Expert Opinion

Chitosan and its salts for mucosal and transmucosal delivery

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This review considers the application of chitosan and its salts in the delivery of drugs intended to act locally towards diseases of the mucosa itself (mucosal delivery), and to undergo systemic absorption by means of transmucosal routes (transmucosal delivery). Those chitosan properties that are particularly useful in mucosal and transmucosal delivery have been reviewed, such as mucoadhesion, penetration enhancement and peptidase inhibition behaviour. Chitosan bioactive properties have also been considered, such as anti-infective, haemostatic, wound healing and immune-stimulating activity. Chitosan is available with a wide range of molecular mass and deacetylation degree: the influence of these properties on polymer performance and solubility has been taken into account. As solubility in particular can strongly limit the results obtained at pH values close to neutrality, particular attention has been paid to chitosan salts and derivatives with modified solubility. Thanks to the presence of positively charged amino groups of the polymer, a subject of increasing interest is the exploitation of its interaction with acidic molecules having potential synergistic behaviour towards bioactive properties, or even with acidic drugs. The aim of the review is to describe not only some properties of chitosan, but also the way they can be modified by the acidic moiety.

Keywords: anti-infective properties, chitosan, ionic interaction, mucoadhesion, penetration enhancement, peptidase inhibition, wound healing

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1. Mucosal and transmucosal delivery

Mucosae are gaining increasing attention as an alternative to other more traditional administration routes, such as oral or parenteral routes, and as a target in the development of new drug delivery systems. Different anatomical districts, such as conjunctiva and oro-oesophageal, respiratory and urogenital tracts are lined by mucosae. These can be considered as administration sites for drugs intended to act locally towards diseases of the mucosa itself, such as anti-inflammatory and anti-infective (antibacterial, antifungal and antiviral) drugs. In these cases the easy accessibility of mucosal districts represents an important advantage. Mucosal sites other than the gastrointestinal tract can also be exploited to deliver drugs that are aimed for systemic absorption by means of transmucosal routes. The absorption surface is in all cases reduced, especially with respect to that of the small intestine, and no structures physiologically aimed at absorption (such as villi and enterocytes) are present. On the other hand, with respect to the gastrointestinal segment, all the above-mentioned mucosal districts present advantages, such as more constant pH conditions, absence of the variability deriving from food, and limited presence of degrading enzymes [1,2].

Another kind of delivery system that is the object of intense research nowadays are those aimed at mucosal vaccination, based on the fact that the mucosal surface is an important way for the pathogens to enter the body, therefore it

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contains the highest concentration of lymphocytes [3]. Both mucosal and systemic immune responses can be induced following mucosal immunisation. For all kinds of drug delivery mucosal application, both aimed at topical action and at systemic delivery, the therapeutic success depends on the possibility of maintaining the delivery system at the administration site for a prolonged period of time; because of the removal mechanisms that act at all the mucosal administration sites, polymers with mucoadhesive properties are useful [4,5].

Moreover, whenever the absorption of the drug is important for its action (in the case of systemic delivery), the use of permeation enhancers can be mandatory to overcome the barrier represented by the mucosal epithelium. Among the permeation enhancers, the attention is more and more focused on polymers. They can be considered safe as they themselves are not absorbed, while they work by transiently opening the paracellular route [6]. Some of these polymers associate the ability to improve permeation and the mucoadhesive behaviour, and are for this reason called 'multifunctional polymers'. A third feature of multifunctional polymers that is especially important in the case of peptidic drug delivery is the ability to inhibit peptidase activity [7]. Among multifunctional polymers, chitosan is one of the more extensively studied materials.

Chitosan is obtained by deacetylation from chitin, that is, a β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranose polysaccharide, a component of insects' and crustaceous species' exoskeletons, industrially derived from crabs, shrimps and prawns. As they can be obtained in a great variety of molecular masses and deacetylation degrees, chitosans can be considered a family of polymers.

Since 2002, chitosan has been reported in *European Pharmacopoeia*, where the most widely used salt (chitosan HCl) is described. Very often also cited in the literature is chitosan glutamate, and among the other possible salts of chitosan are formate, acetate, lactate, malate, citrate, glycoxylate, pyruvate, glycolate and ascorbate [8]. **Figure 1** reports a scheme of the possible applications of chitosan in mucosal and transmucosal delivery.

The aim of this review is to consider the properties of chitosan that make it an interesting material for mucosal/transmucosal delivery, and to discuss, whenever possible, the relevance of the counterion acidic moiety for the exploitation of these properties.

2. Chitosan as multifunctional polymer at different mucosal sites

2.1 Mucoadhesion properties

A gel-like secretion known as mucus, which contains mostly water-insoluble glycoproteins, covers all the mucosal epithelia. Mucus is bound to the apical cell surface and acts as a protective layer to the tissues. It is a viscoelastic hydrogel, and consists primarily of 1 – 5% of glycoproteins, 95 – 99% water, and several other components in small quantities, such as proteins, enzymes, electrolytes and nucleic acids. This composition can vary depending on the origin of the mucus secretion and on the localisation of the mucosa. The interaction of the formulation with mucus can be exploited to improve the residence time at the administration site. This is of particular importance for mucosal application, where removing mechanisms are especially efficient. Among the characteristics of the polymers that are generally considered relevant to the mucoadhesive behaviour is the presence of chemical groups, which may contribute the formation of interactions between the polymer and the mucus. In the case of chitosan, charge and ionisation will be favourable for mucoadhesion. Ionic interactions between positively charged groups and the acidic sialic moieties of mucins can lead to strong mucoadhesion, which is, however, obtained also when a large number of chemical groups that form hydrogen bonds with the mucus gel, such as hydroxyl, amine, sulphate and carboxyl groups, are present [4].

The good mucoadhesion potential of chitosan as cationic polymer was understood early, and in fact the mucoadhesive properties of chitosan have been studied since the early 1990s. A comparison between the mucoadhesion performance of chitosan and other polymers pointed out that only polycarbophil showed better interaction with mucus [9]. Among the tested chitosan grades, the mucoadhesion properties increased with the increase of molecular mass. These results seemed in accordance with the general features indicated in the literature for mucoadhesive polymers [10], considering that the electrostatic interactions between positively charged polymers and anionic sialic acids of mucin are able to strengthen the mucoadhesive boundary. The relevance of polymer molecular mass on mucoadhesion can be explained by considering that the size of the chains determines the number of different functional groups involved in the interaction. By using FITC-labelled chitosans of molecular mass ranging between 70,000 and 2,000,000 Da, some authors showed that high-molecular-mass chitosan offers multiple sites for mucin attachment, whereas low-molecular-mass polymers associate univalently [11]. The effect of molecular mass on mucoadhesion, however, does not always follow a direct relationship. This has been seen for chitosan grades in some other studies, in which low viscosity grades were more adhesive than the higher ones [12]. This result has been confirmed more recently on three viscosity grades of chitosan lactate [13], and it can be explained by considering that shorter chains are able to diffuse more easily within the mucin boundary to allow easier interpenetration. The optimum molecular mass therefore depends on the flexibility and the conformation of the polymer chains [4].

Moreover, it has been demonstrated that the increase in charge density determines an improvement of the adhesive properties of chitosan. In fact, as the extent of mucoadhesion is directly related to the number of free amino groups of chitosan, charge density and mucoadhesion are affected by deacetylation degree and by crosslinking. At the same time, the greater the positive charge density, the stronger the

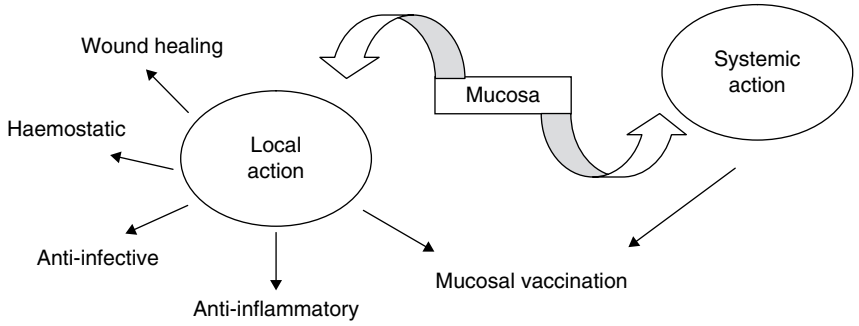

Figure 1. Schematic representation of mucosal and transmucosal delivery.

mucoadhesive joint [14-16]. **Table 1** shows the mucoadhesive properties of chitosans as a function of molecular mass and deacetylation degree. Many studies in the literature confirmed the mucoadhesive potential of chitosan salts at different mucosal sites.

2.1.1 *Nasal application of mucoadhesive chitosan formulations*

Nasal residence time improvement has been described for different chitosan delivery systems. For example, radiolabelled chitosan glutamate microspheres and solution have been compared with starch microspheres and the scintigraphic study demonstrated that chitosan systems were able to control the rate of clearance from the human nasal cavity by increasing the contact time of the delivery system with the nasal mucosa [17]. Nasal delivery of antibiotics such as vancomycin with chitosan lactate, aspartate, glutamate and hydrochloride has been reported as well. The different salts have been compared for their interaction with mucin and for drug release behaviour. It has been shown that the presence of chitosan hydrochloride provides the lowest release of vancomycin [18]. The mucoadhesion properties of chitosan can be exploited also in nasal administration of vaccines. PEG-coated polylactic acid nanospheres, chitosan-coated polylactic–glycolic acid nanospheres and chitosan nanospheres were compared for nasal immunisation with tetanus toxoid. Although PEG-coated nanospheres induced higher levels of tetanus toxoid in the blood compared with chitosan-coated nanospheres, very high IgG titres were obtained 6 months post administration of chitosan nanospheres [19].

Recently, microparticulate drug delivery systems constituted by mannitol or chitosan hydrochloride were prepared, designed for N^6-cyclopentyladenosine (CPA) nasal administration to cerebrospinal fluid (CSF). The CPA appearance in the CSF was rapid and resulted in high concentrations after insufflation of the chitosan formulation. Chitosan gelling and mucoadhesion behaviour can be responsible for these results [20].

2.1.2 *Vaginal application of mucoadhesive chitosan formulations*

The vaginal epithelium is usually considered to be a mucosal surface, although it has no goblet cells that directly release mucin, but it is lined by secretions coming from higher tract, such as uterine fluid or follicular fluid at ovulation. Vaginal discharge is therefore a mixture of transudates through the epithelium, cervical mucus, exfoliating epithelial cells, secretions of the Bartholin's glands, leukocytes, endometrial and tubal fluids [21].

In this vaginal environment, characterised by relatively low pH values, chitosan is generally considered a good mucoadhesive polymer, both alone and associated with other materials in interpolymer complexes with sodium alginate [22] or with polyacrylic acid [23]. Moreover, the positive effect of chitosan lactate in gels based on methylcellulose has been described [24].

Adhesion force studies highlighted that the use of hydroxyethylcellulose (HEC) and chitosan blends or HEC and 5-methylpyrrolidinone-chitosan blends permitted vaginal gels to be obtained with good mucoadhesiveness, confirming that chitosan presence improved gel performance and made it possible for them to be used in vaginal therapy [25]. Chitosan-based formulations have been reported in the literature for vaginal delivery of some anti-infective drugs [26,27].

2.1.3 *Buccal application of mucoadhesive chitosan formulations*

The advantages associated with buccal drug delivery, such as high patient compliance and smooth surface, made this route of administration useful for the application of mucoadhesive delivery systems loaded with a variety of drugs, also biotechnological ones such as peptides and proteins. These systems have been the subject of increasing interest since the early 1980s. Movements of the buccal tissues are, however, present, especially while eating, drinking and talking, although they continue even during sleep, and can potentially lead to the detachment of the dosage form. The production of saliva contributes to the removing of mucoadhesive formulations,

Table 1. chitosan properties as a function of molecular mass and deacetylation degree.

	Solubility	Mucoadhesion	Penetration enhancement	Biocompatibility	Wound healing	Antimicrobial	Immune stimulating
Deacetylation degree	Inversely correlated to DD [8,73]	Directly correlated [14-16,117,118]	Directly correlated [35]	Directly correlated [35,36]	Directly correlated [64-66]	Not proportional to its DD value [61]	It would be expected to affect the antigen release and uptake [72]
Molecular mass	Inversely correlated to molecular mass [8]	Related to flexibility and conformation of polymer chains [4,11-13,118]	Directly correlated [35]	Not crucial [35,36]	Inversely correlated [64,66]	Inversely correlated [61]	Higher for lower molecular mass [71]

DD: Deacetylation degree.

but also affects the behaviour of the polymer. Depending on both the saliva flow rate and the method of determination, the pH of this medium has been estimated to be between 6.5 and 7.5. The pH of the microenvironment surrounding the mucoadhesive polymer can therefore alter its ionisation state and its adhesion properties. Mucin turnover rate is another environmental factor. The residence time of dosage forms is limited by the mucin turnover time, which has been calculated to range between 12 and 24 h in humans [28]. Different examples of mucoadhesive chitosan-based formulations intended for buccal administration are reported in the literature [29-31]. In particular, the mucoadhesive properties of chitosan were useful to prolong the activity of antifungal agents such as nystatin, which is also a prophylactic agent for oral mucositis [30]. Mucoadhesive chitosan-based gels and films also improved the residence time of chlorexidine gluconate against buccal *Candida albicans* [31]. Different chitosan salts have been used, associated with poloxamer 407 to prepare buccal tablets, in a comparative study in which the result was that chitosan lactate was more efficient as a mucoadhesive than chitosan acetate and citrate [32].

2.2 Penetration enhancement properties

Chitosan, like other polymeric enhancers of mucosal epithelia permeability, has some advantages with respect to low-molecular-mass promoters: it is generally not absorbed, reducing toxicity, and the penetration enhancement effect is favoured by the prolonged residence at mucosa surface owing to mucoadhesive properties [6]. In the case of epithelia endowed of tight junctions, penetration enhancement properties are due to the capability of chitosan to open tight junction complexes involving translocation of the proteins ZO-1 and occludin from plasma membranes to cytoplasm [33] and also to induce redistribution of F-actin and so to cause a partial alteration of cytoskeleton [34]. The structural properties of chitosan (both molecular mass and deacetylation degree [DD]) determine absorption-enhancing properties and toxicity [35]. In particular, *in vitro* tests on Caco-2 cell cultures demonstrated that chitosan hydrochloride with low molecular mass (22 kDa) and low DD (< 65%) lacks absorption enhancement activity, whereas chitosan with a high DD and/or high molecular mass increases epithelial permeability. In particular, chitosans with a high DD showed a clear effect on absorption enhancement independently of molecular mass, whereas chitosans with DD of 65 and 51% enhanced the permeation at high molecular mass only. A high DD and a high molecular mass are therefore necessary to improve penetration enhancement, whereas cytotoxicity is strictly related to DD and not to molecular mass [35]. Deacetylation degree is also able to influence chitosan molecule uptake by A549 cells (human lung carcinoma cell line): decreasing the DD results in a decreased uptake of chitosan molecules [36]. **Table 1** shows the penetration enhancement properties of chitosans as a function of molecular mass and deacetylation degree.

2.2.1 *Nasal penetration enhancement*

Although nasal absorption is quite easy for lipophilic drugs, for which bioavailability is close to 100%, the absorption of hydrophilic drugs is impaired by low membrane permeability, and requires absorption promoters that either modify the phospholipid bilayer or open the tight junctions, whose presence is consistent in nasal epithelium [37]. The barrier integrity of epithelial monolayers that are characterised by the presence of tight junctions can be measured directly by variations of the transepithelial electrical resistance (TEER). The effect of chitosan on tight junction opening is well documented in Caco-2 cell cultures where decreases in TEER of up to 80% have been observed with both chitosan hydrochloride and glutamate. It has been demonstrated that the tight junction disruption is not mediated by means of the salt content of chitosan samples, as addition of glutamate alone has no effect on TEER [33,38]. The effect of chitosan on tight junctions, together with the extended contact time resulting from its mucoadhesive behaviour, can explain the ability to improve nasal absorption of drugs and

proteins [39,40]. Transmission electron microscopy (TEM) analyses carried out on excised sheep nasal mucosa confirmed the morphological changes of epithelial cells and tight junctions after application of microparticulate delivery systems based on chitosan hydrochloride and designed for the nasal administration of the antiemetic metoclopramide hydrochloride [41]. This effect was observed further *in vivo* (in sheep) after application of chitosan hydrochloride and glutamate microparticles intended for the systemic nasal administration of carbamazepine. A remarkably improved bioavailability was found with respect to controls [42].

Using *in situ* rat nasal perfusion techniques, both the free amine and two salt forms, hydrochloride and glutamate, of chitosans were effective as nasal absorption enhancers of L-Tyr-D-Arg, a model opioid dipeptide. The soluble salt forms appeared to be less dependent on pH, whereas the enhancing activity of the free amine chitosan increased as the pH was lowered from 6 to 4. According to what had been observed previously [9], chitosan glutamate appeared more readily soluble, and its enhancing effect was even slightly greater at pH 6.0 than at pH 4.0 or 5.0 [43]. These results were confirmed in a subsequent paper where the enhancing effect of 1% chitosan glutamate on salmon calcitonin nasal absorption was maximal at pH 6.0. The authors attributed this observation to the greater solubility of the glutamate salt, which might be able to assume the highly ionised, elongated shape that helped to maintain its enhancing activity at less acidic bulk pH values [44].

A different rank order of the performance of chitosan hydrochloride and glutamate in promoting permeation enhancement was observed by other authors, which found that at pH 6.2, the hydrochloride salt (0.25 – 1.50% w:v) was always more effective than glutamate in lowering the transepithelial electrical resistance and in increasing the *in vitro* transport of hydrophilic markers (mannitol and PEG-4000) across the Caco-2 cell monolayers. They attributed the better efficacy of chitosan hydrochloride to its higher concentration of equivalent chitosan base per weight basis as compared with chitosan glutamate, assuming the same degree of deacetylation [45].

2.2.2 *Vaginal penetration enhancement*

Owing to the rich blood supply and to the folds and microridges that increase surface area, the vagina can be considered a useful application site also for systemic drug delivery. The vaginal administration offers a favourable alternative to the parenteral route, but also to the oral one for some drugs, such as estrogens, which are metabolised extensively by first pass hepatic metabolism, or can induce side effects in the gastrointestinal tract. The epithelium is a non-cornified, stratified squamous epithelium. The absorption mechanisms are transcellular diffusion, vescicular or receptor-mediated transport and paracellular mediated by desmosomes, gap junctions and some tight junctions [46]. A good permeability to a wide range of compounds including peptides and proteins has been shown in vaginal application, although the absorption of high-molecular-mass hydrophilic molecules such as peptides and proteins can take advantage of the use of penetration enhancers. In this respect, however, chitosan salts, although they have been documented extensively for vaginal application as mucoadhesive materials [21], only a few authors have described them as penetration enhancers at the vaginal level [23,47].

2.2.3 *Buccal penetration enhancement*

Buccal delivery is promising for systemic absorption of different drug categories, such as pain relief drugs, hormones and peptidic drugs. The buccal absorption presents the advantage of avoiding the first pass effect, and in general a reduced presystemic degradation with respect to gastrointestinal tract. The vascolarisation is good, but the absorptive surface is limited, so that, especially with high-molecular-mass hydrophilic drugs, the absorption efficiency requires to be improved.

Buccal mucosa is lined by a non-keratinised squamous epithelium supported by a connective tissue. The permeability barrier property of the oral mucosa is predominantly due to intercellular materials derived from the so-called membrane coating granules (MCGs), which are spherical or oval organelles 100 – 300 nm in diameter and found in both keratinised and non-keratinised epithelia [28]. The intercellular spaces of superficial cell layers are therefore rich in neutral lipids and glycolipids. The mechanism of penetration enhancement seems to result from a repackaging of the epithelium cells up to the basal membrane and in a partial disarrangement of desmosomes. The intercellular spaces between contiguous cells were enlarged following contact with the polymers, probably owing to the drainage of fluids from the basal layers to the intermediate and superficial layers. Such behaviour seems to determine a modification or even a disruption of the organised lipid lamellae that represent the principal intercellular barrier in buccal epithelium, causing a permeabilising effect [48-50].

It has been shown that chitosan significantly increases the permeation across the buccal mucosa of different drugs, among them hydrocortisone, but also bioactive peptides such as transforming growth factor-β (TGF-β). This result has been explained by considering the bioadhesion of the chitosan and the prolonged retention of the drug at its application site. However, a permeabilising effect on the intercellular lipid lamellae, which represent the main buccal barrier, can also be supposed. This mechanism could be similar to the interference of chitosan with lipid micelle organisation in the intestine, which explains the chitosan capability of reducing lipid absorption, leading to weight loss [47-50].

2.3 Peptidase inhibition properties

Another contributing factor to the low transport of particularly peptides and proteins across the different mucosal epithelia is the possibility of an enzymatic degradation of

the molecules either at the surface or during the passage across the epithelial barrier. A clear knowledge of the degrading potential of mucosal enzymes has not been achieved so far, owing to variability in experimental procedure. Many studies have in fact been carried out *in vitro* using tissue homogenates. In this case, an overestimation of the enzyme degrading activity is possible owing to the release of intracellular and in particular cytosolic enzymes [51]. In the case of nasal mucosa, an experimental asset useful to elucidate the possible effect of enzymes on the stability of peptides can involve the use of nasal wash in animal models or even in humans. Enzymes present within the lumen of the nasal cavity and in the tissue comprise exopeptidases, such as mono- and diaminopeptidases, and endopeptidases such as serine, cysteine and aspartic proteinases [52]. Analogous considerations have been made in the case of buccal and vaginal mucosa. No carboxypeptidase or dipeptidyl peptidase IV activity was detected on the buccal mucosa, whereas aminopeptidase N activity was detected using Leu-*p*-nitroanilide [51]. In the case of vaginal mucosa, aminopeptidases are believed to play a major role in the degradation of peptides and proteins, and in particular aminopeptidase N is generally regarded as the most abundant metallopeptidase in mucosal tissues. To avoid the leaching of cytosolic enzymes, the aminopeptidase N activity was assessed at the surface of vaginal mucosa by means of a cylinder that was placed on top of a mucosal side of the excised tissue and clamped. In the cylinder substrates and eventually enzyme inhibitors were introduced [53,54].

The largest part of the enzymes responsible for presystemic metabolism of peptidic drugs administrated along a transmucosal route, such as carboxypeptidase and aminopeptidase N, contains metallic ions (e.g., zinc) that are essential for activity. Polycarbophil proved to be a potent inhibitor towards trypsin and carboxypeptidase B, owing to the ability of chelating ions such as calcium and zinc ions. It has also been reported that chitosan does not have enzyme inhibitory properties [55,56]. Derivatives in which chitosan is covalently linked to chelating molecules such as EDTA or to other peptidase inhibitors have been designed and proved to be more efficient [57].

In a quite recent report, it has been observed that chitooligosaccharides are able to inhibit matrix metalloproteinase-2, probably thanks to a binding capacity for zinc ions, which are necessary to the activity of the enzyme. Matrix metalloproteinases are responsible for physiological digestion of extracellular matrix in tissue morphogenesis, tissue repair and angiogenesis, so that the inhibiting effect of chitosans on this class of enzymes could explain the activity of the polymer in wound healing and in the prevention or treatment of pathologies involving excessive degradation in extracellular space [58]. **Figure 2** summarises the properties of chitosan relevant to mucosal (topical) action and transmucosal (systemic) delivery.

3. Chitosan as bioactive polymer

3.1 Anti-infective properties

In the treatment of diseases caused by microorganisms belonging to bacteria, fungi or virus (bacterial vaginitis or fungal infections of the female genital tract, herpes affections or oral candidosis) that affect vaginal or buccal mucosae, topical formulations may exploit antimicrobial and anti fungal activity of chitosan. Some reported experiments confirmed that water-insoluble chitosan in solution increased the permeability of cell membrane and ultimately disrupted bacterial cell membranes, causing release of cellular contents. On the contrary, many research results proved that chitosan oligomers had weak or no antimicrobial activity, although chitosan oligomers were very water-soluble [59].

Chitosan has higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. Antimicrobial activity of chitosan is based on different mechanisms depending on the microorganisms. Some authors have suggested that in the case of Gram-negative bacteria, chitosan as a polymeric macromolecule is unable to pass the outer membrane, as membrane acts as an efficient outer permeability barrier against macromolecules. Therefore, direct access to the intracellular parts of the cells by chitosan is unlikely. A key feature of chitosan is the positive charge of the amino group at C-2 below its pK_a ~ pH 6.5. This creates a polycationic structure, which can be expected to interact with the predominantly anionic components lipopolysaccharides, proteins of the Gram-negative surface. Binding of polycationic molecules has been shown to disrupt the integrity of the outer membrane, resulting in loss of the barrier function but lacking direct bactericidal activity [60].

Other authors proposed the following mechanisms: for Gram-positive bacteria, the chitosan of higher molecular mass forms a film that inhibits nutrient adsorptions. This would explain why the antimicrobial effect was enhanced when the molecular mass of chitosan increased. For Gram-negative bacteria the chitosan antimicrobial effect was enhanced as the molecular mass of chitosan decreased, probably because lower molecular mass enters the microbial cell and disturbs the metabolism of the cell by adsorbing the electronegative substance in the cell and flocculating them [61]. The antifungal activity of chitosan has been explored intensively in the last decade. In particular, chitosan has been demonstrated to possess anti-infective properties towards *C. albicans* in buccal application [30,31,62]. Recently, other authors studied water-soluble chitosans obtained by depolymerisation or by *N*-acetylation and concluded that no antimicrobial activity could be seen without the presence of an acidic environment [59].

Chitosan is also characterised by antiviral properties [63]: in particular, it is able to suppress viral infections in various biological systems. Even if the mechanism of this antiviral activity is poorly understood, the activity seems to be related to the capability of chitosan to prevent the development of

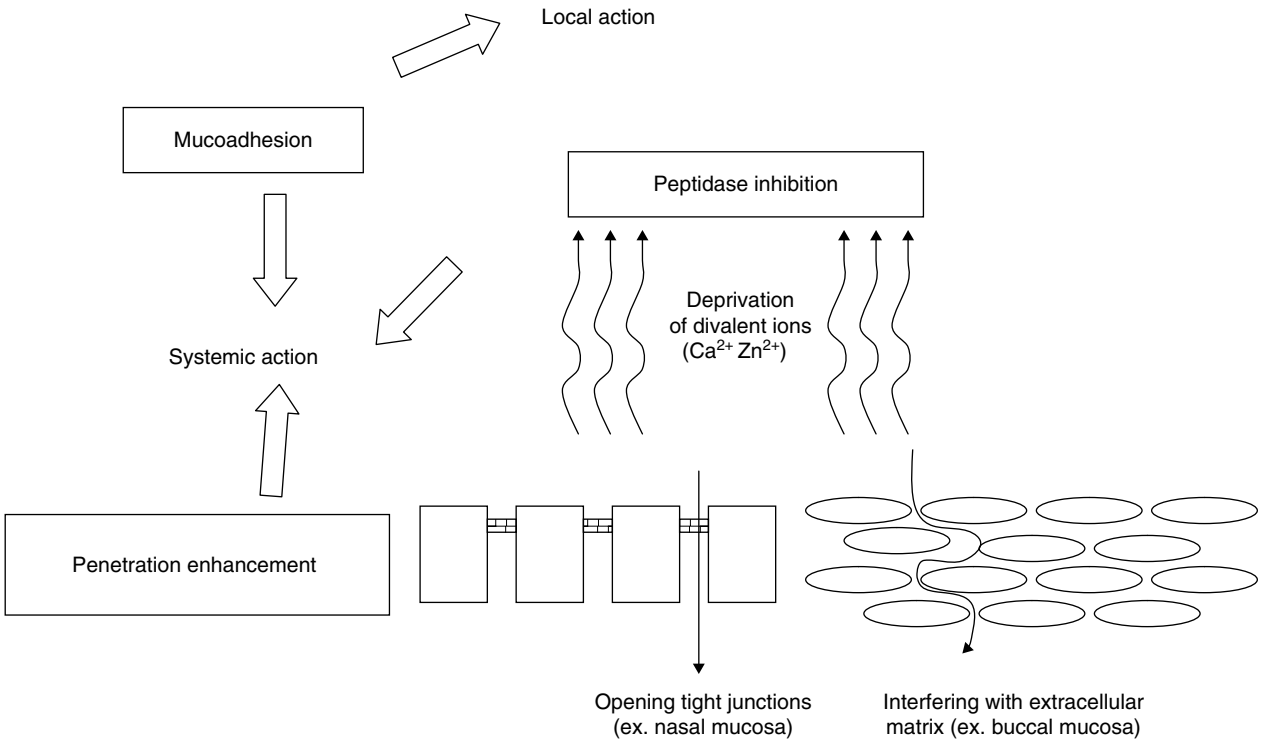

Figure 2. Chitosan properties as multifuctional polymer: mucoadhesion improves local action and together with peptidase inhibition and penetration enhancement contributes to systemic action. Peptidase inhibition occurs through deprivation of divalent ions. A scheme of the main mechanisms of penetration enhancement is illustrated depending on the mucosa: opening of tight junctions or interference with extracellular matrix can occur.

viral infection probably by means of interaction with cell surfaces. This interaction can result in an increase in the membrane permeability and its destruction caused by non-specific binding of polycationic chitosan molecules. As replication of phage DNA and morphogenesis of phage particles are closely related to membranes, changes in properties of cell membranes induced by chitosan may be one of several factors that inhibit the replication of bacteriophages [63].

3.2 Haemostatic and wound-healing properties

Chitosan attracts red blood cells, negatively charged, by means of its positive charge. The contact between chitosan molecules causes a seal over the wound due to red blood cells forming a very tight, coherent seal. The result of chitosan/red blood cell interaction is the activation of the intrinsic coagulation cascade by accelerating the generation of thrombin [64].

Moreover, chitosans have been known as wound-healing accelerators for more than two decades, when it was found that glucosamine, which is a component of shark cartilage, functioned mainly as a healing accelerator. It is reported that a high degree of deacetylation and low molecular mass can improve the chitosan healing process [65].

Chitosan enhances the functions of inflammatory cells such as polymorphonuclear leukocytes (phagocytosis, production of osteopontin and leukotriene B4), macrophages (phagocytosis, production of osteopontin and leukotriene B4, transforming growth factor-f1 and platelet-derived growth factor) and fibroblasts (production of IL-8) [65,66]. As a result, chitosan promotes granulation and tissue organisation [67]. Moreover, chitosan is characterised by radical scavenging activity [62,68,69] that has been related to the wound-healing effect thanks to antioxidant activity.

Several chitosan-based wound dressings are commercially available as haemostatic and wound-healing devices [64]. Some other systems are under evaluation in cinical trials with the same indications, as published by the National Institute of Health [70]. Powders, wound dressings, non-woven dressings, particles and sponge-like dressings are commercially available as formulations. In particular, as far as the chitosan salts are concerned, HemCon® (HemCon Medical Technologies, Inc., OR, USA), a haemostatic dressing based on freeze-dried chitosan acetate, was described as a topical antimicrobial dressing having favourable effects on the healing of excisional wounds in emergency use to stop bleeding. This dressing can be used also in wounds infected with *Staphylococcus aureus* (evaluation in mice): the strongly bactericidal effect and the capability to reduce the number of inflammatory cells in the wound produce an overall beneficial effect on wound healing, especially during the early period where its antimicrobial effect is most important [64].

3.3 Immune-stimulating properties

Chitosan has a great potential to be used in mucosal vaccination because its mucoadhesive properties are useful to carry antigens and adjuvants to the mucosa, and in most cases it acts as adjuvant itself, owing to its immune-stimulating activity. Chitosan has been shown in fact to provide enhanced immune responses through oral (gastrointestinal) delivery and it has been investigated as an effective and safe adjuvant and delivery system for nasal immunisation [71]. A very recent and complete review of chitosan in mucosal vaccination gives many other examples of the use of chitosan in this kind of application. In particular, as far as the chitosan salts are concerned, examples have been reported of chitosan glutamate in intranasal administration of Mutant crossreacting material (CRM)197 of diphtheria toxin, of Neisseria meningitides serogroup C polysaccharide (MCP)-CRM197, and of Tetanus toxoid, and chitosan chloride is reported in intranasal administration of Recombinant H. pylori urease (rUre) [72].

Table 1 reports some properties of chitosans as a function of molecular mass and deacetylation degree.

4. Chitosan salts and chitosan derivatives with modified solubility

The amino groups make chitosan a polycationic polymer, with pK_a ~ 6.3. The amino groups can therefore be protonated by acids to give water-soluble salts. For the above-mentioned properties, chitosan is easily soluble in aqueous media at pH values < 6 – 6.5. Molecular mass and deacetylation degree affect pH-dependent solubility: chitosan solubilisation is related to ionisation of 2-amino-β-D-glucopyranose (GlcN) units, which occur at pH lower than pK_a. Moreover, the charge density of chitosan is also controlled by varying DD because only GlcN units are positively charged [73].

The main drawback with the use of chitosan salts such as hydrochloride and glutamate, and also acetate and lactate, is their pH-dependent solubility. Not only in the intestinal tract, but also in most mucosal applications, such as buccal, nasal and ocular, where the pH is close to neutrality, chitosan loses its positive charge and precipitates. Only the vaginal environment is physiologically characterised by relatively low pH, ranging between 3.5 and 5, where chitosan salts are freely soluble. It has also been shown that only protonated soluble chitosan in its uncoiled configuration can trigger the opening of the tight junctions and facilitate the paracellular transport, probably thanks to the binding of the polymer to the cell membrane, which is mediated by positive charges. Both chitosan hydrochloride and glutamate in fact caused TEER to decrease and significant permeation of C14 mannitol in Caco-2 cell layers at pH 6.2, but not at pH 7.4 [74]. To overcome these limitations, chitosan derivatives with increased basicity and aqueous solubility, such as derivatives with secondary, tertiary and quaternary amino groups, have been designed and synthesised, such as trimethyl chitosan (TMC), dimethylethyl chitosan (DMEC), diethylmethyl chitosan (DEMC) and triethyl chitosan (TEC) [75-78].

The polymer charge density, determined by the substitution degree, is a key factor in obtaining both the mucoadhesion and the penetration enhancement. The comparison of the above-mentioned quaternary derivatives of chitosan in free-soluble forms revealed that all of them were able to open the tight junctions, decreasing the TEER value in the following order: TMC > DMEC > DEMC = TEC > chitosan. A similar rank order (TMC > DMEC > DEMC > TEC > chitosan) was found for the transport of insulin together with the soluble polymers across Caco-2 cell layers. These results are in agreement with the strength of the cationic charge of the polymer.

In comparison with the free-soluble polymers, however, the nanoparticles prepared by tripolyphosphate (TPP) ionic gelation of the chitosan and its quaternised derivatives had a much lower effect on decreasing the TEER by opening of the tight junctions, probably because of the reduced available amount of positive charge at the surface of the nanoparticles [79].

Recently, with the purpose of preparing new derivatives with a potential to improve the transmucosal penetration-enhancing properties of TMC, chitosans of two different molecular masses were reacted with 2-diethylaminoethyl chloride (DEAE-Cl) to obtain partially substituted *N,O*-[*N,N*-diethylaminomethyl(diethyldimethylene ammonium)*n*]methyl chitosans containing different percentages of pendant quaternary ammonium groups. All the derivatives promoted paracellular transport of fluorescein sodium [6,80].

Water-soluble tetraalkylammonium chitosan salts have been also evaluated for their mucoadhesive properties. *N*-trimethylchitosan, *N*-diethylmethylchitosan, *N*-carboxymethylchitosan and *N*-[*N,N*-diethylaminomethyl(diethyldimethylene ammonium) *n*] methyl chitosan were tested along with the parent biopolymer and its citric acid salt, both at neutral and acidic pH, and improved mucoadhesive properties were confirmed [81].

Quaternary ammonium derivatives such as *N,N,N*-trimethyl chitosan, *N-N*-propyl-*N,N*-dimethyl chitosan and *N*-furfuryl-*N,N*-dimethyl chitosan showed antibacterial activity against *Escherichia coli* related to polymer molecular mass. It was also found that the antibacterial activity of quaternised chitosan against *E. coli* is stronger than that of chitosan, and affected by the length of alkyl chains [82].

An opposite approach in modifying the polymer solubility by ionic interaction of acidic moieties with the amino groups of chitosan is aimed at decreasing solubility to obtain nanoparticulate systems. The use of complexation between oppositely charged macromolecules to prepare CS microspheres and nanoparticles has attracted much attention because the process is very simple and mild. One of the earliest and most successful interactions proposed, generally referred to as ionic gelation, represents today a widely used method to obtain chitosan nanoparticles. It involves the reaction of chitosan amino groups with tripolyphosphate, a polyanion that can interact with the cationic CS by electrostatic forces [83-85].

Another crosslinking agent is citrate as it is a multivalent low-molecular-mass ion in certain pH aqueous solutions. It has been described that contact between CS and citrate in aqueous solution immediately induces ionic crosslinking of CS, thus forming gel beads. The stability of complexes formed by this electrostatic interaction is dependent on environmental pH, which means that many of these complexes have pH stimuli–response [86]. This occurs also in citrate crosslinked chitosan films that have been prepared by dipping chitosan film into citrate solution [87].

A reduction of the chitosan solubility can also be obtained by ionic reaction of amino groups of chitosan with ionic hydrophobic molecules. These interactions are the basis for preparing microparticles and nanoparticles aimed at the delivery of hydrophobic drugs and genes.

Some authors have proposed chitosans derivatised by the formation of amide linkages through the EDC-mediated reaction with hydrophobic molecules such as palmitic acid [88,89] and linolenic acid [90]. Also, bile salts such as deoxycholic acid and cholesterol have been covalently linked to chitosan to obtain hydrophobically modified polymers [91]. These polymeric amphiphiles can form monodisperse self-aggregated nanoparticles in aqueous media, whose dimensions and morphology can be controlled by the chemical structures of hydrophobically modified chitosans, such as the molecular mass, and the types and the degree of substitution (DS) of hydrophobic groups.

More recently, microparticles have been obtained by ionic interaction between chitosan and deoxycholic acid (DCA). The particles were loaded with bovine serum albumin and a comparison with chitosan coacervates obtained with sulphate and citrate was performed. Properties such as microparticle morphology, protein protection, incorporation yield, protein integrity during the process and inside the matrix and suitable drug release profile for mucosal administration were considered, and in particular DCA–chitosan-based systems were shown to be highly effective in the formation of microparticles, with good protein encapsulation efficiency size and acceptable shape for mucosal delivery purposes, and prolonged protection of the protein [92].

Nanoparticles have been also prepared by the addition of soybean lecithin alcoholic solution to chitosan water solution. From the supramolecular self-organising interaction of negative lipid material in the presence of the positively charged polysaccharide, a packed and dense structure different from the simple coating of lecithin vesicles was obtained in which hydrophobic drugs such as progesterone could be encapsulated with a good efficiency [93,94].

5. Chitosan salts with peculiar properties coming from the acidic moiety

A peculiar application of the reaction of amino groups of chitosan with oppositely charged moieties involves the choice, as counterions, of molecules that are themselves capable of improvement of the polymer properties. **Table 2** summarises the bioactive properties of some chitosan salts, whose chemical structures are given in **Figure 3**.

5.1 Chitosan citrate

Chitosan citrate was developed as a multifunctional polymer to fulfil mucoadhesive, penetration enhancing and protease inhibition properties [95]. Chitosan citrate was used in gels for the vaginal administration of poorly permeable drugs (drugs with low permeability properties according to BCS and macromolecule). Gel based on chitosan citrate (3% w/v) has been prepared using chitosan with molecular mass 200,000 Da and degree of deacetylation 90% to obtain a viscosity suitable for vaginal application [13]. It showed good absorption enhancement properties towards both acyclovir and ciprofloxacin with respect to chitosan hydrochloride in *ex vivo* tests on pig-excised vaginal mucosa. It also improved the tissue penetration (pig-excised vaginal mucosa) of fluorescein isothiocyanate dextran (molecular mass 4400 Da), a high-molecular-mass hydrophilic molecule. Although specific evidence is not given, this behaviour would suggest the ability of chitosan citrate to enhance the vaginal permeation of peptidic drugs. The mechanism of penetration enhancement is conceivably related to the influence of the citrate moiety on the calcium regulation of tight and gap junctions, particularly important in vaginal absorption. Chitosan citrate is also characterised by stronger inhibition of Zn^{2+}-dependent peptidases, such as carboxypeptidase A and leucine aminopeptidase, the latter one described as characteristic of different mucosal surfaces, among which is the vaginal one [54,96]. In fact citrate is characterised by chelating behaviour of zinc ions, essential cofactors of these peptidases. The chelating properties of the citrate moiety are therefore maintained after interaction with chitosan chains. This interaction is likely to be an electrostatic equilibrium, depending on medium pH and ionic strength. In vaginal environment the pH is physiologically slightly acidic (between 4 and 5), causing the ionisation of both citric acid and chitosan, which should undergo, under these conditions, the maximum interaction. However, citric acid has three carboxylic groups that probably also contribute to the maintenance of chelating properties if the acid is involved not only in salification, but also, at least partially, in reticulation of the chitosan chains [97].

Chitosan citrate is also characterised by wound-healing properties [98]. Chitosan scaffolds were prepared by a freeze-drying technique using a chitosan solution at 1% w/w chitosan (molecular mass 1,000,000 Da and degree of deacetylation 96%) in 2% citric acid solution, and as references 1%w/w chitosan in 0.5 or 2.0% acetic acid solution were used.

The scaffold presented quite a distinct pore structure, depending on the kind of acid used for the preparation. Despite the large difference in the pore structure, both scaffolds were effective in regeneration of the outer skin, although it was noticed that the CS/citrate accelerates wound

Table 2. Properties of some chitosan salts.

	Mucoadhesion	Penetration enhancement	Peptidase inhibition	Antimicrobial	Wound healing
Hydrochloride	Nasal [18,20], buccal [30,31], vaginal [21,31]	Nasal [36,38,39,42,112], buccal [45,46], vaginal [31]	[113]	Antifungal [114]	[60]
Glutamate	Nasal [17,18]	Nasal [42], buccal [119,120], vaginal [120]	[54]		
Citrate	Nasal [115], buccal [116], vaginal [92]	Buccal [119], vaginal [92]	Vaginal [92]	Antimicrobial [104]	[95]
Lactate	Nasal [18], buccal [116], vaginal [13,24]	Buccal [96]	[54]	Antimicrobial [104], antifungal [47]	
Ascorbate	Buccal [96]	Buccal [96]	Vaginal [99]	Antimicrobial [98,99]	[98]

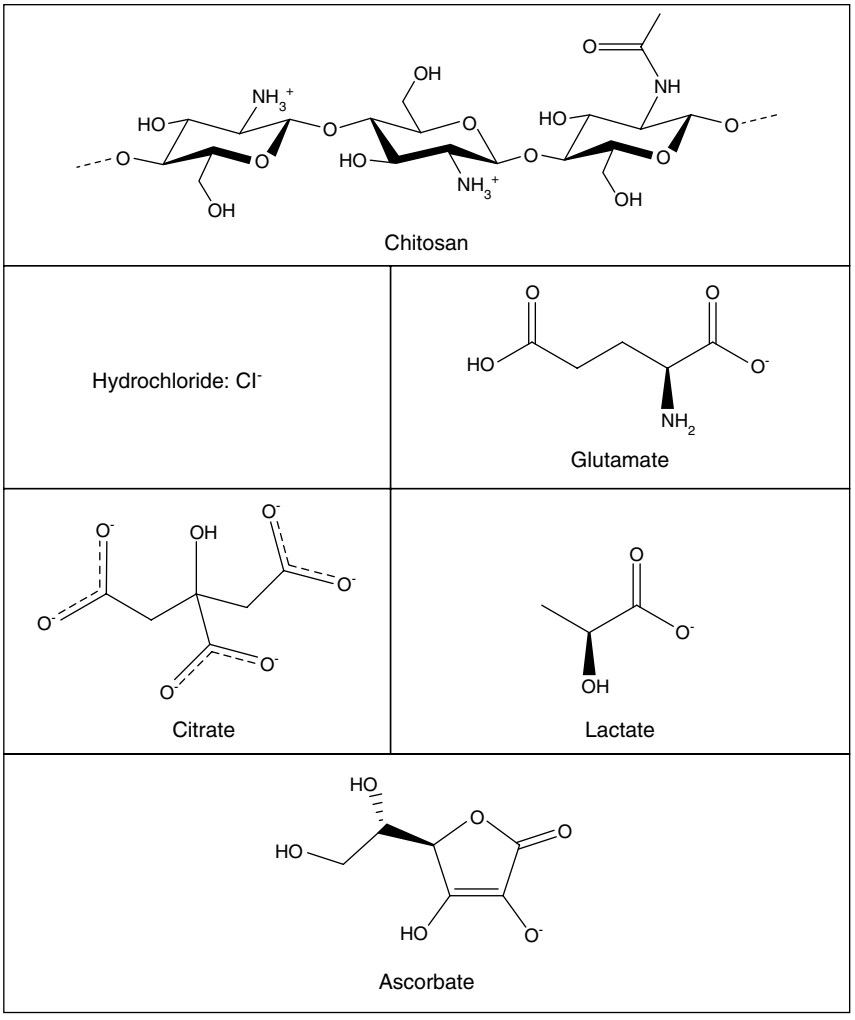

Figure 3. Chemical structure of some chitosan salts.

healing more than the CS/acetate one. The CS/citrate scaffold could adhere close to the wounded part and absorb blood immediately, leading to the bleeding stopping and the rapid regeneration of the skin.

5.2 Chitosan ascorbate

Chitosan ascorbate was proposed for buccal penetration enhancement with increased properties compared with chitosan hydrochloride and lactate [99]. Fluorescein isothiocyanate dextran (FD4), a hydrophilic molecule having a high molecular mass (4400 kDa) similar to that of peptidic drugs, was considered as a model penetrant molecule. The salification of high-molecular-mass chitosan with ascorbic acid produces an increase in penetration enhancement properties of the polymer towards both buccal mucosa and Caco-2 cell monolayer. CS ascorbate salts are able to promote the absorption of the model molecule FD4 (high-molecular-mass hydrophilic molecule) across porcine buccal mucosa; the penetration enhancement properties are particularly marked for high-molecular-mass grade, which permits the permeation of a FD4 amount 13 times higher with respect to that observed for the solution of FD4 alone. The permeation tests were performed also on Caco-2 cell monolayer to investigate whether this kind of salt is able to maintain the properties of chitosan to interact with tight junctions: this was confirmed by a decrease in TEER and the permeation of FD4. The increase in CS penetration enhancement properties in the presence of ascorbic acid was less evident with Caco-2 substrate with respect to those observed when buccal mucosa was used as biological substrate. This is probably because two different interaction mechanisms are involved: an interaction with extracellular lipids for buccal mucosa (poor in TJs [tight junctions]) and an interaction with TJs for the intestinal one.

The penetration enhancement properties are higher when buccal mucosa is used as biological substrate. A hypothesis to explain such behaviour could be that ascorbic acid increases the capability of chitosan to interact with extracellular lipids (the main barrier to drug transport across buccal mucosa). Such a hypothesis is supported by relevant literature: some authors have suggested that chitosan is able to interact with buccal lipids, and it has also been proved that the polymer salification with ascorbic acid produces higher chitosan interaction with diet lipids. In fact, the addition of ascorbic acid to chitosan causes a large increase in faecal fat excretion [99-101]. The mechanism for increasing faecal fat excretion by coexisting chitosan and ascorbic acid seems to be due to a chitosan–ascorbic acid synergic effect that causes an increase in capability of chitosan to interact and entrap dietary lipids by an emulsifying process mediated by ascorbic acid [100].

In recent studies chitosan ascorbate was proved to possess antimicrobial, wound-healing and enzyme inhibitory properties. Thanks to the presence of ascorbic acid, such salt showed antioxidant properties higher than those observed for chitosan hydrochloride [101-103].

5.3 Chitosan and betainic acid complexes

Betains are zwitterionic neutral compounds with a positively charged quaternary ammonium group and a negatively charged carboxylate group that may not be adjacent to the cationic site. Betaines containing a hydrophobic chain of 8 – 20 carbon atoms are surface-active agents. A complex of chitosan acetate with C12 – C18 alkyl amido prophyl dimethylamine betaine (AAPDB) was formed mainly by the electrostatic interaction between $NH3^+$ groups of chitosan and COO^- of AAPDB. The activity against *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus epidermidis* and *C. albicans* was tested. The results showed that the complex could inhibit the growth of all the tested microorganisms, whereas chitosan acetate could not inhibit growth of *C. albicans* and AAPDB could not inhibit growth of *S. epidermidis*, *E. coli*, or *P. aeruginosa*. Following the formation of the complex, the interaction of amino groups in chitosan with anionic components of cell surface could be weakened, but the quaternary ammonium group from AAPDB could in turn denature proteins and interact with phospholipids with disruption of microbial cell membrane. Moreover, the introduction of alkyl chain from AAPDB can bring CS/AAPDB closer to the cell membrane and can cause membrane dissolution. The highest activity of the complex with respect to AAPDB alone can be due to the introduction of a large amount of -OH of chitosan, which may chelate metal elements such as Ca in the cell membrane, and to properties of higher molecular mass of the polymer, which forms a film able to affect the absorption of nutrients [104].

Also, chitosan N-betainates obtained by different degrees of N-alkylation of chitosan were tested for antimicrobial activity against *S. aureus* and *E. coli*, both at pH 7.2 and at pH 5.5 [105]. These chitosan N-betainates showed low activity at pH 7.2, whereas at pH 5.5 the antimicrobial activity increased with decreasing degree of substitution. In the case of non-quaternary chitosan derivatives [106], this effect was explained with the decreasing number of free ionisable amino groups with increasing degree of substitution, leading to a decreased positive charge on the polymer. However, the betaine substituent itself has a positive charge, so an explanation could be that the ammonium moiety of chitosan N-betainate is unfavourably placed in relation to the polymer backbone. For efficient antimicrobial activity, the positive charge should be situated in the amino groups of the chitosan backbone [105]. This is probably the case with the electrostatic complex described previously.

5.4 Chitosan ionic complexes with therapeutic moieties

Some examples can be found in the literature of formulative approaches that involve the complexation of chitosan by ionic interaction with therapeutic moieties. One of these examples is represented by chitosan–lactic acid complexes intended for vaginal delivery not only of drugs, but also of

lactic acid itself, to maintain the physiological environment. Lactobacilli contribute to maintaining the pH of the vaginal fluid in healthy women between 4 and 5. Lactic acid and low pH mediate the natural resistance to the colonisation of pyogenic organisms, and are useful to avoid an increase of the vaginal pH, which may occur, for example, in post-menopausal women with consequent possible colonisation of the vaginal mucosa by pathogenic microorganisms and increased risk of local infections [107].

Among vaginal formulations containing lactic acid, some lactate gels based on methylcellulose mixed with either Eudragit or chitosan have been described in the literature as mucoadhesive vehicles [24]. Particular attention has been given to the influence of the ionic interaction between polymer and lactic acid on acidifying properties of the formulations and on lactate release. Some mucoadhesive chitosan lactate gels were developed that were intended specifically for the controlled release of lactic acid onto vaginal mucosa, and the relevance of polymer molecular mass and lactic acid/polymer ratio to the performance of the formulation was assessed. Two different viscosity grades of chitosan were combined in aqueous environment with lactic acid in 2 lactic acid/polymer ratios. Good mucoadhesive properties were confirmed and were found to depend on molecular mass of the polymer and on the amount of lactic acid in the formulation. Lactic acid release profiles showed the relevance of an ion exchange release mechanism. The influence of the medium ionic strength and of different kinds of counterion on lactate release profiles was in accordance with this mechanism [13].

Antimicrobial chitosan films prepared with different acids (hydrochloric, formic, acetic, lactic and citric acid) have been proposed, considering that organic acids such as lactic and citric acids possess general antimicrobial activities, and chitosan lactate and chitosan glutamate display antagonistic effects against *E. coli*, *S. aureus* and *Saccharomyces cerevisia* [108].

Chitosan-glycyrrethic acid nanoparticles were obtained by complex coacervation under mild conditions. Glycyrrhetic acid (GLA) is an active metabolite of glycyrrhizin, showing anti-inflammatory, antihepatotoxic and interferon-inducing actions. Colloidal polyelectrolyte complexes stable at pH values ranging from 4.0 to 7.0 could be formed when the two polymers were mixed. Optimised production conditions produced spherical nanoparticles with a smooth surface with the smallest mean hydrated size (298 nm). GLA release *in vitro* showed a clear effect of CS encapsulation that provided a continuous release [109].

A taurine-chitosan gel was proposed to release taurine slowly. Taurine, 2-aminoethane sulphonic acid is a strongly acidic amino acid that has an antioxidant effect and influences cell proliferation, inflammation and collagenogenesis. A plain chitosan solution (1.5%) and the same with added taurine (50 mM) were applied to full-thickness skin wounds of mice once a day for 7 days, and the taurine gel was found to be effective in wound healing because it would act synergistically with chitosan [64,110].

In some cases the ionic interaction with chitosan can improve drug stability, as in the case of insulin–chitosan complexes proposed by some authors. Trimethyl chitosan and a PEG-*graft*-TMC copolymer were in fact reacted with insulin to obtain polyelectrolyte complexes (PEC) that were compared with insulin nanoparticles prepared by ionotropic gelation of the same polymers with tripolyphosphate. All PEC were more stable in pH 6.8 simulated intestinal fluid (SIF) than nanoparticles. The PEC also appeared to play some role in protecting insulin from degradation at higher temperature and with proteolytic enzyme more efficiently than nanoparticles [111].

Finally, a further very important field of application of polyelectrolyte complexes involving chitosan is represented by the interaction between chitosan and DNA to obtain non-viral vectors for gene delivery. Properties of the DNA–chitosan complex such as particle size, efficiency of cell uptake and dissociation of DNA from the complex after cell endocytosis have been related with the polymer molecular mass and deacetylation degree, the pH of the medium of interaction, the DNA and chitosan charge ratio. The great importance and the implications of this particular kind of interaction deserve, however, a separate description and discussion, as can be found in specific reviews on the subject [112,113].

6. Conclusions

Chitosan is a polymer endowed with properties particularly useful for mucosal and transmucosal delivery. Besides the well-known mucoadhesive and permeation enhancement properties, peptidase inhibition, anti-infective (antibacterial, antifungal, antiviral) antioxidant, immune-stimulating properties, haemostatic and wound-healing activities have also been described. The exploitation of all these properties is, however, sometimes limited by pH dependence of polymer solubility. This limitation is common to all the salt forms that have been proposed in the literature. The survey of the literature does not give evidence of one salt being better than the others for specific properties. The only way found so far to solve the problem of chitosan pH-dependent solubility is a chemical modification of the polymer chain with quaternisation of the amino groups. Extensive research is therefore proceeding in this respect, especially in the last few years. On the other hand, ionic interaction of chitosan with hydrophobic moieties or with polyions is being studied to obtain polymeric nanoparticles or polymeric micelles that gain the ability of being easily internalised in the cells. In the future it will probably be interesting to investigate how many of the chitosan properties, as multifunctional polymer and as bioactive polymer, will be maintained in these nanoparticulate systems.

The possible combination of chitosan, as cationic polymer, with anionic molecules, which can improve the polymer efficacy as penetration enhancer, as protease inhibitor or also as anti-infective or antioxidant agent, is being investigated more and more in the literature.

7. Expert opinion

The interest of pharmaceutical researchers in chitosan has increased remarkably over the last few years, thanks to its peculiar properties and also to its very good biocompatibility. This is in line with the recent introduction of chitosan monographs in compendia (Eu Pharm. [114], Pharmacopoeial forum [115]), and with the efforts to standardise the evaluation of chitosan salts suitable for use in biomedical or pharmaceutical applications (ASTM standard [116]). This will certainly allow the possibility of development of pharmaceutical formulations based on chitosan to increase, which nowadays are commercially available mainly for wound-healing or haemostatic application. The success of future commercialisation of formulations will depend in fact also on the polymer regulatory acceptance and on the confidence in the reproducibility of its chemical and functional properties.

The precipitation of chitosan salts at neutral pH, resulting from losing of charge, represents the more important limit of the use at most mucosal sites, the only exception being the vaginal one. Positive charge is in fact recognised as the more important feature to establish mucoadhesive interaction, and to efficiently open the paracellular way in absorption promotion. Modification of the chemical structure of the polymer involving the introduction of stronger cationic functions that maintain positive charge also at higher pH values can improve this aspect of chitosan behaviour. In this perspective trimethyl chitosan was described early in the literature as increasing solubility, and a series of different quaternary ammonium derivatives are now under development. It must, however, be kept in mind that any molecule used for the first time either in a new delivery system or for a new route of administration requires safety evaluation. This applies also to excipients if they are subject to any minor change in the chemical composition, and must therefore be considered as new excipients. Considering the unique capability of chitosan to associate properties of mucoadhesion and penetration enhancement to other important features such as anti-infective, immune-stimulating, wound-healing or haemostatic properties, chemical modifications aimed at the improvement of polymer performance can be worth extensive and expensive safety characterisation. This is particularly true in the case of delivery of drug molecules presenting high therapeutic advantages, strong problems of overcoming mucosal barrier, degradability or poor solubility.

Analogous concerns can arise when hydrophobically modified chitosan obtained by covalent derivatisation with hydrophobic moieties is designed to improve drug solubilisation with a micellar mechanism. An important characteristic of these polymeric nanoparticles is low critical micellar concentration and high stability towards dilution in biological fluids. Probably milder in this respect is the modification of chitosan following ionic gelation with TPP or lecitin, as in this case the chemical structure of the polymer is not intrinsically modified. In both cases, however, it must be remembered that the mechanism of interaction with the biological substrate is completely changed with respect to that of polymer solutions. It is definitely recognised that nanoparticles are usually more efficient in favouring absorption, especially of high-molecular-mass and degradable drugs, such as the biotechnological ones. The confidence in higher safety of polymeric enhancers based on the fact that they are not absorbed by the cells, in this case, however, has to be more carefully reconsidered. Particularly when the delivery systems are intended for topical applications, the use of chitosan salts subjected to reversible ionic equilibrium can be advantageous because nanosystems can solubilise or stabilise the loaded drug, whereas their uptake by the epithelial cells is low and drug absorption is less efficient.

A third kind of possible modification of chitosan properties is based on the ionic interaction of the cationic polymer with anionic moieties having particular properties that can be synergic to those of the polymer. Anionic molecules with chelating capacity, or antioxidant or surfactant properties are just examples. This approach is increasingly present in the literature, and again it does not involve an irreversible modification of the polymer structure. It appears evident, however, that the impact of the polymer on the biological substrate changes following this kind of modification and therefore the biocompatibility issues should be carefully considered. Any eventual further biological activity has therefore to be opportunely defended. A key feature is probably the reproducibility and the control of the resulting product characteristics, which are made easier for the relative simplicity of electrostatic reaction between the two interacting species under defined conditions (reciprocal molar ratio, pH and ionic strength control).

Declaration of interest

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