating the protective effects of these polymers against environmental mutagens.

Chitosan has been suggested to be useful for the treatment of lean type non-insulin dependent diabetes mellitus with hypoinsulinemia (Miura et al 1995).

The effects that chitosan might have on calcium metabolism was demonstrated in rats by Wada and coworkers (1997). Urinary excretion of ^{47}Ca was significantly increased in chitosan fed (5% chitosan diet) rats when compared with cellulose fed ones, indicating the possible effect of dietary chitosan on calcium metabolism.

Conclusion

The abundance of chitin and chitosan in nature and its safe toxicological profile has prompted researchers world-wide to investigate the potential pharmaceutical and biological applications of this unique biopolymer. As chitosan is principally obtained by partial deacetylation of chitin, the second most abundant natural polymer, a number of chitosan polymers varying in their physicochemical characteristics may be obtained by changing the degree of deacetylation.

The presence of a number of amino groups permit it to react with a host of anionic drugs or polymeric systems, thereby resulting in modification of the drug release profile. Chitosan may be used as a tablet binder, coating material, disintegrant, mucoadhesive, a vehicle for peptide and gene delivery and for colon targeting. Of special mention is the leverage provided by chitosan in the peroral administration of peptides or peptide based drugs. The ability of chitosan and its derivatives to open the tight junctional proteins makes it a promising candidate for this purpose. Further, chitosan conjugates (EDTA and BBI) have demonstrated enhanced capabilities in inhibiting protease activity in-vivo. The unique swelling characteristic of chitosan in the acidic environment of the gastrointestinal tract may afford protection to the gastric mucosa from the damaging effects of non-steroidal anti-inflammatory drugs, besides providing controlled release of the drug.

Biological applications include possible uses as myriad as cholesterol lowering, wound healing, haemostatic and antimicrobial activity. The potential of using chitosan in minor quantities in toothpastes, mouth-rinses or chewing gums to impair the colonisation of the tooth surface by *Streptococcus mutans* has also been investigated.

In view of the above mentioned, varied, unique pharmaceutical and biological applications of chitosan and its derivatives, it may be pragmatic to say that these polymers have the potential to be used not only as pharmaceutical adjuvants for conventional or novel drug delivery systems, but also as biologically active compounds.

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