

REVIEW

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Chitosan-based nano-objects for drug delivery: a review of their chemical modifications, supramolecular organization and biological fate

Gabriela Pereira Parchen,^a Marion Quaillet,^{†b} Rilton Alves de Freitas^a and Hervé Hillaireau^{*,b}

Chitosan is a widely applied polysaccharide in different fields due to its versatility, biocompatibility and low toxicity. Its structure possesses reactive functional groups that can be modified without involving the chain backbone, which improves its physicochemical and biochemical properties. Several chemical modifications such as alkylation, acylation, thiolation, and grafting with polymers and active molecules can be combined with various supramolecular chemistry approaches such as crosslinking, self-assembly, polyelectrolyte-complex formation, ionic gelation, and polymerization to formulate chitosan-based nano-objects that can encapsulate many active pharmaceutical ingredients, eventually enabling new applications of chitosan in the pharmaceutical, biomedical and biotechnological fields. This review summarizes the critical findings of some recent works published in the last years on the chemical modification of chitosan; the design of chitosan-based nano-objects for the encapsulation and controlled delivery of active pharmaceutical ingredients; and the biodistribution, biodegradation and toxicology of the nano-objects.

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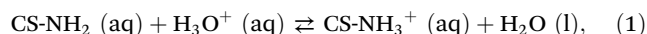
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1. Introduction

Chitosan (CS) is a pseudo-natural polysaccharide, obtained *via* the deacetylation of chitin in an alkaline medium, resulting in a random arrangement of β -D-glucosamine (GlcN) and N-acetyl- β -D-glucosamine (GlcNAc) units linked by (1-4) glycosidic bonds (Fig. 1). Chitosan is one of the few cationic polysaccharides known to date, possessing many advantages for application in different fields such as food,¹⁻³ crops,^{4,5} cosmetics,^{6,7} and, in particular, drug delivery.⁸⁻¹⁰ The control of the physicochemical properties of chitosan, such as its degree of deacetylation and molar mass, is crucial for its biomedical applications.¹¹ In particular, chitosan is characterized by the mole fraction of its N-acetyl groups, called degree of acetylation (DA), or by the mole fraction of its N-acetyl groups removed from the chitin macromolecule during deacetylation, which is called the degree of deacetylation (DD, with DD = 1 – DA).^{12,13} The term ‘chitosan’ is used when the DA of the macromolecule is less than 50%.¹¹

The behavior of chitosan in solution is directly linked to its acid–base properties. Indeed, chitosan is a cationic poly-

electrolyte in an acidic medium, whose state of ionization is described by the equilibrium given in eqn (1), with a pK_a value generally in the range of 6–6.5.¹⁴⁻¹⁶ However, this pK_a is not constant and varies according to the degree of dissociation (α) of chitosan according to Katchalsky's relation¹⁷ (eqn (2)):



$$pK_a = \text{pH} + \log\left(\frac{(1-\alpha)}{\alpha}\right) = pK_0 - \frac{\varepsilon\Delta\Psi(\alpha)}{kT}, \quad (2)$$

where pK_0 is the intrinsic pK_a of an isolated and non-protonated amine function, ε is the dielectric constant of the medium, $\Delta\Psi(\alpha)$ is the potential difference between an ion placed on the surface of the polyelectrolyte and at an infinite distance, k is the Boltzmann constant, and T is the temperature.

The pK_a of chitosan also depends on its DA. Sorlier *et al.* demonstrated that for a wide range of DA (5.2%–89.0%) and from a chitosan colloidal solution concentration and ionic strength, it is possible to obtain a polynomial equation to deduce pK_a based only on the DA and medium ionic strength.¹⁸

Chitosan is a weak base and insoluble in alkaline solutions and organic solvents, but soluble in acidic aqueous media when its DA is less than 50%.¹⁹⁻²¹ It is commonly solubilized

^aBioPol, Pharmacy Department, Universidade Federal do Paraná (UFPR), Av. Prefeito Lothário Meissner, 632 – Jardim Botânico, Curitiba-81531-980, PR, Brazil

^bInstitut Galien Paris-Saclay, Université Paris-Saclay, CNRS, 91400 Orsay, France.
E-mail: herve.hillaireau@universite-paris-saclay.fr

[†]These authors contributed equally to this work.

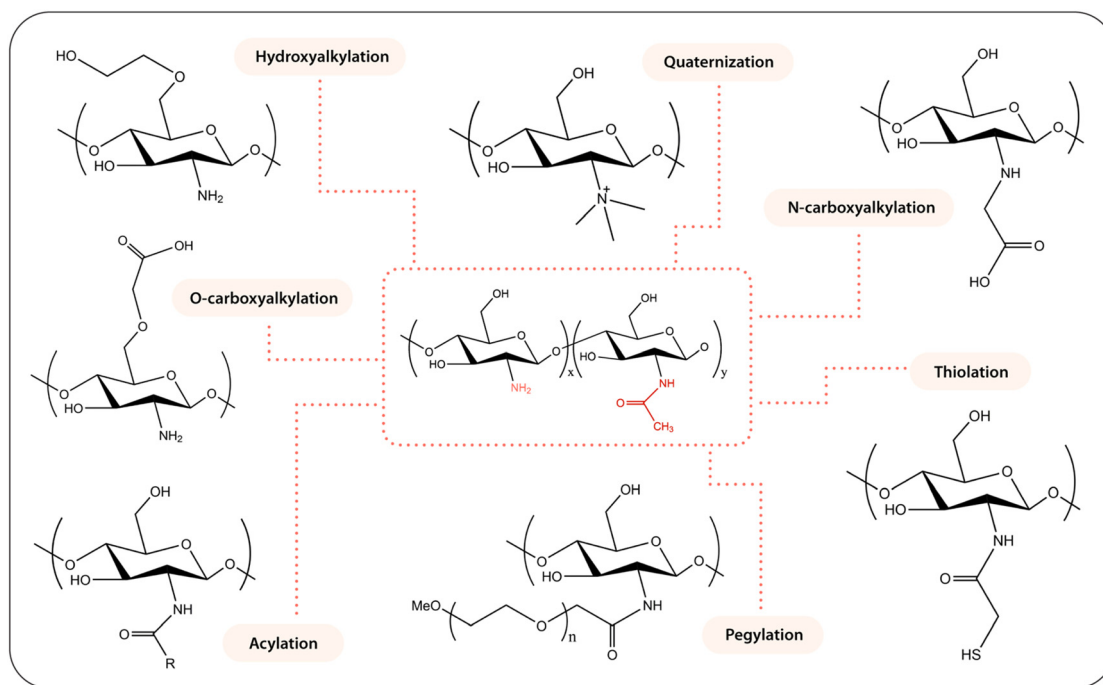


Fig. 1 Overview of the main chemical modifications in the structure of chitosan described for its use in drug delivery, as reviewed below.

in aqueous acetic acid solution (0.1 mol L^{-1} or $1\% \text{ w v}^{-1}$)²² or strong acid solutions such as hydrochloric acid.²³ The solubility of chitosan is related at the same time to its DA, ionic strength, pH, the nature of the acid used and the distribution of the acetyl groups on the polymer chain. The protonation of amino groups generates electrostatic repulsions between the polymer chains segments, allowing the solvation of the chains and their subsequent solubilization and expansion. Dissolution at neutral or basic pH can only be accessed with a pre-step of dissolution in an acid medium to protonate the amine groups; the pH can then be increased by adding a base without causing precipitation up to its amine group pK_a .

Chitosan is generally considered soluble up to a pH of 6^{14,22,24} to 6.5.^{25–27} Rinaudo *et al.* studied the role of amino group protonation on the solubility of chitosan in the presence of a weak acid (acetic acid, AcOH) or strong acid (HCl) at different acid concentrations. Independent of the polymer concentration ($[\text{N}] = \text{CS-NH}_2 + \text{CS-NH}_3^+$) or the type of acid, the complete solubilization of chitosan was obtained for $\alpha \geq 0.5$, corresponding to a stoichiometric ratio $[\text{AcOH}]/[\text{CS-NH}_2] = 0.6$ or $[\text{HCl}]/[\text{CS-NH}_2] = 0.5\text{--}0.6$. The ion concentration required for complete solubilization of chitosan is proportional to its number of amine groups (CS-NH_2).^{22,23} The solubility of chitosan is an obstacle that needs to be circumvented to modulate or give it new physicochemical properties, especially for the encapsulation of active substances. Chemical modification of chitosan has been carried out at its deacetylated units, on its C6 hydroxyl group, and/or its C2 primary amine group, usually without involving the glycosidic bonds and depolymerization.

Chitosan, in its native or a chemically modified form, has been widely used to produce nano-objects that can encapsulate drug molecules to improve their delivery and targeting. This review provides an overview of the most important chemical modification of chitosan, the different supramolecular chemistry approaches to produce nano-objects and encapsulate drug molecules, their resulting biological fate, relevance for drug delivery and potential toxicity, and especially how these aspects are connected. Other recent reviews also cover its chemical modification,²⁸ formulation on a larger scale²⁹ and other biomedical applications (wound dressings, dental materials, *etc.*).^{30–32}

2. Chemical modification of chitosan

Chitosan has been modified in various ways to introduce numerous hydrophilic or hydrophobic moieties, owing to the reactivity of its primary amine, and to a lesser extent to hydroxyl groups. The main chemical modifications described in the literature are summarized in Fig. 1 and discussed below with a focus on their relevance to drug delivery (Table 1).

2.1. Alkylation of chitosan

2.1.1. Quaternization. Alkylation was achieved by bonding carbon chains to the amino or hydroxyl groups of chitosan. Rinaudo's group^{89,90} described the quaternization of chitosan *via* the alkylation of its amine groups with methyl iodide under alkaline conditions (NaOH). The iodide ion was subsequently replaced by a chloride ion using an ion exchange



Table 1 Summary of major chitosan derivatives used in drug delivery and the encapsulated drugs

Chitosan derivative	Encapsulated drug	Ref.
Trimethyl chitosan	Camptothecin	33–35 36 37 38 39
	Candesartan-cilexetil	
	Insulin	
	Resveratrol	
	Vancomycin	
	Vitamins	
<i>N</i> -Octyl- <i>O</i> -sulfate chitosan	Docetaxel	40
	Paclitaxel	41 and 42
Glycol-chitosan	Camptothecin	43
	Cisplatin	44
	Dexamethasone	45
	Docetaxel	46
	Doxorubicin	47 and 48
	Paclitaxel	49
<i>N</i> -Carboxymethyl chitosan	Idebenone	50
	Ofloxacin	51
<i>O</i> -Carboxymethyl chitosan	Camptothecin	52
	Curcumin	53
	Gatifloxacin	54
	Metformin	55
	Methotrexate	56
	Tetracycline	57
<i>N,O</i> -Carboxymethyl chitosan	Dopamine	58
Oleoyl-chitosan	Doxorubicin	59
<i>N</i> -Succinyl-chitosan	5-Aminosalicylic acid	60
	5-Fluorouracil	61
	Hydroxycamptothecin	62
Chitosan- <i>g</i> -stearic acid	Acyclovir	63
	Doxorubicin	64
	Tamoxifen	65
Chitosan-thioglycolic acid	Cyclobenzaprine	66
	Gemcitabine	67
	Leuprolide	68
	Theophylline	69
	Tizanidine	70
Chitosan-6-mercaptopnicotinic acid	Insulin	71
Chitosan-2-iminothiolane	Insulin	72
Chitosan- <i>N</i> -acetyl-L-cysteine	Insulin	73
Chitosan- <i>N</i> -(4-hydroxyphenyl)-methacrylamide	Curcumin	74
	Gene transfection	
PEGylated chitosan	Insulin	75
	Methotrexate,	76
	mitomycin C	
	Ormeloxifene	77
	Resveratrol	78
	Rosuvastatin	79
	Indole-3-carbinol	80
Chitosan- <i>g</i> - β -cyclodextrin	Etoposide	81
	Ketoprofen	82
	Ovalbumin	83
Chitosan- <i>g</i> -polycaprolactone	Paclitaxel	84
Chitosan- <i>g</i> -poly(lactic-co-glycolic acid)	Paclitaxel	85 and 86
	Tamoxifen	87
Quaternized chitosan	Ketoconazole	88

process to obtain a more stable chitosan salt, *N*-trimethyl chitosan chloride (TMC). This quaternization allows the introduction of permanent positive charges, enabling the polymer to be a cationic polyelectrolyte regardless of the pH. Its solubility directly depends on its degree of methylation.⁹¹ Pardeshi *et al.* synthesized a TMC using the same technique and evaluated its mucoadhesive strength and bioadhesive potential. Due to the greater cationic nature of TMC, the mucoadhesive strength increased by 3.4-fold compared to unmodified chitosan.⁹²

Du Plessis *et al.* compared chitosan with different degrees of quaternization for the nasal and rectal administration of insulin to rats. Highly quaternized TMC (61.2%) increased insulin absorption at neutral pH, while chitosan hydrochloride and low quaternized TMC (12.3%) were ineffective.⁹³ This effect was correlated with the more efficient interaction of TMC with the anionic components of the cell membrane, potentiating the absorption at different values of pH. TMC was also applied as a drug delivery agent for DNA⁹⁴ and used in wound dressing,⁹⁵ as antibacterial⁹⁶ and antioxidant.⁹⁷

2.1.2. *N*-Alkylation. The *N*-alkylation of chitosan has been achieved by grafting it with alkyl chains of different lengths.⁹⁸ The main parameters influencing the hydrophobic interactions by van der Waals forces are the polymer concentration, the number of carbons grafted, temperature, and the ionic strength of the medium. Karam *et al.* described *N*-alkyl chitosan derivatives through reductive amination reactions using 1-dodecanal (C12) or 1-tetradecanal (C14). The *N*-dodecyl derivatives exhibit lower viscosities compared to *N*-tetradecyl derivatives, both measured in 0.3 mol L⁻¹ acetate buffer. The viscosity affects the hydrophobic interactions between the alkyl chains. *N*-Tetradecyl products result in crosslink networks, whilst in the case of *N*-dodecyl derivatives, the hydrophobic interactions facilitate the self-aggregation process to self-assemble into a nano-object (see section 3.3).⁹⁹

Dang *et al.* modified chitosan with decanoic acid with different degrees of substitution with amino groups. The derivatives with a higher degree of substitution showed low toxicity to L929 cells, mainly due to the insertion of the alkyl chains into the lipid bilayer without destroying the cell membrane, promoting cell adhesion. Increasing the content of alkyl chains also reduced the hemolysis rates of these conjugates in cells and promoted faster wound healing compared to chitosan grafted with a low degree of substitution.¹⁰⁰ Liu *et al.* demonstrated an increase in the transfection efficiency of plasmids mediated by the *N*-alkylation of chitosan depending on the number of carbons in the alkyl side chains, which is directly related to the hydrophobicity of *N*-alkyl chitosan.¹⁰¹

2.1.3. Hydroxyalkylation. The hydroxyalkylation of chitosan was achieved *via* the reaction of epoxides (ethylene oxide, propylene oxide, butylene oxide, *etc.*) with its primary amine and/or hydroxyl group, depending on the pH, solvent, and reaction temperature.¹⁰² Due to its water solubility, chitosan glycol, a chitosan derivative conjugated with hydrophilic ethylene glycol branches, has been extensively studied for additional chemical modifications, particularly for the addition of hydrophobic groups to obtain amphiphilic derivatives (see section 2.2).



Mallick *et al.* described glycol chitosan-dequalinium, a branched chitosan glycol with a quaternary ammonium cation that contains two quaternary quinolinium units linked by an *N*-decylene chain, which can form micelles exhibiting a low toxicity towards HeLa and HDF cells.¹⁰³ Glycol chitosan was also studied by Yu *et al.*, where dexamethasone-loaded glycol chitosan nanoparticles showed an increase in the precorneal duration of action due to their ionic interactions with the surface of the cornea compared to free dexamethasone.⁴⁵ Other amphiphilic derivatives of chitosan glycol have also been developed, in particular with tocopherol,¹⁰⁴ palmitoyl,^{105–107} hexadecyl,^{108,109} cholesterol,¹¹⁰ and *N*-acetyl-histidine.¹¹¹

2.1.4. Carboxyalkylation. Carboxyalkylation allows the introduction of carboxyalkyl groups on chitosan, giving it zwitterionic properties. By electrostatic repulsion, the carboxylic groups prevent potential intramolecular interactions between the polymer chains and improve their solubilization in water.¹¹² By varying the degree of carboxyalkylation, various charge densities on the polymer chains can be obtained, and therefore control the solubility of chitosan at various pH values. *N*- and *O*-carboxyalkyl chitosan are obtained by reaction with monohalocarboxylic acids under different conditions to control the selectivity of the reaction in the C2 or C6 position.^{113,114}

The most common reaction to form *O*-carboxyalkyl chitosan is with monochloroacetic acid and sodium hydroxide. Glyoxylic acid can also be used to selectively produce *N*-carboxyalkyl chitosan derivatives by reductive amination.^{115,116} Adnan *et al.* synthesized different *O*-carboxymethylated chitosan using different ratios of monochloroacetic acid in isopropanol. The samples were tested to evaluate their analgesic and anti-inflammatory activity *in vivo*. An increase in analgesia was noted and the paw volume did not present significant difference after treatment with the polymers. *O*-CMC inhibited the release of pro-inflammatory mediators and did not present significant difference with the paracetamol-treated group, suggesting a strong analgesic effect.¹¹⁷

Carboxymethyl chitosan is mainly used for the delivery of gatifloxacin,⁵⁴ camptothecin,⁵² methotrexate,⁵⁶ tetracycline,⁵⁷ curcumin,^{53,118} idebenone,⁵⁰ and metformin.⁵⁵

2.2. Acylation of chitosan

The acylation of chitosan introduces hydrophobic groups at its C2 and/or C6 position *via* an ester bond by reaction with chlorides or acyl anhydrides. The hydrophobic groups are generally fatty acid (C6–C18) chains such as oleic acid,⁵⁹ linoleic acid,¹¹⁹ lauric acid,¹²⁰ palmitoyl acid,¹²¹ stearic acid,⁶⁴ and 5 β -cholic acid,^{122,123} which increase the hydrophobic nature of chitosan, and therefore allow the polymer, under certain conditions, to self-assemble into nano-objects in aqueous medium (see section 3.3). This type of nanoparticle has also been developed for the encapsulation of hydrophobic active substances. Due to their amphoteric properties, short-chain acylated chitosan has also been developed, which is soluble in both acidic and basic

media.¹²⁴ One of them is *N*-succinyl-chitosan, a carboxyacylated derivative obtained by introducing a succinyl group,²⁷ which has been investigated for the formulation of many nano-objects^{125–127} and the encapsulation of anti-cancer drugs such as mitomycin C,¹²⁸ doxorubicin,¹²⁹ camptothecin,⁶² and paclitaxel.¹³⁰

Cho *et al.* investigated the impact of acyl chain length on drug encapsulation and delivery. Chitosan grafted with acyl chains of 1 to 16 carbons was used to formulate nanoparticles by self-assembly to encapsulate vitamin C, with the drug loading efficiency increasing from C3 to C12. With longer *N*-acyl side chains, the resistance in acidic media also becomes stronger, leading to slower drug release, which is attributed to the stronger hydrophobic interaction within *N*-acyl chitosan-based nanoparticles.¹³¹ Echazú *et al.* modified chitosan with dodecenylsuccinic anhydride to develop hydrogels for the buccal delivery of thymol. Rheological measurements showed that the elastic behaviour was predominant, and acylation modified both the storage and loss modulus of the chitosan hydrogel. Moreover, the swelling test indicated an increase in the hydrophobicity of the modified chitosan compared to the unmodified one, facilitating the affinity of poorly water soluble compounds, such as the thymol.¹³²

2.3. Chitosan thiolation

The Bernkop-Schnürch group described the synthesis of various thiolated chitosans (thiomers). The first was a chitosan-thioglycolic acid (TGA) conjugate obtained by forming an amide bond between the primary amine group of chitosan and the carboxylic acid group of TGA activated by a carbodiimide.^{133–135} Subsequently, other thiolated chitosans were synthesized by modifying the amine group with 2-iminothiolane^{136,137} or glutathione.¹³⁸ Thiolated chitosan conjugates have *in situ* gelation properties, given that the thiol groups oxidize between pH = 5 and 6.8, forming inter- and intramolecular disulfide bonds.¹³⁶ The gelling behavior of thiolated chitosan depends on the polymer chain entanglement and the rearrangement of disulfide bonds.¹³⁹ These properties of mucoadhesion and *in situ* gelation have been exploited for the formulation of liquid and semi-liquid forms as well as for the formulation of nanocarriers.

A correlation was found between the degree of thiolation and the adhesion properties of the polymer. For example, chitosan-thioglycolic acid demonstrates a 5- to 10-times greater mucoadhesive effect than unmodified chitosan.^{136,137} The mucoadhesion was also found to be influenced by the formation of disulfide bonds between its thiol groups and cysteine residues of the glycoproteins,^{69,140} as illustrated by Krauland *et al.* in the case of nasal absorption of insulin by chitosan-4-thiobutylamidine conjugates¹⁴¹ and Lee *et al.* in the case of drug delivery to the bronchial epithelium.⁶⁹

Other applications covered the P-glycoprotein (P-gp) inhibitory properties of these conjugates. Sakloetsakun *et al.* showed that the permeation enhancement of thiolated chitosan was achieved due to its capacity to form a disulfide bond between the cysteine of protein tyrosine phosphatase (PTPase), acceler-



ating the reduction of GSH. Furthermore, the absorptive apparent permeability across the rat intestine was directly dependent on the pK_a of the ligands and the amount of thiol group conjugates on chitosan, impacting the P-gp inhibitory properties of the derivative.¹⁴² Usually, thiolated chitosan nanoparticles were prepared by the ionic gelation method,^{70,143–145} and this polymer was employed in the delivery of tizanidine,⁷⁰ curcumin,¹⁴⁵ cyclobenzaprine,⁶⁶ leuprolide,¹⁴⁶ and insulin.^{71,73}

2.4. Other chemical functionalization of chitosan

In addition to the chemical modification of chitosan described above, the functionalization of chitosan by other polymers or by active substances has great interest to improve the physicochemical properties of chitosan or the active substance.

2.4.1 Polyethylene glycol (PEG). PEG is a nonionic hydrophilic polymer approved by several drug safety agencies (FDA, EMA) that can be synthesized with different molar masses and different functionalizable groups in its terminal position. For the functionalization of chitosan, PEG must be first chemically modified by adding a terminal group such as an aldehyde, a carboxylic acid, a carbonate, or an acrylate, which can react with the amine or hydroxyl groups of chitosan. Methoxy-PEG (mPEG) is predominantly used instead of PEG to prevent cross-linking between polymers.^{147–149} PEGylation of chitosan generally takes place at the level of the amine group.¹⁵⁰ However, some studies described PEG grafting at the level of the hydroxyl group at the C6 position of chitosan.^{151–153} Different methods have also been developed, such as radical polymerization,¹⁵⁴ click chemistry,¹⁵⁵ and crosslinking.^{156,157}

Through its hydrophilic properties, PEG improves the solubility of chitosan depending on the degree of substitution and the chain length of PEG.^{147,150,158} Depending on the degree of PEGylation, PEGylated chitosan can form complexes with polyanions,^{159–164} or self-assemble in aqueous medium thanks to the formation of intermolecular hydrogen bonds (see sections 3.2 and 3.3 for the mechanisms of assembly, respectively).¹⁵⁷ In particular, the high molar mass of PEG improves the self-assembly of nanoparticles with smaller sizes.

The molar mass of PEG modulates the rheological properties of PEGylated chitosan hydrogels. Del Olmo *et al.* developed several hydrogels based on 400 and 1000 g mol⁻¹ PEG. The decrease in the molar mass of PEG and the increase in CS : PEG weight ratio (from 1 : 0.4 up to 1 : 1) directly interfered with the increase in viscosity and both the storage and loss moduli, and also increased the stability of the hydrogels.¹⁶⁵ Other effects of molecular weight were reported by Anraku *et al.*, who showed that low M_w chitosan had more pronounced scavenging activities for DPPH radicals, with the corresponding nanoparticles with a low particle size presenting better activity in the DPPH test than that with a larger particle size. Furthermore, low M_w PEGylated chitosan was able to protect the liver against damage.¹⁶⁶

PEGylated chitosan nanostructures were developed to reduce toxicity and enhance drug delivery (see section 6.1 for the detailed toxicology). In cell culture, non-modified chitosan

was observed in a large amount on the cell membrane, while chitosan grafted with 5 to 10 kg mol⁻¹ PEG and complexed to siRNA was observed within cells, increasing their enhanced transfection efficiency.¹⁶⁷ Bae *et al.* produced PEG-grafted chitosan micelles with heparin, which showed good cytotoxic activity against B16F10 cells and increased the intracellular transport of the treatment across the cell membrane.¹⁶⁰ *In vivo*, Malhotra *et al.* found that the absorption of nanoparticles based on chitosan-PEG derivatives occurred across the nasal epithelial tissue 4 h after administration to mice and cleared after 16 h, allowing a measurable biodistribution in the cerebral cortex, which disappeared before 16 h.¹⁶⁸ PEGylated chitosan nanostructures were also applied for the delivery of doxorubicin,¹⁶⁹ insulin,¹⁶³ methotrexate, mitomycin C,⁷⁶ puerarin,¹⁷⁰ and ibuprofen.¹⁷¹

2.4.2 Other polymers. Other less studied polymers have been grafted to chitosan such as dextran,^{172,173} poly(vinylpyrrolidone) (PVP),¹⁷⁴ poly(caprolactone) (PCL),^{175–178} or even poly(ethylenimine) (PEI),^{179–183} a cationic polymer used as a non-viral vector in gene therapy. Chitosan copolymers can also form complexes with polyanions (see section 3.2) or self-assemble in an aqueous medium (see section 3.3). Different groups have also been interested in synthesizing copolymers of CS-PEI-PEG¹⁸⁴ and CS-PCL-PEG,^{185–187} allowing the combination of the physicochemical properties of different polymers.

2.4.3 Cyclodextrins. With their hydrophobic cavity, cyclodextrins can non-covalently bind aromatic molecules and other small organic molecules and form inclusion complexes. Grafting cyclodextrins with chitosan allows obtaining a molecular transporter system that protects against degradation, increases the apparent solubility, reduces the undesirable effects, and controls the release of the substance active. Furusaki *et al.* described the grafting of β -cyclodextrin on chitosan by coupling carboxymethyl- β -cyclodextrin in the presence of carbodiimide.¹⁸⁸ To a lesser extent, cyclodextrin grafting can be achieved by nucleophilic substitution, reductive amination, or in the presence of other chemical groups such as tosyl chloride or hexamethylene diisocyanate.¹¹⁴

Many systems have been developed for the encapsulation of active substances or macromolecules such as heparin,¹⁸⁹ glutathione,¹⁹⁰ ketoprofen,⁸² insulin,¹⁹¹ doxorubicin,^{192,193} BSA,¹⁹⁴ triclosan and furosemide.¹⁹⁵

2.4.4 Metal ions. Through its chelating properties, chitosan can form organometallic complexes with different metal ions. Chitosan forms coordination complexes with metals on the free electron pairs present on its oxygen and nitrogen atoms.¹⁹⁶ Reynaud *et al.* developed organometallic complexes of chitosan with different metals (Fe²⁺, Fe³⁺ and Zn²⁺) formulated in the form of microparticles to eliminate residual medicinal active substances in the digestive tract.¹⁹⁷ Other groups have used chitosan-iron complexes in water treatment to remove chromium¹⁹⁸ or chitosan-zinc complexes to enhance the antimicrobial activity of chitosan.¹⁹⁹ Giacalone *et al.* used organometallic complexes of chitosan-iron to stabilize nano-objects based on chitosan and polyphosphate molecules (see section 3.2.1).²⁰⁰



2.4.5 Targeting ligands. Chitosan can also be functionalized with a variety of ligands to enhance the cell-specific targeting and internalization of nano-objects by receptor-mediated endocytosis pathways. Conjugation can take place on native chitosan or on chitosan already chemically modified, before or after the formation of chitosan nanoparticles. The different types of ligands studied as well as their targets of interest are summarized in Table 2.

2.4.5.1 Saccharides. Saccharides play an important role in biological recognition at the cell surface level. They can interact specifically with membrane receptors, allowing active targeting. For example, galactose conjugation has been studied to improve hepatic targeting due to the high affinity for the asialoglycoprotein receptors (ASGR) mainly present on the surface of hepatocytes. These receptors bind and internalize glycoproteins with a terminal galactose group.²⁴⁵ Galactosylated chitosan was obtained by coupling with lactobionic acid carrying galactose groups *via* a carbodiimide or directly with D-galactose.²⁰⁴ Lactose conjugation has also been reported in the literature.^{246,247}

Another saccharide of interest for active targeting is mannose. Mannose receptors are mainly present at the level of antigen presenting cells such as dendritic cells and macrophages, playing an important role in the immune system. These cells are overexpressed at pathological sites such as tumors, atherosclerotic plaques, arthritic joints and infection sites.²⁴⁸ Mannosylated chitosan is mainly obtained by coupling mannopyranosyl phenyl-isothiocyanate with the amine groups of chitosan.^{211–213,249}

2.4.5.2 Proteins and peptides. Transferrin is a protein involved in the cellular transport of iron in the body by interaction with cell surface receptors to facilitate iron absorption. Transferrin receptors have been shown to be overexpressed in cancer cells of various malignancies.^{250,251} Mao *et al.* developed two methods for the post-formation conjugation of transferrin to chitosan nanoparticles. The first involved introducing aldehyde groups by periodate oxidation, and thus the modified transferrin can react with the amine groups on the surface of the nanoparticles. This method minimizes steric hindrance

and loss of protein activity. The second method is based on reversible conjugation, where transferrin is linked to the nanoparticles by disulphide bonds. These bonds allow the cleavage of transferrin after cell capture as well as the degradation of nanoparticles and the release of DNA. However, *in vitro*, the nanoparticles conjugated with transferrin did not show an enhancement in DNA transfection. Therefore, Mao *et al.* developed nanoparticles with another protein, the KNOB protein, globular domain C-terminus of adenovirus capsid fibrous protein, which was conjugated to chitosan in the same way by disulfide bond. These conjugated nanoparticles improved transfection.²¹⁶

RGD peptides are peptides containing arginine–glycine–aspartate with the ability to target cells expressing integrin receptors at their cell membranes,^{252,253} such as cancer cells.^{254,255} The RGD peptide is mainly conjugated to chitosan by reaction with carbodiimides^{228–230} but it has also been physically encapsulated within chitosan-based nanoparticles.^{256,257}

To improve the cellular uptake of different molecular structures (from small molecules to DNA fragments and nanoparticles), different groups have synthesized conjugates of chitosan with cell penetrating peptides (CPP). They are short sequence peptides (5 to 30 amino acids) that can interact and cross membranes.²¹⁹ Chitosan has been modified for nucleic acid (DNA and small interfering RNA and siRNA) delivery with various CPPs including TAT,^{218,221,223,224,258} penetratin,²¹⁹ and oligoarginines (7–9 units).^{220,226,227}

2.4.5.3 Small molecules. Different small molecules have been conjugated to chitosan mainly by reaction with carbodiimides to target certain cell types. For example, folic acid has been conjugated to chitosan for its ability to target overexpressed folate receptors, particularly in cancer cells.²⁵⁹ In the case of urocanic acid, a molecule bearing an imidazole group, it can act as a proton sponge and improve the release of active substances into the cytoplasm after endocytosis.²⁶⁰ Another example, glycyrrhizin, the main compound extracted from the root of *Glycyrrhiza glabra* (liquorice), has shown specific affinity for ASGR receptors present on the surface of

Table 2 Chitosan-grafted ligands and their applications

Ligands	Targets of interest	Molecules delivered
Galactose	Liver cells expressing asialoglycoprotein receptors	Oridonin, ²⁰¹ norcantharidin, ²⁰² glycyrrhizin, ²⁰³ curcumin, ¹⁷⁸ DNA ^{173,174,204–209}
Mannose	Antigen-presenting cells, <i>e.g.</i> , macrophages and dendritic cells	Rifampicin, ²¹⁰ DNA, ^{211,212} oligonucleotide ²¹³
Transferrin protein	Targeting cancer cells <i>e.g.</i> , HEK293 and HeLa cells. Increased transfection and transcytosis	Doxorubicin, ²¹⁴ methotrexate, ²¹⁵ DNA ^{216,217}
KNOB	Increased transfection	DNA, ^{218–222} siRNA ^{223–227}
CPP	Targeting cells expressing integrin receptors $\alpha\beta 3$ and $\alpha\beta 5$	Doxorubicin, ^{228,229} siRNA ²³⁰
Peptide RGD	Membrane targeting of overexpressed folate receptors on cancer cells (KB, OV2008, MCR-7)	Paclitaxel, ²³¹ 5-ALA, ²³² doxorubicin, ²³³ DNA ^{234,235}
Folic acid	Liver cells (asialoglycoprotein receptor)	Doxorubicin, ^{236,240} Paclitaxel, ²³⁷ Atorvastatin, ²³⁸ Lamivudine ²³⁹
Glycyrrhizin	Proton sponge enhancing cytoplasmic release	DNA, ^{241,242} siRNA, ²⁴³ p53 gene ²⁴⁴
Urocanic acid		Doxorubicin, mitomycin C, paclitaxel
Active substances		

CPP: cell penetrating cells; RGD: arginine–glycine–aspartate tripeptide; 5-ALA: 5-aminolevulinic acid; PTEN: phosphatase and tensin homolog.



hepatocytes.²⁶¹ Glycyrrhizin was grafted directly onto chitosan^{236–238} or grafted post-formation of chitosan nanoparticles.^{239,240}

2.4.6 Active substances. To increase the quantity of active substances delivered and their retention at the site of action, different groups investigated their conjugation to chitosan. As a result, numerous conjugates have been developed to deliver generally hydrophobic, anti-cancer, active substances. The chitosan-active substance conjugates consisting of a hydrophilic part (the polymer) and a hydrophobic part (the active substance) can behave as amphiphilic polymers and self-assemble as nanoparticles in aqueous media (see section 3.3).²⁶²

The active substance can be conjugated to chitosan *via* a biodegradable linker, which is stable in physiological medium but cleavable at the site of action by hydrolysis or enzymatic degradation. For example, a chitosan glycol-doxorubicin conjugate (DOX-GC) has been obtained by a chemical reaction between *N*-cis-aconityl-doxorubicin and chitosan glycol by reaction with carbodiimides. The *N*-cis-aconityl linker is stable at physiological pH but is hydrolyzed at acidic pH (4.5–6.5), allowing the release of the active substance at the endosomes and lysosomes of tumor cells. Son *et al.* described DOX-GC nanoparticles with a high yield (97%) and charge rate (38% m m⁻¹). These intravenously administered DOX-GC nanoparticles accumulated preferentially in the tumor with the release of the active substance (see section 4).⁴⁷

2.5 Combinations of chemical modifications

The combination of several chemical modifications of chitosan has been reported on numerous occasions. For example, the synthesis of alkylated and thiolated chitosan has been developed to improve the solubility and mucoadhesion of

chitosan.^{263,264} The synthesis of chitosan alkylated with fatty acid chains or PEG has made it possible to obtain nano-objects by self-assembly for the encapsulation of active substances.^{159,265,266} The conjugation of PEGylated chitosan with folic acid increases the solubility of chitosan, while improving cell targeting.¹⁵⁸

The thiolation of carboxymethyl chitosan was synthesized in a self-assembled particle *via* a disulfide bond crosslinked to encapsulate methotrexate for a tumor-specific drug release,²⁶⁷ gene carriers,²⁶⁴ and insulin orally delivered.²⁶³ The thiolation of glycol chitosan enhances the pulmonary absorption of calcitonin compared to a glycol chitosan nanoparticle.²⁶⁸ The octanoylation of a glycol chitosan derivative stimulates cell proliferation, metabolism, and differentiation due to the increase in the duration of the G1 phase.²⁶⁹

3 Formation of nano-objects based on chitosan

Due to its various physicochemical and biological properties, native and chemically modified chitosan has been widely used to formulate nano-objects encapsulating active substances for multiple routes of administration. These nano-objects can be obtained in different ways including chemical crosslinking, ionic crosslinking (ionic gelation or polyelectrolyte complexes), and even self-assembly (Fig. 2).

3.1 Formation of nano-objects by chemical crosslinking

The first chitosan nanoparticles described were obtained by chemical crosslinking (Fig. 2A). The most common crosslinking agent used is glutaraldehyde, which binds covalently by its aldehyde groups to the primary amine groups of chito-

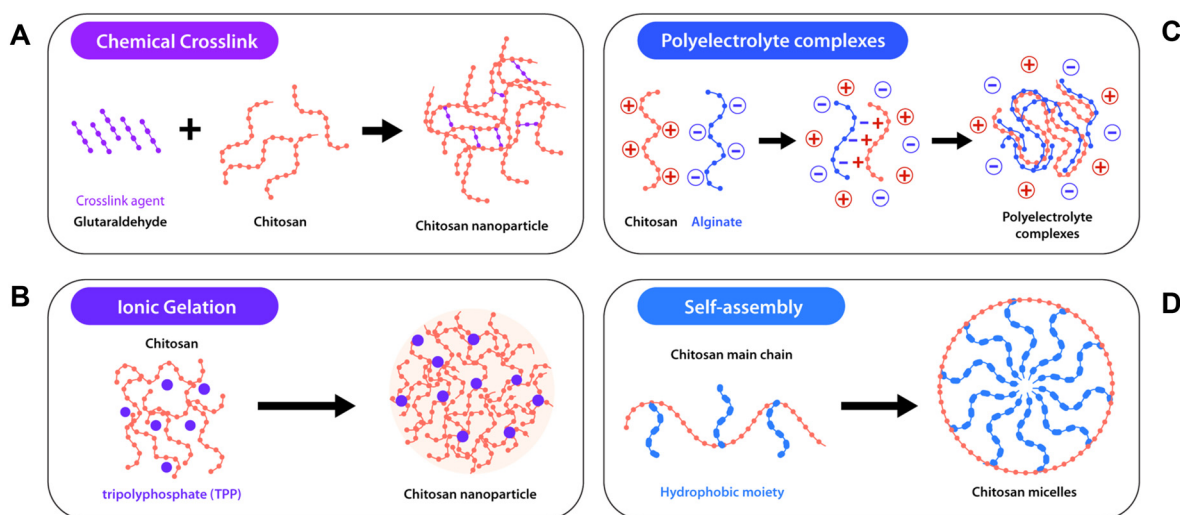


Fig. 2 Main strategies for the formulation of nano-objects based on native or modified chitosan chains: (A) chemical crosslinking by formation of covalent bounds between chitosan chains; (B) ionic gelation by formation of ionic bounds between chitosan chains and small polyions such as TPP; (C) formation of polyelectrolyte complexes by mixing chitosan with anionic polymer chains; (D) self-assembly of specific amphiphilic derivatives of chitosan.



san and, in some cases, to the primary amine groups of the active substance (Schiff reaction), allowing the formation of nanoparticles at specific concentrations and ratios. Different manufacturing methods have been developed and described on numerous occasions in different reviews.^{270–274}

Paclitaxel has also been encapsulated by chemical cross-linking.²⁷⁵ However, because of the cytotoxicity of glutaraldehyde, recent studies have focused on using other crosslinking agents such as genipin, an agent of natural origin isolated from the fruit of *Gardenia jasminoides* Ellis. Due to its better stability (resistance to enzymatic degradation) and lower cytotoxicity than glutaraldehyde,^{276,277} genipin has been used for the encapsulation of various active ingredients for targeting enteropathogenic bacteria.^{278–280} In addition, other naturally occurring crosslinking agents have been investigated with di- and tri-carboxylic acids (tartaric, malic, succinic, citric, and PEG-COOH).^{281–283}

Del Olmo *et al.* used chitosan crosslinked with genipin hydrogels for the sustained release of several drugs. The rheological parameters of viscosity and storage and loss moduli were obtained using a 1 : 0.2 weight ratio of CS : genipin due to the greater number of chemical linkages formed between them. The reaction time was also an important parameter given that the hydrogels showed a decrease in rheological parameters with an increase in the reaction time. The hydrogels also enabled the healing of ulcerated wounds, an improvement in metabolic activity and an increase in collagen and elastin levels compared to the negative control.²⁸⁴

The nanoparticles obtained by chemical crosslinking exhibit good encapsulation of the active substance and good stability in physiological medium due to the intermolecular chemical bonds involved. However, these nanoparticles are obtained under aggressive experimental conditions, which may alter the active substance. Moreover, solvent and reagent removal problems may be encountered, prompting interest for manufacturing methods under milder conditions.

3.2 Formation of nano-objects by ionic crosslinking

Protonated chitosan chains can interact with the negative charges carried by an ionic crosslinking agent, an active substance, or a polymer. Two main approaches rely on this idea, ionic gelation (Fig. 2B) and complexation of polyelectrolytes (Fig. 2C).

3.2.1 Ionic gelation. The group of Alonso *et al.* was the first to formulate chitosan-based nanoparticles by the ionic gelation process,^{285,286} (US6649192B2 patent). This simple technique relies on the spontaneous formation of nano-objects by adding a solution of sodium polyanion triphosphate (TPP), used as an ionic crosslinking agent, dropwise to a (protonated) chitosan solution. A colloidal suspension is obtained by inter and intramolecular electrostatic interactions between the phosphate groups of the negatively charged TPP and the amine groups of the positively charged chitosan. The resulting nano-objects are referred to as ‘nanogels’ or ‘nanoparticles’ in the literature.

Due the nature of the reversible electrostatic interactions involved between chitosan chains and TPP, several parameters

can affect the physicochemical properties of the nano-objects (size, surface charge, and compactness), such as the concentration of chitosan and TPP as well as the CS/TPP ratio,^{287–294} the molar mass and the DD of chitosan,^{289,290,293–296} the initial pH of the chitosan solution,^{287,289,296} the incorporation of another polymer such as PEG,²⁸⁸ and most importantly, ionic strength, which plays a crucial role in the colloidal stability of CS/TPP nanoparticles. The colloidal instability of these nanoparticles results in either the dissociation of the nanoparticles or their aggregation.²⁹⁷ An important source of dissociation of CS/TPP nanoparticles prepared in a salt-free acetic acid solution is mere dilution in isotonic (150 mmol L^{−1}) NaCl,²⁹⁸ which highlights the importance of competitive ionic interactions in a salt-rich environment. Huang *et al.* also demonstrated that in NaCl (150 mmol L^{−1}, pH = 4), a reduction in DD leads to a decrease in the aggregation but also an increase in the dissociation of nanoparticles, reflecting weaker electrostatic interactions with the most deacetylated chitosan. Conversely, in phosphate buffered saline PBS (pH = 7.2), the most deacetylated chitosan-based nanoparticles do not dissociate but precipitate because of the low solubility of chitosan. Therefore, the dissociation or aggregation of nanoparticles is correlated with the strength of the interactions between chitosan and TPP and dependent on the ionic strength and pH.²⁹⁴

Several strategies have addressed the potential instability of CS/TPP nanoparticles in physiological ionic media. Increasing the ionic strength of the nanoparticle formation medium by adding salt (150 mmol L^{−1} NaCl) results in the formation of more compact and stable nanoparticles that resist dilution in physiological media.^{292,299,300} Giacalone *et al.* also successfully improved the colloidal stability of chitosan nanoparticles by incorporating iron(III) ions during their preparation. Iron(III) forms coordination complexes with chitosan and phosphate groups and allows additional and overall stronger interactions (less sensitive to ionic strength) to be formed between chitosan and the active substance, improving the colloidal stability.²⁰⁰

These studies were performed on CS/TPP nanoparticles that do not encapsulate any active substance. However, depending on the nature of the active substance, the electrostatic interactions can be stronger. Exemplifying this, Kalam *et al.* developed tedizolid phosphate-encapsulated chitosan nanoparticles *via* the ionic gelation method for ocular delivery.³⁰¹ The particles formed using a low weight ratio of CS/TPP obtained optimum-sized particles with high encapsulation (82%) and a good drug loading capacity (7%), indicating ionic interaction between the anionic groups of TPP with the amine groups of CS in the weight ratio (CS was 3.12-fold higher than TPP). When the weight ratio decreased (CS was 2.77-fold TPP), the ionic interaction between them was not sufficient. The nanoparticles containing tedizolid did not show symptoms of discomfort in the ocular irritation study in rabbits, and the transcorneal permeation of tedizolid by the particles resulted in a 1.6-fold increase in flux and the permeability coefficient, indicating their higher permeation compared to the free drug.

CS/TPP nanoparticles prepared *via* the ionic gelation process have been used to encapsulate many active substances,



showing their versatility as a drug delivery system.^{302,303} The potential of an active substance itself to induce the ionic gelation of chitosan has been proposed by Giacalone *et al.* as a strategy to improve the drug loading (Fig. 3). The nucleotide adenosine triphosphate (ATP) and the nucleotide analog azidothymidine triphosphate (AzT-TP), both of which have a triphosphate group similar to TPP, formed nanoparticles by mere mixing with chitosan in the absence of TPP, resulting in a drug loading as high as 44% by weight.²⁹⁸ Russo *et al.* also demonstrated this with foscarnet, a molecule also having a triphosphate group.³⁰⁴

3.2.2 Polyelectrolyte complexes (PEC). Polyelectrolytes are macromolecules carrying numerous positive (polycation) or negative (polyanion) charges on their structure. By simply mixing two or more oppositely charged polyelectrolytes, PECs can be obtained spontaneously through the formation of electrostatic interactions as well as hydrogen and hydrophobic bonds.^{305,306} In an acidic medium, chitosan behaves like a cationic polyelectrolyte, and therefore can interact with anionic polyelectrolytes to form complexes. Many of these complexes have reported with chitosan and various polyanions for the design of drug delivery systems including alginate,^{297,307–315} carrageenan,^{310,316,317} glucomannan,^{318,319} pectin,^{320,321} hyaluronic acid,^{322–325} carboxymethylcellulose,³²⁶ chondroitin sulfate,^{327,328} polyglutamic acid,^{329–331} heparin,^{322,332–334} and dextran sulfate.^{333,335–339}

The formation of PECs depends on many parameters, particularly: (i) the nature of the polyelectrolytes, (ii) the degree of ionization of each polyelectrolyte, (iii) the density and distribution of charges on the chains of polyelectrolytes, (iv) the concentration of polyelectrolytes, and (v) the ratio between the two polyelectrolytes.^{308,321,324,325,332,335} Quiñones *et al.* showed the effect of the charge ratio of polyelectrolytes on the size and charge of the formed polyelectrolyte complexes. As the charge ratio moves away from neutrality, the nanoparticles formed are

charged with excess polyelectrolyte. Alternatively, if the charge ratio is close to 1, uncharged particles are obtained, forming aggregates.³⁰⁵ Their formation also depends on the reaction parameters including temperature, ionic strength, and pH. The colloidal stability of PECs heavily depends on their ionic strength and is controlled by the dissociation or aggregation of the complexes.³⁴⁰ The colloidal stability of PECs has been improved with the use of zinc.^{324,341} Different groups have developed systems combining these two manufacturing processes, using a chemical or ionic crosslinking agent to formulate PECs.³⁰⁵

3.3 Formation of nano-objects by self-assembly

Native chitosan has difficulty in self-assembling into nano-objects in aqueous media, unlike some chitosan derivatives. The grafting of hydrophobic fragments such as fatty acids by acylation (see section 2.2) or certain polymers (see section 2.4) allows the conjugates, by hydrophobic interactions, to self-assemble into nano-objects in an aqueous medium (Fig. 2D). By varying the type and degree of substitution of the hydrophobic moiety, the size and zeta potential of nano-objects and the release profile of the active substance can be modulated. This type of nanoparticle has been mainly used for the encapsulation of anti-cancer drugs including epirubicin,³⁴² paclitaxel,^{41,42,49,84,111,343} doxorubicin,^{46–48,129,344} mitomycin C,³⁴⁵ and camptothecin.^{43,346}

3.4 Formation of nano-objects by polymerization

Chitosan-based nanoparticles have also been obtained by grafting a polymerizable group onto chitosan, leading to the formation of polymer chains. Then, polymerization generates inter- and intra-molecular bonds between the carboxylic groups of the polymer and the amine groups of the chitosan-forming nanoparticles. The most widely used copolymerization method is controlled or uncontrolled radical polymeriz-

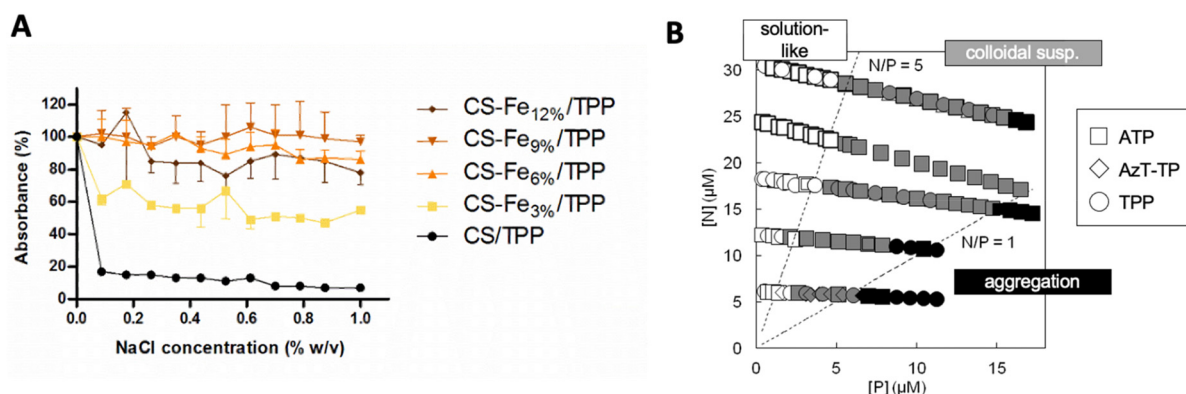


Fig. 3 Illustration of drug delivery challenges and opportunities associated with the ionic gelation of chitosan. (A) Dilution of CS/TPP nanogels in electrolytes may cause their rapid disassembly, as shown by turbidity measurements following nanogel dilution in a 0–150 mM (~0–0.9% w/v) NaCl medium. This effect can be reversed by the introduction of Fe³⁺ coordination complexes within the nanogels (reproduced from ref. 200 with permission from Elsevier, copyright 2014). (B) Active drug molecules containing a triphosphate group can induce chitosan gelation similarly to TPP, leading to drug loading values higher than typically obtained in nanocarrier formulations, as shown in the case of the nucleotide adenosine triphosphate (ATP) and the nucleotide analogue azidothymidine triphosphate (AzT-TP) (reproduced from ref. 298 with permission from the American Chemical Society, copyright 2013).



ation by free radicals with vinyl monomers. By this method, various copolymers have been synthesized with acrylates^{347–351} or styrenes.³⁵²

The chemical flexibility of chitosan, due to its numerous possible chemical modifications, is important for obtaining biomaterials suitable for different therapeutic applications. However, this diversity of chitosan leads to new chemical and particulate entities whose biodistribution, biodegradation and toxicology profiles are still poorly understood to date.

4 Biodistribution

The biodistribution of chitosan-based systems strongly depends on their route of administration, which originates in the diversity of the chemical modifications of the polymer and the nano-formulation types based on them. The understand-

ing of their *in vivo* fate after administration is not only crucial to evaluate their potential to improve drug delivery, but also to understand the potential toxicity of these drug delivery systems. In this respect, most studies have used the main parenteral routes.

4.1 Intravenous route

He *et al.* investigated the impact of the size and surface charge of nanoparticles on their biodistribution in H-22 tumor-bearing mice (Fig. 4). To do this, two types of chitosan conjugated with rhodamine, carboxymethyl-chitosan (RhB-CMC) and chitosan hydrochloride (RhB-CH), were associated with FITC-labeled protamine sulfate (FITC-PS) and camptothecin (CPT) to yield nanoparticles with variable physicochemical properties in terms of size (150–500 nm) and zeta potential (–40 to +35 mV).³⁵³ Overall, this study demonstrates that the surface charge influences the biodistribution of chitosan-based nanoparticles, with negatively charged nanoparticles

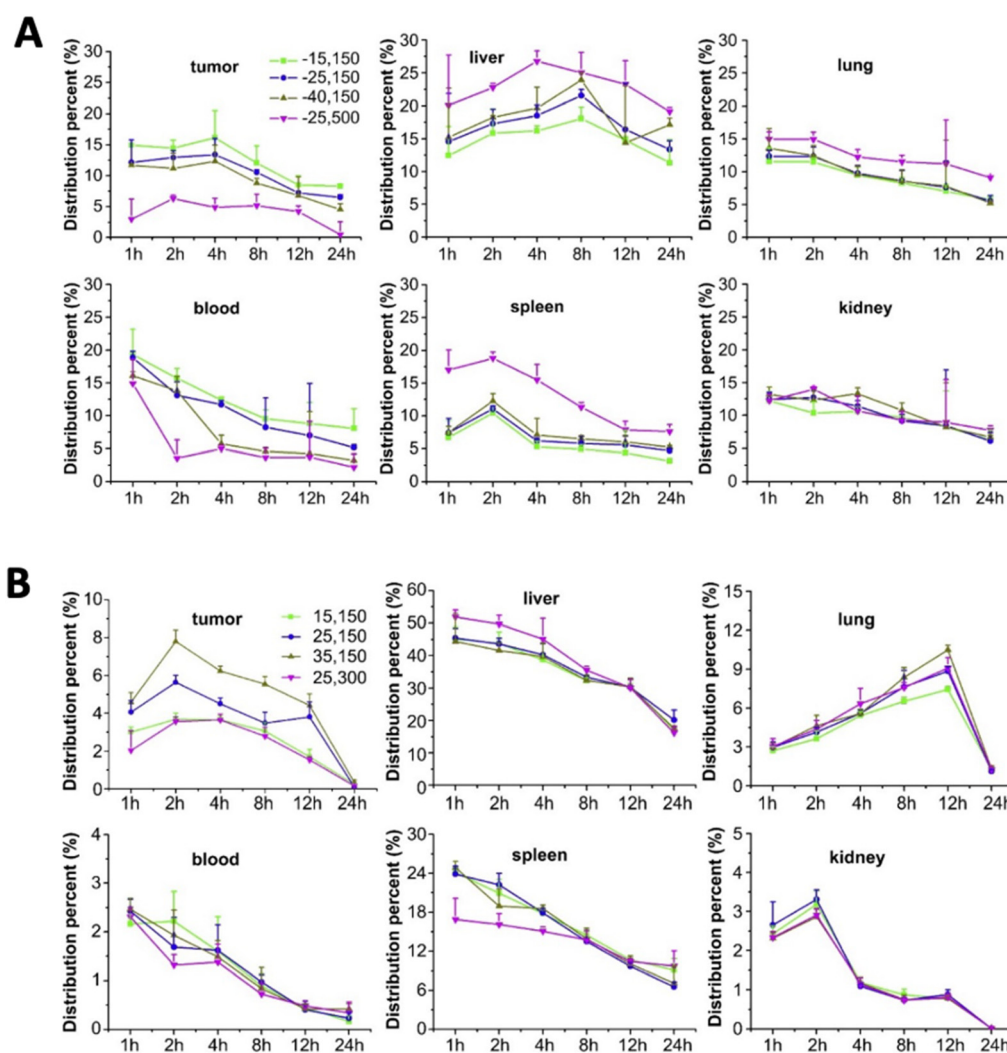


Fig. 4 Biodistribution of chitosan-based nanoparticles displaying various size and surface charge (legend: zeta potential [mV], size [nm]) obtained from (A) carboxymethyl-chitosan and (B) chitosan hydrochloride, following intravenous administration to H-22 tumor-bearing mice (reproduced from ref. 353 with permission from Elsevier, copyright 2010).



accumulating more in the tumor compared to positively charged nanoparticles, which are cleared more quickly from the bloodstream and accumulate in the liver and spleen. The size of the nanoparticles also has an impact, where the larger the nanoparticles, the more they are captured by the liver. In particular, small and negatively charged (150 nm, -15 mV) nanoparticles showed the greatest accumulation in the tumor due to their increased circulation time in the bloodstream. Higher surface charges and nanoparticle size caused a decrease in the circulation time in the blood and a higher accumulation in the liver and spleen. The renal elimination of nanoparticles was not found to be influenced by their charge and size.³⁵³ A higher hepatic accumulation of RhB-CH nanoparticles (+25 mV, 300 nm) compared to RhB-CH (+25 mV, 150 nm) was also observed by Hu *et al.*, while the opposite trend was observed in the spleen. In the lungs, the accumulation of nanoparticles depends on their load, probably resulting from the formation of aggregates in the pulmonary capillaries.

The biodistribution of chitosan glycol-based nanoparticles with different anti-cancer agents (cisplatin,⁴⁴ doxorubicin,⁴⁸ and docetaxel⁴⁶) has also been described in the literature. Again, the accumulation of nanoparticles in the tumor was observed as well as small amounts in the organs, decreasing over time. Anti-cancer drugs in nanoparticles exhibited lower toxicity and maintained or improved anti-cancer activity depending on the active substance. The impact of the physicochemical properties of nanoparticles of chitosan glycol-based nanoparticles has been investigated in terms of molar mass of the polymer (20–250 kg mol⁻¹) in mice carrying subcutaneous tumors (SCC7). Regardless of the molar mass, fluorescence was detected in the liver, lung, kidneys, spleen, and heart, and gradually decreasing over time. All types of nanoparticles showed accumulation at the tumor level depending on the molar mass of chitosan glycol-cholic acid. With 20 kg mol⁻¹ and 100 kg mol⁻¹ chitosan-glycol, tumor accumulation was low and decreased after 6 h, while in the case of 250 kg mol⁻¹, higher and prolonged tumor fluorescence were recorded. The nanoparticles with lower molar masses were eliminated more quickly. According to Park *et al.*, this difference is linked to the difference in the stability of nanoparticles in the bloodstream. However, this parameter has not been extensively studied.³⁵⁴

4.2 Intra-peritoneal (IP) route

The group of Machida *et al.*³⁴⁵ compared the biodistribution of nanoparticles of *N*-succinyl-chitosan labeled with FITC and conjugated with mitomycin C, injected by IV or IP routes in mice bearing subcutaneous tumors (Sarcoma 180). Depending on the route of administration, a difference in kinetics was observed, whereas the biodistribution remained unchanged. The nanoparticles accumulated preferentially at the level of the tumor, and few have been found in the organs (kidney, spleen, liver, and lungs). The amounts determined in the blood and the tumor were higher after IV administration, where 15% of the dose reached the tumor after 24 h, while after IP administration, the nanoparticles remained close to

the point of injection, causing potential toxicity. The distribution and accumulation at the tumor level were similar to that obtained with *N*-succinyl-chitosan in solution with varying degrees of substitution.¹²⁷

4.3 Subcutaneous route

Lu *et al.* described chitosan-based nanoparticles for the encapsulation of mitoxantrone, an anti-cancer drug indicated against breast cancer.³⁵⁵ After subcutaneous injection, the nanoparticles showed slow absorption from the injection site and better accumulation in the lymph nodes than a solution of the active substance. These nanoparticles have also made it possible to reduce the toxicity of the anti-cancer drug.³⁵⁵

The majority of biodistribution studies reported in the literature were carried out in mice bearing subcutaneous tumors, impacting the biodistribution of chitosan nanoparticles. These studies have shown the accumulation of nanoparticles in the tumor due to their increased circulation time in the blood. However, the biodistribution studies carried out do not compare the chitosan-based nanosystems and the polymer in solution. In addition, the impact of colloidal stability and physicochemical properties of nanoparticles (size, surface charge, and molar mass) remains to be fully understood.

5 Biodegradation

Once in the form of a polymer in solution, chitosan is degraded by hydrolysis of β (1-4) glycosidic bonds chemically in the stomach by acid catalysis.³⁵⁶ The DA of chitosan indeed plays an essential role in the degradation. Zhang *et al.* studied the degradation of chitosan by isolated β -glucosidase and enzymes from the cecum and rat colon, whose activity is comparable to that of the human colon. Chitosan having a molar mass of 600 kg mol⁻¹ and a low DA (77%) showed a faster degradation rate, and the correlation between the *in vitro* and *ex vivo* results has also been demonstrated.^{357,358}

From study to study, the degradation rates vary depending on the type of chitosan, enzymes, enzyme concentration, study time, and degradation conditions.³⁵⁹ The availability of amine groups also impacts the rate of degradation. Indeed, the chemical modification of chitosan can limit the accessibility of its amino groups for the hydrolysis of β -1-4-glycosidic bonds. For example, thiolated chitosan with thioglycolic acid (CS-TGA) is between 12.9–24.7% less degraded than native chitosan. This reduction is correlated with the degree of grafting of thiol groups on chitosan. When CS-TGA is crosslinked (presence of a disulfide bridge), the degradation of chitosan is also reduced.³⁶⁰

Oppositely, porous microparticles formulated with different acetylated chitosan (DA of 10–50%) have shown an increased degradation rate depending on their DA.³⁶¹ This was also observed when the chitosan was in the form of matrix³⁶² and fibers. Yang *et al.* have shown *in vitro* and *in vivo* that acetylated chitosan fibers degrade more than chitosan fibers.³⁶³ Numerous studies on the degradation of acetylated chitosan



have been carried out in films or fibers, which have great prospects in tissue engineering.³⁶⁴

The presence of a crosslinking agent also significantly impacts the degradation of chitosan by preventing the access of enzymes to its $\beta(1-4)$ -glycosidic bonds. McConnell *et al.* evaluated this with different chitosan films prepared in the presence or absence of different concentrations of chemical or ionic crosslinking agent, glutaraldehyde, or TPP, respectively. The non-crosslinked films were degraded by pancreatic and colonic enzymes from human colonic bacteria and porcine pancreatic enzymes in less than 4 h, while the films cross-linked with glutaraldehyde resisted any type of degradation. In contrast, the films crosslinked with only TPP resisted degradation by pancreatic enzymes. This difference is linked to the nature of the crosslinking, where glutaraldehyde forms covalent bonds, while TPP forms ionic and more 'flexible' bonds between chitosan chains.³⁶⁵

Therefore, the rate of degradation of chitosan depends on the molar mass, the DA, and chemical modifications. Thus far, the *in vivo* degradation pathways of chitosan and its derivatives have not been fully determined. Despite this, the modulation of the biodegradation of chitosan has many interests, more precisely in the field of the delivery of active substances for controlled and/or prolonged release.

6 Toxicology of chitosan

6.1 *In vitro* toxicity

Many studies have investigated the effect of chitosan on cell viability, its modulation on its polymer physicochemical properties, and chemical modifications (Table 3). In general, it is difficult to conclude simply on the toxicity of chitosan. Not all cytotoxicity studies presented are comparable, each using different cell lines and incubation times. However, compared to cationic reference polymers such as PEI ($IC_{50} < 0.03 \text{ mg mL}^{-1}$ (ref. 366)) or poly-L-lysine, chitosan and its derivatives are not very toxic with an IC_{50} varying from 0.2 to 2 mg mL^{-1} in most cell models,³⁰² with their cytotoxicity depending on their concentration and incubation time.^{266,367}

The impact of molar mass and degree of substitution (deacetylation, trimethylation, and PEGylation) must also be considered. According to Thanou *et al.*, there is a threshold at which the molecule and the components of the cell have enough contact to have significant toxicity. This threshold would be between 40% and 60% degree of substitution. In terms of molar mass, chitosan with low molar masses ($<10 \text{ kg mol}^{-1}$) would not exhibit significant toxicity,³⁵⁶ while its toxicity may increase with higher molar masses.^{266,366} In particular, a study carried out on chitosan of 3 to 100 kg mol^{-1} exhibiting different degrees of trimethylation (DTM) showed that the toxicity of the polymer increases with trimethylation regardless of its molar mass and more significantly with polymers of higher molar masses. For identical DTMs, chitosan with the highest molar masses also showed the highest toxicities.³⁶⁶ Mao *et al.* compared trimethylated (TMC) and

Table 3 Cytotoxicity of chitosan and its derivatives measured by the MTT test

Chitosan (DD, M_w)	IC_{50} (cells, incubation time)	Ref.
Trimethylated chitosan, PEGylated 84.7% DD, 40% DTM 400 kg mol^{-1} PEG 5000 g mol^{-1}	L929 cells, 24 h	266
0% DP	0.015 mg mL^{-1}	
12% DP	0.04 mg mL^{-1}	
25.7% DP	$>0.5 \text{ mg mL}^{-1}$	
27.4% DP	$>0.5 \text{ mg mL}^{-1}$	
Chitosan salts	Caco-2 cells, 2 h	367
87% DD, 20–460 kg mol^{-1}		
Aspartate	$0.67\text{--}0.72 \text{ mg mL}^{-1}$	
Glutamate	$0.35\text{--}0.46 \text{ mg mL}^{-1}$	
Lactate	$0.31\text{--}0.38 \text{ mg mL}^{-1}$	
Hydrochloride	$0.22\text{--}0.27 \text{ mg mL}^{-1}$	
Chitosan lactate	B16F10 cells, 72 h	368
78% DD, $<50 \text{ kg mol}^{-1}$	2.50 mg mL^{-1}	
82% DD, $150\text{--}170 \text{ kg mol}^{-1}$	$2.00 \pm 0.18 \text{ mg mL}^{-1}$	
Chitosan glutamate		
$>80\%$ DD, $60\text{--}90 \text{ kg mol}^{-1}$	$2.47 \pm 0.14 \text{ mg mL}^{-1}$	
77% DD, $180\text{--}230 \text{ kg mol}^{-1}$	$1.73 \pm 1.39 \text{ mg mL}^{-1}$	
Chitosan hydrochloride	B16F10 cells, 72 h	368
85% DD, $60\text{--}90 \text{ kg mol}^{-1}$	$2.24 \pm 0.16 \text{ mg mL}^{-1}$	
81% DD, $100\text{--}130 \text{ kg mol}^{-1}$	$0.21 \pm 0.04 \text{ mg mL}^{-1}$	
Chitosan glycol		
100% DD, 152 kg mol^{-1}	$2.47 \pm 0.15 \text{ mg mL}^{-1}$	
Trimethylated chitosan 3–6 kg mol^{-1} (oligomer)	MCF-7 cells, 24 h	366
20% DTM	$>10 \text{ mg mL}^{-1}$	
44% DTM	$>10 \text{ mg mL}^{-1}$	
55% DTM	5.959 mg mL^{-1}	
94% DTM	0.417 mg mL^{-1}	
100 kg mol^{-1}		
36% DTM	$0.285 \pm 0.1 \text{ mg mL}^{-1}$	
57% DTM	$0.265 \pm 0.05 \text{ mg mL}^{-1}$	
76% DTM	$0.059 \pm 0.03 \text{ mg mL}^{-1}$	
93% DTM	$0.118 \pm 0.28 \text{ mg mL}^{-1}$	

Adapted from ref. 356. DD: degree of deacetylation; DP: degree of PEGylation, DTM: degree of trimethylation.

PEGylated chitosan. The copolymers studied were synthesized with different molar masses and a DTM set at 40%. The cytotoxicity of TMC (400 kg mol^{-1}) significantly decreased with an increasing degree of PEGylation (DP). The cytotoxicity is also dependent on the molar masses of the constituents of the copolymer TMC-PEG. For a fixed degree of PEGylation (DP = 6%), the 100 kg mol^{-1} TMC and 50 kg mol^{-1} TMC pegylated with PEG (5 kg mol^{-1}) showed 10 times less toxicity ($IC_{50} > 0.5 \text{ mg mL}^{-1}$) than the TMC 400 kg mol^{-1} -PEG (5 kg mol^{-1}) ($IC_{50} = 0.04 \text{ mg mL}^{-1}$). Conversely, for a molar mass of fixed TMC, lower cytotoxicity was obtained with PEG of higher molar mass (5 kg mol^{-1}) compared to PEG (550 g mol^{-1}), despite the higher degree of PEGylation, *i.e.*, 36.7% for PEG (550 g mol^{-1}) against 6.4% for PEG (5 kg mol^{-1}). PEG grafting makes it possible to reduce the cationic surface charge of chitosan, leading to a reduction in interactions with cells and, therefore its cytotoxicity.²⁶⁶



All the *in vitro* studies presented show that the toxicity of chitosan is directly related to its charge density. Chemical modifications that do not induce an increase in charge, such as PEGylation, may reduce the toxicity of chitosan. The counterions present in the chitosan salts may increase the cationic charge of the chitosan and cause an increase in toxicity. Opanasopit *et al.* showed that the cytotoxicity of chitosan varied according to the type of chitosan salts (hydrochloride > lactate > glutamate > aspartate), with hydrochloride chitosan as the most toxic with an IC_{50} of 0.22–0.27 $mg\ mL^{-1}$.³⁶⁷

A few more studies have focused on the impact of the physical–chemical properties of nano-objects (size, zeta potential, and composition) based on chitosan on the cytotoxicity, knowing that these nano-formulations have already demonstrated a significant role in cellular capture.³⁵³ Huang *et al.* showed that chitosan and chitosan nanoparticles crosslinked with TPP exhibited comparable cytotoxicity with similar IC_{50} values. However, the cytotoxicity of chitosan and nanoparticles was reduced with a decrease in the polymer DD from 88% to 61%.³⁶⁹

Qi *et al.* studied the impact of the surface charge and the size of CS/TPP nanoparticles, loaded or not with Cu(II). CS/TPP nanoparticles (40 nm, +51 mV) and CS/TPP/Cu nanoparticles (257 nm, +96 mV) showed more significant toxicity than that of chitosan in solution with an IC_{50} of 15 and 6 $\mu g\ mL^{-1}$, respectively. The surface charge of the nanoparticles increased the electrostatic interactions with the negatively charged membrane components of cells, resulting in increased cytotoxicity, which was also impacted by the size of the nanoparticles. A variation in cytotoxicity was also observed depending on the

cell line studied. The nanoparticles showed high cytotoxicity towards tumor cells and lower cytotoxicity towards hepatic cells³⁷⁰ (Fig. 5D and E).

Nasti *et al.* also studied the cytotoxicity profiles of CS/TPP nanoparticles on macrophages and fibroblasts. Two types of nanoparticles were analyzed in terms of size, nanoparticles of about 240 nm, coated or not with hyaluronic acid (HA), and nanoparticles of around 360 nm. The HA coating allows the surface charge of the smaller nanoparticles to be changed; however, the surface charge value of CS/TPP/HA nanoparticles has not been reported.³⁷¹ Higher nanoparticle cytotoxicity was obtained for all types of nanoparticles on macrophages compared to fibroblasts ($IC_{50} > 2\ mg\ mL^{-1}$). At the level of macrophages, a difference in cytotoxicity was also observed depending on the surface charge of the nanoparticles, with CS/TPP/HA nanoparticles showing less cytotoxicity compared to CS/TPP nanoparticles. However, depending on the size of the nanoparticles, the variation in cytotoxicity observed may not be statistically significant. This study demonstrated that the observed cytotoxicity is mainly linked to the internalization of nanoparticles, which is directly related to the surface charge of the nanoparticles.³⁷¹

Other studies have also shown the impact of the size and surface charge of nanoparticles on different cell lines including L929 cells²⁶⁶ (Fig. 5B and C), COS-7 and MCF-7 cells,³⁶⁶ and hematopoietic stem cells.³⁷² All the studies show that the cytotoxicity of chitosan nanoparticles is directly related to their surface charge density resulting from amine groups, the size of the nanoparticles and the three-dimensional arrangement of the polymer. Depending on the conformation and the cat-

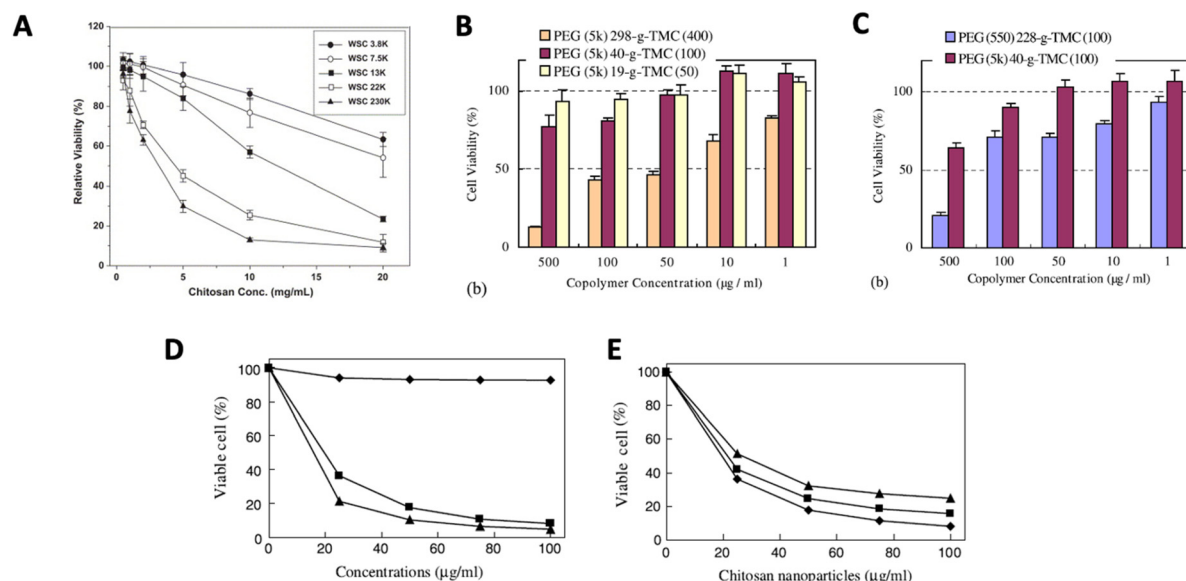


Fig. 5 Impact of chitosan chemistry and supramolecular organization on *in vitro* toxicity. (A) Cytotoxicity of soluble chitosan of various M_w : ● 3.8 $kg\ mol^{-1}$; ○ 7.5 $kg\ mol^{-1}$; ■ 13 $kg\ mol^{-1}$; □ 22 $kg\ mol^{-1}$ and ▲ 230 $kg\ mol^{-1}$ (reproduced from ref. 373 with permission from Elsevier, copyright 2005). (B and C) Cytotoxicity of trimethyl chitosan-poly(ethylene glycol) (TMC-PEG) conjugates displaying various M_w of the TMC and PEG segments (reproduced from ref. 266 with permission from Elsevier, copyright 2005). (D) Cytotoxicity of ◆ free CS, ■ CS/TPP nanoparticles and ▲ CS/TPP/Cu nanoparticles and (E) influence of CS/TPP nanoparticle size: ▲ 100 nm, ■ 70 nm and ◆ 40 nm (reproduced from ref. 370 with permission from Elsevier, 2005).



ionic charge of the nanoparticles, a decrease or increase in interactions with the anionic components of the glycoproteins present at the level of the cell membranes can be observed, impacting the cytotoxicity of the chitosan-based nanoparticles.

6.2 In vivo toxicity

6.2.1 Parenteral route. Depending on its route of administration, chitosan does not present the same toxicity profile (Table 4). Intravenously, through the interaction of its cationic groups with plasma proteins or blood cells, chitosan can cause complement activation.^{374,375}

Regarding the toxicity of chitosan in the form of nano-objects, Hu *et al.* studied it with chitosan nanoparticles cross-linked with TPP in a zebrafish embryo model, which is commonly used for genotoxicity studies. High concentrations of

nanoparticles (20–40 mg mL⁻¹) with a size of 200 or 340 nm were tested. A decrease in hatching and an increase in concentration-dependent mortality have been demonstrated. However, malformations were only observed in the embryos treated with the 200 nm nanoparticles, showing an impact on the size of the nanoparticles. At the cellular level, the nanoparticles caused physiological stress. These toxic effects were observed only for high concentrations of nanoparticles. The embryos exposed to lower concentrations (5 mg mL⁻¹) exhibited oxidative cellular stress but no tissue toxicity.³⁸⁴

N-Octyl-*O*-sulfate chitosan micelles encapsulating paclitaxel did not induce anaphylactic reactions, histopathological or hemolytic effects after intravenous injection with 6 mg kg⁻¹ day in mice. These micelles have also made it possible to reduce the toxicity of the active molecule, where the median

Table 4 In vivo toxicity of chitosan and its derivatives after parenteral and oral administration

Chitosan (DD, M_w)	Route	Study	Observations	Ref.
Chitosan oligosaccharides 304–1.162 kg mol ⁻¹ Chitosan LMW 3 kg mol ⁻¹	Parenteral route	Rabbit, IV injection 7.1–8.6 mg kg ⁻¹ for 5 days	Increased lysozyme activity after two or more injections	377 378
Chitosan 80% DD, 30–40 kg mol ⁻¹	Parenteral route	Mouse, IP or SC injection 1–5 mg Every 15 days for 84 days	Clinical signs and cellular abnormalities observed only after IP administration	379
<i>N</i> -Succinyl chitosan (Suc-CS) nanoparticles Mitomycin C (MMC) 72% DS, 300 kg mol ⁻¹	Parenteral route	Mouse, IP injection	Suc-CS: LD ₅₀ = 2000 mg kg ⁻¹ Suc-CS/MMC: LD ₅₀ = 25 mg kg ⁻¹ No clinical sign	345
<i>N</i> -Octyl-nanoparticles Chitosan <i>O</i> -sulfate Paclitaxel	Parenteral route	Mouse, IV injection Rats, IV injection Rabbit, IV injection 6 mg per kg per day for 3 days Rabbit, IV injection 2 mg kg ⁻¹	92% DD, 65 kg mol ⁻¹ IV: LD ₅₀ = 72.16 mg kg ⁻¹ PI: LD ₅₀ = 81.28 mg kg ⁻¹ 97% DD, 65 kg mol ⁻¹ IV: LD ₅₀ = 102.59 mg kg ⁻¹ PI: LD ₅₀ = 130.53 mg kg ⁻¹ No pathological changes observed No hypersensitivity	42 and 380
Chitosan 3 kg mol ⁻¹	Oral route	Rabbits, hens 700–800 mg kg ⁻¹ for 239 days	No pathological changes	378
Chitosan 80% DD, 30–40 kg mol ⁻¹	Oral route	Mouse Food containing between 0.5–5% chitosan for 28 days	Weight loss and reduction of bacteria in the intestinal flora at the 5% dose	379
Chitosan oligomer 85% DD, 1.86 kg mol ⁻¹	Oral route	Mouse 1000–10 000 mg kg ⁻¹ Rats 750–3000 mg kg ⁻¹ for 30 days	LD ₅₀ > 10 000 mg kg ⁻¹ No mutagenicity No clinical or pathological signs LD ₅₀ > 3000 mg kg ⁻¹ No clinical, hematological or pathological signs	381
Chitosan n/a	Oral route	Rats 0–2545 mg kg ⁻¹ for 364 days 0–2545 mg kg ⁻¹ for 728 days	LD ₅₀ > 2323–2545 mg kg ⁻¹ No toxic effects (clinical, hematological, biochemical, pathological) No carcinogenicity	382
Chitosan/TPP nanoparticles 85% DD, 80 kg mol ⁻¹	Oral route	Mouse 100 mg kg ⁻¹ for 14 days	No clinical signs (diarrhea, fever, weight loss, etc.), pathological and inflammatory Non-significant hematological and biochemical abnormalities	383

Adapted from ref. 376. DD: degree of deacetylation; M_w : molar mass; LMW: low molecular weight IV: intravenous; IP: intraperitoneal; LD₅₀: median lethal dose; DS: degree of *N*-succinylation.



lethal doses (LD_{50}) after intravenous or intraperitoneal administration were between 1.14 and 1.52 times higher than that of paclitaxel in the free form.⁴²

Overall, the use of chitosan-based nanosystems may be more challenging by parenteral route than oral or other local ones. In particular, subcutaneous administration of chitosan-based nanoparticles³⁸⁵ faces less risks compared to the intravenous route, for which a lack of sufficient hemocompatibility and the formation of deadly emboli, despite the safe administration in several animal models, have been raised as concerns.³¹

6.2.2 Oral route. Orally, chitosan is mainly used as an absorption promoter in the gastrointestinal tract due to its mucoadhesion properties and ability to modulate the permeability of active molecules due to the reversible opening of tight junctions between epithelial cells,³⁸⁶ which facilitates the paracellular transport of hydrophilic macromolecules.^{105,387–390}

The *in vivo* toxicity profile of chitosan after oral administration has been established in different species (mice, rats, rabbits, and chickens) (Table 4). Absorption through the intestinal barrier is dependent on the molar mass of chitosan, which increases as its molar mass decreases and its water solubility increases.³⁹¹ Therefore, the oral route is not the preferred route of administration for the design of chitosan-based nano-objects for targeting tissues other than the gastrointestinal tract. Overall, chitosan exhibits no significant toxicity or minimal toxic effects regardless of its route of administration, which encourages its use as a biomaterial for the administration of active drug substances.

Many examples can be found in relation to the permeability enhancement of drugs. Yin *et al.* developed micelles of *N*-octyl-*O,N*-carboxymethyl chitosan to investigate their role in enhancing the oral absorbance of silybin.³⁹² When incorporated into modified chitosan micelles, the drug in plasma concentrations was improved and remained detectable for more time than in free solution. Krauland *et al.* treated non-diabetic rats with thiolated chitosan tablets containing insulin orally, and the decrease of blood glucose level was higher when the insulin was administered in the tablets compared to free insulin and insulin in the control tablets.³⁹³ Sudhakar *et al.* observed a difference in the blood glucose level in rats between the free insulin administered subcutaneously and insulin encapsulated in thiolated chitosan nanoparticles administered orally. The free insulin decreased the blood glucose level in 30 min, while the encapsulated insulin showed a prolonged reduction in blood glucose. The presence of insulin in the plasma was also modified by the nanoparticles. After 1 h injection of free insulin, the maximum was reached, and in the case of the insulin-loaded nanoparticles, this maximum was observed at 2–3 h after administration.³⁹⁴

However, each *in vivo* toxicity study uses its own experimental conditions; thus, the standardization of procedures should be considered (*cf.* section 7). In addition, most toxicity studies described in the literature aim to show the effectiveness of the nanoparticles developed. This lack of toxicological knowledge is also due to the system itself. Chitosan forms

nano-objects in the presence of other components such as a chemical and ionic crosslinking agents or active substance, which may have their own toxicity.

7 Conclusions and future perspectives

Nanoparticles composed of chitosan have emerged as an excellent option to compose biodegradable, biocompatible and versatile drug delivery systems with overall low toxicity. Restrictions in chitosan solubility draws attention and chitosan derivatives were developed to become excellent alternatives to overcome this issue. The modification of chitosan may lead to more suitable and less toxic delivery systems, improving the properties of chitosan. In addition, numerous nanoscale architectures have been described, with various levels of complexity, drug encapsulation abilities and targeting properties. The application of chitosan derivatives in industry can be a promising perspective in the use of polymers in different fields, such as food, agriculture, and especially health care.

Several challenges remain to be addressed for the full development of chitosan-based nanomedicine. As highlighted above, the variety of chitosan biopolymers used in the literature spans a wide range of molar masses, DAs, sources, *etc.*, which complicates comparisons and consolidation of the published data (with occasional inconsistencies in the data and a limited understanding of the underlying mechanisms, as highlighted by some authors³⁰). The translation from laboratory studies to industrial scale also faces several significant challenges, including the scaling-up and economic feasibility of chemical modifications and processes of nano-formulations under GMP conditions,²⁹ in addition to the cost of the chitosan raw material depending on its source,³⁹⁵ and the need to find more environmentally friendly extraction methods.³⁰ There is also an important need for standardization, as already identified in the nanomedicine field, in particular in the development of standard test methods and reference materials, and unified approaches among the community,³⁹⁶ as addressed by several European initiatives.³⁹⁷

Despite these hurdles, it should be noted that several commercial products have received approval for use as wound dressings, hemostatic sealants, and even as nerve conduits.^{28,30} The scale up of chitosan-based nanoparticle fabrication has also been investigated to identify relevant formulation parameters.³⁹⁸ Finally, another specificity of chitosan lies in its immunomodulatory effects.³⁹⁹ It has shown potential immune-triggering capacity and potential to induce both cellular and humoral immunity,^{400–403} which holds promise for application as an adjuvant in vaccines, but should be taken into account for other drug delivery purposes.

Conflicts of interest

There are no conflicts to declare.



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References

- 1 R. Priyadarshi and R. J. Whan, Chitosan-based biodegradable functional films for food packaging applications, *Innovative Food Sci. Emerging Technol.*, 2020, **62**, 102346.
- 2 M. Hadidi, S. Pouramin, F. Adinepour, S. Haghani and S. M. Jafari, Chitosan nanoparticles loaded with clove essential oil: Characterization, antioxidant and antibacterial activities, *Carbohydr. Polym.*, 2020, **236**, 116075.
- 3 A. Pavinatto, A. V. D. A. Mattos, A. C. G. Malpass, M. H. Okura, D. T. Balogh and R. C. Sanfelice, Coating with chitosan-based edible films for mechanical/biological protection of strawberries, *Int. J. Biol. Macromol.*, 2020, **151**, 1004–1011.
- 4 M. E. I. Badawy and E. I. Rabea, A Biopolymer Chitosan and Its Derivatives as Promising Antimicrobial Agents against Plant Pathogens and Their Applications in Crop Protection, *Int. J. Carbohydr. Chem.*, 2011, **2011**, 1–29.
- 5 G. Betchem, N. A. N. Johnson and Y. Wang, The application of chitosan in the control of post-harvest diseases: a review, *J. Plant Dis. Prot.*, 2019, **126**(6), 495–507.
- 6 E. Yenilmez, E. Basaran and Y. Yazan, Release characteristics of vitamin E incorporated chitosan microspheres and in vitro – in vivo evaluation for topical application, *Carbohydr. Polym.*, 2011, **84**, 807–811.
- 7 Q. Ta, J. Ting, S. Harwood, N. Browning, A. Simm, K. Ross, I. Olier and R. Al-Kassas, Chitosan nanoparticles for enhancing drugs and cosmetic components penetration through the skin, *Eur. J. Pharm. Sci.*, 2021, **160**, 105765.
- 8 W. Xia, P. Liu, J. Zhang and J. Chen, Biological activities of chitosan and chitooligosaccharides, *Food Hydrocolloids*, 2011, **25**(2), 170–179.
- 9 C. Muanprasat and V. Chatsudthipong, Chitosan oligosaccharide: Biological activities and potential therapeutic applications, *Pharmacol. Ther.*, 2017, **170**, 80–97.
- 10 G. P. Parchen, J. Jacumazo, H. S. Koop, S. M. P. Biscaia, E. S. Trindade, J. L. M. Silveira and R. A. De Freitas, Modulation of Epidermal Growth Factor Release by Biopolymer-Coated Liposomes, *J. Pharm. Sci.*, 2020, 1–8.
- 11 M. Rinaudo, Chitin and chitosan: Properties and applications, *Prog. Polym. Sci.*, 2006, **31**(7), 603–632.
- 12 M. Lavertu, Z. Xia, A. N. Serreqi, M. Berrada, A. Rodrigues, D. Wang, M. D. Buschmann and A. Gupta, A validated ^1H NMR method for the determination of the degree of deacetylation of chitosan, *J. Pharm. Biomed. Anal.*, 2003, **32**(6), 1149–1158.
- 13 A. Hirai, H. Odani and A. Nakajima, Determination of degree of deacetylation of chitosan by ^1H NMR spectroscopy, *Polym. Bull.*, 1991, **26**(1), 87–94.
- 14 A. Domard, pH and c.d measurements on a fully deacetylated, chitosan: application to CuII-polymer interactions, *Int. J. Biol. Macromol.*, 1987, **9**, 98–104.
- 15 M. W. Anthonsen and O. Smidsrod, Hydrogen ion titration of chitosans with varying degrees of N-acetylation by monitoring induced ^1H -NMR chemical shifts, *Carbohydr. Polym.*, 1995, **26**, 303–305.
- 16 J. W. Park, K. Choi and K. K. Park, Acid-Base Equilibria and Related Properties of Chitosan, *Bull. Korean Chem. Soc.*, 1983, **4**(2), 68–72.
- 17 A. Katchalsky, Polyelectrolytes, *Pure Appl. Chem.*, 1971, **26**, 327–373.
- 18 P. Sorlier, A. Denuzie, C. Viton and A. Domard, Relation between the Degree of Acetylation and the Electrostatic Properties of Chitin and Chitosan, *Biomacromolecules*, 2001, **2**, 765–772.
- 19 K. M. Varum, M. H. Ottoy and O. Smidsrod, Water-solubility of partially N-acetylated chitosans as a function of pH: effect of chemical composition and depolymerisation, *Carbohydr. Polym.*, 1994, **25**, 65–70.
- 20 S. Aiba, Studies on chitosan : 3 . Evidence for the presence of random and block copolymer structures in partially N-acetylated chitosans, *Int. J. Biol. Macromol.*, 1990, **13**, 40–44.
- 21 N. Kubota and Y. Eguchi, Facile Preparation of Water-Soluble N-Acetylated Chitosan and Molecular Weight Dependence of Its Water-Solubility, *Polym. J.*, 1997, **29**(2), 123–127.
- 22 M. Rinaudo, G. Pavlov and J. Desbrières, Influence of acetic acid concentration on the solubilization of chitosan, *Polymer*, 1999, **40**, 7029–7032.
- 23 M. Rinaudo, G. Pavlov and J. Desbrières, Solubilization of Chitosan in Strong Acid Medium, *Int. J. Polym. Anal. Charact.*, 1999, **5**, 267–276.
- 24 C. K. S. Pillai, W. Paul and C. P. Sharma, Chitin and chitosan polymers: Chemistry , solubility and fiber formation, *Prog. Polym. Sci.*, 2009, **34**, 641–678.
- 25 M. N. V. R. Kumar, R. A. A. Muzzarelli, C. Muzzarelli, H. Sashiwa and A. J. Domb, Chitosan Chemistry and Pharmaceutical Perspectives, *Chem. Rev.*, 2004, **104**, 6017–6084.
- 26 L. Illum, Chitosan and its use as a pharmaceutical excipient, *Pharm. Res.*, 1998, **15**(9), 1326–1331.
- 27 C. Yan, D. Chen, J. Gu, H. Hu, X. Zhao and M. Qiao, Preparation of N-Succinyl-chitosan and Their Physical-



- Chemical Properties as a Novel Excipient, *Pharm. Soc. Jpn.*, 2006, **126**(9), 789–793.
- 28 A. Elizalde-Cárdenas, R. M. Ribas-Aparicio, A. Rodríguez-Martínez, G. Leyva-Gómez, C. Ríos-Castañeda and M. González-Torres, Advances in chitosan and chitosan derivatives for biomedical applications in tissue engineering: An updated review, *Int. J. Biol. Macromol.*, 2024, **262**, 129999.
 - 29 Y. Yu, Z. Su, Y. Peng, Y. Zhong, L. Wang, M. Xin and M. Li, Recent advances in modifications, biotechnology, and biomedical applications of chitosan-based materials: A review, *Int. J. Biol. Macromol.*, 2025, **289**, 138772.
 - 30 G. I. Edo, E. Yousif and M. H. Al-Mashhadani, Modified chitosan: Insight on biomedical and industrial applications, *Int. J. Biol. Macromol.*, 2024, **275**, 133526.
 - 31 H. Yadav, R. Malviya and N. Kaushik, Chitosan in biomedicine: A comprehensive review of recent developments, *Carbohydr. Polym. Technol. Appl.*, 2024, **8**, 100551.
 - 32 S. Manna, A. Seth, P. Gupta, G. Nandi, R. Dutta, S. Jana and S. Jana, Chitosan Derivatives as Carriers for Drug Delivery and Biomedical Applications, *ACS Biomater. Sci. Eng.*, 2023, **9**(5), 2181–2202.
 - 33 X. P. Liu, S. T. Zhou, X. Y. Li, X. C. Chen, X. Zhao, Z. Y. Qian, L. N. Zhou, Z. Y. Li, Y. M. Wang, Q. Zhong, Z. Y. Li, X. He and Y. Q. Wei, Anti-tumor activity of N-trimethyl chitosan-encapsulated camptothecin in a mouse melanoma model, *J. Exp. Clin. Cancer Res.*, 2010, **29**(76), 1–9.
 - 34 L. Zhou, X. Li, X. Chen, Z. Li, X. Liu, S. Zhou, Q. Zhong, T. Yi, Y. Wei, X. Zhao and Z. Qian, In vivo antitumor and antimetastatic activities of camptothecin encapsulated with N-trimethyl chitosan in a preclinical mouse model of liver cancer, *Cancer Lett.*, 2010, **297**(1), 56–64.
 - 35 A. Geçer, N. Yildiz and A. Çalimli, Trimethyl Chitosan Nanoparticles Enhances Dissolution of the Poorly Water Soluble Drug Candesartan-Cilexetil, *Macromol. Res.*, 2010, **18**(10), 986–991.
 - 36 M. Jamshidi, N. Ziamajidi, I. Khodadadi, A. Dehghan, G. Kalantarian and R. Abbasalipourkabir, The effect of insulin-loaded trimethylchitosan nanoparticles on rats with diabetes type I, *Biomed. Pharmacother.*, 2018, **97**, 729–735.
 - 37 J. B. Min, E. S. Kim, J. Lee and H. G. Lee, Preparation, characterization, and cellular uptake of resveratrol-loaded trimethyl chitosan nanoparticles, *Food Sci. Biotechnol.*, 2018, **27**(2), 441–450.
 - 38 J. Xu, B. Xu, D. Shou, X. Xia and Y. Hu, Preparation and Evaluation of Vancomycin-Loaded N -trimethyl Chitosan Nanoparticles, *Polymers*, 2015, **7**, 1850–1870.
 - 39 D. de Britto, M. R. de Moura, F. A. Aouada, F. G. Pinola, L. M. Lundstedt, O. B. G. Assis and L. H. C. Mattoso, Entrapment Characteristics of Hydrosoluble Vitamins Loaded into Chitosan and N, N, N-Trimethyl Chitosan Nanoparticles, *Macromol. Res.*, 2014, **22**(12), 1261–1267.
 - 40 G. Qu, X. Wu, L. Yin and C. Zhang, N-octyl-O-sulfate chitosan-modified liposomes for delivery of docetaxel: Preparation, characterization, and pharmacokinetics, *Biomed. Pharmacother.*, 2012, **66**(1), 46–51.
 - 41 R. Mo, X. Jin, N. Li, C. Ju, M. Sun, C. Zhang and Q. Ping, The mechanism of enhancement on oral absorption of paclitaxel by N-octyl-O-sulfate chitosan micelles, *Biomaterials*, 2011, **32**(20), 4609–4620.
 - 42 C. Zhang, G. Qu, Y. Sun, X. Wu, Z. Yao, Q. Guo, Q. Ding, S. Yuan, Z. Shen, Q. Ping and H. Zhou, Pharmacokinetics, biodistribution, efficacy and safety of N-octyl-O-sulfate chitosan micelles loaded with paclitaxel, *Biomaterials*, 2008, **29**, 1233–1241.
 - 43 K. H. Min, K. Park, Y. S. Kim, S. M. Bae, S. Lee, H. G. Jo, R. W. Park, I. S. Kim, S. Y. Jeong, K. Kim and I. C. Kwon, Hydrophobically modified glycol chitosan nanoparticles-encapsulated camptothecin enhance the drug stability and tumor targeting in cancer therapy, *J. Controlled Release*, 2008, **127**, 208–218.
 - 44 J. H. Kim, Y. S. Kim, K. Park, S. Lee, H. Y. Nam, K. H. Min, H. G. Jo, J. H. Park, K. Choi, S. Y. Jeong, R. W. Park, I. S. Kim, K. Kim and I. C. Kwon, Antitumor efficacy of cis-platin-loaded glycol chitosan nanoparticles in tumor-bearing mice, *J. Controlled Release*, 2008, **127**, 41–49.
 - 45 A. Yu, H. Shi, H. Liu, Z. Bao, D. Lin, D. Lin, X. Xu, X. Li and Y. Wang, Mucoadhesive dexamethasone-glycol chitosan nanoparticles for ophthalmic drug delivery, *Int. J. Pharm.*, 2020, **575**, 118943.
 - 46 H. Y. Hwang, I. S. Kim, I. C. Kwon and Y. H. Kim, Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles, *J. Controlled Release*, 2008, **128**, 23–31.
 - 47 Y. J. Son, J. S. Jang, Y. W. Cho, H. Chung, R. W. Park, I. C. Kwon, I. S. Kim, J. Y. Park, S. B. Seo, C. R. Park and S. Y. Jeong, Biodistribution and anti-tumor efficacy of doxorubicin loaded glycol-chitosan nanoaggregates by EPR effect, *J. Controlled Release*, 2003, **91**, 135–145.
 - 48 J. H. Park, S. Kwon, M. Lee, H. Chung, J. H. Kim, Y. S. Kim, R. W. Park, I. S. Kim, S. B. Seo, I. C. Kwon and S. Y. Jeong, Self-assembled nanoparticles based on glycol chitosan bearing hydrophobic moieties as carriers for doxorubicin: In vivo biodistribution and anti-tumor activity, *Biomaterials*, 2006, **27**, 119–126.
 - 49 J. H. Kim, Y. S. Kim, S. Kim, J. H. Park, K. Kim, K. Choi, H. Chung, S. Y. Jeong, R. W. Park, I. S. Kim and I. C. Kwon, Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel, *J. Controlled Release*, 2006, **111**, 228–234.
 - 50 C. D. M. Amorim, A. G. Couto, D. J. A. Netz, R. A. de Freitas and T. M. B. Bresolin, Antioxidant idebenone-loaded nanoparticles based on chitosan and N-carboxymethylchitosan, *Nanomedicine*, 2010, **6**(6), 745–752.
 - 51 G. Di Colo, Y. Zambito, S. Burgalassi, I. Nardini and M. F. Saettone, Effect of chitosan and of N-carboxymethylchitosan on intraocular penetration of topically applied ofloxacin, *Int. J. Pharm.*, 2004, **273**, 37–44.



- 52 Z. Aiping, L. Jianhong and Y. Wenhui, Effective loading and controlled release of camptothecin by O-carboxymethylchitosan aggregates, *Carbohydr. Polym.*, 2006, **63**, 89–96.
- 53 A. Anitha, S. Maya, N. Deepa, K. P. Chennazhi, S. V. Nair, H. Tamura and R. Jayakumar, Efficient water soluble O-carboxymethyl chitosan nanocarrier for the delivery of curcumin to cancer cells, *Carbohydr. Polym.*, 2011, **83**(2), 452–461.
- 54 A. Zhu, W. Jin, L. Yuan, G. Yang, H. Yu and H. Wu, O-Carboxymethylchitosan-based, novel gatifloxacin delivery system, *Carbohydr. Polym.*, 2007, **68**, 693–700.
- 55 K. S. Snima, R. Jayakumar, A. G. Unnikrishnan, S. V. Nair and V. K. Lakshmanan, O-Carboxymethyl chitosan nanoparticles for metformin delivery to pancreatic cancer cells, *Carbohydr. Polym.*, 2012, **89**(3), 1003–1007.
- 56 Y. Wang, X. Yang, J. Yang, Y. Wang, R. Chen, J. Wu, Y. Liu and N. Zhang, Self-assembled nanoparticles of methotrexate conjugated O-carboxymethyl chitosan: Preparation, characterization and drug release behavior in vitro, *Carbohydr. Polym.*, 2011, **86**(4), 1665–1670.
- 57 S. Maya, S. Indulekha, V. Sukhithasri, K. T. Smitha, S. V. Nair, R. Jayakumar and R. Biswas, Efficacy of tetracycline encapsulated O-carboxymethyl chitosan nanoparticles against intracellular infections of *Staphylococcus aureus*, *Int. J. Biol. Macromol.*, 2012, **51**(4), 392–399.
- 58 A. Trapani, S. Cometa, E. De Giglio, F. Corbo, R. Cassano, M. L. Di Gioia, S. Trombino, M. N. Hossain, S. Di Gioia, G. Trapani and M. Conese, Novel Nanoparticles Based on N,O-Carboxymethyl Chitosan-Dopamine Amide Conjugate for Nose-to-Brain Delivery, *Pharmaceutics*, 2022, **14**(1), 147.
- 59 J. Zhang, X. G. Chen, Y. Y. Li and C. S. Liu, Self-assembled nanoparticles based on hydrophobically modified chitosan as carriers for doxorubicin, *Nanomedicine*, 2007, **3**, 258–265.
- 60 C. Mura, A. Nácher, V. Merino, M. Merino-Sanjuán, M. Manconi, G. Loy, A. M. Fadda and O. Díez-Sales, Design, characterization and in vitro evaluation of 5-aminosalicylic acid loaded N-succinyl-chitosan microparticles for colon specific delivery, *Colloids Surf., B*, 2012, **94**, 199–205.
- 61 C. Yan, D. Chen, J. Gu and J. Qin, Nanoparticles of 5-fluorouracil (5-FU) loaded N-succinyl-chitosan (Suc-Chi) for cancer chemotherapy: preparation, characterization—*in vitro* drug release and anti-tumour activity, *J. Pharm. Pharmacol.*, 2006, **58**, 1177–1181.
- 62 Z. Hou, J. Han, C. Zhan, C. Zhou, Q. Hu and Q. Zhang, Synthesis and evaluation of N-succinyl-chitosan nanoparticles toward local hydroxycamptothecin delivery, *Carbohydr. Polym.*, 2010, **81**(4), 765–768.
- 63 S. T. Huang, Y. Z. Du, H. Yuan, X. G. Zhang, J. Miao, F. D. Cui and F. Q. Hu, Synthesis and anti-hepatitis B virus activity of Acyclovir conjugated stearic acid-g-chitosan oligosaccharide micelle, *Carbohydr. Polym.*, 2011, **83**(4), 1715–1722.
- 64 Y. T. Xie, Y. Z. Du, H. Yuan and F. Q. Hu, Brain-targeting study of stearic acid – grafted chitosan micelle drug-delivery system, *Int. J. Nanomed.*, 2012, **7**, 3235–3244.
- 65 N. Thotakura, M. Dadarwal, P. Kumar, G. Sharma, S. K. Guru, S. Bhushan, K. Raza and O. P. Katare, Chitosan-Stearic Acid Based Polymeric Micelles for the Effective Delivery of Tamoxifen: Cytotoxic and Pharmacokinetic Evaluation, *AAPS PharmSciTech*, 2017, **18**(3), 759–768.
- 66 D. Patel, S. Naik, K. Chuttani, R. Mathur, A. K. Mishra and A. Misra, Intranasal delivery of cyclobenzaprine hydrochloride-loaded thiolated chitosan nanoparticles for pain relief, *J. Drug Targeting*, 2013, **2330**, 1–11.
- 67 A. Kaur, P. Kumar, L. Kaur, R. Sharma and P. Kush, Thiolated chitosan nanoparticles for augmented oral bioavailability of gemcitabine: Preparation, optimization, in vitro and in vivo study, *J. Drug Delivery Sci. Technol.*, 2021, **61**, 102169.
- 68 G. Shahnaz, A. Vetter, J. Barthelmes, D. Rahmat, F. Laffleur, J. Iqbal, G. Perera, W. Schlocker, S. Dünnhaupt, P. Augustijns and A. Bernkop-Schnürch, Thiolated chitosan nanoparticles for the nasal administration of leuprolide: Bioavailability and pharmacokinetic characterization, *Int. J. Pharm.*, 2012, **428**(1–2), 164–170.
- 69 D. W. Lee, S. A. Shirley, R. F. Lockey and S. S. Mohapatra, Thiolated chitosan nanoparticles enhance anti-inflammatory effects of intranasally delivered theophylline, *Respir. Res.*, 2006, **7**(112), 1–10.
- 70 D. Patel, S. Naik and A. Misra, Improved Transnasal Transport and Brain Uptake of Tizanidine HCl-Loaded Thiolated Chitosan Nanoparticles for Alleviation of Pain, *J. Pharm. Sci.*, 2012, **101**(2), 690–706.
- 71 G. Millotti, G. Perera, C. Vigl, K. Pickl, F. M. Sinner and A. Bernkop-Schnürch, The use of chitosan-6-mercaptopyridine acid nanoparticles for oral peptide drug delivery, *Drug Delivery*, 2011, **18**(3), 190–197.
- 72 A. H. Krauland, V. M. Leitner, V. Grabovac and A. Bernkop-Schnürch, In Vivo Evaluation of a Nasal Insulin Delivery System Based on Thiolated Chitosan, *J. Pharm. Sci.*, 2006, **95**(11), 2463–2472.
- 73 X. Wang, C. Zheng, Z. Wu, D. Teng, X. Zhang, Z. Wang and C. Li, Chitosan-NAC Nanoparticles as a Vehicle for Nasal Absorption Enhancement of Insulin, *J. Biomed. Mater. Res., Part B*, 2008, 150–161.
- 74 S. Jaiswal, P. K. Dutta, S. Kumar, J. Koh, M. C. Lee, J. W. Lim, S. Pandey and P. Garg, Synthesis, characterization and application of chitosan-N-(4-hydroxyphenyl)-methacrylamide derivative as a drug and gene carrier, *Int. J. Biol. Macromol.*, 2022, **195**, 75–85.
- 75 X. Zhang, H. Zhang, Z. Wu, Z. Wang, H. Niu and C. Li, Nasal absorption enhancement of insulin using PEG-grafted chitosan nanoparticles, *Eur. J. Pharm. Biopharm.*, 2008, **68**, 526–534.
- 76 M. Jia, Y. Li, X. Yang, Y. Huang, H. Wu, Y. Huang, J. Lin, Y. Li, Z. Hou and Q. Zhang, Development of Both Methotrexate and Mitomycin C Loaded PEGylated Chitosan Nanoparticles for Targeted Drug Codelivery and Synergistic Anticancer Effect, *ACS Appl. Mater. Interfaces*, 2014, **6**, 11413–11423.



- 77 S. Agrawal, H. Ahmad, M. Dwivedi, M. Shukla, A. Arya, K. Sharma, J. Lal and A. K. Dwivedi, PEGylated chitosan nanoparticles potentiate repurposing of ormeloxifene in breast cancer therapy, *Nanomedicine*, 2016, **11**(16), 2147–2169.
- 78 S. Pandian, V. Jeevanesan, C. Ponnusamy and S. Natesan, RES-loaded pegylated CS NPs: for efficient ocular delivery, *IET Nanobiotechnol.*, 2017, **11**(1), 32–39.
- 79 M. Rameshbhai, J. Manikkath, K. Sivakumar, R. S. Managuli, K. Gourishetti, N. Krishnadas, R. R. Shenoy, B. Jayaprakash, C. Mallikarjuna and S. Mutalik, Long circulating PEGylated-chitosan nanoparticles of rosuvastatin calcium: Development and in vitro and in vivo evaluations, *Int. J. Biol. Macromol.*, 2018, **107**, 2190–2200.
- 80 M. N. Melo, F. M. Pereira, M. A. Rocha, J. G. Ribeiro, A. Junges, W. F. Monteiro, F. M. Diz, R. A. Ligabue, F. B. Morrone, P. Severino and A. T. Fricks, Chitosan and chitosan/PEG nanoparticles loaded with indole-3-carbinol: Characterization, computational study and potential effect on human bladder cancer cells, *Mater. Sci. Eng., C*, 2021, **124**, 112089.
- 81 J. Wang, Z. Guo, J. Xiong, D. Wu, S. Li, Y. Tao, Y. Qin and Y. Kong, Facile synthesis of chitosan-grafted beta-cyclodextrin for stimuli-responsive drug delivery, *Int. J. Biol. Macromol.*, 2019, **125**, 941–947.
- 82 Z. Yuan, Y. Ye, F. Gao, H. Yuan, M. Lan, K. Lou and W. Wang, Chitosan-graft- β -cyclodextrin nanoparticles as a carrier for controlled drug release, *Int. J. Pharm.*, 2013, **446**(1–2), 191–198.
- 83 M. He, C. Zhong, H. Hu, Y. Jin, Y. Chen, K. Lou and F. Gao, Cyclodextrin/chitosan nanoparticles for oral ovalbumin delivery: Preparation, characterization and intestinal mucosal immunity in mice, *Asian J. Pharm. Sci.*, 2019, **14**(2), 193–203.
- 84 A. Almeida, D. Silva, V. Gonçalves and B. Sarmiento, Synthesis and characterization of chitosan-grafted-polycaprolactone micelles for modulate intestinal paclitaxel delivery, *Drug Delivery Transl. Res.*, 2018, **8**, 387–397.
- 85 B. Lu, X. Lv and Y. Le, Chitosan-Modified PLGA Nanoparticles for Control-Release Drug Delivery, *Polymers*, 2019, **11**, 304.
- 86 S. S. Chakravarthi and D. H. Robinson, Enhanced cellular association of paclitaxel delivered in chitosan-PLGA particles, *Int. J. Pharm.*, 2011, **409**(1–2), 111–120.
- 87 C. Kiran, N. Thotakura, R. Kumar, P. Kumar, B. Singh, D. Chitkara and K. Raza, Chitosan-modified PLGA polymeric nanocarriers with better delivery potential for tamoxifen, *Int. J. Biol. Macromol.*, 2016, **93**, 381–389.
- 88 P. Dhiman and M. Bhatia, Ketoconazole loaded quaternized chitosan nanoparticles-PVA film: preparation and evaluation, *Polym. Bull.*, 2022, **79**(2), 1001–1019.
- 89 A. Domard, M. Rinaudo and C. Terrassin, New method for the quaternization of chitosan, *Int. J. Biol. Macromol.*, 1986, **8**, 105–107.
- 90 P. L. Dung, M. Milas, M. Rinaudo and J. Desbrières, Water soluble derivatives obtained by controlled chemical modifications of chitosan, *Carbohydr. Polym.*, 1994, **24**, 0–5.
- 91 A. Jintapattanakit, S. Mao, T. Kissel and V. B. Junyaprasert, Physicochemical properties and biocompatibility of N-trimethyl chitosan: Effect of quaternization and dimethylation, *Eur. J. Pharm. Biopharm.*, 2008, **70**, 563–571.
- 92 C. V. Pardeshi and V. S. Belgamwar, Controlled synthesis of N, N, N-trimethyl chitosan for modulated bioadhesion and nasal membrane permeability, *Int. J. Biol. Macromol.*, 2016, **82**, 933–944.
- 93 L. H. du Plessis, A. F. Kotzé and H. E. Junginger, Nasal and rectal delivery of insulin with chitosan and N-trimethyl chitosan chloride, *Drug Delivery*, 2010, **17**(6), 399–407.
- 94 H. Ren, S. Liu, J. Yang, X. Zhang, H. Zhou, J. Chen and T. Guo, N, N, N-trimethylchitosan modified with well defined multifunctional polymer modules used as pDNA delivery vector, *Carbohydr. Polym.*, 2016, **137**, 222–230.
- 95 Y. Zhou, H. Yang, X. Liu, J. Mao, S. Gu and W. Xu, Potential of quaternization-functionalized chitosan fiber for wound dressing, *Int. J. Biol. Macromol.*, 2013, **52**, 327–332.
- 96 G. K. Khaira, R. Kumariya, M. Chibber and M. Ghosh, Development of a quaternized chitosan with enhanced antibacterial efficacy, *J. Water Health*, 2013, **11.3**, 410–418.
- 97 J. Zhang, W. Tan, G. Wang, X. Yin, Q. Li, F. Dong and Z. Guo, Synthesis, characterization, and the antioxidant activity of N, N, N-trimethyl chitosan salts, *Int. J. Biol. Macromol.*, 2018, **118**, 9–14.
- 98 J. Desbrières, C. Martinez and M. Rinaudo, Hydrophobic derivatives of chitosan: Characterization and rheological behaviour, *Int. J. Biol. Macromol.*, 1996, **19**, 21–28.
- 99 T. K. Karam, S. Ortega, T. U. Nakamura, R. Auzély-velty and C. V. Nakamura, Development of chitosan nanocapsules containing essential oil of *Matricaria chamomilla* L. for the treatment of cutaneous leishmaniasis, *Int. J. Biol. Macromol.*, 2020, **162**, 199–208.
- 100 Q. Dang, Q. Zhang, C. Liu, J. Yan, G. Chang, Y. Xin, X. Cheng, Y. Cao, H. Gao and Y. Liu, Decanoic acid functionalized chitosan: Synthesis, characterization, and evaluation as potential wound dressing material, *Int. J. Biol. Macromol.*, 2019, **139**, 1046–1053.
- 101 W. G. Liu, X. Zhang, S. J. Sun, G. J. Sun, K. De Yao, D. C. Liang, G. Guo and J. Y. Zhang, N-Alkylated Chitosan as a Potential Nonviral Vector for Gene, *Bioconjugate Chem.*, 2003, **14**, 782–789.
- 102 V. K. Mourya and N. N. Inamdar, Chitosan modifications and applications: Opportunities galore, *React. Funct. Polym.*, 2008, **68**, 1013–1051.
- 103 S. Mallick, S. J. Song, Y. Bae and J. S. Choi, Self-assembled nanoparticles composed of glycol chitosan-dequalinium for mitochondria-targeted drug delivery, *Int. J. Biol. Macromol.*, 2019, **132**, 451–460.
- 104 N. Duhem, J. Rolland, R. Riva, P. Guillet, J. M. Schumers, C. Jérôme, J. F. Gohy and V. Préat, Tocol modified glycol chitosan for the oral delivery of poorly soluble drugs, *Int. J. Pharm.*, 2012, **423**, 452–460.



- 105 A. Siew, H. Le, M. Thiovolet, P. Gellert, A. Schatzlein and I. Uchegbu, Enhanced Oral Absorption of Hydrophobic and Hydrophilic Drugs Using Quaternary Ammonium Palmitoyl Glycol Chitosan Nanoparticles, *Mol. Pharm.*, 2012, **9**, 14–28.
- 106 M. C. Bonferoni, G. Sandri, E. Dellera, S. Rossi, F. Ferrari, Y. Zambito and C. Caramella, Palmitoyl Glycol Chitosan Micelles for Corneal Delivery of Cyclosporine, *J. Biomed. Nanotechnol.*, 2016, **12**(1), 231–240.
- 107 I. F. Uchegbu, L. Sadiq, M. Arastoo, A. I. Gray, W. Wang, R. D. Waigh and A. G. Schätzleina, Quaternary ammonium palmitoyl glycol chitosan—a new polysoap for drug delivery, *Int. J. Pharm.*, 2001, **224**, 185–199.
- 108 I. F. Uchegbu, L. Sadiq, A. Pardakhty, M. El-hammadi, A. I. Gray, L. Tetley, W. Wang, B. Zinselmeyer and A. Schätzlein, Gene Transfer with Three Amphiphilic Glycol Chitosans—the Degree of Polymerisation is the Main Controller of Transfection Efficiency, *J. Drug Targeting*, 2004, **12**(8), 527–539.
- 109 A. Lalatsa, N. L. Garrett, T. Ferrarelli, J. Moger, A. G. Schätzlein and I. F. Uchegbu, Delivery of Peptides to the Blood and Brain after Oral Uptake of Quaternary Ammonium Palmitoyl Glycol Chitosan Nanoparticles, *Mol. Pharm.*, 2012, **9**, 1764–1774.
- 110 J. M. Yu, Y. J. Li, L. Qiu and Y. Jin, Self-aggregated nanoparticles of cholesterol-modified glycol chitosan conjugate: Preparation, characterization, and preliminary assessment as a new drug delivery carrier, *Eur. Polym. J.*, 2008, **44**, 555–565.
- 111 J. S. Park, T. H. Han, K. Y. Lee, S. S. Han, J. J. Hwang, D. H. Moon, S. Y. Kim and Y. W. Cho, N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles for intracytoplasmic delivery of drugs: Endocytosis, exocytosis and drug release, *J. Controlled Release*, 2006, **115**, 37–45.
- 112 R. A. A. Muzzarelli, Carboxymethylated Chitins and Chitosans, *Carbohydr. Polym.*, 1988, **8**, 1–21.
- 113 X. G. Chen and H. J. Park, Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions, *Carbohydr. Polym.*, 2003, **53**, 355–359.
- 114 R. Jayakumar, M. Prabakaran, S. V. Nair, S. Tokura, H. Tamura and N. Selvamurugan, Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications, *Prog. Mater. Sci.*, 2010, **55**(7), 675–709.
- 115 M. Rinaudo, P. Le Dung, C. Gey and M. Milas, Substituent distribution on O, N-carboxymethylchitosans by ¹H and ¹³C N.M.R., *Int. J. Biol. Macromol.*, 1992, **14**, 122–128.
- 116 R. A. A. Muzzarelli, F. Tanfani, M. Emanuelli and S. Mariotti, N-(Carboxymethylidene)Chitosans and N-(Carboxymethyl)-Chitosans: Novel Chelating Polyampholytes Obtained From Chitosan Glyoxylate, *Carbohydr. Res.*, 1982, **107**, 199–214.
- 117 S. Adnan, N. M. Ranjha, M. Hanif and S. Asghar, O-Carboxymethylated, chitosan; A promising tool with *in vivo* anti-inflammatory and analgesic properties in albino rats, *Int. J. Biol. Macromol.*, 2020, **156**, 531–536.
- 118 Y. C. Huang and T. H. Kuo, O-carboxymethyl chitosan/fucoidan nanoparticles increase cellular curcumin uptake, *Food Hydrocolloids*, 2016, **53**, 261–269.
- 119 C. G. Liu, X. G. Chen and H. J. Park, Self-assembled nanoparticles based on linoleic-acid modified chitosan: Stability and adsorption of trypsin, *Carbohydr. Polym.*, 2005, **62**, 293–298.
- 120 M. R. Rekha and C. P. Sharma, Synthesis and evaluation of lauryl succinyl chitosan particles towards oral insulin delivery and absorption, *J. Controlled Release*, 2009, **135**(2), 144–151.
- 121 Y. L. Chiu, Y. C. Ho, Y. M. Chen, S. F. Peng, C. J. Ke, K. J. Chen, F. L. Mi and H. W. Sung, The characteristics, cellular uptake and intracellular trafficking of nanoparticles made of hydrophobically-modified chitosan, *J. Controlled Release*, 2010, **146**, 152–159.
- 122 Y. H. Kim, S. H. Gihm, C. R. Park, K. Y. Lee, T. W. Kim, I. C. Kwon, H. Chung and S. Y. Jeong, Structural Characteristics of Size-Controlled Self-Aggregates of Deoxycholic Acid-Modified Chitosan and Their Application as a DNA Delivery Carrier, *Bioconjugate Chem.*, 2001, **122**, 932–938.
- 123 K. Y. Lee, I. C. Kwon, Y. Kim, W. H. Jo and S. Y. Jeong, Preparation of chitosan self-aggregates as a gene delivery system, *J. Controlled Release*, 1998, **51**, 213–220.
- 124 S. Bashir, Y. Y. Teo, S. Ramesh, K. Ramesh and A. A. Khan, N-succinyl chitosan preparation, characterization, properties and biomedical applications: a state of the art review, *Rev. Chem. Eng.*, 2015, **31**(6), 563–597.
- 125 Z. Aiping, C. Tian, Y. Lanhua, W. Hao and L. Ping, Synthesis and characterization of N-succinyl-chitosan and its self-assembly of nanospheres, *Carbohydr. Polym.*, 2006, **66**, 274–279.
- 126 Y. Kato, H. Onishi and Y. Machida, Biological characteristics of lactosaminated N-succinyl-chitosan as a liver-specific drug carrier in mice, *J. Controlled Release*, 2001, **70**, 295–307.
- 127 Y. Kato, H. Onishi and Y. Machida, Evaluation of N-succinyl-chitosan as a systemic long-circulating polymer, *Biomaterials*, 2000, **21**, 2–4.
- 128 Y. Kato, H. Onishi and Y. Machida, N-succinyl-chitosan as a drug carrier: water-insoluble and water-soluble conjugates, *Biomaterials*, 2004, **25**(1), 907–915.
- 129 X. Xiangyang, L. Ling, Z. Jianping, L. Shiyue, Y. Jie, Y. Xiaojin and R. Jinsheng, Preparation and characterization of N-succinyl-N'-octyl chitosan micelles as doxorubicin carriers for effective anti-tumor activity, *Colloids Surf., B*, 2007, **55**, 222–228.
- 130 B. Nanda, A. S. Manjappa, K. Chuttani, N. H. Balasinar, A. K. Mishra and R. S. R. Murthy, Acylated chitosan anchored paclitaxel loaded liposomes: Pharmacokinetic and biodistribution study in Ehrlich ascites tumor bearing mice, *Int. J. Biol. Macromol.*, 2019, **122**, 367–379.
- 131 Y. Cho, T. J. Kim and H. J. Park, Size-controlled self-aggregated N-acyl chitosan nanoparticles as a vitamin C carrier, *Carbohydr. Polym.*, 2012, **88**(3), 1087–1092.



- 132 M. I. Alvarez Echazú, M. E. Antona, O. Perna, C. E. Olivetti, G. S. Alvarez, E. V. Macri, C. J. Perez, M. Czerner, S. M. Friedman and M. F. Desimone, Dodecenylsuccinic anhydride modified chitosan hydrogels for the sustained delivery of hydrophobic drugs. The case of thymol buccal delivery, *J. Appl. Polym. Sci.*, 2022, **139**(1), 1–13.
- 133 C. E. Kast and A. Bernkop-Schnürch, Thiolated polymers - thiomers: development and in vitro evaluation of chitosan - thioglycolic acid conjugates, *Biomaterials*, 2001, **22**, 2345–2352.
- 134 A. Bernkop-Schnürch and T. E. Hopf, Synthesis and in Vitro Evaluation of Chitosan- Thioglycolic Acid Conjugates, *Sci. Pharm.*, 2001, **69**, 109–118.
- 135 D. Sakloetsakun, J. M. R. Hombach and A. Bernkop-Schnürch, In situ gelling properties of chitosan-thioglycolic acid conjugate in the presence of oxidizing agents, *Biomaterials*, 2009, **30**, 6151–6157.
- 136 M. Roldo, M. Hornof, P. Caliceti and A. Bernkop-Schnürch, Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation, *Eur. J. Pharm. Biopharm.*, 2004, **57**, 115–121.
- 137 A. Bernkop-Schnürch, M. Hornof and T. Zoidl, Thiolated polymers—thiomers: synthesis and in vitro evaluation of chitosan-2-iminothiolane conjugates, *Int. J. Pharm.*, 2003, **260**, 229–237.
- 138 K. Kafedjiiski, F. Föger, M. Werle and A. Bernkop-Schnürch, Synthesis and in vitro evaluation of a novel chitosan-glutathione conjugate, *Pharm. Res.*, 2005, **22**(9), 1480–1488.
- 139 W. Zhao, M. Kong, C. Feng, X. Cheng, Y. Liu and X. Chen, Investigation of gelling behavior of thiolated chitosan in alkaline condition and its application in stent coating, *Carbohydr. Polym.*, 2016, **136**, 307–315.
- 140 V. M. Leitner, G. F. Walker and A. Bernkop-Schnürch, Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins, *Eur. J. Pharm. Biopharm.*, 2003, **56**, 207–214.
- 141 A. H. Krauland, D. Guggi and A. Bernkop-Schnürch, Thiolated chitosan microparticles: A vehicle for nasal peptide drug delivery, *Int. J. Pharm.*, 2006, **307**, 270–277.
- 142 D. Sakloetsakun, J. Iqbal, G. Millotti, A. Vetter and A. Bernkop-Schnürch, Thiolated chitosans: influence of various sulfhydryl ligands on permeation-enhancing and P-gp inhibitory properties, *Drug Dev. Ind. Pharm.*, 2011, **37**(6), 648–655.
- 143 R. Esquivel, J. Juárez, M. Almada, J. Ibarra and M. A. Valdez, Synthesis and Characterization of New Thiolated Chitosan Nanoparticles Obtained by Ionic Gelation Method, *Int. J. Polym. Sci.*, 2015, **2015**, 18.
- 144 S. Maria, H. S. Sarwar, M. F. Sohail, M. Imran, O. S. Qureshi, A. Raza, N. M. Ahmad, A. Iqbal and G. Shahnaz, Synthesis and characterization of pre-activated thiolated chitosan nanoparticles for oral delivery of octreotide, *J. Drug Delivery Sci. Technol.*, 2020, 58.
- 145 A. Anitha, N. Deepa, K. P. Chennazhi, V. K. Lakshmanan and R. Jayakumar, Combinatorial anticancer effects of curcumin and 5-fluorouracil loaded thiolated chitosan nanoparticles towards colon cancer treatment, *Biochim. Biophys. Acta*, 2014, **1840**(9), 2730–2743.
- 146 J. Iqbal, G. Shahnaz, G. Perera, F. Hintzen, F. Sarti and A. Bernkop-schnürch, Thiolated chitosan: Development and in vivo evaluation of an oral delivery system for leuprolide, *Eur. J. Pharm. Biopharm.*, 2012, **80**(1), 95–102.
- 147 Y. I. Jeong, D. G. Kim, M. K. Jang and J. W. Nah, Preparation and spectroscopic characterization of methoxy poly(ethylene glycol)-grafted water-soluble chitosan, *Carbohydr. Res.*, 2008, **343**, 282–289.
- 148 L. Deng, H. Qi, C. Yao, M. Feng and A. Dong, Investigation on the properties of methoxy poly(ethylene glycol)/chitosan graft co-polymers, *J. Biomater. Sci.*, 2012, **18**(12), 1575–1589.
- 149 L. Casettari, D. Vllasaliu, E. Castagnino, S. Stolnik, S. Howdle and L. Illum, PEGylated chitosan derivatives: Synthesis, characterizations and pharmaceutical applications, *Prog. Polym. Sci.*, 2012, **37**(5), 659–685.
- 150 Y. Hu, H. Jiang, C. Xu, Y. Wang and K. Zhu, Preparation and characterization of poly (ethylene glycol)-g-chitosan with water- and organosolubility, *Carbohydr. Polym.*, 2005, **61**, 472–479.
- 151 N. Gorochovceva and R. Makuska, Synthesis and study of water-soluble chitosan-O-poly (ethylene glycol) graft copolymers, *Eur. Polym. J.*, 2004, **40**, 685–691.
- 152 R. Makuska and N. Gorochovceva, Regioselective grafting of poly (ethylene glycol) onto chitosan through C-6 position of glucosamine units, *Carbohydr. Polym.*, 2006, **64**, 319–327.
- 153 M. Malhotra, C. Lane, C. Tomaro-Duchesneau, S. Saha and S. Prakash, A novel method for synthesizing PEGylated chitosan nanoparticles: strategy, preparation, and in vitro analysis, *Int. J. Nanomed.*, 2011, **6**, 485–494.
- 154 X. Li, X. Kong, S. Shi, X. Wang, Y. Gu, G. Guo, Y. Mao, F. Luo, X. Zhao, Y. Wei and Z. Qian, Preparation, Characterization, and Self-assembly Behavior of a Novel MPEG/PCL-g-Chitosan Copolymer, *Soft Mater.*, 2010, **8**(4), 320–327.
- 155 R. Kulbokaite, G. Ciuta, M. Netopilik and R. Makuska, N-PEGylation of chitosan via “click chemistry” reactions, *React. Funct. Polym.*, 2009, **69**(10), 771–778.
- 156 A. R. Kulkarni, V. I. Hukkeri, H. W. Sung and H. F. Liang, A Novel Method for the Synthesis of the PEG-Crosslinked Chitosan with a pH-Independent Swelling Behavior, *Macromol. Biosci.*, 2005, **5**, 925–928.
- 157 X. Yang, Q. Zhang, Y. Wang, H. Chen, H. Zhang, F. Gao and L. Liu, Self-aggregated nanoparticles from methoxy poly(ethylene glycol)-modified chitosan: Synthesis; characterization; aggregation and methotrexate release in vitro, *Colloids Surf., B*, 2008, **61**, 125–131.
- 158 P. Chan, M. Kurisawa, J. E. Chung and Y. Y. Yang, Synthesis and characterization of chitosan-g-poly(ethylene glycol) -folate as a non-viral carrier for tumor-targeted gene delivery, *Biomaterials*, 2007, **28**, 540–549.



- 159 S. Mao, O. Germershaus, D. Fischer, T. Linn, R. Schnepf and T. Kissel, Uptake and Transport of PEG-Graft-Trimethyl-Chitosan Copolymer – Insulin Nanocomplexes by Epithelial Cells, *Pharm. Res.*, 2005, **22**(12), 2058–2068.
- 160 K. H. Bae, C. W. Moon, Y. Lee and T. G. Park, Intracellular Delivery of Heparin Complexed with Chitosan-g-Poly (Ethylene Glycol) for Inducing Apoptosis, *Pharm. Res.*, 2009, **26**(1), 93–100.
- 161 Y. Aktas, M. Yemisci, K. Andrieux, R. N. Gursoy, M. J. Alonso, E. Fernandez-Megia, R. Novoa-Carballal, E. Quiñoá, R. Riguera, M. F. Sargon, H. H. Çelik, A. S. Demir, A. A. Hincal, T. Dalkara, Y. Çapan and P. Couvreur, Development and Brain Delivery of Chitosan - PEG Nanoparticles Functionalized with the Monoclonal Antibody OX26, *Bioconjugate Chem.*, 2005, **16**(6), 1503–1511.
- 162 W. Sun, S. Mao, Y. Wang, V. B. Junyaprasert, T. Zhang, L. Na and J. Wang, Bioadhesion and oral absorption of enoxaparin nanocomplexes, *Int. J. Pharm.*, 2010, **386**, 275–281.
- 163 X. G. Zhang, D. Y. Teng, Z. M. Wu, X. Wang, Z. Wang, D. M. Yu and C. X. Li, PEG-grafted chitosan nanoparticles as an injectable carrier for sustained protein release, *J. Mater. Sci.: Mater. Med.*, 2008, **19**, 3525–3533.
- 164 Y. I. Jeong, S. H. Kim, T. Y. Jung, I. Y. Kim, S. S. Kang, Y. H. Jin, H. H. Ryu, H. S. Sun, S. Jin, K. K. Kim, K. Y. Ahn and S. Jung, Polyion Complex Micelles Composed of All-Trans Retinoic Acid and Poly(Ethylene Glycol)-Grafted-Chitosan, *J. Pharm. Sci.*, 2006, **95**(11), 2348–2360.
- 165 J. A. del Olmo, J. M. Alonso, V. Sáez-Martínez, S. Benito-Cid, I. Moreno-Benítez, M. Bengoa-Larrauri, R. Pérez-González, J. L. Vilas-Vilela and L. Pérez-Álvarez, Self-healing, antibacterial and anti-inflammatory chitosan-PEG hydrogels for ulcerated skin wound healing and drug delivery, *Biomater. Adv.*, 2022, **139**, 212992.
- 166 M. Anraku, A. Hiraga, D. Iohara, K. Uekama, H. Tomida, M. Otagiri and F. Hirayama, Preparation and antioxidant activity of PEGylated chitosans with different particle sizes, *Int. J. Biol. Macromol.*, 2014, **70**, 64–69.
- 167 C. Yang, S. Gao, F. Dagnæs-hansen, M. Jakobsen and J. Kjems, Impact of PEG Chain Length on the Physical Properties and Bioactivity of PEGylated Chitosan/siRNA Nanoparticles in Vitro and in Vivo, *ACS Appl. Mater. Interfaces*, 2017, **9**, 12203–12216.
- 168 M. Malhotra, C. Tomaro-Duchesneau, S. Saha and S. Prakash, Intranasal, siRNA Delivery to the Brain by TAT/MGF Tagged PEGylated Chitosan Nanoparticles, *J. Pharm.*, 2013, 812387.
- 169 G. Liu, K. Li and H. Wang, Polymeric micelles based on PEGylated chitosan-g-lipoic acid as carrier for efficient intracellular drug delivery, *J. Biomater. Appl.*, 2017, **31**(7), 1039–1048.
- 170 X. Hu, Y. Zhang, H. Zhou and H. Wan, PEGylated chitosan microspheres as mucoadhesive drug-delivery carriers for puerarin, *J. Appl. Polym. Sci.*, 2015, **42623**, 1–9.
- 171 A. H. Najafabadi, M. Abdouss and S. Faghihi, Synthesis and evaluation of PEG-O-chitosan nanoparticles for delivery of poor water soluble drugs: Ibuprofen, *Mater. Sci. Eng., C*, 2014, **41**, 91–99.
- 172 U. Janciauskaite, V. Rakutyte, J. Miskinis and R. Makuska, Synthesis and properties of chitosan-N-dextran graft copolymers, *React. Funct. Polym.*, 2008, **68**, 787–796.
- 173 Y. K. Park, Y. H. Park, B. A. Shin, E. S. Choi, Y. R. Park, T. Akaike and C. S. Cho, Galactosylated chitosan-graft-dextran as hepatocyte-targeting DNA carrier, *J. Controlled Release*, 2000, **69**, 97–108.
- 174 I. K. Park, J. E. Ihm, Y. H. Park, Y. J. Choi, S. I. Kim, W. J. Kim, T. Akaike and C. S. Cho, Galactosylated chitosan (GC)-graft-poly (vinyl pyrrolidone) (PVP) as hepatocyte-targeting DNA carrier Preparation and physicochemical characterization of GC-graft-PVP/DNA complex (1), *J. Controlled Release*, 2003, **86**, 349–359.
- 175 S. Tang, Z. Huang, H. Zhang, Y. Wang, Q. Hu and H. Jiang, Design and formulation of trimethylated chitosan-graft- poly(e-caprolactone) nanoparticles used for gene delivery, *Carbohydr. Polym.*, 2014, **101**, 104–112.
- 176 C. Gu, V. Le, M. Lang and J. Liu, Preparation of polysaccharide derivatives chitosan-graft-poly(e-caprolactone) amphiphilic copolymer micelles for 5-fluorouracil drug delivery, *Colloids Surf., B*, 2014, **116**, 745–750.
- 177 X. Guan, D. Quan, X. Shuai, K. Liao and K. Mai, Chitosan-graft-Poly (e-caprolactone)s : An Optimized Chemical Approach Leading to a Controllable Structure and Enhanced Properties, *J. Polym. Sci., Part A: Polym. Chem.*, 2007, **45**, 2556–2568.
- 178 N. Zhou, X. Zan, Z. Wang, H. Wu, D. Yin, C. Liao and Y. Wan, Galactosylated chitosan-polycaprolactone nanoparticles for hepatocyte-targeted delivery of curcumin, *Carbohydr. Polym.*, 2013, **94**(1), 420–429.
- 179 J. Q. Gao, Q. Q. Zhao, T. F. Lv, W. P. Shuai, J. Zhou, G. P. Tang, W. Q. Liang, Y. Tabata and Y. L. Hu, Gene-carried chitosan-linked-PEI induced high gene transfection efficiency with low toxicity and significant tumor-suppressive activity, *Int. J. Pharm.*, 2010, **387**(1–2), 286–294.
- 180 Z. T. Li, J. Guo, J. S. Zhang, Y. P. Zhao, L. Lv, C. Ding and X. Z. Zhang, Chitosan-graft-polyethylenimine with improved properties as a potential gene vector, *Carbohydr. Polym.*, 2010, **80**(1), 254–259.
- 181 H. L. Jiang, Y. K. Kim, R. Arote, J. W. Nah, M. H. Cho, Y. J. Choi, T. Akaike and C. S. Cho, Chitosan-graft-polyethylenimine as a gene carrier, *J. Controlled Release*, 2007, **117**, 273–280.
- 182 B. Lu, X. D. Xu, X. Z. Zhang, S. X. Cheng and R. X. Zhuo, Low Molecular Weight Polyethylenimine Grafted N-Maleated Chitosan for Gene Delivery: Properties and In Vitro Transfection Studies, *Biomacromolecules*, 2008, **9**, 2594–2600.
- 183 B. Lu, Y. X. Sun, Y. Q. Li, X. Z. Zhang and R. X. Zhuo, N-Succinyl-chitosan grafted with low molecular weight polyethylenimine as a serum-resistant gene vector, *Mol. Biosyst.*, 2009, **5**, 629–637.
- 184 X. Wan, J. Chen, C. H. I. Cheng, H. Zhang, S. Zhao, J. Li, X. Lv, Z. Wang and R. Gao, Improved expression of recom-



- binant fusion defensin gene plasmids packed with chitosan-derived nanoparticles and effect on antibacteria and mouse immunity, *Exp. Ther. Med.*, 2018, **16**, 3965–3972.
- 185 L. Liu, X. Xu, S. Guo and W. Han, Synthesis and self-assembly of chitosan-based copolymer with a pair of hydrophobic/hydrophilic grafts of polycaprolactone and poly(ethylene glycol), *Carbohydr. Polym.*, 2009, **75**(3), 401–407.
 - 186 Y. Lu, L. Liu and S. Guo, Novel Amphiphilic Ternary Polysaccharide Derivates Chitosan-g-PCL-b-MPEG: Synthesis, Characterization, and Aggregation in Aqueous Solution, *Biopolymers*, 2007, **86**(5), 403–408.
 - 187 C. Chen, G. Cai, H. Zhang, H. Jiang and L. Wang, Chitosan-poly(ϵ -caprolactone)-poly(ethylene glycol) graft copolymers: Synthesis, self-assembly, and drug release behavior, *J. Biomed. Mater. Res., Part A*, 2010, 116–124.
 - 188 E. Furusaki, Y. Ueno, N. Sakairi, N. Nishi and S. Tokura, Facile preparation and inclusion ability of a chitosan derivative bearing carboxymethyl- β -cyclodextrin, *Carbohydr. Polym.*, 1996, **29**(1), 29–34.
 - 189 A. H. Krauland and M. J. Alonso, Chitosan/cyclodextrin nanoparticles as macromolecular drug delivery system, *Int. J. Pharm.*, 2007, **340**, 134–142.
 - 190 A. Trapani, A. Lopodota, M. Franco, N. Cioffi, E. Ieva, M. Garcia-Fuentes and M. J. Alonso, A comparative study of chitosan and chitosan/cyclodextrin nanoparticles as potential carriers for the oral delivery of small peptides, *Eur. J. Pharm. Biopharm.*, 2010, **75**(1), 26–32.
 - 191 X. Zhang, Z. Wu, X. Gao, S. Shu, H. Zhang, Z. Wang and C. Li, Chitosan bearing pendant cyclodextrin as a carrier for controlled protein release, *Carbohydr. Polym.*, 2009, **77**(2), 394–401.
 - 192 L. Lu, X. Shao, Y. Jiao and C. Zhou, Synthesis of Chitosan-graft- β -Cyclodextrin for Improving the Loading and Release of Doxorubicin in the Nanoparticles, *J. Appl. Polym. Sci.*, 2014, 41033–41040.
 - 193 H. Izawa, K. Yamamoto, S. Yoshihashi, S. Ifuku, M. Morimoto and H. Saimoto, Facile preparation of cyclodextrin-grafted chitosans and their conversion into nanoparticles for an anticancer drug delivery system, *Polym. J.*, 2016, 203–207.
 - 194 M. Song, L. Li, Y. Zhang, K. Chen, H. Wang and R. Gong, Carboxymethyl- β -cyclodextrin grafted chitosan nanoparticles as oral delivery carrier of protein drugs, *React. Funct. Polym.*, 2017, **117**, 10–15.
 - 195 F. Maestrelli, M. Garcia-Fuentes, P. Mura and M. J. Alonso, A new drug nanocarrier consisting of chitosan and hydroxypropylcyclodextrin, *Eur. J. Pharm. Biopharm.*, 2006, **63**, 79–86.
 - 196 S. C. Bhatia and N. Ravi, A magnetic study of an Fe-chitosan complex and its relevance to other biomolecules, *Biomacromolecules*, 2000, **1**(3), 413–417.
 - 197 F. Reynaud, N. Tsapis, M. Deyme, T. G. Vasconcelos, C. Gueutin, S. S. Guterres, A. R. Pohlmann and E. Fattal, Spray-dried chitosan-metal microparticles for ciprofloxacin adsorption: Kinetic and equilibrium studies, *Soft Matter*, 2011, **7**(16), 7304–7312.
 - 198 A. C. Zimmermann, A. Mecabô, T. Fagundes and C. A. Rodrigues, Adsorption of Cr(VI) using Fe-crosslinked chitosan complex (Ch-Fe), *J. Hazard. Mater.*, 2010, **179**(1–3), 192–196.
 - 199 X. Wang, Y. Du and H. Liu, Preparation, characterization and antimicrobial activity of chitosan-Zn complex, *Carbohydr. Polym.*, 2004, **56**(1), 21–26.
 - 200 G. Giacalone, H. Hillaireau, P. Capiou, H. Chacun, F. Reynaud and E. Fattal, Stabilization and cellular delivery of chitosan-polyphosphate nanoparticles by incorporation of iron, *J. Controlled Release*, 2014, **194**, 211–219.
 - 201 D. Zheng, C. Duan, D. Zhang, L. Jia, G. Liu, Y. Liu, F. Wang, C. Li, H. Guo and Q. Zhang, Galactosylated chitosan nanoparticles for hepatocyte-targeted delivery of oridonin, *Int. J. Pharm.*, 2012, **436**(1–2), 379–386.
 - 202 Q. Wang, L. Zhang, W. Hu, Z. H. Hu, Y. Y. Bei, J. Y. Xu, W. J. Wang, X. N. Zhang and Q. Zhang, Norcantharidin-associated galactosylated chitosan nanoparticles for hepatocyte-targeted delivery, *Nanomedicine*, 2010, **6**(2), 371–381.
 - 203 H. Zheng, X. Zhang, F. Xiong, Z. Zhu, B. Lu, Y. Yin, P. Xu and Y. Du, Preparation, characterization, and tissue distribution in mice of lactosaminated carboxymethyl chitosan nanoparticles, *Carbohydr. Polym.*, 2011, **83**(3), 1139–1145.
 - 204 B. Song, W. Zhang, R. Peng, J. Huang, T. Nie, Y. Li, Q. Jiang and R. Gao, Synthesis and cell activity of novel galactosylated chitosan as a gene carrier, *Colloids Surf., B*, 2009, **70**(2), 181–186.
 - 205 S. Gao, J. Chen, L. Dong, Z. Ding, Y. H. Yang and J. Zhang, Targeting delivery of oligonucleotide and plasmid DNA to hepatocyte via galactosylated chitosan vector, *Eur. J. Pharm. Biopharm.*, 2005, **60**(3), 327–334.
 - 206 S. Gao, J. Chen, X. Xu, Z. Ding, Y. H. Yang, Z. Hua and J. Zhang, Galactosylated low molecular weight chitosan as DNA carrier for hepatocyte-targeting, *Int. J. Pharm.*, 2003, **255**(1–2), 57–68.
 - 207 I. K. Park, T. H. Kim, Y. H. Park, B. A. Shin, E. S. Choi, E. H. Chowdhury, T. Akaike and C. S. Cho, Galactosylated chitosan-graft-poly(ethylene glycol) as hepatocyte-targeting DNA carrier, *J. Controlled Release*, 2001, **76**(3), 349–362.
 - 208 H. L. Jiang, J. T. Kwon, Y. K. Kim, E. M. Kim, R. Arote, H. J. Jeong, J. W. Nah, Y. J. Choi, T. Akaike, M. H. Cho and C. S. Cho, Galactosylated chitosan-graft-polyethylenimine as a gene carrier for hepatocyte targeting, *Gene Ther.*, 2007, **14**(19), 1389–1398.
 - 209 H. K. Tae, I. K. Su, T. Akaike and S. C. Chong, Synergistic effect of poly(ethylenimine) on the transfection efficiency of galactosylated chitosan/DNA complexes, *J. Controlled Release*, 2005, **105**(3), 354–366.
 - 210 P. Chaubey and B. Mishra, Mannose-conjugated chitosan nanoparticles loaded with rifampicin for the treatment of visceral leishmaniasis, *Carbohydr. Polym.*, 2014, **101**, 1101–1108.



- 211 T. H. Kim, H. Jin, H. W. Kim, M. H. Cho and C. S. Cho, Mannosylated chitosan nanoparticle-based cytokine gene therapy suppressed cancer growth in BALB/c mice bearing CT-26 carcinoma cells, *Mol. Cancer Ther.*, 2006, **5**(7), 1723–1732.
- 212 T. H. Kim, J. W. Nah, M. H. Cho, T. G. Park and C. S. Cho, Receptor-mediated gene delivery into antigen presenting cells using mannosylated chitosan/DNA nanoparticles, *J. Nanosci. Nanotechnol.*, 2006, **6**(9), 2796–2803.
- 213 G. Shilakari Asthana, A. Asthana, D. V. Kohli and S. P. Vyas, Mannosylated chitosan nanoparticles for delivery of antisense oligonucleotides for macrophage targeting, *BioMed Res. Int.*, 2014, 2014.
- 214 Y. I. Jeong, Y. W. Kim, S. Jung, J. Pei, M. Wen, S. Y. Li, H. H. Ryu, J. C. Lim, W. Y. Jang, I. Y. Kim, K. S. Moon and T. Y. Jung, Delivery of transferrin-conjugated polysaccharide nanoparticles in 9L gliosacoma cells, *J. Nanosci. Nanotechnol.*, 2015, **15**(1), 125–129.
- 215 H. Zhang, S. Mardiyani, W. C. W. Chan and E. Kumacheva, Design of biocompatible chitosan microgels for targeted pH-mediated intracellular release of cancer therapeutics, *Biomacromolecules*, 2006, **7**(5), 1568–1572.
- 216 H. Q. Mao, K. Roy, V. L. Troung-Le, K. A. Janes, K. Y. Lin, Y. Wang, J. T. August and K. W. Leong, Chitosan-DNA nanoparticles as gene carriers: Synthesis, characterization and transfection efficiency, *J. Controlled Release*, 2001, **70**(3), 399–421.
- 217 I. Kadiyala, Y. Loo, K. Roy, J. Rice and K. W. Leong, Transport of chitosan-DNA nanoparticles in human intestinal M-cell model versus normal intestinal enterocytes, *Eur. J. Pharm. Sci.*, 2010, **39**(1–3), 103–109.
- 218 L. Liu, X. Dong, D. Zhu, L. Song, H. Zhang and X. G. Leng, TAT-LHRH conjugated low molecular weight chitosan as a gene carrier specific for hepatocellular carcinoma cells, *Int. J. Nanomed.*, 2014, **9**(1), 2879–2889.
- 219 B. Layek and J. Singh, Cell penetrating peptide conjugated polymeric micelles as a high performance versatile non-viral gene carrier, *Biomacromolecules*, 2013, **14**(11), 4071–4081.
- 220 X. Zhao, Z. Li, W. Liu, W. Lam, P. Sun, R. Y. T. Kao, K. D. K. Luk and W. W. Lu, Octaarginine-modified chitosan as a nonviral gene delivery vector: Properties and in vitro transfection efficiency, *J. Nanopart. Res.*, 2011, **13**(2), 693–702.
- 221 D. Rahmat, M. I. Khan, G. Shahnaz, D. Sakloetsakun, G. Perera and A. Bernkop-Schnürch, Synergistic effects of conjugating cell penetrating peptides and thiomers on non-viral transfection efficiency, *Biomaterials*, 2012, **33**(7), 2321–2326.
- 222 C. Y. Yan, J. W. Gu, D. P. Hou, H. Y. Jing, J. Wang, Y. Z. Guo, H. Katsumi, T. Sakane and A. Yamamoto, Synthesis of Tat tagged and folate modified N -succinyl-chitosan self-assembly nanoparticles as a novel gene vector, *Int. J. Biol. Macromol.*, 2015, **72**, 751–756.
- 223 M. Malhotra, C. Tomaro-Duchesneau, S. Saha, I. Kahouli and S. Prakash, Development and characterization of chitosan-PEG-TAT nanoparticles for the intracellular delivery of siRNA, *Int. J. Nanomed.*, 2013, **8**, 2041–2052.
- 224 H. Katas, N. N. S. Nik Dzulkefli and S. Sahudin, Synthesis of a new potential conjugated TAT-peptide-chitosan nanoparticles carrier via disulphide linkage, *J. Nanomater.*, 2012, 2012.
- 225 W. Xie, J. Liu, M. Qiu, J. Yuan and A. Xu, Design, synthesis and biological activity of cell-penetrating peptide-modified octreotide analogs, *J. Pept. Sci.*, 2010, **16**(2), 105–109.
- 226 S. Park, E. J. Jeong, J. Lee, T. Rhim, S. K. Lee and K. Y. Lee, Preparation and characterization of nonaarginine-modified chitosan nanoparticles for siRNA delivery, *Carbohydr. Polym.*, 2013, **92**(1), 57–62.
- 227 S. M. Noh, M. O. Park, G. Shim, S. E. Han, H. Y. Lee, J. H. Huh, M. S. Kim, J. J. Choi, K. Kim, I. C. Kwon, J. S. Kim, K. H. Baek and Y. K. Oh, Pegylated poly-l-arginine derivatives of chitosan for effective delivery of siRNA, *J. Controlled Release*, 2010, **145**(2), 159–164.
- 228 L. L. Cai, P. Liu, X. Li, X. Huang, Y. Ye, F. Y. Chen, H. Yuan, F. Q. Hu and Y. Z. Du, RGD peptide-mediated chitosan-based polymeric micelles targeting delivery for integrin-overexpressing tumor cells, *Int. J. Nanomed.*, 2011, **6**, 3499–3508.
- 229 L. Ge, X. You, K. Huang, Y. Kang, Y. Chen, Y. Zhu, Y. Ren, Y. Zhang, J. Wu and H. Qian, Screening of novel RGD peptides to modify nanoparticles for targeted cancer therapy, *Biomater. Sci.*, 2018, **6**(1), 125–135.
- 230 H. D. Han, L. S. Mangala, J. W. Lee, M. M. K. Shahzad, H. S. Kim, D. Shen, E. J. Nam, E. M. Mora, R. L. Stone, C. Lu, S. J. Lee, J. W. Roh, A. M. Nick, G. Lopez-Berestein and A. K. Sood, Targeted gene silencing using RGD-labeled chitosan nanoparticles, *Clin. Cancer Res.*, 2010, **16**(15), 3910–3922.
- 231 F. Wang, Y. Chen, D. Zhang, Q. Zhang, D. Zheng, L. Hao, Y. Liu, C. Duan, L. Jia and G. Liu, Folate-mediated targeted and intracellular delivery of paclitaxel using a novel deoxycholic acid-O-carboxymethylated chitosan-folic acid micelles, *Int. J. Nanomed.*, 2012, **7**, 325–337.
- 232 S. J. Yang, F. H. Lin, K. C. Tsai, M. F. Wei, H. M. Tsai, J. M. Wong and M. J. Shieh, Folic acid-conjugated chitosan nanoparticles enhanced protoporphyrin IX accumulation in colorectal cancer cells, *Bioconjugate Chem.*, 2010, **21**(4), 679–689.
- 233 S. K. Sahu, S. K. Mallick, S. Santra, T. K. Maiti, S. K. Ghosh and P. Pramanik, In vitro evaluation of folic acid modified carboxymethyl chitosan nanoparticles loaded with doxorubicin for targeted delivery, *J. Mater. Sci.: Mater. Med.*, 2010, **21**(5), 1587–1597.
- 234 P. Chan, M. Kurisawa, J. E. Chung and Y. Y. Yang, Synthesis and characterization of chitosan-g-poly(ethylene glycol)-folate as a non-viral carrier for tumor-targeted gene delivery, *Biomaterials*, 2007, **28**(3), 540–549.
- 235 S. Mansouri, Y. Cuie, F. Winnik, Q. Shi, P. Lavigne, M. Benderdour, E. Beaumont and J. C. Fernandes, Characterization of folate-chitosan-DNA nanoparticles for gene therapy, *Biomaterials*, 2006, **27**(9), 2060–2065.



- 236 Q. Tian, X. H. Wang, W. Wang, C. N. Zhang, P. Wang and Z. Yuan, Self-assembly and liver targeting of sulfated chitosan nanoparticles functionalized with glycyrrhethinic acid, *Nanomedicine*, 2012, **8**(6), 870–879.
- 237 L. Shi, C. Tang and C. Yin, Glycyrrhizin-modified O-carboxymethyl chitosan nanoparticles as drug vehicles targeting hepatocellular carcinoma, *Biomaterials*, 2012, **33**(30), 7594–7604.
- 238 R. Rohilla, T. Garg, J. Bariwal, A. K. Goyal and G. Rath, Development, optimization and characterization of glycyrrhethinic acid–chitosan nanoparticles of atorvastatin for liver targeting, *Drug Delivery*, 2016, **23**(7), 2290–2297.
- 239 D. Mishra, N. Jain, V. Rajoriya and A. K. Jain, Glycyrrhizin conjugated chitosan nanoparticles for hepatocyte-targeted delivery of lamivudine, *J. Pharm. Pharmacol.*, 2014, **66**(8), 1082–1093.
- 240 A. Lin, Y. Liu, Y. Huang, J. Sun, Z. Wu, X. Zhang and Q. Ping, Glycyrrhizin surface-modified chitosan nanoparticles for hepatocyte-targeted delivery, *Int. J. Pharm.*, 2008, **359**(1–2), 247–253.
- 241 H. Jin, T. H. Kim, S. K. Hwang, S. H. Chang, H. W. Kim, H. K. Anderson, H. W. Lee, K. H. Lee, N. H. Colburn, H. S. Yang, M. H. Cho and C. S. Cho, Aerosol delivery of urocanic acid-modified chitosan/programmed cell death 4 complex regulated apoptosis, cell cycle, and angiogenesis in lungs of K-ras null mice, *Mol. Cancer Ther.*, 2006, **5**(4), 1041–1049.
- 242 Y. S. Hsueh, S. Subramaniam, Y. C. Tseng, T. M. Chiang, O. Mestak, T. K. Cheng, T. F. Kuo, S. Sivasubramanian, F. H. Lin and M. J. Shieh, In vitro and in vivo assessment of chitosan modified urocanic acid as gene carrier, *Mater. Sci. Eng., C*, 2017, **70**, 599–606.
- 243 B. Xiao, P. Ma, E. Viennois and D. Merlin, Urocanic acid-modified chitosan nanoparticles can confer anti-inflammatory effect by delivering CD98 siRNA to macrophages, *Colloids Surf., B*, 2016, **143**, 186–193.
- 244 W. Wang, J. Yao, J. P. Zhou, Y. Lu, Y. Wang, L. Tao and Y. P. Li, Urocanic acid-modified chitosan-mediated p53 gene delivery inducing apoptosis of human hepatocellular carcinoma cell line HepG2 is involved in its antitumor effect in vitro and in vivo, *Biochem. Biophys. Res. Commun.*, 2008, **377**(2), 567–572.
- 245 A. A. D'Souza and P. V. Devarajan, Asialoglycoprotein receptor mediated hepatocyte targeting - Strategies and applications, *J. Controlled Release*, 2015, **203**, 126–139.
- 246 P. Erbacher, S. Zou, T. Bettinger, A. M. Steffan and J. S. Remy, Chitosan-based vector/DNA complexes for gene delivery: Biophysical characteristics and transfection ability, *Pharm. Res.*, 1998, **15**(9), 1332–1339.
- 247 M. Hashimoto, M. Morimoto, H. Saimoto, Y. Shigemasa and T. Sato, Lactosylated chitosan for DNA delivery into hepatocytes: The effect of lactosylation on the physicochemical properties and intracellular trafficking of pDNA/chitosan complexes, *Bioconjugate Chem.*, 2006, **17**(2), 309–316.
- 248 M. Hashimoto, M. Morimoto, H. Saimoto, Y. Shigemasa, H. Yanagie, M. Eriguchi and T. Sato, Gene transfer by DNA/mannosylated chitosan complexes into mouse peritoneal macrophages, *Biotechnol. Lett.*, 2006, **28**(11), 815–821.
- 249 H. L. Jiang, M. L. Kang, J. S. Quan, S. G. Kang, T. Akaike, H. S. Yoo and C. S. Cho, The potential of mannosylated chitosan microspheres to target macrophage mannose receptors in an adjuvant-delivery system for intranasal immunization, *Biomaterials*, 2008, **29**(12), 1931–1939.
- 250 T. R. Daniels, E. Bernabeu, J. A. Rodríguez, S. Patel, M. Kozman, D. A. Chiappetta, E. Holler, J. Y. Ljubimova, G. Helguera and M. L. Penichet, The transferrin receptor and the targeted delivery of therapeutic agents against cancer, *Biochim. Biophys. Acta*, 2012, **1820**(3), 291–317.
- 251 P. T. Gomme and K. B. McCann, Transferrin: Structure, function and potential therapeutic actions, *Drug Discovery Today*, 2005, **10**(4), 267–273.
- 252 R. O. Hynes, Integrins: Bidirectional, allosteric signaling machines, *Cell*, 2002, **110**, 673–687.
- 253 T. G. Kapp, F. Reichenmacher, S. Neubauer, O. V. Maltsev, E. A. Cavalcanti-Adam, R. Zarka, U. Reuning, J. Notni, H. J. Wester, C. Mas-Moruno, J. Spatz, B. Geiger and H. Kessler, A comprehensive evaluation of the activity and selectivity profile of ligands for RGD-binding integrins, *Sci. Rep.*, 2017, **7**.
- 254 W. Arap, R. Pasqualini and E. Ruoslahti, Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model, *Science*, 1998, **279**(5349), 377–380.
- 255 R. Pasqualini, E. Koivunen and E. Ruoslahti, α Integrins as Receptors for Tumor Targeting by Circulating Ligands, *Nat. Biotechnol.*, 1997, **15**(6), 542–546.
- 256 J. H. Park, S. Kwon, J. O. Nam, R. W. Park, H. Chung, S. B. Seo, I. S. Kim, I. C. Kwon and S. Y. Jeong, Self-assembled nanoparticles based on glycol chitosan bearing 5 β -cholanic acid for RGD peptide delivery, *J. Controlled Release*, 2004, **95**(3), 579–588.
- 257 J. H. Kim, Y. S. Kim, K. Park, E. Kang, S. Lee, H. Y. Nam, K. Kim, J. H. Park, D. Y. Chi, R. W. Park, I. S. Kim, K. Choi and I. C. Kwon, Self-assembled glycol chitosan nanoparticles for the sustained and prolonged delivery of anti-angiogenic small peptide drugs in cancer therapy, *Biomaterials*, 2008, **29**(12), 1920–1930.
- 258 C. Y. Yan, J. W. Gu, D. P. Hou, H. Y. Jing, J. Wang, Y. Z. Guo, H. Katsumi, T. Sakane and A. Yamamoto, Synthesis of TAT tagged and folate modified N-succinyl-chitosan self-assembly nanoparticles as a novel gene vector, *Int. J. Biol. Macromol.*, 2015, **72**, 751–756.
- 259 J. Sudimack and R. J. Lee, Targeted drug delivery via the folate receptor, *Adv. Drug Delivery Rev.*, 2000, **41**(2), 147–162.
- 260 T. H. Kim, J. E. Ihm, Y. J. Choi, J. W. Nah and C. S. Cho, Efficient gene delivery by urocanic acid-modified chitosan, *J. Controlled Release*, 2003, **93**(3), 389–402.
- 261 M. Negishi, A. Irie, N. Nagata and A. Ichikawa, Specific binding of glycyrrhethinic acid to the rat liver membrane, *Biochim. Biophys. Acta, Biomembr.*, 1991, **1066**(1), 77–82.



- 262 J. H. Park, G. Saravanakumar, K. Kim and I. C. Kwon, Targeted delivery of low molecular drugs using chitosan and its derivatives, *Adv. Drug Delivery Rev.*, 2010, **62**(1), 28–41.
- 263 L. Yin, J. Ding, C. He, L. Cui, C. Tang and C. Yin, Drug permeability and mucoadhesion properties of thiolated trimethyl chitosan nanoparticles in oral insulin delivery, *Biomaterials*, 2009, **30**(29), 5691–5700.
- 264 X. Zhao, L. Yin, J. Ding, C. Tang, S. Gu, C. Yin and Y. Mao, Thiolated trimethyl chitosan nanocomplexes as gene carriers with high in vitro and in vivo transfection efficiency, *J. Controlled Release*, 2010, **144**(1), 46–54.
- 265 Y. L. Tan and C. G. Liu, Self-aggregated nanoparticles from linoleic acid modified carboxymethyl chitosan: Synthesis, characterization and application in vitro, *Colloids Surf., B*, 2009, **69**, 178–182.
- 266 S. Mao, X. Shuai, F. Unger, M. Wittmar, X. Xie and T. Kissel, Synthesis, characterization and cytotoxicity of poly(ethylene glycol)-graft-trimethyl chitosan block copolymers, *Biomaterials*, 2005, **26**, 6343–6356.
- 267 C. Gao, T. Liu, Y. Dang, Z. Yu, W. Wang, J. Guo, X. Zhang, G. He, H. Zheng, Y. Yin and X. Kong, pH/redox responsive core cross-linked nanoparticles from thiolated carboxymethyl chitosan for in vitro release study of methotrexate, *Carbohydr. Polym.*, 2014, **111**, 964–970.
- 268 A. Makhlof, M. Werle, Y. Tozuka and H. Takeuchi, Nanoparticles of glycol chitosan and its thiolated derivative significantly improved the pulmonary delivery of calcitonin, *Int. J. Pharm.*, 2010, **397**(1–2), 92–95.
- 269 K. E. Lee, D. H. Choi, C. Joo, S. W. Kang, K. M. Huh and Y. S. Park, Octanoyl glycol chitosan enhances the proliferation and differentiation of tonsil-derived mesenchymal stem cells, *Carbohydr. Polym.*, 2021, **264**, 1–12.
- 270 T. A. Ahmed and B. M. Aljaeid, Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery, *Drug Des., Dev. Ther.*, 2016, **10**, 483–507.
- 271 J. J. Wang, Z. W. Zeng, R. Z. Xiao, T. Xie, G. L. Zhou, X. R. Zhan and S. L. Wang, Recent advances of chitosan nanoparticles as drug carriers, *Int. J. Nanomed.*, 2011, **6**, 765–774.
- 272 H. Peniche and C. Peniche, Chitosan nanoparticles: a contribution to nanomedicine, *Polym. Int.*, 2011, **60**, 883–889.
- 273 M. Hamidi, A. Azadi and P. Rafiei, Hydrogel nanoparticles in drug delivery, *Adv. Drug Delivery Rev.*, 2008, **60**, 1638–1649.
- 274 S. A. Agnihotri, N. N. Mallikarjuna and T. M. Aminabhavi, Recent advances on chitosan-based micro- and nanoparticles in drug delivery, *J. Controlled Release*, 2004, **100**(1), 5–28.
- 275 J. Xu, L. Ma, Y. Liu, F. Xu, J. Nie and G. Ma, Design and characterization of antitumor drug paclitaxel-loaded chitosan nanoparticles by W/O emulsions, *Int. J. Biol. Macromol.*, 2012, **50**(2), 438–443.
- 276 F. L. Mi, H. W. Sung and S. S. Shyu, Synthesis and Characterization of a Novel Chitosan-Based Network Prepared Using Naturally Occurring Crosslinker, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 2804–2814.
- 277 F. L. Mi, Y. C. Tan, H. F. Liang and H. W. Sung, In vivo biocompatibility and degradability of a novel injectable-chitosan-based implant, *Biomaterials*, 2002, **23**, 181–191.
- 278 M. A. Pujana, L. Pérez-Álvarez, L. Carlos, C. Iturbe and I. Katime, Biodegradable chitosan nanogels crosslinked with genipin, *Carbohydr. Polym.*, 2013, **94**(2), 836–842.
- 279 Y. H. Lin, S. C. Tsai, C. H. Lai, C. H. Lee, Z. Sian and G. C. Tseng, Genipin-cross-linked fucose e chitosan/heparin nanoparticles for the eradication of *Helicobacter pylori*, *Biomaterials*, 2013, **34**(18), 4466–4479.
- 280 G. V. Kumar, C. H. Su and P. Velusamy, Ciprofloxacin loaded genipin cross-linked chitosan/heparin nanoparticles for drug delivery application, *Mater. Lett.*, 2016, **180**, 119–122.
- 281 M. A. Pujana, L. Pérez-Álvarez, L. C. C. Iturbe and I. Katime, Water dispersible pH-responsive chitosan nanogels modified with biocompatible crosslinking-agents, *Polymer*, 2012, **53**, 3107–3116.
- 282 M. A. Pujana, L. Pérez-Álvarez, L. Carlos, C. Iturbe and I. Katime, pH-sensitive chitosan-folate nanogels cross-linked with biocompatible dicarboxylic acids, *Eur. Polym. J.*, 2014, **61**, 215–225.
- 283 M. Bodnar, J. F. Hartmann and J. Borbely, Preparation and Characterization of Chitosan-Based Nanoparticles, *Biomacromolecules*, 2005, **6**, 2521–2527.
- 284 J. A. del Olmo, L. Pérez-Álvarez, V. Sáez-Martínez, S. Benito-Cid, L. Ruiz-Rubio, R. Pérez-González, J. L. Vilas-Vilela and J. M. Alonso, Wound healing and antibacterial chitosan-genipin hydrogels with controlled drug delivery for synergistic anti-inflammatory activity, *Int. J. Biol. Macromol.*, 2022, **203**, 679–694.
- 285 A. Fernandez, P. C. Salve, C. R. Lopes and J. L. V. Jato, *Application of Nanoparticles Based on Hydrophilic Polymers as Pharmaceutical Forms*, 2003, vol. 2.
- 286 P. Calvo, C. Remuñán-López, J. L. Vila-Jato and M. J. Alonso, Novel Hydrophilic Chitosan – Polyethylene Oxide Nanoparticles as Protein Carriers, *J. Appl. Polym. Sci.*, 1997, **63**, 125–132.
- 287 H. Liu and C. Gao, Preparation and properties of ionically cross-linked chitosan nanoparticles, *Polym. Adv. Technol.*, 2009, **20**, 613–619.
- 288 S. Shah, A. Pal, V. K. Kaushik and S. Devi, Preparation and Characterization of Venlafaxine Hydrochloride-Loaded Chitosan Nanoparticles and In Vitro Release of Drug, *J. Appl. Polym. Sci.*, 2009, **112**, 2876–2887.
- 289 Q. Gan, T. Wang, C. Cochrane and P. McCarron, Modulation of surface charge, particle size and morphological properties of chitosan-TPP nanoparticles intended for gene delivery, *Colloids Surf., B*, 2005, **44**, 65–73.
- 290 Y. Wu, W. Yang, C. Wang, J. Hu and S. Fu, Chitosan nanoparticles as a novel delivery system for ammonium glycyrhizinate, *Int. J. Pharm.*, 2005, **295**, 235–245.
- 291 H. C. Yang and M. H. Hon, The effect of the molecular weight of chitosan nanoparticles and its application on drug delivery, *Microchem. J.*, 2009, **92**(1), 87–91.



- 292 H. Jonassen, A. L. Kjøniksen and M. Hiorth, Effects of ionic strength on the size and compactness of chitosan nanoparticles, *Colloid Polym. Sci.*, 2012, **290**, 919–929.
- 293 S. Sreekumar, F. M. Goycoolea, B. M. Moerschbacher and G. R. Rivera-Rodriguez, Parameters influencing the size of chitosan-TPP nano- and microparticles, *Sci. Rep.*, 2018, 1–11.
- 294 Y. Huang, Y. Cai and Y. Lapitsky, Factors affecting the stability of chitosan/tripolyphosphate micro- and nanogels: resolving the opposing findings, *J. Mater. Chem. B*, 2015, **3**, 5957–5970.
- 295 K. A. Janes and M. J. Alonso, Depolymerized Chitosan Nanoparticles for Protein Delivery: Preparation and Characterization, *J. Appl. Polym. Sci.*, 2003, **88**, 2769–2776.
- 296 H. Zhang, M. Oh, C. Allen and E. Kumacheva, Monodisperse Chitosan Nanoparticles for Mucosal Drug Delivery, *Biomacromolecules*, 2004, **5**, 2461–2468.
- 297 T. López-León, E. L. S. Carvalho, B. Seijo, J. L. Ortega-Vinuesa and D. Bastos-González, Physicochemical characterization of chitosan nanoparticles: electrokinetic and stability behavior, *J. Colloid Interface Sci.*, 2005, **283**, 344–351.
- 298 G. Giacalone, A. Bochot, E. Fattal and H. Hillaireau, Drug-Induced Nanocarrier Assembly as a Strategy for the Cellular Delivery of Nucleotides and Nucleotide Analogues, *Biomacromolecules*, 2013, **14**, 737–742.
- 299 H. Jonassen, A. L. Kjøniksen and M. Hiorth, Stability of Chitosan Nanoparticles Cross-Linked with Tripolyphosphate, *Biomacromolecules*, 2012, **13**, 3747–3756.
- 300 Y. Huang and Y. Lapitsky, Monovalent Salt Enhances Colloidal Stability during the Formation of Chitosan/Tripolyphosphate Microgels, *Langmuir*, 2011, **27**, 10392–10399.
- 301 M. A. Kalam, M. Iqbal, A. Alshememry, M. Alkholief and A. Alshamsan, Development and Evaluation of Chitosan Nanoparticles for Ocular Delivery of Tedizolid Phosphate, *Molecules*, 2022, **27**(7), 1–22.
- 302 M. Garcia-Fuentes and M. J. Alonso, Chitosan-based drug nanocarriers: Where do we stand?, *J. Controlled Release*, 2012, **161**(2), 496–504.
- 303 L. Bugnicourt and C. Ladavière, Interests of chitosan nanoparticles ionically cross-linked with tripolyphosphate for biomedical applications, *Prog. Polym. Sci.*, 2016, **60**, 1–17.
- 304 E. Russo, N. Gaglianone, S. Baldassari, B. Parodi, S. Cafaggi, C. Zibana, M. Donalisio, V. Cagno, D. Lembo and G. Caviglioli, Preparation, characterization and in vitro antiviral activity evaluation of foscarnet-chitosan nanoparticles, *Colloids Surf., B*, 2014, **118**, 117–125.
- 305 J. Quiñones, H. Peniche and C. Peniche, Chitosan Based Self-Assembled Nanoparticles in Drug Delivery, *Polymers*, 2018, **10**, 235.
- 306 Y. Luo and Q. Wang, Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery, *Int. J. Biol. Macromol.*, 2014, **64**, 353–367.
- 307 M. G. Sankalia, R. C. Mashru, J. M. Sankalia and V. B. Sutariya, Reversed chitosan-alginate polyelectrolyte complex for stability improvement of alpha-amylase: Optimization and physicochemical characterization, *Eur. J. Pharm. Biopharm.*, 2007, **65**(2), 215–232.
- 308 H. Saether, H. K. Holme, G. Maurstad, O. Smidsrød and B. T. Stokke, Polyelectrolyte complex formation using alginate and chitosan, *Carbohydr. Polym.*, 2008, **74**, 813–821.
- 309 S. Cafaggi, E. Russo, R. Stefani, R. Leardi, G. Caviglioli, B. Parodi, G. Bignardi, D. Toterò, C. Aiello and M. Viale, Preparation and evaluation of nanoparticles made of chitosan or N-trimethyl chitosan and a cisplatin–alginate complex, *J. Controlled Release*, 2007, **121**, 110–123.
- 310 J. H. Hamman, Chitosan Based Polyelectrolyte Complexes as Potential Carrier Materials in Drug Delivery Systems, *Mar. Drugs*, 2010, **8**, 1305–1322.
- 311 S. K. Motwani, S. Chopra, S. Talegaonkar, K. Kohli, F. J. Ahmad and R. K. Khar, Chitosan–sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimisation and in vitro characterisation, *Eur. J. Pharm. Biopharm.*, 2008, **68**, 513–525.
- 312 P. Liu and X. Zhao, Facile preparation of well-defined near-monodisperse chitosan/sodium alginate polyelectrolyte complex nanoparticles (CS/SAL NPs) via ionotropic gelification: A suitable technique for drug delivery systems, *Biotechnol. J.*, 2013, **8**, 847–854.
- 313 N. P. Katuwavila, A. D. L. C. Perera, S. R. Samarakoon, P. Soysa, V. Karunaratne, G. A. J. Amaratunga and D. N. Karunaratne, Chitosan-Alginate Nanoparticle System Efficiently Delivers Doxorubicin to MCF-7 Cells, *J. Nanomater.*, 2016, 3178904.
- 314 M. A. Azevedo, A. I. Bourbon, A. A. Vicente and M. A. Cerqueira, Alginate/chitosan nanoparticles for encapsulation and controlled release of vitamin B2, *Int. J. Biol. Macromol.*, 2014, **71**, 141–146.
- 315 X. Zhu, M. Su, S. Tang, L. Wang, X. Liang, F. Meng, Y. Hong and Z. Xu, Synthesis of thiolated chitosan and preparation nanoparticles with sodium alginate for ocular drug delivery, *Mol. Vision*, 2012, **18**, 1973–1982.
- 316 A. Grenha, M. E. Gomes, V. E. Santo, J. F. Mano, N. M. Neves and R. L. Reis, Development of new chitosan/carrageenan nanoparticles for drug delivery applications, *J. Biomed. Mater. Res., Part A*, 2009, 1265–1272.
- 317 A. V. Briones and T. Sato, Encapsulation of glucose oxidase (GOD) in polyelectrolyte complexes of chitosan–carrageenan, *React. Funct. Polym.*, 2010, **70**(1), 19–27.
- 318 M. Alonso-Sande, M. Cunã, C. Remuñán-López, D. Teijeiro-Osorio, J. L. Alonso-Lebrero and M. J. Alonso, Formation of New Glucomannan-Chitosan Nanoparticles and Study of Their Ability To Associate and Deliver Proteins, *Macromolecules*, 2006, **39**, 4152–4158.
- 319 J. Du, J. Dai, J. L. Liu and T. Dankovich, Novel pH-sensitive polyelectrolyte carboxymethyl Konjac glucomannan-chitosan beads as drug carriers, *React. Funct. Polym.*, 2006, **66**, 1055–1061.



- 320 N. P. Birch and J. D. Schiffman, Characterization of Self-Assembled Polyelectrolyte Complex Nanoparticles Formed from Chitosan and Pectin, *Langmuir*, 2014, **40**, 3441–3447.
- 321 V. B. V. Maciel, C. M. P. Yoshida, S. M. S. S. Pereira, F. M. Goycoolea and T. T. Franco, Electrostatic Self-Assembled Chitosan-Pectin Nano- and Microparticles for Insulin Delivery, *Molecules*, 2017, **22**, 1707–1728.
- 322 S. Boddohi, N. Moore, P. A. Johnson and M. J. Kipper, Polysaccharide-Based Polyelectrolyte Complex Nanoparticles from Chitosan, Heparin, and Hyaluronan, *Biomacromolecules*, 2009, **10**, 1402–1409.
- 323 H. D. Lu, H. Q. Zhao, K. Wang and L. L. Lv, Novel hyaluronic acid–chitosan nanoparticles as non-viral gene delivery vectors targeting osteoarthritis, *Int. J. Pharm.*, 2011, **420**, 358–365.
- 324 D. Wu and T. Delair, Stabilization of chitosan/hyaluronan colloidal polyelectrolyte complexes in physiological conditions, *Carbohydr. Polym.*, 2015, **119**, 149–158.
- 325 G. Lalevée, G. Sudre, A. Montembault, J. Meadows, S. Malaise, A. Crépet, L. David and T. Delair, Polyelectrolyte complexes via desalting mixtures of hyaluronic acid and chitosan—Physicochemical study and structural analysis, *Carbohydr. Polym.*, 2016, **154**, 86–95.
- 326 J. Jacumazo, M. M. de Carvalho, G. P. Parchen, I. M. F. Campos, M. J. Ballesteros Garcia, T. Brugnari, G. M. Maciel, F. A. Marques and R. A. de Freitas, Development, characterization and antimicrobial activity of sodium dodecyl sulfate-polysaccharides capsules containing eugenol, *Carbohydr. Polym.*, 2020, 230.
- 327 V. E. Santo, M. E. Gomes, J. F. Mano and R. L. Reis, Chitosan-chondroitin sulphate nanoparticles for controlled delivery of platelet lysates in bone regenerative medicine, *J. Tissue Eng. Regen. Med.*, 2012, **6**, 47–59.
- 328 C. S. Hu, C. H. Chiang, P. Hong and M. K. Yeh, Influence of charge on FITC-BSA-loaded chondroitin sulfate-chitosan nanoparticles upon cell uptake in human Caco-2 cell monolayers, *Int. J. Nanomed.*, 2012, **7**, 4861–4872.
- 329 I. Hajdu, M. Bodnár, G. Filipcsei, J. F. Hartmann, L. Daróczi, M. Zrínyi and J. Borbély, Nanoparticles prepared by self-assembly of Chitosan and poly- γ -glutamic acid, *Colloid Polym. Sci.*, 2008, **286**, 343–350.
- 330 Z. Keresztesy, M. Bodnár, E. Ber, I. Hajdu, M. Zhang, J. F. Hartmann, T. Minko and J. Borbély, Self-assembling chitosan/poly- γ -glutamic acid nanoparticles for targeted drug delivery, *Colloid Polym. Sci.*, 2009, **297**, 759–765.
- 331 Y. H. Lin, C. T. Chen, H. F. Liang, A. R. Kulkarni, P. W. Lee, C. H. Chen and H. W. Sung, Novel nanoparticles for oral insulin delivery via the paracellular pathway, *Nanotechnology*, 2007, **18**, 105102.
- 332 W. Sun, S. Mao, D. Mei and T. Kissel, Self-assembled polyelectrolyte nanocomplexes between chitosan derivatives and enoxaparin, *Eur. J. Pharm. Biopharm.*, 2008, **69**, 417–425.
- 333 M. Costalat, P. Alcouffe, L. David and T. Delair, Controlling the complexation of polysaccharides into multi-functional colloidal assemblies for nanomedicine, *J. Colloid Interface Sci.*, 2014, **430**, 147–156.
- 334 A. F. Martins, J. F. Piai, I. Schuquel, A. Rubira and E. C. Muniz, Polyelectrolyte complexes of chitosan/heparin and N, N, N-trimethyl chitosan/heparin obtained at different pH: I. Preparation, characterization, and controlled release of heparin, *Colloid Polym. Sci.*, 2011, **289**, 1133–1144.
- 335 C. Schatz, J. M. Lucas, C. Viton, A. Domard, C. Pichot and T. Delair, Formation and Properties of Positively Charged Colloids Based on Polyelectrolyte Complexes of Biopolymers, *Langmuir*, 2004, **20**(6), 7766–7778.
- 336 T. Delair, Colloidal polyelectrolyte complexes of chitosan and dextran sulfate towards versatile nanocarriers of bioactive molecules, *Eur. J. Pharm. Biopharm.*, 2011, **78**(1), 10–18.
- 337 M. Huang, S. N. Vitharana, L. J. Peek, T. Coop and C. Berkland, Polyelectrolyte Complexes Stabilize and Controllably Release Vascular Endothelial Growth Factor, *Biomacromolecules*, 2007, **8**, 1607–1614.
- 338 W. Chaiyasan, S. Praputbut, U. B. Kompella, S. P. Srinivas and W. Tiyaboonchai, Penetration of mucoadhesive chitosan-dextran sulfate nanoparticles into the porcine cornea, *Colloids Surf., B*, 2017, **149**, 288–296.
- 339 B. Sarmento, A. Ribeiro, F. Veiga and D. Ferreira, Development and characterization of new insulin containing polysaccharide nanoparticles, *Colloids Surf., B*, 2006, **53**, 193–202.
- 340 H. Dautzenberg and J. Kriz, Response of Polyelectrolyte Complexes to Subsequent Addition of Salts with Different Cations, *Langmuir*, 2003, **19**(6), 5204–5211.
- 341 W. Tiyaboonchai and N. Limpeanchob, Formulation and characterization of amphotericin B–chitosan–dextran sulfate nanoparticles, *Int. J. Pharm.*, 2007, **329**, 142–149.
- 342 Y. S. Wang, L. R. Liu, Q. Jiang and Q. Q. Zhang, Self-aggregated nanoparticles of cholesterol-modified chitosan conjugate as a novel carrier of epirubicin, *Eur. Polym. J.*, 2007, **43**, 43–51.
- 343 G. Qu, Z. Yao, C. Zhang, X. Wu and Q. Ping, PEG conjugated N-octyl-O-sulfate chitosan micelles for delivery of paclitaxel: In vitro characterization and in vivo evaluation, *Eur. J. Pharm. Sci.*, 2009, **37**, 98–105.
- 344 M. A. Raja, M. Arif, C. Feng, S. Zeenat and C. G. Liu, Synthesis and evaluation of pH-sensitive, self-assembled chitosan-based nanoparticles as efficient doxorubicin carriers, *J. Biomater. Appl.*, 2017, **31**(8), 1182–1195.
- 345 Y. Kato, H. Onishi and Y. Machida, Biological Fate of Highly-Succinylated N-Succinyl-chitosan and Antitumor Characteristics of Its Water-soluble Conjugate with Mitomycin C at I.V and I.P Administration into Tumor-Bearing Mice, *Biol. Pharm. Bull.*, 2000, **23**(12), 1497–1503.
- 346 P. Opanasopit, T. Ngawhirunpat, A. Chaidedgumjorn, T. Rojanarata, A. Apirakaramwong, S. Phongying, C. Choochottiros and S. Chirachanchai, Incorporation of camptothecin into N-phthaloyl chitosan-g-mPEG self-assembly micellar system, *Eur. J. Pharm. Biopharm.*, 2006, **64**, 269–276.
- 347 F. Qian, F. Cui, J. Ding, C. Tang and C. Yin, Chitosan Graft Copolymer Nanoparticles for Oral Protein Drug



- Delivery: Preparation and Characterization, *Biomacromolecules*, 2006, **7**, 2722–2727.
- 348 M. R. De Moura, F. A. Aouada and L. H. C. Mattoso, Preparation of chitosan nanoparticles using methacrylic acid, *J. Colloid Interface Sci.*, 2008, **321**, 477–483.
- 349 Y. Hu, X. Jiang, Y. Ding, H. Ge, Y. Yuan and C. Yang, Synthesis and characterization of chitosan-poly(acrylic acid) nanoparticles, *Biomaterials*, 2002, **23**, 3193–3201.
- 350 I. Bravo-Osuna, C. Vauthier, A. Farabollini, G. Filippo and G. Ponchel, Mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core-shell nanoparticles, *Biomaterials*, 2007, **28**, 2233–2243.
- 351 A. Heidari, H. Younesi, Z. Mehraban and H. Heikkinen, Selective adsorption of Pb(II), Cd(II), and Ni(II) ions from aqueous solution using chitosan – MAA nanoparticles, *Int. J. Biol. Macromol.*, 2013, **61**, 251–263.
- 352 R. Francis, D. K. Baby and Y. Gnanou, Synthesis and self-assembly of Chitosan-g-Polystyrene copolymer: A new route for the preparation of heavy metal nanoparticles, *J. Colloid Interface Sci.*, 2015, **438**, 110–115.
- 353 C. He, Y. Hu, L. Yin, C. Tang and C. Yin, Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles, *Biomaterials*, 2010, **31**(13), 3657–3666.
- 354 K. Park, J. H. Kim, Y. S. Nam, S. Lee, H. Y. Nam, K. Kim, J. H. Park, I. S. Kim, K. Choi, S. Y. Kim and I. C. Kwon, Effect of polymer molecular weight on the tumor targeting characteristics of self-assembled glycol chitosan nanoparticles, *J. Controlled Release*, 2007, **122**, 305–314.
- 355 B. Lu, S. B. Xiong, H. Yang, X. D. Yin and R. B. Zhao, Mitoxantrone-loaded BSA nanospheres and chitosan nanospheres for local injection against breast cancer and its lymph node metastases II: Tissue distribution and pharmacodynamics, *Int. J. Pharm.*, 2006, **307**, 175–181.
- 356 T. Kean and M. Thanou, Biodegradation, biodistribution and toxicity of chitosan, *Adv. Drug Delivery Rev.*, 2010, **62**(1), 3–11.
- 357 H. Zhang and S. H. Neau, In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents, *Biomaterials*, 2002, **23**, 2761–2766.
- 358 H. Zhang and S. H. Neau, In vitro degradation of chitosan by a commercial enzyme preparation: effect of molecular weight and degree of deacetylation, *Biomaterials*, 2001, **22**, 1653–1658.
- 359 K. Kofuji, C. J. Qian, M. Nishimura, I. Sugiyama, Y. Murata and S. Kawashima, Relationship between physicochemical characteristics and functional properties of chitosan, *Eur. Polym. J.*, 2005, **41**, 2784–2791.
- 360 K. Kafedjiiski, F. Föger, H. Hoyer, A. Bernkop-Schnürch and M. Werle, Evaluation of In Vitro Enzymatic Degradation of Evaluation of In Vitro Enzymatic Degradation of Various Thiomers and Cross-Linked Thiomers, *Drug Dev. Ind. Pharm.*, 2017, **33**, 199–208.
- 361 S. M. Lim, D. K. Song, S. H. Oh, D. S. Lee-Yoon, E. H. Bae and J. H. Lee, In vitro and in vivo degradation behavior of acetylated chitosan porous beads, *J. Biomater. Sci., Polym. Ed.*, 2008, **19**(4), 453–466.
- 362 D. Ren, H. Yi, W. Wang and X. Ma, The enzymatic degradation and swelling properties of chitosan matrices with different degrees of N-acetylation, *Carbohydr. Res.*, 2005, **340**, 2403–2410.
- 363 Y. M. Yang, W. Hu, X. D. Wang and X. S. Gu, The controlling biodegradation of chitosan fibers by N-acetylation in vitro and in vivo, *J. Mater. Sci.: Mater. Med.*, 2007, **18**, 2117–2121.
- 364 L. Guangyuan, S. Baiyang, W. Gan, W. Yujun, G. Yandao, Z. Xiufang and Z. Lihai, Controlling the Degradation of Covalently Cross-linked Carboxymethyl Chitosan Utilizing Bimodal Molecular Weight Distribution, *J. Biomater. Appl.*, 2009, **23**, 435–451.
- 365 E. L. McConnell, S. Murdan and A. W. Basit, An Investigation into the Digestion of Chitosan (Noncrosslinked and Crosslinked) by Human Colonic Bacteria, *J. Pharm. Sci.*, 2008, **97**(9), 3820–3829.
- 366 T. Kean, S. Roth and M. Thanou, Trimethylated chitosans as non-viral gene delivery vectors: Cytotoxicity and transfection efficiency, *J. Controlled Release*, 2005, **103**, 643–653.
- 367 P. Opanasopit, P. Aumklad, J. Kowapradit, T. Ngawhiranpat, A. Apirakaramwong and T. Rojanarata, Effect of Salt Forms and Molecular Weight of Chitosans on In Vitro Permeability Enhancement in Intestinal Epithelial Cells (Caco-2), *Pharm. Dev. Technol.*, 2007, **12**, 447–455.
- 368 B. Carreño-Gómez and R. Duncan, Evaluation Of the biological properties of soluble chitosan and chitosan microspheres, *Int. J. Pharm.*, 1997, **148**(2), 231–240.
- 369 M. Huang, E. Khor and L. Y. Lim, Uptake and Cytotoxicity of Chitosan Molecules and Nanoparticles: Effects of Molecular Weight and Degree of Deacetylation, *Pharm. Res.*, 2004, **21**(2), 344–353.
- 370 L. Qi, Z. Xu, X. Jiang, Y. Li and M. Wang, Cytotoxic activities of chitosan nanoparticles and copper-loaded nanoparticles, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1397–1399.
- 371 A. Nasti, N. M. Zaki, P. De Leonardis, S. Ungphaiboon, P. Sansongsak, M. G. Rimoli and N. Tirelli, Chitosan/TPP and Chitosan/TPP-hyaluronic Acid Nanoparticles: Systematic Optimisation of the Preparative Process and Preliminary Biological Evaluation, *Pharm. Res.*, 2009, **26**(8), 1918–1930.
- 372 S. S. O. Zaki, M. N. Ibrahim and H. Katas, Particle Size Affects Concentration-Dependent Cytotoxicity of Chitosan Nanoparticles towards Mouse Hematopoietic Stem Cells, *J. Nanotechnol.*, 2015, 919658.
- 373 C. Sy, M. K. Jang and J. W. Nah, Influence of molecular weight on oral absorption of water soluble chitosans, *J. Controlled Release*, 2005, **102**(2), 383–394.
- 374 Y. Suzuki, K. Miyatake, Y. Okamoto, E. Muraki and S. Minami, Influence of the chain length of chitosan on complement activation, *Carbohydr. Polym.*, 2003, **54**, 465–469.



- 375 C. Marchand, J. Bachand, E. Baraghis, M. Lamarre, G. E. Rivard, G. De Crescenzo and C. D. Hoemann, C3, C5, and factor B bind to chitosan without complement activation, *J. Biomed. Mater. Res., Part A*, 2009, 1429–1441.
- 376 P. Baldrick, The safety of chitosan as a pharmaceutical excipient, *Regul. Toxicol. Pharmacol.*, 2010, 56(3), 290–299.
- 377 S. Hirano, M. Iwata, K. Yamanaka, H. Tanaka, T. Toda and H. Inui, Enhancement of serum lysozyme activity by injecting a mixture of chitosan oligosaccharides intravenously in rabbits, *Agric. Biol. Chem.*, 1991, 55(10), 2623–2625.
- 378 S. Hirano, H. Seino, Y. Akiyama and N. Isao, Biocompatibility of chitosan by oral and intravenous administrations, *Polym. Mater. Sci. Eng.*, 1988, 59, 897–901.
- 379 Y. Tanaka, S. I. Tanioka, M. Tanaka, T. Tanigawa, Y. Kitamura, S. Minami, Y. Okamoto, M. Miyashita and M. Nanno, Effects of chitin and chitosan particles on BALB/c mice by oral and parenteral administration, *Biomaterials*, 1997, 18(8), 591–595.
- 380 C. Zhang, G. Qu, Y. Sun, T. Yang, Z. Yao, W. Shen, Z. Shen, Q. Ding, H. Zhou and Q. Ping, Biological evaluation of N-octyl-O-sulfate chitosan as a new nano-carrier of intravenous drugs, *Eur. J. Pharm. Sci.*, 2008, 33(4–5), 415–423.
- 381 C. Qin, J. Gao, L. Wang, L. Zeng and Y. Liu, Safety evaluation of short-term exposure to chito oligomers from enzymic preparation, *Food Chem. Toxicol.*, 2006, 44(6), 855–861.
- 382 M. Takahashi, K. Inoue, M. Yoshida, T. Morikawa, M. Shibutani and A. Nishikawa, Lack of chronic toxicity or carcinogenicity of dietary N-acetylglucosamine in F344 rats, *Food Chem. Toxicol.*, 2009, 47(2), 462–471.
- 383 K. Sonaje, Y. H. Lin, J. H. Juang, S. P. Wey, C. T. Chen and H. W. Sung, In vivo evaluation of safety and efficacy of self-assembled nanoparticles for oral insulin delivery, *Biomaterials*, 2009, 30(12), 2329–2339.
- 384 Y. L. Hu, W. Qi, F. Han, J. Z. Shao and J. Q. Gao, Toxicity evaluation of biodegradable chitosan nanoparticles using a zebrafish embryo model, *Int. J. Nanomed.*, 2011, 6, 3351–3359.
- 385 G. Giacalone, M. Quaillet, N. Huang, V. Nicolas, C. Boulogne, C. Gillet, E. Fattal, A. Bochot and H. Hillaireau, An injectable, nanostructured implant for the delivery of adenosine triphosphate: towards long-acting formulations of small, hydrophilic drugs, *Drug Delivery Transl. Res.*, 2024, 14(8), 2146–2157.
- 386 M. Thanou, J. C. Verhoef and H. E. Junginger, Chitosan and its derivatives as intestinal absorption enhancers, *Adv. Drug Delivery Rev.*, 2001, 50, 91–101.
- 387 A. M. M. Sadeghi, F. A. Dorkoosh, M. R. Avadi, M. Weinhold, A. Bayat, F. Delie, R. Gurny, B. Larijani, M. Rafiee-Tehrani and H. E. Junginger, Permeation enhancer effect of chitosan and chitosan derivatives: Comparison of formulations as soluble polymers and nanoparticulate systems on insulin absorption in Caco-2 cells, *Eur. J. Pharm. Biopharm.*, 2008, 70, 270–278.
- 388 M. Thanou, M. T. Nihot, M. Jansen, J. C. Verhoef and H. E. Junginger, Mono-N-Carboxymethyl Chitosan (MCC), a Polyampholytic Chitosan Derivative, Enhances the Intestinal Absorption of Low Molecular Weight Heparin Across Intestinal Epithelia, *J. Pharm. Sci.*, 2001, 90(1), 38–46.
- 389 G. Sandri, M. Cristina, S. Rossi, F. Ferrari, S. Gibin, Y. Zambito, G. Di and C. Caramella, Nanoparticles based on N-trimethylchitosan: Evaluation of absorption properties using in vitro (Caco-2 cells) and ex vivo (excised rat jejunum) models, *Eur. J. Pharm. Biopharm.*, 2007, 65, 68–77.
- 390 A. Bernkop-Schnürch, D. Guggi and Y. Pinter, Thiolated chitosans: development and in vitro evaluation of a mucoadhesive, permeation enhancing oral drug delivery system, *J. Controlled Release*, 2004, 94, 177–186.
- 391 L. Zeng, C. Qin, W. Wang, W. Chi and W. Li, Absorption and distribution of chitosan in mice after oral administration, *Carbohydr. Polym.*, 2008, 71, 435–440.
- 392 T. Yin, Y. Zhang, Y. Liu, Q. Chen, Y. Fu, J. Liang, J. Zhou, X. Tang, J. Liu and M. Huo, The efficiency and mechanism of N-octyl-O,N-carboxymethyl chitosan-based micelles to enhance the oral absorption of silybin, *Int. J. Pharm.*, 2018, 536(1), 231–240.
- 393 A. H. Krauland, D. Guggi and A. Bernkop-Schnürch, Oral insulin delivery: the potential of thiolated chitosan-insulin tablets on non-diabetic rats, *J. Controlled Release*, 2004, 95, 547–555.
- 394 S. Sudhakar, S. V. Chandran, N. Selvamurugan and R. A. Nazeer, Biodistribution and pharmacokinetics of thiolated chitosan nanoparticles for oral delivery of insulin in vivo, *Int. J. Biol. Macromol.*, 2020, 150, 281–288.
- 395 T. Huq, A. Khan, D. Brown, N. Dhayagude, Z. He and Y. Ni, Sources, production and commercial applications of fungal chitosan: A review, *J. Bioresour. Bioprod.*, 2022, 7(2), 85–98.
- 396 F. Caputo, G. Favre, G. Borchard, L. Calzolari, P. Fiscaro, E. Frejafon, N. Günday-Türel, D. Koltsov, C. Minelli, B. C. Nelson, J. Parot, A. Prina-Mello, S. Zou and F. X. Ouf, Toward an international standardisation roadmap for nanomedicine, *Drug Delivery Transl. Res.*, 2024, 14(9), 2578–2588.
- 397 European Commission H2020 Projects: Metrological Evaluation and Testing of Robots in International CompetitionS (<https://cordis.Europa.eu/project/id/871252>); Pharmaceutical Open Innovation Test Bed for Enabling Nano-pharmaceutical Innovative Products (<https://cordis.Europa.eu/project/id/953110>).
- 398 S. Sreekumar, F. M. Goycoolea, B. M. Moerschbacher and G. R. Rivera-Rodriguez, Parameters influencing the size of chitosan-TPP nano- and microparticles, *Sci. Rep.*, 2018, 8(1), 4695 [cited 2025 July 23]; available from: <https://www.nature.com/articles/s41598-018-23064-4>.



- 399 R. Masimov and E. K. Wasan, Chitosan non-particulate vaccine delivery systems, *J. Pharm. Pharm. Sci.*, 2024, 27, [cited 2025 July 23]. Available from: <https://www.frontierspartnerships.org/articles/10.3389/jpps.2024.12921/full>.
- 400 A. Vila, A. Sánchez, M. Tobío, P. Calvo and M. J. Alonso, Design of biodegradable particles for protein delivery, *J. Controlled Release*, 2002, 78(1–3), 15–24.
- 401 E. C. Carroll, L. Jin, A. Mori, N. Muñoz-Wolf, E. Oleszycka, H. B. T. Moran, S. Mansouri, C. P. McEntee, E. Lambe, E. M. Agger, P. Andersen, C. Cunningham, P. Hertzog, K. A. Fitzgerald, A. G. Bowie and E. C. Lavelle, The Vaccine Adjuvant Chitosan Promotes Cellular Immunity via DNA Sensor cGAS-STING-Dependent Induction of Type I Interferons, *Immunity*, 2016, 44(3), 597–608.
- 402 D. A. Zaharoff, C. J. Rogers, K. W. Hance, J. Schlom and J. W. Greiner, Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination, *Vaccine*, 2007, 25(11), 2085–2094.
- 403 F. Khademi, R. A. Taheri, Y. A. Arshid, H. Vaez, A. A. Momtazi-Borojeni and S. Soleimanpour, Are chitosan natural polymers suitable as adjuvant/delivery system for anti-tuberculosis vaccines?, *Microb. Pathog.*, 2018, 121, 218–223.

