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CHITOSAN AS A BOON TO DRUG DELIVERY SYSTEMS

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ABSTRACT

Within the past 20 years, a considerable amount of work has been published on chitosan and its potential use in drug delivery systems. In contrast to all other polysaccharides having a monograph in a pharmacopeia, chitosan has a cationic character because of its primary amino groups. These primary amino groups are responsible for properties such as controlled drug release, mucoadhesion, in situ gellation, transfection, permeation enhancement, and efflux pump inhibitory properties. Due to chemical modifications, most of these properties can even be further improved.

Key words: Chitosan, mucoadhesive, depolymerization, permeation enhancer.

1. INTRODUCTION

1.1 Chitosan

Chitosan is a polysaccharide comprising copolymers of glucosamine (β-(1–4)-linked 2-amino-2-deoxy-D-glucose) and N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) as shown in below Fig. 1 and can be derived by partial deacetylation of chitin from crustacean shells. Due to its specific properties, chitosan has found a number of applications in drug delivery including that of as an absorption enhancer of hydrophilic macromolecular drugs[1]-[3] and as gene delivery system.[4],[5] The term chitosan embraces a series of polymers, which vary in molecular weight (MW) and degree of deacetylation (DD). Generally, low molecular weight chitosans can be prepared from high molecular weight chitosan by depolymerization using enzymatic degradation[6] oxidative degradation[7], acidic cleavage and ultrasonic degradation.[8] The rate of molecular weight degradation was irregular during the time course

of ultrasound treatment. Liu studied the depolymerization of chitosan using $NaNO_2$, H_2O_2, and HCl. They found that $NaNO_2$ showed the best performance.[9]

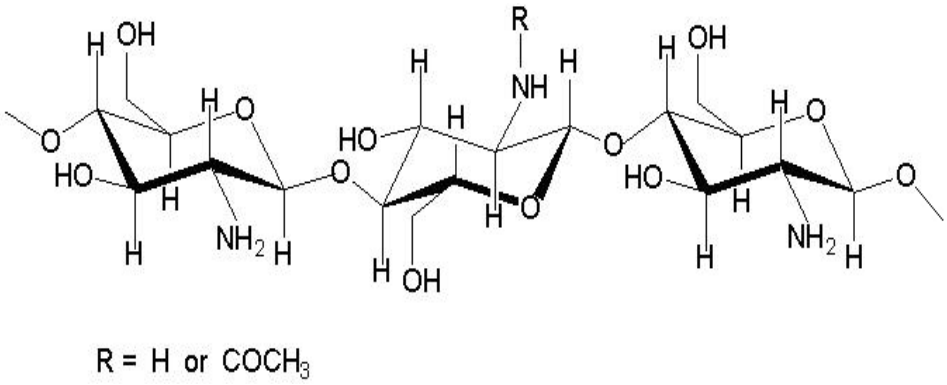

Fig. 1 structure of chitosan

2. PROPERTIES OF CHITOSAN

2.1. Controlled drug release

When sustained drug release cannot be provided by making use of a simple drug dissolution process, by diffusion, by erosion, by membrane control, or by osmotic systems, retardation mediated by ionic interactions is often the ultimate ratio. Such a controlled release can be achieved for cationic drugs by using anionic polymeric excipients such as polyacrylates, sodium carboxymethylcellulose, or alginate. In case of anionic drugs, however, chitosan is the only choice. Bhise et al [10] designed sustained release systems for the anionic drug naproxen using chitosan as drug carrier matrix. Using polyanionic drugs, the interactions between chitosan and the therapeutic agent are more pronounced, and based on an ionic cross-linking in addition, even stable complexes are formed from which the drug can be released even over a more prolonged time period. Sun et al.[11] designed enoxaparin chitosan nanoparticulate delivery systems, providing very stable complexes that led to a significantly improved drug uptake. In addition, chitosans can be homogenized with anionic polymeric excipients such as polyacrylates, hyaluronic acid, alginate, pectin, or carrageenan, resulting in comparatively stable complexes of high density. Mainly based on diffusion and erosion processes, incorporated drugs are released in a sustained manner from such complexes.[12] Alternative to anionic polymers, multivalent inorganic anions such as tripolyphosphate or sulfate can be used in order to achieve the same effect.[13]

2.2. Mucoadhesive properties

The mucoadhesive properties are likely also based on its cationic character. The mucus gel

layer exhibits anionic substructures in the form of sialic acid and sulfonic acid substructures. Based on ionic interactions between the cationic primary amino groups of chitosan and these anionic substructures of the mucus, mucoadhesion can be achieved. In addition, hydrophobic interactions might contribute to its mucoadhesive properties. In comparison with various anionic polymeric excipients such as carbomer, polycarbophil, and hyaluronic acid, however, its mucoadhesive properties are weak.[14] Furthermore, in order to achieve high mucoadhesive properties, the polymer needs to exhibit also high cohesive properties as the adhesive bond otherwise fails within the mucoadhesive polymer rather than between the mucus gel layer and the polymer. In case of chitosans, however, these cohesive properties are also comparatively weak. Although they can be strongly improved by the formation of complexes with multivalent anionic drugs, multivalent anionic polymeric excipients, and multivalent inorganic anions, this strategy is only to a quite limited extent effective, as the cationic substructures of chitosan being responsible for mucoadhesion via ionic interactions with the mucus are in this way blocked. Lueßen et al., for instance, demonstrated a significantly improved oral bioavailability of buserelin when being administered with mucoadhesive polymers such as chitosan and carbomer to rats. This effect, however, could not be observed anymore when chitosan was combined with the polyanionic carbomer in the same formulation.[15] Trimethylation of the primary amino group of chitosan provides an even more cationic character of the polymer. When trimethylated chitosan (TMC) is additionally PEGylated, its mucoadhesive properties are even up to 3.4-fold improved.[16]

2.3 In situ gelling properties

In case of hydrogels, chitosan offers the advantage of in situ gelling properties when its pH-dependent hydratability is addressed properly from the formulation point of view. Gupta et al., for instance, developed an in situ gelling delivery system by the combination of polyacrylic acid and chitosan. The resulting formulation was in liquid state at pH 6.0 and underwent rapid transition into the viscous gel phase at physiological pH of 7.4.[17]

2.4 Transfection enhancing properties

In contrast to small molecules, where a controlled release of anionic drugs can be achieved as described above, comparatively large polyanionic molecules such as DNA-based drugs and siRNA form stable complexes with chitosan. In this way, nanoparticles exhibiting a positive zeta potential can be formed, when the ratio of the cationic polymer is sufficiently high. Because of this positive net charge and the small size of these particles, endocytosis can be

achieved in particular when these particles are below 100 nm in size.[18] As chitosan is comparatively less toxic than other cationic polymers such as polyethyleneimine, polylysine, or polyarginine[19], it is therefore a promising excipient for non-viral gene delivery systems. In addition, chitosan–DNA-based drug complexes protect at least to some extend towards degradation by DNAses in this way improving the bioavailability of DNA-based drugs delivered into the body.[20]

2.5 Permeation enhancing properties

The mechanism being responsible for the permeation enhancing effect of chitosan is also based on the positive charges of the polymer, which seems to interact with the cell membrane resulting in a structural reorganization of tight junction-associated proteins.[21] A more pronounced cationic character being achieved by trimethylation of the primary amino group, however, did not lead to further improved permeation enhancing properties, suggesting that the underlying mechanism described above is obviously more complex than assumed.[22] Schipper et al.[23] could demonstrate that the structural properties of chitosan, that is, molecular mass and degree of deacetylation, dictate the permeation enhancing properties and toxicity to a large extent. Chitosans of high degree of deacetylation and of high molecular mass exhibit the comparatively highest increase in epithelial permeability. This increase in permeation enhancing effect with increasing molecular mass could also be observed for other permeation enhancing polymers such as polyacrylates[24], providing another piece for the overall puzzle of the underlying mechanism. As these parameters determining permeation enhancement do not correlate with those determining toxicity, chitosans with maximal permeation enhancing effect and minimal toxicity are available.

2.6 Efflux pump inhibitory properties

In 2002, Carreno-Gomez and Duncan demonstrated efflux pump inhibitory properties for various polysaccharides[25]. Although they did not evaluate chitosan in their studies, it was quite obvious that this polysaccharide will exhibit the same effect. A proof-of-principle for this theory was then provided by Föger et al.[26] demonstrating a significantly improved oral uptake of the P-gp substrate rhodamine 123 due to the co-administration of (thiolated) chitosan in rats. Although this effect is in comparison with other efflux pump inhibitors not that pronounced, it seems, nevertheless, useful to improve the mucosal uptake of various efflux pump substrate drugs. In case of permeation enhancement, the effect of chitosan increased with increasing molecular mass of the polymer, whereas in case of efflux pump

inhibition, a molecular mass in the range of 150 kDa led at least for a chitosan derivative to the most pronounced inhibition.[27]

2.7 Colon targeting

In the same way as numerous other polysaccharides, chitosan is degraded in the colon. By making use of this colon-specific degradation, chitosan has been discovered as useful coating in order to guarantee a site specific delivery. Radiolabelled (99mTc) tablets coated with a combination of pectin/chitosan/hydroxypropyl methylcellulose (3 + 1 + 1), for instance, were administered orally to human volunteers.[28] Within this study, gamma scintigraphy was used to evaluate the gastrointestinal transit of these tablets, showing that they remain intact through the stomach and small intestine. In the colon, the bacteria degraded the coat, and thus, the tablets disintegrated. In another study, our own research group developed a sustained dosage form for alpha-lipoic acid making use of ionic interactions between this anionic drug and chitosan used as carrier matrix. Studies in human volunteers showed a release maximum once the formulation had reached the colon.[29]

3. SAFETY OF CHITOSAN

As stated in the introduction, chitosan is a collective term applied to deacetylated chitins in various stages of deacetylation and depolymerization. Almost all functional properties of chitosan depend on the chain length, charge density and charge distribution. Numerous studies have demonstrated that the salt-form[30]-[34] molecular weight,[35] degree of deacetylation[36] as well as the pH at which chitosan is used influence the properties of this polymer in drug delivery systems. Therefore, these factors must be considered carefully during formulation optimization of dosage forms. In addition, regulatory requirements concerning the use of chitosan in humans will be far more demanding. It has been reported that the purity of chitosan influences its toxicological profile. Dornish *et al.*[37] have demonstrated the safety of an ultrapure grade of chitosan salts in various biological and physiological systems. Therefore, it would stand to reason that only the highest purity of chitosan would satisfy the standards set by regulatory agencies.

4. MECHANISMS OF ACTION OF CHITOSAN

The mechanism of action of chitosan was suggested to be a combination of mucoadhesion and an effect on tight-junction (TJ) regulation.[35] Using a human colon carcinoma cell line (Caco-2) as an *in vitro* model of intestinal epithelium, cell permeability was shown to increase following treatment with chitosans of various salt forms and molecular

weights.[35][38][39] Epithelial permeability can be assessed by measuring transepithelial electrical resistance (TER), which is inversely proportional to the permeability of the epithelial layer to organic ions. Measurements of TER revealed that chitosan's effects were concentration-dependent and reversible . However, additional studies showed no significant difference in the resistance values obtained between 0.1 and 0.5% (w/v), suggesting a threshold effect of chitosan above 0.1%78. This observation is in accordance with the data obtained by several groups with chitosan glutamate.[35],[40] The apparent permeability coefficient of mannitol, a marker of the paracellular pathway, reached a plateau at polymer concentrations of 0.25 and 0.5% (w/v)73. A comparable effect was obtained by Lueben *et al.*[40] where 0.4 and 1% (w/v) chitosan elicited similar values for the transport rate of DGAVP peptide (9-desglycinamide, 8-Larginine vasopressin.

Transmission electron micrographs of cells exposed to 0.1% chitosan for 30 minutes resulted in the appearance of large intracellular vacuoles and swollen endoplasmic reticulum cisternae (V. Dodane, M.A. Khan and J.R. Merwin, unpublished). However, the cells displayed a continuous apical membrane, normal microvilli, intact organelles and TJ as observed in control cells. The absence of apparent changes in the junctional morphology accompanied by an increased paracellular permeabilithas been previously reported85. This observation reinforces the existence of additional factors in TJ modulation, such as: the number and length of strands in the junctions; the existence of channels; the biochemical state of the junctional components; and the regulation of the junctional complex by the cytoskeleton or secondary messenger systems. Simultaneous addition of cycloheximide, a protein-synthesis inhibitor, prevented full recovery, implying protein synthesis is required for the TER to return to baseline levels.[41]

5. CHITOSAN DRUG DELIVERY SYSTEMS

These properties of chitosan resulted in the development of numerous drug delivery systems for various application sites, which are described in more detail below (**Table-1**).

The drug can thereby be homogenized with chitosan and directly compressed to tablets. As chitosan precipitates at pH above 6.5, it loses its mucoadhesive and permeation enhancing properties in distal segments of the intestine. This effect reduces its applicability to drugs having their absorption window in the proximal segment of the GI tract. Dhaliwal et al.[42], In case of nanoparticulate delivery systems, chitosan is in most cases ionically cross-linked. Precipitation at higher pH is therefore not anymore an issue at all, as the polymer is already

‘‘co-precipitated’’ with the multivalent anionic cross-linker. Although precipitated and homogenized with a polyanion blocking, its cationic groups to a high extend such nanoparticulate delivery systems exhibit surprisingly still potential. Chitosan-based formulations used for ophthalmic drug delivery are hydrogels,[43] nanoparticles,[44] and coated colloidal systems.[45] Chitosan based systems have the potential for improving the retention and biodistribution of drugs applied topically onto the eye. Fisher et al.[46] developed fentanyl nasal spray formulations with pectin, chitosan, and chitosan–poloxamer 188 for clinical evaluation to provide rapid absorption and subsequently increased bioavailability. Sandri et al.[47] evaluated the mucoadhesive and permeation enhancing properties of four different chitosan derivatives: 5-methyl-pyrrolidinone chitosan, two low molecular mass chitosans, and a partially re-acetylated chitosan via the vaginal and buccal mucosa using acyclovir as model drug. Methyl-pyrrolidinone chitosan showed the highest mucoadhesive and permeation enhancing properties in both vaginal and buccal environments. The capability of enhancing the permeation/ penetration of acyclovir was decreased by partial depolymerization of chitosan and disappeared after partial re-acetylation. The antimicrobial properties of chitosan, however, might have a negative impact on the vaginal microflora.[48] Its vaginal use for treatment of chronic diseases has therefore to be seen with caution.

Table-1	**Chitosan based drug delivery systems**		
Route of administration		**Delivery systems**	**References**
	Oral	Microparticulate	49
		Liposomes	50
		Buccal discs	51
		Solutions	52
		Vesicle	53
		Film coating	54
		Tablets	55
		Spray dried particles	56
		Capsules	57
	Parenteral	Microspheres	58
		Solutions	59
	Nasal	Solutions	60-64
	Ocular	Suspensions	65,66
	Gene therapy		67,68
	Others	Gel systems	69,70

6. CONCLUSIONS

Outstanding scientific progress has been made, demonstrating the application of chitosan in drug delivery systems. As stated in the introduction, the properties of chitosan make it a versatile excipient, not only for controlled release applications but also as a bioadhesive polymer, depending on the route of delivery.

In addition, it appears to have potential as an absorption enhancer promoting drug uptake across the mucosal barrier. To date, the lack of an approved product containing chitosanhas probably inhibited the universal acceptance of this polymer as a pharmaceutical excipient. However, extensive research has been devoted to the demonstration of the safety of chitosan by performing toxicity studies and elucidating its mechanisms of action. Clinical trials are currently in progress to optimize chitosan-based formulations for drug delivery systems with a broad range of therapeutic applications.

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