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REVIEW PAPER

Chitosan: A Unique Polysaccharide for Drug Delivery

Olivia Felt, Pierre Buri, and Robert Gurny*

School of Pharmacy, University of Geneva, CH-1211 Geneva 4, Switzerland

ABSTRACT

The aim of this review is to give an insight into the many potential applications of chitosan as a pharmaceutical drug carrier. The first part of this review concerns the principal uses of chitosan as an excipient in oral formulations (particularly as a direct tableting agent) and as a vehicle for parenteral drug delivery devices. The use of chitosan to manufacture sustained-release systems deliverable by other routes (nasal, ophthalmic, transdermal, and implantable devices) is discussed in the second part.

Key Words: *Chitosan; Drug delivery; Review.*

INTRODUCTION

Chitin is one of the most abundant polysaccharides in nature, second only to cellulose. Its sugar backbone consists of β-1,4-linked glucosamine with a high degree of *N*-acetylation, a structure very similar to that of cellulose, the only difference being the replacement of the hydroxyl moieties by amino groups (1).

This polycationic biopolymer is a structural component of the exoskeleton of crustacea and insects, but also occurs in some fungi (1) (Table 1). Main industrial sources of chitin are the shell wastes of shrimp, lobster, and crab, for which it represents about 70% of the organic compounds (2). The principal derivative of chitin, namely chitosan, is usually obtained by alkaline deacetylation, the two polymers being distinguished by insolubility or solubility in dilute aqueous acid solutions (3) (Fig. 1).

Since chitosan exhibits favorable biological properties such as nontoxicity (4), biocompatibility (5,6), and biodegradability (7), it has attracted great attention in the pharmaceutical and biomedical fields. Biomedically, chitosan is reported to have pharmacological properties such as hypocholesterolemic action (8–11), wound-healing properties (12–14), antacid and antiulcer activity (15). In addition, its polycationic character gives chitosan the ability

* To whom correspondence should be addressed.

Table 1

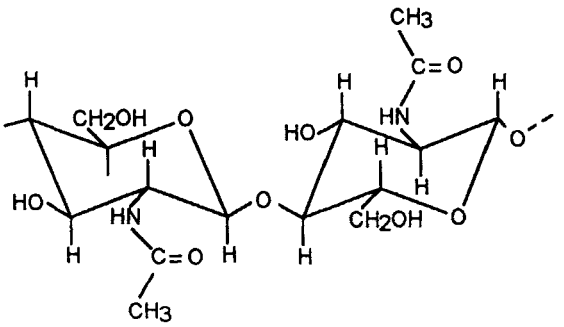
Principal Sources of Chitin

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

Adapted from Ref. 2.
[a]Organic weight of cuticle.
[b]Dry weight of the cell wall.

to bind strongly to several mammalian cells, leading to many potential uses, including hemostatic (16–18) and spermicidal use (19).

The cationic character, along with the presence of reactive functional group in chitosan, has given it particular



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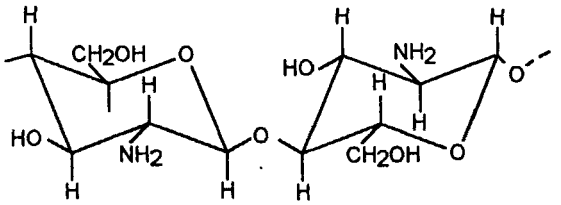

(1.2)

Figure 1. Structures of chitin (1.1) and chitosan (1.2).

Table 2

Analytical Methods for the Characterization of Chitosan

Property	Analytical Method	Reference No.
Molecular weight	HPLC	20
	Laser light scattering	21
	GPC	22
	High-performance GPC	23
Deacetylation degree	UV	24
	IR	25
	Titrimetry	25

possibilities for utilization in controlled-release technologies. Characteristics of the polymer, such as the molecular weight and the degree of deacetylation, greatly influence the properties of pharmaceutical formulations based on chitosan. Some useful physicochemical methods to determine these properties are listed in Table 2.

The objective of this review is to provide an overview of various pharmaceutical applications of chitosan. Previous investigations have led to the use of chitosan as an excipient for oral drug formulations. Currently, several drug delivery systems based on chitosan for other routes of administration are also being investigated.

ORAL ROUTE

The oral route is the most popular and the most practical way to administer a therapeutic agent, particularly from the point of view of the patient. However, it is not always the most suitable route for some active compounds, such as nonsteroidal antiinflammatory drugs, which cause gastric mucosal damage; for drugs poorly absorbed, such as peptides; or for drugs that undergo an extensive first-past effect (e.g., nitroglycerin, alprenolol, fluorouracil, and desipramine) (26). Furthermore, the possibility to control drug delivery after oral administration is very limited since it depends on the residence time of the dosage form in the gastrointestinal tract. Indeed, the drug will follow the gastric emptying rate, a physiological parameter that is subject to significant interindividual variability (27).

For these reasons, researchers have tried new excipients for manufacturing tablets or to develop drug carrier systems capable of controlling drug delivery after oral administration (e.g., microparticles).

For several years, chitosan has been largely evaluated as a potential vehicle for drugs administered orally.

Table 3

Chitosan Tablets Obtained by Direct Compression

Chitosan DD[a] (%)	Drug	Assays Reported	Reference No.
92.7	No drug	Disintegration test	31
92.7	No drug	Disintegration test	32
92.7	Propranolol hydrochloride	In vitro drug release	30
92.7	No drug	Disintegration test	33
N.A.	Chlorpheniramine maleate	In vitro drug release	29
N.A.	Theophylline	In vitro drug release	34
80.0	Ketoprofen	In vivo drug absorption	35
		In vitro and in vivo adhesion	35
80.0	Diltiazem	In vitro drug release	36
		In vitro adhesion	36

[a]Deacetylation degree.

Tablets

Since tablets are still considered as the dosage form of choice for reasons such as low cost of manufacturing or good stability, early studies have led to the use of chitosan as a tableting excipient.

As shown in Table 3, chitosan has been mainly used as a direct tableting agent. However, Henriksen et al. (28) suggested preparing chitosan formulations by standard wet granulation. They found that a chitosan salt (chitosan malate) was retarding the drug release rate, whereas chitosan base had the opposite effect by enhancing the dissolution of the active compound. This is probably due to the fact that chitosan base does not swell rapidly enough in the release medium to be the rate-determining step; hence, the drug release is controlled by the dissolution of the drugs. This observation is not in agreement with the results of Brine (29) and Sawayanagi et al. (30), who have noticed that water-soluble drug release from tablets based on chitosan followed a zero-order or a pseudo-zero-order pattern and provided controlled release. This difference could be explained either by the manufacturing process or by chitosan origin.

Sawayanagi et al. (31–33) observed that addition of chitosan to conventional excipients (mannitol, lactose, or potato starch) decreased the angle of repose and, as a consequence, improved the fluidity of the powder mixtures. Although chitosan has been mostly used as a diluent in tablet manufacturing, it has also been proposed as a binder (37) and a lubricant (33) and has been reported to be a potential disintegrant owing its water uptake property (34).

A further advantage of chitosan is the possibility to administer ulcerogenic drugs such as aspirin. In fact, the gel-forming property of the polysaccharide at low pH, along with its antacid and antiulcer properties (15), makes this polymer an interesting agent to prevent irritation in the stomach induced by some active compounds. Kawashima et al. (38) have studied the efficacy of chitosan in preparing tablets containing aspirin. Some characteristics on the drug release from tablets with or without chitosan are shown in Table 4. The presence of chitosan obviously provides a sustained release of aspirin compared with tablets based on a conventional diluent. The ulcerogenic index is an interesting feature, which ought to be measured to verify the assumption that chitosan is able to reduce the most common side effect of aspirin, gastric irritation. Such a study has been conducted by Acikgöz et al. (39), who demonstrated the potential of chitosan to weaken gastric mucosal injury associated with another antiinflammatory drug, diclofenac sodium.

Table 4

Drug Release of Aspirin from Tablets Based on Chitosan or Tablets Based on Conventional Excipients

Chitosan Content (%)	Dissolution Medium (pH)	$T_{50\%}$ (hr)
0	1.2	2
	6.8	1
11.1	1.2	>6
	6.8	>4

From Ref. 38.

More recently, Miyazaki et al. (35,36) conducted in vitro and in vivo assays of oral mucoadhesive tablets based on mixtures of chitosan and sodium alginate. In vitro bioadhesion force measurements were higher as the alginate content increased, demonstrating the strong adhesive properties of the alginate. In vivo tests have shown, on one hand, the tight adhesion of the tablets to the mucosa in the sublingual site and, on the other hand, the great improvement of bioavailability of drugs after sublingual administration. In addition, since the bioadhesive systems produced neither irritation nor unpleasant taste or discomfort, it has been suggested that they would be well accepted by patients.

Chitosan derivatives (e.g., chitosan glutamate) are also studied for their ability to enhance intestinal peptide drug delivery (40,41) by opening epithelial tight junctions, thus allowing paracellular peptide drug transport. The influence of chitosan glutamate on the permeability of epithelial cell monolayers in vitro (using Caco-2 cells) has been demonstrated by measuring the transepithelial electrical resistance. The effect has been presumed to be due to a direct interaction of the cationic polymer with the negatively charged cell membrane.

Microparticles

Over the last few decades, research workers have tried to develop systems capable of delivering active compounds at a specific target site. Microspheres and microcapsules belong to this category of drug delivery systems. These microparticulate carriers are considered as good potential oral delivery systems to provide a constant therapeutic level of the drug and, in this way, to avoid frequent administration of the solid oral dosage form.

Many materials have been proposed for preparing microspheres and microcapsules, including synthetic polymers (polylactic acid, copolymers of lactic and glycolic acids) and natural polymers such as chitosan. The amino groups of the polysaccharide are responsible for its pH-dependent solubility, which could represent a potential problem for oral delivery. In fact, chitosan microspheres formed by electrostatic interaction between a polyion and counterion become unstable in gastric fluid. A potential way to overcome this problem is irreversible chemical cross-linkage, which has been demonstrated by Berthold et al. (42). Furthermore, a large number of oral chitosan microsphere preparations described in the literature have been prepared using a suspension or emulsion cross-linking procedure (43–47). Nevertheless, authors (48) have chosen to prepare chitosan microspheres by a novel precipitation process using sodium sulfate as the precipitant.

As shown in Table 5, many active compounds have been entrapped in chitosan microspheres in order to study their drug release. Since the release from a drug delivery device is influenced by a number of parameters, some researchers (43,44,49) have analyzed in detail factors that are supposed to modify drug release. For example, Thanoo et al. (43) pointed out the importance of cross-linking density, particle size, and drug loading. Moreover, Akbuga and Durmaz (44) have found that the pharmacokinetic properties of microspheres were also affected by the type of chitosan, the drug concentration, and the viscosity of the lipophilic phase. Recently, Acikgöz et al. (49) performed an interesting study that showed the effect of various conditions on release kinetics. A factorial design experiment was used that considered concentrations of chitosan and tripolyphosphate (counterion) and stabilization time as independent variables. The advantage of such a method is that it gives the optimal combinations of parameters at different levels, which cannot be obtained by an empirical optimization. Acikgöz et al. (49) established the best conditions to prolong the residence time of diclofenac sodium and to minimize gastrointestinal side effects.

Table 5

Examples of Drugs Entrapped in Chitosan Microspheres

Chitosan DD (%)	Drug(s)	Reference No.
80.0	Diclofenac sodium	49
>70.0	Furosemide	44
N.A.	Indomethacin	46
N.A.	Methotrexate	45
88.7 and 81.0	Nifedipine	47
87.0	Prednisolone sodium phosphate	48
N.A.	Theophylline, aspirin, griseofulvine	43

Regarding pharmacokinetic performances of chitosan microspheres, it can be noted that a zero-order drug release has often been observed. For example, Narayani and Panduranga Rao (45) have shown that the in vitro release of methotrexate (MTX) from chitosan microspheres followed a zero-order pattern. Furthermore, drug release was controlled for 5–7 days in gastric fluid and for 7–10 days in intestinal fluid. The more prolonged effect appearing at intestinal pH is the result of the insolubility of the polymer at pH values up to 6. In fact, the intestinal dissolution medium penetrated the polymeric matrix, causing gradual swelling of chitosan, which acted as a rate-controlling barrier to the diffusion of MTX. However, although the results of Narayani and Panduranga Rao (45) seem to be promising, complementary in vivo experiments would be necessary to confirm the in vitro data. In fact, the controlled release of MTX from a chitosan matrix has been demonstrated in simple dissolution medium (0.1 N HCl at pH 1.2 and 0.01 M phosphate buffer at pH 7.4). It can be expected that in vivo release of MTX will be much more rapid regarding the susceptibility of chitosan to enzymatic degradation. In all cases, correlation between in vitro and in vivo results is very approximate. For this reason, the usefulness of chitosan as a polymer for the preparation of oral microspheres deserves further investigations to evaluate in more detail their in vivo efficacy.

Some authors (50,51) have suggested preparing microcapsules by a mild chitosan/calcium alginate microencapsulation process. The polyelectrolyte chitosan has been reacted with a counterion, sodium alginate, in the presence of calcium chloride to form microcapsules. In fact, this method is more commonly used to manufacture oral microcapsules than other processes such as precipitation, interfacial polymerization, and spray drying, which involve organic solvents. As shown in Table 6, the release rate of the drug is much slower in simulated gastric medium than in simulated intestinal medium, suggesting that alginate calcium chitosan microcapsules could act as a good gastrointestinal sustained delivery system.

Polk et al. (52) have examined different variables believed to be significant for encapsulation of bovine serum albumin (BSA). Among the several parameters studied (reaction time, chitosan molecular weight, alginate and chitosan concentration, and solution pH), both molecular weight of chitosan and alginate concentration have been found to affect BSA release. High molecular weight or a blend of high and low molecular weight chitosan was found to be very efficient in releasing the bioactive agent. Okhamafe et al. (51) have incorporated the same protein in chitosan-alginate microcapsules in order to protect it from gastric degradation. The results were unconvincing since almost all the BSA was released over 24 hr in a medium of pH 1.2. Nevertheless, release profiles had been modified by addition of a pH-sensitive polymer (hydroxypropylmethylcellulose acetate succinate). The resulting microcapsules were able to retain up to 60% of the protein over a period of 24 hr and, hence, would be suitable for oral administration of therapeutic proteins.

The last studies suggest that chitosan-alginate membranes enclosing a protein could be an interesting approach to control the release rate of the drug and, at the same time, to protect the product from denaturation and degradation in gastric fluid.

Granules and Beads

Matrices based on polysaccharides have been widely used for therapeutic drug targeting. Owing to its gel-forming ability at low pH, chitosan has been thought to be suitable to provide oral sustained release. Thus, the use of chitosan to develop a bioerodible matrix, granules and/or beads, has been reported by some authors (Table 7).

Table 6

Microcapsules Based on Chitosan

Chitosan DD (%)	Drug	In Vitro Drug Release[a] SGM[b]	SIM[c]	Reference No.
>85	Nitrofurantoin	>24 hr	4 hr	50
N.A.	Bovine serum albumin	1 hr	<1 hr	51

[a]50% release time.
[b]Simulated gastric medium.
[c]Simulated intestinal medium.

Table 7

Granules and Beads Based on Chitosan

Year	Chitosan DD (%)	Drug	Release Studies	Reference No.
1984	N.A.	Prednisolone	In vitro and in vivo	53
1985	N.A.	Indomethacin	In vitro	54
1988	N.A.	Indomethacin	In vitro and in vivo	55
1989	81.0 84.0 80.4	Sulfadiazine	In vitro	58
1992	>85.0	Nifedipine	In vitro	56
1993	>85.0	Ampicillin	In vitro	57
1993	N.A.	Acetaminophen, theophylline	In vitro	59

Among the several approaches explored to prolong the residence time of pharmaceuticals in the stomach, intragastric floating chitosan granules have been proposed (53–55). In vitro data of Chandy and Sharma (56,57) have demonstrated a near zero-order release of nifedipine and ampicillin from chitosan beads and a first-order release of the same compounds from granules. The mechanism of drug release was probably diffusion from beads, whereas it was disintegration of the matrix in the granules. Some in vivo experiments using dogs (53) or rabbits (55) have been described in the literature. In spite of the different animal models selected and the different drugs tested, the conclusions were quite similar, revealing delayed, prolonged, and higher plasma levels of drugs after administration of chitosan granules compared with conventional solid oral dosage forms.

In vitro and in vivo results confirm that chitosan granules are systems well suited to provide sustained release for oral administration.

Liposomes

The ability of chitosan to interact with liposomes has been reported (60,61) to have two positive consequences: the stabilization of the liposomes and the possibility of targeting the vesicle to a specific site due to its mucoadhesion property.

These properties have been exploited by Takeuchi et al. (62), who prepared mucoadhesive chitosan-coated liposomes to improve oral absorption of insulin. After in vivo administration of the liposomes to male Wistar rats, the hypoglycemic response was prolonged over a period of up to 12 hr. This sustained effect has been explained by the mucoadhesiveness of the system, which increased the duration of contact of insulin with the intestinal mucosa, thus leading to an increased probability of insulin absorption. Hence, chitosan-coated liposomes could represent an interesting concept for the administration of poorly absorbed drugs as is the case for peptides.

Others

Whereas the different oral dosage forms discussed above are quite commonly studied, some other systems based on chitosan appear rarely in the literature. One can mention hard capsules (63), chitosan-gelatin coacervates (64), and semi-interpenetrating polymer networks (IPNs) (65).

In the last study, the authors (65) proposed the use of a semi-IPN based on chitosan and poly(ethylene oxide) (PEO) to localize antibiotic delivery in the stomach. Studying in vitro release profiles of amoxicillin and metronidazole, they suggested that a freeze-dried chitosan-PEO semi-IPN could be well suited for site-specific antibiotic delivery in gastric fluid for the treatment of diseases such as *H. pylori* infection.

INJECTABLE ROUTE

The parenteral administration of drug products offers a number of advantages over the oral route (66). Principally, the injectable route is a viable alternative for drugs that are strong irritants when taken orally and for substances not absorbed or inactivated in the gastrointestinal tract.

Although pharmaceuticals administered parenterally generally exert a rapid effect, some injectable systems, such as microspheres (67) and macromolecular conjugates, are intended to provide sustained drug release.

Microspheres are well known to be useful as drug delivery systems due to their possible localization to the target site (70). Consequently, biodegradable polymeric microspheres have received particular attention in cancer chemotherapy, for which it is imperative to have efficient delivery to the desired site of action due to the high toxicity, poor stability, and short biological half-life of antitumoral drugs.

Since chitosan is well tolerated by living tissues and is biodegradable, it has been recently envisaged for the controlled release of drugs administered parenterally in the form of cross-linked microspheres. It has been shown (43,71,72) that drug diffusion from a chitosan matrix could be effectively controlled using a cross-linking agent such as glutaraldehyde, which leads to a diminished susceptibility of the polymer to lysozyme. As the polysaccharide per se has been reported to possess an antitumor property, chitosan microspheres loaded with antineoplasic agents appear to be promising drug delivery systems for the treatment of cancer. As shown in Table 8, most of the published information concerns only the physical characterization of the microspheres and the release pattern of entrapped drug. However, Jameela and coworkers (73) have evaluated in vivo efficacy of cross-linked chitosan microspheres loaded with mitoxantrone against Ehrlich carcinoma after intraperitoneal injections in mice. Monitoring parameters such as the survival time and changes in body weight, they have concluded that their formulation could minimize drug toxicity and enhance the therapeutic efficacy. They observed that administration of chitosan microspheres lead to a mean survival time about 25 times superior compared to that for animals that received free mitoxantrone.

Regarding some features of microspheres based on chitosan, gelatin, fibrinogen, or albumin (Table 9) loaded with 5-fluorouracil (5-FU), it can be noticed in all cases studied that drug release is characterized by an initial burst effect. This initial effect is probably due to the release of the drug, which is adsorbed on the surface of the microspheres (76). In the case of chitosan microspheres, Akbuga and Bergisadi (75) have demonstrated that this rapid initial phase could be retarded by addition of substances such as chitin, alginic acid, caprylic acid sodium salt, and stearic acid. Pharmacokinetic performance seems to be more satisfactory for gelatin than for chitosan microspheres. In fact, 5-FU entrapped in a gelatin matrix follows a zero-order release, except for the initial burst effect, and exerts a prolonged effect over 8 days, whereas about 70–80% of the drug entrapped in chitosan microspheres was released within the first hour. A further advantage of gelatin microspheres is the incorporation efficiency, which ranges from 3 to 20 times higher for gelatin microspheres compared to chitosan.

However, morphological and biopharmaceutical characteristics of microspheres are greatly affected by preparation parameters such as the nature and the ratio of polymeric material; the size, the shape, and the density of the microparticle; the nature and the amount of any cross-linking agent; the presence of adjuvants; and the eventual physicochemical interaction between the drug and the matrix. Since the conditions of production are different from one study to another, comparison among the different polymeric materials is somewhat difficult.

It has been suggested that magnetic chitosan microspheres might be able to localize drugs by both biochemical and physical means (79). Magnetic microspheres are expected to be retained in target site capillaries under the force of an external magnetic field. The advantage of a cationic polymer such as chitosan is that a strong interaction can occur between cationic microspheres and anionic glycosaminoglycan receptors and, in this way, retain the microspheres in the capillary region. Recently, Hassan and colleagues (80) have studied the influence of several factors on the fabrication of oxantrazole-loaded mag-

Table 8

Injectable Chitosan Microspheres

Drug	Drug Loading (%)	Physical Characterization	Tests		Reference No.
			In Vitro	In Vivo	
Cisplatin	N.A.	Drug dispersion in the matrix	In vitro drug release	None	74
5-Fluorouracil	3.5–20.6	Morphology Particles size	In vitro drug release	None	75
Mitoxantrone	~4	Morphology Particles size	In vitro drug release	Tumor inhibitory effect	73

Table 9

Characteristics of 5-Fluorouracil-Loaded Microspheres

Matrix Material	Drug Loading (%)	Mean Particle Size (μm)	Release Characteristics	Reference No.
Chitosan	3.50–20.60[a]	490–751[a]	Initial burst effect, then slow release	75
Gelatin	67.28	10–20	Initial burst effect, then zero-order release	77
Fibrinogen	6.10–16.10[b]	1.1–3.9[b]	Initial burst effect, then gradual release	76
Albumin	4.00	0.66	N.A.	78

[a]Variations with preparation parameters: drug concentration, chitosan concentration, type of chitosan, concentration of glutaraldehyde.
[b]Variations with temperature of fabrication.

netic microspheres by statistical optimization procedures. They have found that critical factors affecting the percentage of drug entrapped and the mean particle size were the amount of drug and glutaraldehyde, as well as the cross-linking time. Expanding from these results, Wang et al. (81) have explored the influence of other parameters, such as molecular weight and deacetylation degree of chitosan, as variables that might influence particle size, drug content, and entrapment efficiency. It has been pointed out that the type of chitosan shows a significant influence on the particle size distribution.

Macromolecular drug carrier systems have been largely studied (82), particularly in the field of cancer chemotherapy, in an attempt to improve therapeutic effects and to reduce nonselective toxic effects associated with antitumor agents. The majority of carrier-conjugate systems are composed of biological macromolecules such as antibodies (83), albumin (84), and DNA (82). The rationale for the utilization of chitosan lies, in part, in the presence of free amino groups, which allow its conjugation with some drugs.

Ouchi and coworkers (85) have synthesized several chitosan–5-FU conjugates using four kinds of chitosan and fixing 5-FU through various spacers via ether, amide, ester, or carbamoyl bonds. Since none of the tested conjugates displayed acute toxicity in mice, it has been suggested that covalent attachment of 5-FU to chitosan could afford the depression of side effects. The results of Ichikawa and colleagues (86) are in agreement with the above study. They confirmed the lack of acute toxicity of chitosan conjugates after intraperitoneal administration by testing 1-β-D-arabinofuranosylcytosine (ara-C). However, the conjugate, along with its high antitumor effectiveness, still demonstrated some side effects.

Among the different MTX conjugates described in the literature, the study of Sanzgiri and coworkers (87) seems to be of particular interest. In fact, they have evaluated the in vitro ability of the ionic chitosan-MTX conjugate to complex heparin (as a model for anionic glycosaminoglycan receptors), supposing that it could interact with the vascular endothelium. However, it has been observed that, in a neutral medium, the chitosan-heparin complex did not occur, which was probably due to the loss of the ionic state of amino groups. Thus, further investigations are essential to obtain a formulation capable of maintaining the cationic character of chitosan and, in this way, overcoming the actual limitation to the in vivo interaction of the conjugate with endothelial receptors.

A derivative of chitosan, *N*-succinyl chitosan (suc-chitosan), has also been proposed for the preparation of macromolecular conjugates with mitomycin C (MMC) (88,89). Suc-chitosan–MMC conjugate has been submitted to in vitro (88) and in vivo (89) tests. These assays have indicated that suc-chitosan–MMC was water insoluble, but swelled in aqueous solutions, enabling the gradual regeneration of free drug at physiological pH. From these tests, it has been shown that the conjugate was less toxic than free MMC (LD_{50} of the conjugate is about three times less than that of MMC) and has a high antitumor activity against P388 leukemia.

An original approach is that of Onishi et al. (90), who have advocated combining microspheres and conjugate devices rather than using simple microspheres (or simple conjugates) to carry 5-fluorouridine. Some characteristics of the conjugate microspheres and conventional microspheres (prepared with the same dry-in-oil method) are compared in Table 10.

Table 10

Characteristics of 5-Fluorouridine-Loaded Conjugate Microspheres and Microspheres

Carrier	Drug Content (%)	Particle Shape	Particle Size (μm)	Swelling Ratio (%)	$T_{50\%}$
Conjugate microspheres	2.5	Spherical	30	57.7	61 hr
Microspheres	1.9	Spherical	22	52.4	<15 min

Adapted from Ref. 90.

Both types of preparations present morphological similarities, with microspheres alone exhibiting an average diameter somewhat smaller. Drug-loading efficacy and drug-release behavior have been improved using the conjugate microspheres. In fact, simple microspheres released the drug very rapidly, whereas the conjugate microspheres showed a slow disintegration and a gradual release, which could be useful for chemoembolization therapy.

OTHER ADMINISTRATION ROUTES

Nasal Route

Nasal drug delivery represents an interesting alternative to the parenteral route for administration of drugs that show poor oral bioavailability, such as peptides and proteins. In addition, the nose has a further advantage for the absorption of drugs in that it has a large epithelial surface area available due to the presence of numerous microvilli. On the other hand, one of the major drawbacks of the nasal cavity is its rapid mucociliary clearance mechanism, which can reduce the bioavailability of drugs given intranasally. A possible strategy to circumvent this problem, without using absorption enhancers, is to prevent the clearance of the delivery system from the nasal cavity and thereby prolong contact between the drug and the mucosa. This aim can be reached by employing bioadhesive systems that form a gel-like structure at the contact of the mucus. For example, Illum and coworkers (91) have suggested the use of three bioadhesive systems (albumin, starch, and DEAE–dextran microspheres) to increase the absorption of sodium cromoglycate.

The good mucoadhesive properties of chitosan, probably mediated by ionic interaction between the positive amino groups of the polysaccharide and the negative residues of sialic acid in the mucus, make it a promising candidate for development of nasal delivery systems that exert drug controlled release. In fact, chitosan is able to swell and form a gel-like layer in an aqueous environment (here, for example by absorbing water from the mucous layer in the nasal cavity), which is favorable for the interpenetration of polymer and glycoprotein chains of the mucus. However, the use of chitosan in the nasal formulations still remains under investigation, and few experimental data are available in the literature.

Recently, Aspden and colleagues (92) studied the effect of five types of chitosan (with varying molecular weights and degrees of deacetylation) on the nasal epithelium and the beat frequency of the cilia using a frog palate model. They concluded that chitosan affected only transiently the mucociliary clearance, with the recovery of normal mucociliary transport velocity appearing about 180 min after administration of 0.25% solutions of chitosan; therefore, it can be considered nontoxic for the nasal epithelium. These results have been confirmed by testing ex vivo and in vivo chitosan solutions in human tissue (93). An experiment conducted by Zhou and Donovan (94) demonstrated in a rat model that incorporating chitosan in nasal formulations resulted in a significant increase of the residence time of the preparations in the nasal cavity compared with a control suspension of Fluospheres® (Fig. 2).

The only experiment based on a chitosan nasal delivery system containing a model drug has been described by Illum and colleagues (95). The main goal was the enhancement of the systemic bioavailability of insulin after intranasal instillation in animals models (rats and sheep). The authors have shown that a concentration of 0.5% polymer is sufficient to improve plasma insulin levels greatly (Fig. 3).

Ophthalmic Route

Conventional aqueous solutions topically applied to the eye have the disadvantage that most of the instilled drug is lost within the first 15–30 sec after instillation (96) due to reflex tearing and to drainage via the nasolacrimal duct. One of the goals in ophthalmic research has been directed toward an increase of drug absorption and duration of contact time. The most frequent approach to achieve improved drug efficacy is exemplified by the use of viscosified solutions. Nevertheless, if viscosity alone

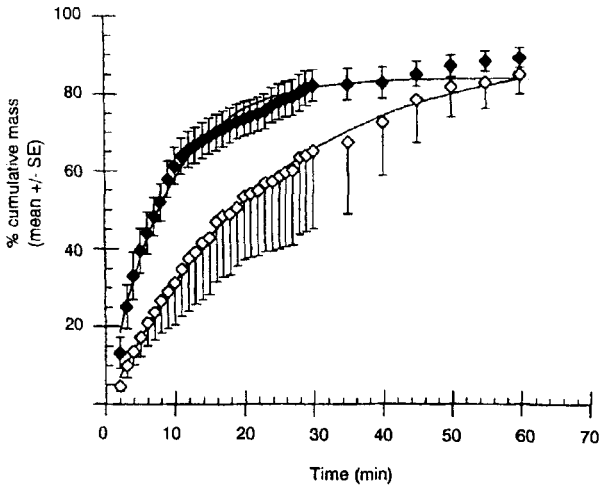

Figure 2. Intranasal clearance profiles of Fluospheres® suspension (◇) and of 3% chitosan glutamate gel (◆). (Adapted from Ref. 94.)

may explain to a large extent the prolonged residence times of polymeric solutions, it is not as significant as it was originally believed. In fact, comparing several isoviscous polymer vehicles, Saettone et al. (97,98) found that each polymer has a unique effect on the cornea independent of its viscosity, thus influencing corneal drug absorption differently. This can be considered, in part, as the premise of using bioadhesive polymers to enhance drug absorption. Mucoadhesive polymers, both synthetic and natural, have been screened to optimize ocular drug delivery.

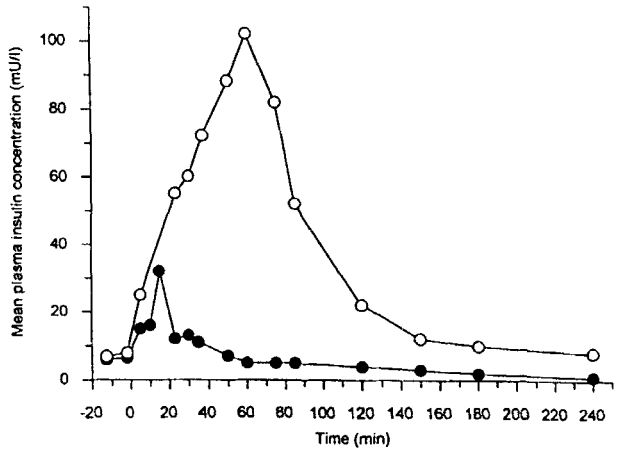

Figure 3. Mean plasma concentration of insulin following intranasal administration to sheep of 2 IU/kg of insulin (●) as a simple solution and (○) in combination with 0.5% chitosan. (Adapted from Ref. 95.)

Park and coworkers (99) and Lehr and coworkers (100) have suggested that cationic polymers were probably superior mucoadhesives owing to their ability to develop molecular attraction forces by electrostatic interactions with the negative charges of the mucus. In this context, the polycation chitosan appears as an attractive vehicle. In addition, Lehr et al. (100) demonstrated that the mucoadhesive performance of chitosan was significantly higher in a neutral or slightly alkaline medium; the pH values of tear fluid reported in the literature range from 7.0 to 7.4 (101).

At this time, the use of chitosan in the ophthalmic field is still in the preliminary stages of investigation. Since chitosan is endowed with an appreciable film-forming capacity, it has been suggested as a biopolymer of choice for the development of contact lenses. Principally, it has been evaluated for the manufacture of ocular bandage lenses used as protective devices for acutely or chronically traumatized eyes (102,103). The lenses obtained have been claimed to possess desirable physical properties such as tensile strength, water content, protein deposition, and oxygen permeability (102).

The susceptibility of chitosan to the lysozyme produced by the apical portion of the lachrymal acinar cells represents another favorable characteristic, which avoids the necessity of removing the lens. In addition, the degradation product of chitosan, *N*-acetylglucosamine, is a monomeric unit present in the core of certain human glycoproteins, such as heparin and hyaluronic acid (104). For this reason, it can be considered as a very safe agent. Yomota et al. (105) found that the enzymatic degradation rate of chitosan films was pH dependent, being slower in a neutral environment. A problem that may be encountered concerns the absorption rate of chitosan, which is linked to the lysozyme and depends partially on the degree of *N*-acetylation. Shah and Mowbray (103) estimated that a satisfactory *N*-acetylation range was between 30% and 50%, which can be reached by reacetylation with acetic anhydride. From this study, it would be expected that chitosan films may provide sustained drug delivery, and that they can be kept in place on the eye without causing discomfort to the patient.

The administration of ophthalmic preparations should influence as little as possible the pseudoplastic character of the precorneal tear film. This can be achieved using polymeric solutions exhibiting pseudoplastic behavior (106). Since the ocular shear rate is very large, ranging from 0.03 sec^{-1} during interblinking periods to 4250–28,500 sec^{-1} during blinking, viscoelastic fluids, with a

viscosity that is high under conditions of low shear rate and low under conditions of high shear, should be preferred (107). Chitosan solutions show pseudoplastic and viscoelastic properties (108,109) and, as a consequence, seem to be a potential vehicle for the instillation of drugs to the eye.

El-Samaligy et al. (110) tested three biodegradable polymers, including chitosan, to prepare ganciclovir nanoparticles for the treatment of cytomegalovirus retinitis. In vitro data have shown that drug release from chitosan nanoparticles was encouraging. In fact, ganciclovir was released for up to 4 days following a first-order pattern. Furthermore, the release rate was slower for chitosan devices than for the two other polymers (BSA and polyethylcyanoacrylate [PEC]). However, intravitreal injections have been conducted with PEC only. In vivo evaluations with PEC have demonstrated that the drug was present in the retina and the vitreous 10 days after intravitreal administration. Since the in vitro release rate is slower with chitosan than PEC, one can imagine that a corresponding prolongation of in vivo drug release could take place.

Recently, Henriksen et al. (111) evaluated the possibility of using chitosan-coated liposomes containing ^{125}I-labeled BSA for topical ocular administration. Comparing the retention of simple liposomes and chitosan-coated liposomes after in vivo administration to anesthetized rats, they observed that retention of the radioactive marker was not significantly improved with chitosan-coated liposomes.

Transdermal Route

It has been increasingly recognized that intact skin represents an interesting way to provide controlled delivery of drugs to the systemic circulation. Drugs administered via transdermal devices approach a zero-order input, which is quite equivalent to the administration of therapeutic agents after a constant intravenous infusion (112). In addition, transdermal administration represents a reliable alternative to oral administration for substances that are subject to an extensive hepatic first-pass metabolism (112).

In spite of its well-known film-forming property, only a few studies have been performed on the usefulness of chitosan membranes as transdermal devices. Thacharodi and Panduranga Rao (113–115) evaluated the efficacy of chitosan membranes as rate-controlling membranes by testing series of hydrophilic and hydrophobic drugs. They concluded that water-soluble drugs such as propranolol could be transported through chitosan membranes principally via a pore mechanism (114), whereas hydrophobic solutes such as nifedipine would be influenced by both partition and pore mechanisms operating concurrently (113). The data of Nakatsuka and Andrady (116) are in agreement with these results since they have suggested that the transport of the hydrosoluble vitamin B-12 through cross-linked or blended chitosan films followed predominantly a pore mechanism.

Comparing the capacities of chitosan versus cellulose membranes, Sawayanagi and coworkers (117) found that the diffusion constant for acidic substances such as nonsteroidal antiinflammatory drugs was two to three times larger through the chitosan membrane.

Regarding the kinetic characteristics of several films described in the literature, it can be seen that permeability coefficients are affected by different parameters, including the degree of cross-linking (113,114), the acid or base nature of the drug (117), the molecular volume of the drug (117), the pH of the environmental medium (118), and the thickness of the membrane (116).

Until now, little information has been available concerning the influence of the site of application of such systems. Furthermore, the majority of the literature consists of in vitro release tests, which provide information on pharmacokinetic parameters affected by the biopolymeric membrane solely. It could be interesting to conduct in vitro skin permeation tests using excised skin mounted on a diffusion cell such as the Franz diffusion apparatus (119). This type of test could provide useful indications of the pharmaceutical flux from the membrane to the skin sample, which in turn may provide information on in vivo performance of the transdermal system (120).

Implants

A possible approach to provide sustained release of a medication is to place implants in body tissues surgically (121). Biodegradable polymers have become increasingly important in the development of such devices. The constant contact between the systems and living tissues implies specific requirements. Dunn et al. (122) have stated some requirements that the polymer should meet, such as nontoxicity, sterilizability, good mechanical properties, harmless degradation products, and ease of film formation.

Since chitosan possesses these characteristics, attempts have been made to develop implantable dosage forms of antitumoral drugs based on chitosan or its derivatives. Several of these studies are listed in Table 11.

The biodegradability of chitosan after implantation has been confirmed by Jameela and Jayakrishnan (72)

Table 11

Examples of Implants Based on Chitosan or Derivatives

Carrier	Dosage Forms	Drug	Reference No.
Chitosan and hydroxypropylchitosan	Sphere Membrane Fiber Stick	Uracil	123
Chitosan	Microsphere	5-Fluorouracil	124
Chitosan	Microsphere	Mitoxantrone	72
N-Succinylchitosan	Conjugate	Mitomycin C	125

and Machida et al. (123). In vivo data (72) have shown that chitosan microspheres were not degraded to a significant extent over a period of 3 months after implantation in the skeletal muscle of rat.

Machida et al. (123) compared enzymatic degradation of the polysaccharide and hydroxypropylchitosan (HP-chitosan) in vitro (using lysozyme) and in vivo (subcutaneous implantation of both materials in rats). They concluded that in vitro experiments seemed to provide a relatively good model for in vivo behavior, even though degradation was somewhat faster in vivo than in vitro. Positive results have been also reported by Song et al. (125), who found that *N*-succinyl–chitosan–mitomycin C (suc-chitosan-MMC) implanted subcutaneously (in tablet form) was able to supply mitomycin C over 1 week. Furthermore, the authors have investigated the in vivo efficacy of suc-chitosan-MMC against Sarcoma 180 solid tumor after direct intratumoral injection. The results have indicated that the conjugate was more effective than free MMC. Such a localization of the drug could be an advantage for achieving high efficacy and reducing side effects of the drug.

From these studies, chitosan appears as a promising drug carrier to control delivery over very long periods, avoiding repeated administration of certain drugs, which is unpleasant for the patient.

CONCLUSION

Since chitosan combines unique physicochemical characteristics, in vivo biodegradability, biocompatibility, and antimicrobial action, it has been widely investigated over the last few years for potential applications as a drug carrier for many possible routes of administration.

As mentioned in the first part of this review, chitosan offers several advantages for various parenteral formulations and as a vehicle in oral drug delivery systems, enabling, for example, prolonged drug release or improved bioavailability of poorly absorbed compounds such as peptides.

However, other promising possibilities for application exist, but have not been extensively developed. For example, the use of chitosan to enhance ocular or nasal absorption of drugs by prolonging contact with the mucosa is still poorly investigated. This could be explained, in part, by the fact that commercially available chitosan is not always well characterized, particularly regarding molecular weight.

Finally, it will obviously be necessary to make further investigations to study the in vivo capacity of formulations based on chitosan.

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