Advanced Drug Delivery Reviews 60 (2008) 1650–1662

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

# Polysaccharides-based nanoparticles as drug delivery systems

Zonghua Liu [a], Yanpeng Jiao [a], Yifei Wang [b], Changren Zhou [a], Ziyong Zhang [a,*]

[a] Department of Materials Science and Engineering, Jinan University, Guangzhou 510632, China
[b] Biomedicine Research and Development Center, Jinan University, Guangzhou 510632, China

ARTICLE INFO

*Article history:*
Received 7 July 2008
Accepted 8 August 2008
Available online 17 September 2008

*Keywords:*
Polysaccharide
Nanoparticle
Drug delivery system

ABSTRACT

Natural polysaccharides, due to their outstanding merits, have received more and more attention in the field of drug delivery systems. In particular, polysaccharides seem to be the most promising materials in the preparation of nanometeric carriers. This review relates to the newest developments in the preparation of polysaccharides-based nanoparticles. In this review, four mechanisms are introduced to prepare polysaccharides-based nanoparticles, that is, covalent crosslinking, ionic crosslinking, polyelectrolyte complex, and the self-assembly of hydrophobically modified polysaccharides.



**Contents**



## 1. Introduction

Nanoparticle drug delivery systems are nanometeric carriers used to deliver drugs or biomolecules. Generally, nanometeric carriers also comprise sub-micro particles with size below 1000 nm and with various morphologies, including nanospheres, nanocapsules, nanomicelles, nanoliposomes, and nanodrugs, etc. [1,2].

Nanoparticle drug delivery systems have outstanding advantages [1]: (1) they can pass through the smallest capillary vessels because of their ultra-tiny volume and avoid rapid clearance by phagocytes so that their duration in blood stream is greatly prolonged; (2) they can penetrate cells and tissue gap to arrive at target organs such as liver, spleen, lung, spinal cord and lymph; (3) they could show controlled-release properties due to the biodegradability, pH, ion and/or temperature sensibility of materials; (4) they can improve the utility of drugs and reduce toxic side effects; etc.

As drug delivery system, nanoparticles can entrap drugs or biomolecules into their interior structures and/or absorb drugs or biomolecules onto their exterior surfaces. Presently, nanoparticles have been widely used to deliver drugs, polypeptides, proteins, vaccines, nucleic acids, genes and so on. Over the years, nanoparticle drug delivery systems have shown huge potential in biological, medical and pharmaceutical applications [3].

Currently, the researches on nanoparticle drug delivery system focus on: (1) the selectness and combination of carrier materials to obtain suitable drug release speed; (2) the surface modification of nanoparticles

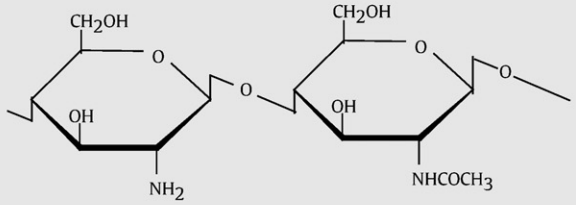
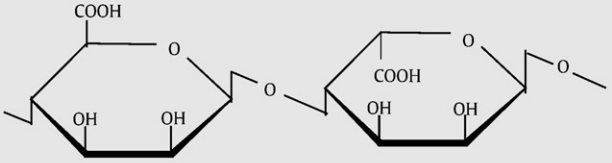
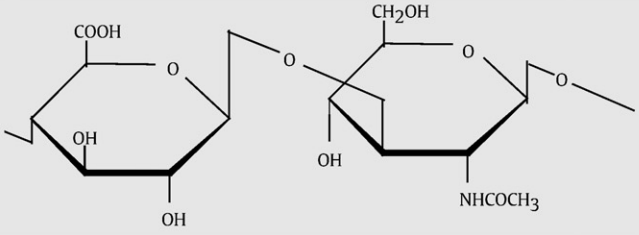
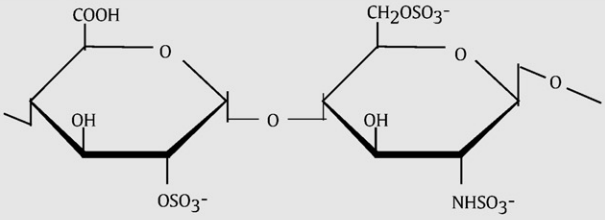
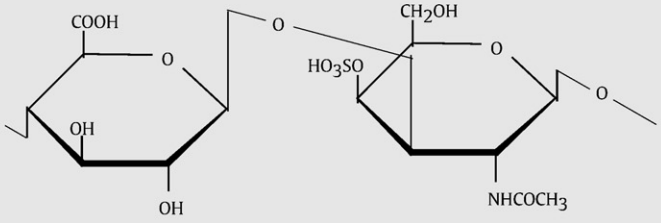
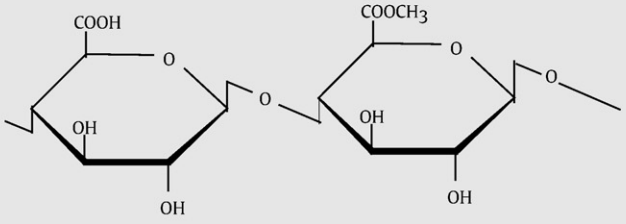
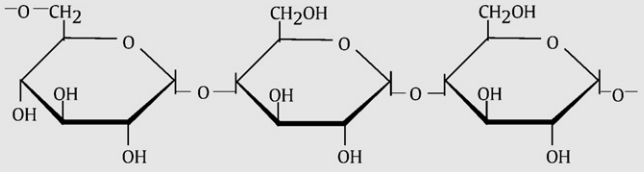
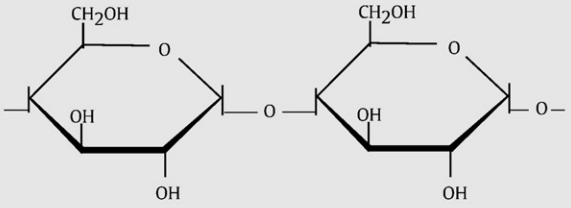
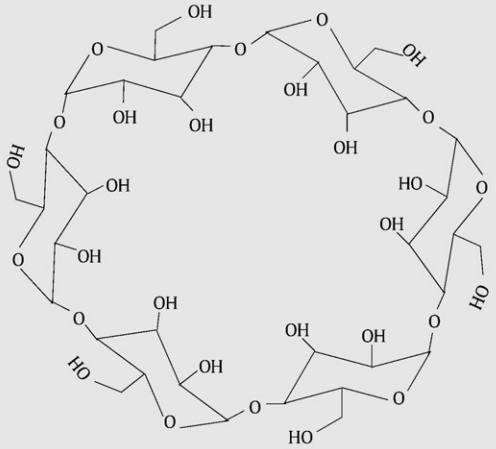
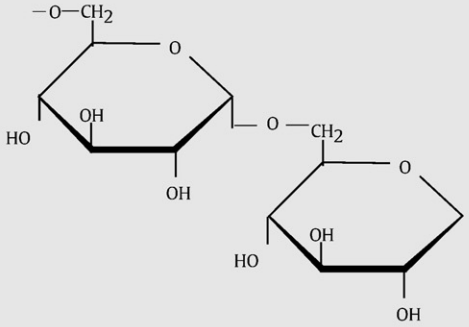
* Corresponding author.
*E-mail address:* tzhangzy@163.com (Z. Zhang).

0169-409X/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2008.09.001

to improve their targeting ability; (3) the optimization of the preparation of nanoparticles to increase their drug delivery capability, their application in clinics and the possibility of industrial production; (4) the investigation of *in vivo* dynamic process to disclose the interaction of nanoparticles with blood and targeting tissues and organs, etc.

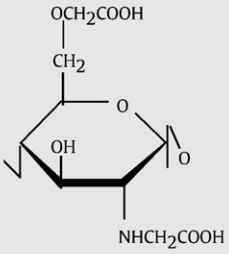
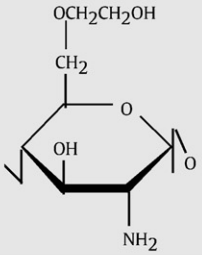
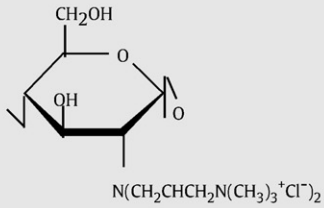
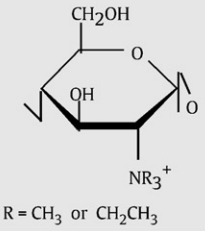
Polymeric materials used for preparing nanoparticles for drug delivery must be biocompatible at least and biodegradable best. To this aim, many polymeric materials have been applied, including poly(lactic acid), poly(glycolic acid), polycaprolactone, polysaccharides (particularly chitosan), poly(acrylic acid) family, proteins or polypeptides (such as

**Table 1**
Chemical Structures of polysaccharides

| | |
|---|---|
| Chitosan | Alginate |
|  |  |
| Hyaluronic acid | Heparin |
|  |  |
| Chondroitin sulphate | Pectin |
|  |  |
| Pullulan | Amylose |
|  |  |
| Cyclodextrin | Dextran |
|  |  |

*(continued on next page)*

**Table 1** (*continued*)

| | |
|---|---|
| Carboxymethyl chitosan | Glycol chitosan |
|  |  |
| N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride | N-trimethyl chitosan ( R = $CH_3$ )<br>N-triethyl chitosan ( R = $CH_2CH_3$ ) |
|  |  |

gelatin), etc. Among them, polysaccharides are the most popular polymeric materials to prepare nanoparticles for drug delivery.

## 2. Polysaccharides

Polysaccharides are the polymers of monosaccharides. In nature, polysaccharides have various resources from algal origin (e.g. alginate), plant origin (e.g. pectin, guar gum), microbial origin (e.g. dextran, xanthan gum), and animal origin (chitosan, chondroitin) [4]. Polysaccharides have a large number of reactive groups, a wide range of molecular weight (MW), varying chemical composition, which contribute to their diversity in structure and in property. The chemical structures of usual polysaccharides and important chitosan derivatives are listed in Table 1 [4]. From the viewpoint of polyelectrolyte, polysaccharides can be divided into polyelectrolytes and non-polyelectrolytes, the former can be further divided into positively charged polysaccharides (chitosan) and negatively charged polysaccharides (alginate, heparin, hyaluronic acid, pectin, etc.).

Due to the presence of various derivable groups on molecular chains, polysaccharides can be easily modified chemically and biochemically, resulting in many kinds of polysaccharide derivatives. As natural biomaterials, polysaccharides are highly stable, safe, non-toxic, hydrophilic and biodegradable. In addition, polysaccharides have abundant resources in nature and low cost in their processing. Particularly, most of natural polysaccharides have hydrophilic groups such as hydroxyl, carboxyl and amino groups, which could form non-covalent bonds with biological tissues (mainly epithelia and mucous membranes), forming bioadhesion [5]. For example, chitosan, starch, alginate and so on are good bioadhesive materials. Nanoparticle carriers made of bioadhesive polysaccharides could prolong the residence time and therefore increase the absorbance of loaded drugs. All these merits endow polysaccharides a promising future as biomaterials. For the application of these naturally occurring polysaccharides for drug carriers, issues of safety, toxicity and availability are greatly simplified. In recent years, a large number of studies have been conducted on polysaccharides and their derivatives for their potential application as nanoparticle drug delivery systems [4,6–8].

## 3. Polysaccharide-based nanoparticles

As for polysaccharide-based nanoparticles, Alonso et al. [9] and Prabaharan et al. [10] have ever made excellent reviews in 2001 and 2005, respectively, focusing on the preparation and application of chitosan nanoparticle carriers. As time goes on, more polysaccharide-based nanoparticles emerge, which greatly enriches the versatility of nanoparticle carriers in terms of category and function. According to structural characteristics, these nanoparticles are prepared mainly by four mechanisms, namely covalent crosslinking, ionic crosslinking, polyelectrolyte complexation, and self-assembly of hydrophobically modified polysaccharides.

### 3.1. Covalently crosslinked polysaccharide nanoparticles

The early preparation of polysaccharide nanoparticles was by means of covalent crosslinking. Among various polysaccharides, chitosan is the early one to be used to prepare nanoparticles. As a usual crosslinker, glutaraldehyde was ever used to crosslink chitosan-based nanoparticles. Recently, some chitosan nanoparticles were still crosslinked by glutaradehyde [11,12]. Unfortunately, the toxicity of glutaraldehyde on cell viability limits its utility in the field of drug delivery.

Along with the use of biocompatible crosslinkers, biocompatible covalent crosslinking is promising. With the aid of water-soluble condensation agent of carbodiimide, natural di- and tricarboxylic acids, including succinic acid, malic acid, tartaric acid and citric acid, were used for intermolecular crosslinking of chitosan nanoparticles [13,14]. The condensation reaction was performed between the carboxylic groups of natural acids and the pendant amino groups of chitosan, through which biodegradable chitosan nanoparticles were obtained. This method allows the formation of polycations, polyanions, and polyampholyte nanoparticles. The prepared nanoparticles were stable in aqueous media at low pH, neutral, and mild alkaline conditions. In the swollen state, the average size of the particles was in the range of 270–370 nm depending on the pH.

### 3.2. Ionically crosslinked polysaccharide nanoparticles

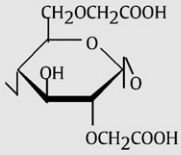
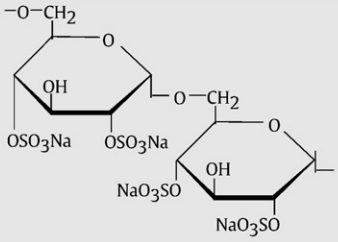
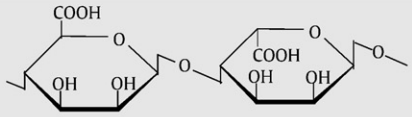
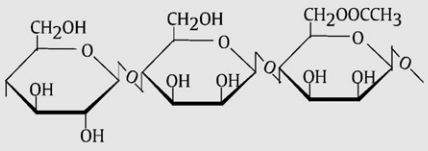
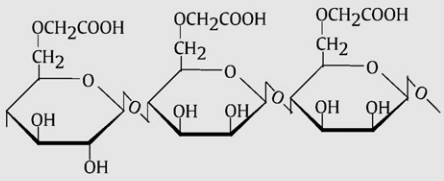
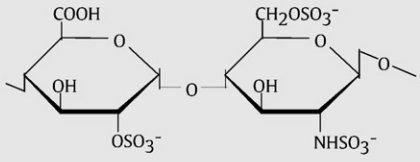
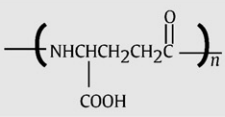
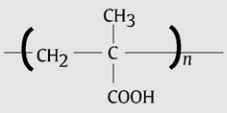
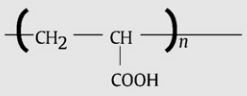
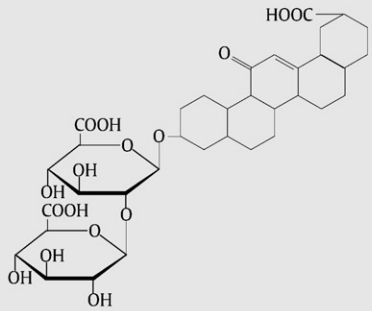
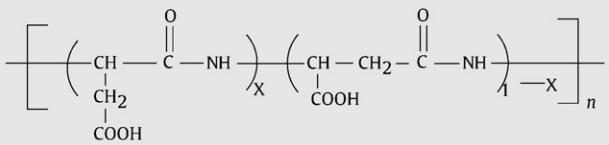
Compared with covalent crosslinking, ionic crosslinking has more advantages: mild preparation conditions and simple procedures. For charged polysaccharides, low MW of polyanions and polycations could act as ionic crosslinkers for polycationic and polyanionic polysaccharides, respectively. To date, the most widely used polyanion crosslinker is tripolyphosphate (TPP). Alonso et al. [15,16] first reported TPP-

crosslinked chitosan nanoparticles in 1997. TPP is non-toxic and has multivalent anions. It can form a gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counterions of TPP [17]. From then on, TPP-chitosan nanoparticles have been widely used to deliver various drugs and macromolecules [18–30].

Recently, water-soluble chitosan derivatives were also be ionically crosslinked to prepare nanoparticles. Compared with chitosan itself, its derivatives can easily dissolve in neutral aqueous media, avoiding the potential toxicity of acids and hence protecting the bioactivity of loaded biomacromolecules. Xu et al. [31] synthesized water-soluble chitosan derivative, N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (the chemical structure shown in Table 1) by the reaction between glycidyl-trimethyl-ammonium chloride and chitosan. Nanoparticles of 110–180 nm in size were formed based on ionic gelation process of the derivative and TPP. Bovine serum albumin, as a model protein drug, was incorporated into the nanoparticles with encapsulation efficiency up to 90%. In addition, Amidi et al. [32] prepared N-trimethyl chitosan nanoparticles by ionic crosslinking of N-trimethyl chitosan (the chemical structure shown in Table 1) with TPP and evaluated their potential as a carrier system for the nasal delivery of proteins, ovalbumin. The nanoparticles had an average size of about 350 nm and a positive zeta potential. They showed a loading efficiency up to 95% and a loading capacity up to 50% (w/w). The integrity of the entrapped ovalbumin was preserved. Cytotoxicity tests with Calu-3 cells showed no toxic effects of the nanoparticles. In vivo uptake studies indicated the transport of fluorescent isothiocyanate (FITC)-albumin-associated nanoparticles across the nasal mucosa. Sandri et al. [33] evaluated the absorption properties of N-trimethyl-chitosan/TPP nanoparticles using in vitro (Caco-2 cells) and ex vivo (excised rat jejunum) models. Shi et al. prepared carboxymethyl chitosan (the chemical structure shown in Table 1) nanoparticles (200–300 nm and in a narrow distribution) through ionic gelification with calcium ion and evaluated the potential of the nanoparticles as carriers for anticancer drug, doxorubicin. Effects of degree of substitution (DS) and MW of carboxymethyl chitosan on doxorubicin delivery were discussed.

Besides, calcium-crosslinked negatively charged polysaccharide nanoparticles have recently found utility as drug carriers. Some polysaccharides bearing carboxylic groups on molecular chains can be crosslinked by bivalent calcium ion to form nanoparticles. You et al. [34] prepared Ca-crosslinked alginate nanoparticles by water-in-oil reverse microemuldion method. To examine the potency of the nanoparticles for gene delivery, green fluorescent protein-encoding plasmids were encapsulated in the nanoparticles to investigate the degree of endocytosis by NIH 3T3 cells and ensuing transfection rate. Results showed that Ca-alginate nanoparticles with an average size around 80 nm in diameter were very efficient gene carriers. Zahoor

**Table 2**
Chemical structures of negative polymers complexed with chitosan

| | | |
|---|---|---|
| Carboxymethyl cellulose [37] | Dextran sulfate [38-41] | Alginate [40-43] |
|  |  |  |
| Glucomannan [44] | Carboxymethyl konjac glucomannan [45-47] | Heparin [48] |
|  |  |  |
| Poly-γ-glutamic acid [50, 51] | Polymethacrylic acid [52] | Polyacrylic acid [53, 54] |
|  |  |  |
| Glycyrrhetic acid [55] | Polyaspartic acid sodium salt [57] | |
|  |  | |

et al. [35] also prepared Ca-alginate nanoparticles (235.5±0 nm in size) by ion-induced gelification. Drug encapsulation efficiencies in the nanoparticles were 70–90% for isoniazid, pyrazinamide and 80–90% for rifampicin. The relative bioavailabilities of all drugs encapsulated were significantly higher compared with oral free drugs. All drugs were detected in organs (lungs, liver and spleen) above the minimum inhibitory concentration until 15 days post nebulisation, whilst free drugs stayed up to day 1. These inhalable nanoparticles could serve as an ideal carrier for the controlled release of anti-tubercular drugs.

In addition, Kim et al. [36] encapsulated retinol into chitosan nanoparticles and reconstituted it into aqueous solution for cosmetic and pharmaceutical applications. Solubility of retinol is able to increase by encapsulation into chitosan nanoparticles more than 1600-fold. It was suggested that retinol was encapsulated into chitosan nanoparticles by ion complex due to the electrostatic interaction between amine group of chitosan and hydroxyl group of retinol.

### 3.3. Polysaccharide nanoparticles by polyelectrolyte complexation (PEC)

Polyelectrolyte polysaccharides can form PEC with oppositely charged polymers by intermolecular electrostatic interaction. Polysaccharide-based PEC nanoparticles can be obtained by means of adjusting the MW of component polymers in a certain range. In theory, any polyelectrolyte could interact with polysaccharides to fabricate PEC nanoparticles. However, in practice, these polyelectrolytes are restricted to those water-soluble and biocompatible polymers in view of safety purpose. In this sense, chitosan is the only natural polycationic polysaccharide that satisfies the needs. There are many negative polymers (with chemical structures shown in Table 2) complexed with chitosan to form PEC nanoparticles, which can be divided into polysaccharides, peptides, polyacrylic acid family and so on.

#### 3.3.1. Negative polysaccharides

Cui et al. [37] used carboxymethyl cellulose to complex chitosan to form stable cationic nanoparticles and investigated the topical application of these nanoparticles containing plasmid DNA as a potential approach to genetic immunization. Plasmid DNA was coated on pre-formed cationic chitosan/carboxymethylcellulose nanoparticles. Selected plasmid DNA-coated nanoparticles (with plasmid DNA up to 400 mg/ml) were stable to challenge with serum. Several different chitosan-based nanoparticles containing plasmid DNA resulted in both detectable and quantifiable levels of luciferase expression in mouse skin 24 h after topical application, and significant antigen-specific IgG titer to expressed β-galactosidase at 28 days. Chen et al. [38] developed chitosan/dextran sulfate nanoparticle delivery system by employing a simple coacervation process. The study investigated the effect of the weight ratio of the two polymers on particle size, surface charge, entrapment efficiency and release characteristics of anti-angiogenesis peptide. Particles of 223 nm mean diameter were produced under optimal conditions with a zeta potential of approximately −32.6 mV. The physicochemical and release characteristics of the nanoparticles could be modulated by changing ratios of two ionic polymers. Tiyaboonchai et al. [39] developed a nanoparticulate delivery system for amphotericin B with chitosan and dextran sulfate together with zinc sulfate as a cross-linking and hardening agent. The nanoparticles obtained possessed a mean particle size of 600–800 nm with a polydispersity index of 0.2, indicating a narrow size distribution. The measured zeta potential of the nanoparticle surface was approximately −32 mV, indicating a strong negative charge at the particle's surface. Drug association efficacy of up to 65% was achieved. Sarmento et al. [40,41] studied dextran sulfate or alginate complexation with chitosan on mean particle size, insulin association efficiency, loading capacity and release profile. Nanoparticles were formed by ionotropic complexation and coacervation between polyanions (dextran sulfate and alginate) and chitosan. Mean nanoparticle diameter ranged from 423 to 850 nm, insulin association efficiency from 63 to 94% and loading capacity from 5 to 13%. Dextran sulfate/chitosan nanoparticle system provided highest insulin association efficiency and retention of insulin in gastric simulated conditions. Sarmento et al. [42] also prepared insulin-loaded nanoparticles by ionotropic pre-gelation of alginate with calcium chloride followed by complexation between alginate and chitosan. The same group probed the structural integrity of insulin after being entrapped into chitosan/alginate nanoparticles [43]. The results confirmed that no significant conformational changes of insulin occurred in terms of α-helix and β-sheet content. Alonso-Sande et al. [44] used two different types of glucomannan (non-phosphorylated and phosphorylated) and two different approaches to prepare nanoparticles. These procedures involved the interaction of chitosan and glucomannan in the presence or absence of sodium tripolyphosphate, which acted as an ionic cross-linking agent for chitosan. Depending on the formulation conditions, it was possible to obtain nanoparticles with size from 200 to 700 nm and zeta potential from −2 to +39 mV. The nanoparticles exhibited a great capacity for the association of insulin and the immunomodulatory protein P1, reaching association efficiency values as high as 89%. Du et al. [45–47] prepared carboxymethyl konjac glucomannan/chitosan nanoparticles under very mild conditions via polyelectrolyte complexation. Bovine serum albumin, as a model protein drug, was incorporated into the nanoparticles and the encapsulation efficiency and in vitro release behavior of the bovine serum albumin were investigated. The nanoparticles not only exhibited pH-responsive properties, but ionic strength-sensitive properties. Liu et al. [48] prepared heparin/chitosan nanoparticles by polyelectrolyte complexation. Entrapment studies of the nanoparticles were conducted using bovine serum albumin as a model protein. Specifically, the effects of the pH value of chitosan solution, chitosan MW, chitosan concentration, heparin concentration, and the protein concentration on the nanoparticle size, the nanoparticle yield, and the protein entrapment were studied in detail. Li et al. [49] prepared quaternized chitosan/alginate nanoparticles in neutral condition for the oral delivery of protein. The diameter of the nanoparticles with a positive surface charge was about 200 nm.

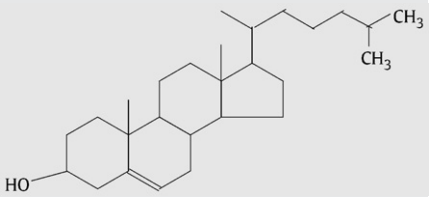
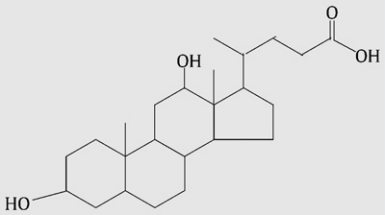
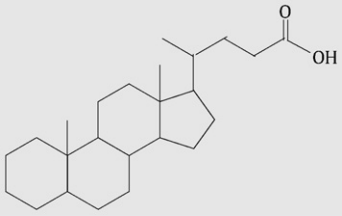
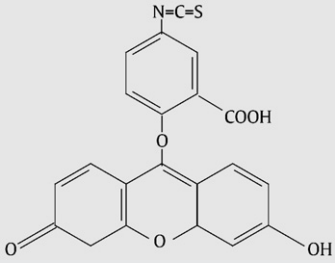
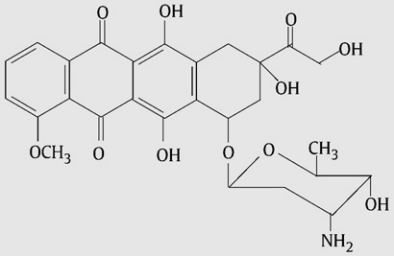
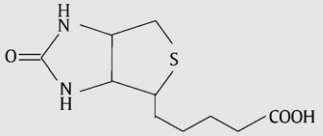
#### 3.3.2. Negative peptides

Lin et al. [50] prepared poly-γ-glutamic acid/chitosan nanoparticle system using ionic-gelation method. Evaluation of the prepared nanoparticles in enhancing intestinal paracellular transport was investigated in vitro in Caco-2 cell monolayers. It was found that the nanoparticles with chitosan dominated on the surfaces could effectively reduce the transepithelial electrical resistance of Caco-2 cell monolayers and opened the tight junctions between Caco-2 cells and allowed transport of the nanoparticles via the paracellular pathways. Moreover, the nanoparticles were further used for transdermal gene delivery. As compared with chitosan/DNA, chitosan/poly-γ-glutamic acid/DNA improved their penetration depth into the mouse skin and enhanced gene expression. These observations may be attributed to the fact that chitosan/poly-γ-glutamic acid/DNA were more compact in their internal structures and had a greater density than their chitosan/DNA counterparts, thus having a larger momentum to penetrate into the skin barrier [51].

#### 3.3.3. Polyacrylic acid family

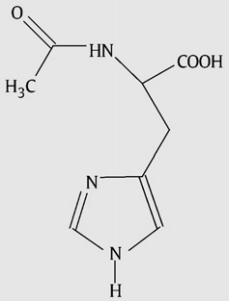
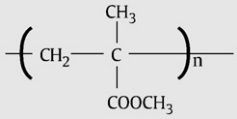
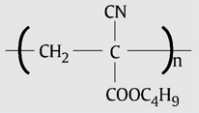
Sajeesh et al. [52] prepared pH sensitive polymethacrylic acid/chitosan/polyethylene glycol nanoparticles under mild aqueous. Free radical polymerization of methacrylic acid was carried out in presence of chitosan and polyethylene glycol using a water-soluble initiator and particles were obtained spontaneously during polymerization without using organic solvents or surfactants/steric stabilizers. Insulin and bovine serum albumin as model proteins were incorporated into the nanoparticles by diffusion filling method and their in vitro release characteristics were evaluated at pH 1.2 and 7.4. The nanoparticles exhibited good protein encapsulation efficiency and pH responsive

**Table 3**
Hydrophobic molecules used to modify polysaccharides

| Polysaccharides | Hydrophobic molecules | Chemical structure of hydrophobic molecules |
|---|---|---|
| Chitosan [69–74] | Poly(ethylene glycol) | $HO–CH_2CH_2–O–(CH_2CH_2O)_n–CH_3$ |
| β-Cyclodextrin [75] | Hexanoic acid | $CH_3\ (CH_2)_4COOH$ |
| | Decanoic acid | $CH_3\ (CH_2)_8COOH$ |
| Chitosan [76], Amylose [82] | Linoleic acid | $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$ |
| Chitosan [77,78] | Linolenic acid | $CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$ |
| Chitosan [79] | Palmitic acid | $CH_3(CH_2)_{14}COOH$ |
| Chitosan [80] | Stearic acid | $CH_3(CH_2)_{16}COOH$ |
| Chitosan [81] | Oleic acid | $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ |
| Dextran [83–86], chitosan [87] | Poly(ε-caprolactone) | $–[O–(CH_2)_5–CO]_n–$ |
| Heparin [88], Hyaluronic acid [89] | Pluronic | $–(CH_2CH_2O)_n–(CH_2CH(CH_3)O)_m–$ |
| Pullulan [97] | Hexadecanol | $CH_3(CH_2)_{15}OH$ |
| Chitosan [90], Carboxymethyl chitosan [91], Pullulan [92–96] | Cholesterol |  |
| Chitosan [98–101], heparin [102], Glycol chitosan [103] | Deoxycholic acid |  |
| Glycol chitosan [104–108] | 5β-Cholanic acid |  |
| Glycol chitosan [109,111,113] | Fluorescein isothiocyanate (FITC) |  |
| Glycol chitosan [110–112] | Doxorubicin |  |
| Pullulan [114] | Vitamin H |  |

(continued on next page)

**Table 3** (*continued*)

| Polysaccharides | Hydrophobic molecules | Chemical structure of hydrophobic molecules |
|---|---|---|
| Glycol chitosan [116] | N-Acetyl histidine |  |
| Heparin [117, 118], Dextran [118] | Poly(methyl methacrylate) |  |
| Chitosan [119,120,125], Dextran [120,125,127,128], Dextran sulfate [120,125], Thiolated chitosan [121–124], Heparin [125,126,128], Hyaluronic acid [125], Pectin [125] | Poly(isobutyl Cyanoacrylate) |  |

release profile was observed under in vitro conditions. Chen et al. [53] reported the formation of chitosan/poly(acrylic acid) nanoparticles. When polyanion poly(acrylic acid) was dropped into polycation chitosan solution, nanoparticles with diverse microstructure would be formed under different experimental conditions. The influence of MW of chitosan and poly(acrylic acid), shell cross-linking, dropping temperature on the size, stability and morphology of the nanoparticles were also studied. These nanoparticles could encapsulate plasmid DNA very well, which makes them have great potential in gene delivery. The same team also synthesized hollow polymeric nanospheres by polymerization of acrylic acid monomers inside the chitosan-acrylic acid assemblies. The effects of polymerization concentration, shell cross-linking, pH, salt concentration and temperature on the size and stability of hollow polymeric nanospheres were investigated [54].

### 3.3.4. Others

Zheng et al. [55] prepared chitosan/glycyrrhetic acid nanoparticles by polyelectrolyte complexation and studied the glycyrrhetic acid encapsulation efficiency and in vitro release. Stoilova et al. [56] prepared PEC nanoparticles between chitosan and poly(2-acryloylamido-2-methylpropanesulfonic acid) by mixing aqueous solutions of its components or by free radical polymerization on chitosan template. The nanoparticles (mean diameter 250 nm and monomodal distribution) were stable in acidic and neutral medium and dissociated at pH>8. Zheng et al. [57] prepared anionic or cationic nanoparticles based on chitosan and polyaspartic acid sodium salt. A hydrophilic drug, 5-fluorouracil, was contained in the nanoparticles.

In addition, the nanoparticle formed by complexion between chitosan or its derivative and DNA or RNA is a special type of delivery system, which has been well studied [58–67].

Noteworthy is that, the category of this kind of nanoparticles will continuously increase because: (1) chitosan has a lot of water-soluble positively charged derivatives, such as glycol chitosan, N-trimethyl chitosan and N-triethyl chitosan, which can be used as polycation instead of chitosan; (2) more biocompatible negative polymers will be exploited, in particular, various polyanionic polysaccharides.

## 3.4. Self-assembly of hydrophobically modified polysaccharides

When hydrophilic polymeric chains are grafted with hydrophobic segments, amphiphilic copolymers are synthesized. Upon contact with an aqueous environment, polymeric amphiphiles spontaneously form micelles or micellelike aggregates via undergoing intra- or intermolecular associations between hydrophobic moieties, primarily to minimize interfacial free energy. These polymeric micelles exhibit unique characteristics, depending on hydrophilic/hydrophobic constituents, such as unusual rheological feature, small hydrodynamic radius (less than microsize) with core-shell structure, and thermodynamic stability. In particular, polymeric micelles have been recognized as a promising drug carrier, since their hydrophobic domain, surrounded by a hydrophilic outer shell, can serve as a preservatory for various hydrophobic drugs [68]. In recent years, numerous studies have been carried out to investigate the synthesis and the application of polysaccharide-based self-aggregate nanoparticles as drug delivery systems. The hydrophobic molecules used to modify polysaccharides are listed in Table 3. Generally, these hydrophobic molecules can be divided into linear, cyclic hydrophobic molecules, hydrophobic drug, polyacrylate family, etc.

### 3.4.1. Linear hydrophobic molecules

Poly(ethylene glycol) has been employed extensively in pharmaceutical and biomedical fields because of its outstanding physicochemical and biological properties including hydrophilic property, solubility, non-toxicity, ease of chemical modification and absence of antigenicity and immunogenicity. Therefore, poly(ethylene glycol) has been often used as a soluble polymeric modifier in organic synthesis; it is also widely used as a pharmacological polymer with high hydrophilicity, biocompatibility and biodegradability. It is ideal for prevention of bacterial surface growth, decrease of plasma protein binding and erythrocyte aggregation, and prevention of recognition by the immune system. In recent years, poly(ethylene glycol)-g-chitosan has been studied by many researchers [69]. Yoksan et al. [70] grafted poly(ethylene glycol) methyl ether onto N-Phthaloyl chitosan chains. The graft-copolymer aggregated to obtain sphere-like nanoparticles. When the chain length of poly(ethylene glycol) methyl ether was as high as $5\times10^3$ Da, the sphere size became as small as 80–100 nm. By simply adjusting the hydrophobicity/hydrophilicity of the chitosan chain, stable nanospheres could be obtained directly. Jeong et al. [71] synthesized methoxy poly(ethylene glycol)-grafted chitosan to develop polymeric micelles for the drug delivery to brain tumor. The micelles encapsulated all-trans retinoic acid based on polyion complex formation. The loading efficiency of the micelles was higher

than 80% (w/w) for all formulations. Park et al. [72] found the all-trans retinoic acid-incorporated nanoparticles were more effective to inhibit invasion of tumor cells than free all-trans retinoic acid at invasion test using matrigel. Yang et al. [73] synthesized methoxy poly (ethylene glycol)-grafted-chitosan conjugates by formaldehyde linking method. The critical aggregation concentration (CMC) of the conjugates was 0.07 mg/ml in water. The conjugates formed monodisperse self-aggregated nanoparticles with a roughly spherical shape and a mean diameter of 261.9 nm. A poorly water-soluble anticancer drug, methotrexate was physically entrapped inside the nanoparticles. Opanasopit et al. [74] synthesized amphiphilic grafted copolymers, N-phthaloylchitosan-grafted poly(ethylene glycol) methyl ether. These copolymers could form micelle-like nanoparticles. The CMC of these nanoparticles in water was similar (28 μg/ml). The nanoparticles exhibited a regular spherical shape with core–shell structure with sizes in the range of 100–250 nm. Camptothecin as a model drug was loaded into the inner core of the micelles.

Some long-chain fatty acids such as hexanoic acid, decanoic acid, linoleic acid, linolenic acid, palmitic acid, stearic acid, and oleic acid have been used for modifying polysaccharides. Choisnard et al. [75] synthesized decanoate β-cyclodextrin esters (DS, 2–7) and hexanoate β-cyclodextrin esters (DS, 4–8) biocatalyzed by thermolysin from native β-cyclodextrin and vinyl hexanoate or vinyl decanoate used as acyl donors. Both esters self-organized into nanoparticles by a nanoprecipitation technique. Chen et al. [76] modified chitosan by coupling with linoleic acid through the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-mediated reaction to increase its amphipathicity for improved emulsification. The micelle formation of linoleic acid-modified chitosan in the 0.1 M acetic acid solution was enhanced by O/W emulsification with methylene chloride, an oil phase, the self-aggregation concentration from 1.0 g/L to 2.0 g/L. The addition of 1 M sodium chloride promoted the self-aggregation of linoleic acid-chitosan molecules both with and without emulsification. The micelles formed nanosize particles ranging from 200 to 600 nm. The nanoparticles encapsulated a lipid soluble model compound, retinal acetate, with 50% efficiency. The same group modified chitosan with linolenic acid (the DS 1.8%) using the same reaction. The CMC of linolenic acid-chitosan in pH7.4 PBS was $5\times10^{-2}$ mg/ml, the self-aggregates with average particle size of 210.8 nm with a unimodal size distribution ranging from 100 to 500 nm. The loading capacity of bovine serum albumin in self-aggregated nanoparticles increased (19.85±0.04 to 37.57±0.25%) with an increasing concentration of bovine serum albumin (0.1–0.5 mg/ml) [77]. The self-aggregated nanoparticles of linolenic acid-chitosan were also used to immobilize trypsin using glutaraldehyde as crosslinker. Results indicated that the activity of trypsin immobilized onto the nanoparticles increased with increasing concentration of glutaraldehyde up to 0.07% (v/v) and then decreased with increasing amount of glutaraldehyde. On the other hand, particle size increased (from 523 to 1372 nm) with the increasing concentration of glutaraldehyde (from 0.03 to 0.1% v/v). The kinetic constant value of trypsin immobilized on nanoparticle (71.9 mg/ml) was higher than that of pure trypsin (50.2 mg/ml). And the thermal stability and optimum temperature of trypsin immobilized on nanoparticles improved, which makes it more attractive in the application aspect [78]. Jiang et al. [79] prepared water-soluble N-palmitoyl chitosan by swollen chitosan coupling with palmitic anhydride in dimethyl sulfoxide, which could form micelles in water. The DS of N-palmitoyl chitosan was in the range of 1.2–14.2%, and the CMC of N-palmitoyl chitosan micelles was in the range of $2.0\times10^{-3}$ to $37.2\times10^{-3}$ mg/ml. The loading capacity of hydrophobic model drug ibuprofen in the micelles was approximately 10%. The drug release strongly depended on pH and temperature: low pH and high temperature accelerated drug release markedly. Hu et al. [80] synthesized stearic acid grafted chitosan oligosaccharide by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-mediated coupling reaction. The CMC of the copolymer was about 0.06, 0.04, 0.01 mg/ml, respectively. To increase the stability of the micelle in vivo and controlled drug release, the shells of micelles were cross-linked by glutaraldehyde. Paclitaxel was used as a model drug to incorporate into the micelles, and the surfaces of the micelles were further cross-linked by glutaraldehyde to form drug loaded and shell cross-linked nanoparticles. The higher drug entrapment efficiencies (above 94%) were observed in all cases. Zhang et al. [81] developed self-assembled nanoparticles based on oleoyl-chitosan with a mean diameter of 255.3 nm. The hemolysis rates of the nanoparticles came well within permissible limits (5%). The nanoparticles showed no cytotoxicity to mouse embryo fibroblasts. Doxorubicin was efficiently loaded into the nanoparticles with an encapsulation efficiency of 52.6%. The drug was rapidly and completely released from the nanoparticles at pH 3.8, whereas at pH 7.4 there was a sustained release after a burst release. The inhibitory rates of doxorubicin-loading nanoparticle suspension to different human cancer cells (A549, Bel-7402, HeLa, and SGC-7901) significantly outperformed that of doxorubicin solution. Amylose-conjugated linoleic acid complexes were synthesized to serve as molecular nanocapsules for the protection and the delivery of linoleic acid [82].

Poly(ε-caprolactone) (PCL) is a biodegradable industrial polyester with excellent mechanical strength, biocompatibility, and non-toxicity. It has been frequently used as implantable carriers for drug delivery systems or as surgical repair materials. It is promising to combine chitosan with the biodegradable polyester to produce amphiphilic copolymer applicable to drug delivery systems. Gref et al. [83,84] synthesized amphiphilic dextran by coupling between carboxylic function present on preformed PCL monocarboxylic acid and the hydroxyl groups on dextran. The comb-like copolymers (dextran-$PCL_n$) consisted of a dextran backbone onto which n preformed PCL blocks were grafted. Nanoparticles of less than 200 nm were successfully prepared by using the new materials. Further, bovine serum albumin and lectin were incorporated in the nanoparticles. Lectins could also be adsorbed onto the surface of the nanoparticles. Surface-bound lectin conserved its hemagglutinating activity, suggesting the possible application of this type of surface-modified nanoparticles for targeted oral administration. Caco-2 cellular viability was higher than 70% when put in contact with the nanoparticles, even at concentrations as high as 660 mg/ml [85]. In addition, they investigated the ability of the dextran coating to modify the interactions with the biological media. They first studied the influence of the dextran coating on the phagocytosis of the nanoparticles by human TPH-1 and J774 murine macrophage-like cell lines. Then, the activation of the complement system (CH50 measurement) at the surface of the nanoparticles and the adsorption of plasma proteins (2D-PAGE) were investigated. It was found that the modification of the surface with dextran significantly reduced the cytotoxicity towards J774 macrophages: the IC50 was increased from 10 to 600 mg/ml. However, the dextran coating could activate complement, probably due to a loop-like conformation of dextran similar to that of cross-linked dextran in Sephadex (a strong complement activator). In addition, depending on whether the dextran loops were large or compact, preferential adsorption, apolipoproteins or immunoglobulins, was observed [86]. Yu et al. [87] synthesized biodegradable amphiphilic PCL-graft-chitosan copolymers. The copolymers could form spherical or elliptic nanoparticles in water.

Pluronic tri-block copolymers composed of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) show lower critical solution temperature behaviors over a broad temperature range depending on the composition and MW. They self-assemble to form a spherical micellar structure above the lower critical solution temperature by hydrophobic interaction of the poly(propylene oxide) middle block in the structure. At high concentration above about 25% (v/v), they exhibit a sol–gel transition behavior when raising the temperature above the lower critical solution temperature. Choi et al.

[88] prepared Pluronic/heparin composite nanocapsules, which exhibited a 1000-fold volume transition (ca. 336 nm at 25 °C; ca. 32 nm at 37 °C), and a reversible swelling and de-swelling behavior when the temperature was cycled between 20 and 37 °C. Han et al. [89] prepared core/shell nanoparticles with the poly(lactide-co-glycolide) core and the polymeric shell composed of pluronics and hyaluronic acid. Lysozyme was loaded into the polymeric shell up to 7 wt.% via ionic interaction between hyaluronic acid and lysozyme and the sustained release pattern was observed, which was due to the stable immobilization of lysozyme in the polymeric shell.

*3.4.2. Cyclic hydrophobic molecules*

Cholesterol is an indispensable lipid in animals, which not only participates the formation of cell membranes but also works as a raw material for the synthesis of bile acids, vitamin D and steroid hormones. Conjugating hydrophobic cholesterol to hydrophilic polysaccharides may form amphiphilic copolymer which may further form self-assembly nanoparticles in aqueous solution. Wang et al. [90] synthesized cholesterol-modified chitosan conjugate with succinyl linkages. The CMC was $1.16\times10^{-2}$ mg/ml in 0.1 M acetic acid solution. The conjugates formed monodisperse self-aggregated nanoparticles with a roughly spherical shape and a mean diameter of 417.2 nm by probe sonication in aqueous media. Epirubicin, as a model anticancer drug, was physically entrapped inside the nanoparticles by the remote loading method. Epirubicin-loaded nanoparticles were almost spherical in shape and their size increased from 338.2 to 472.9 nm with the epirubicin-loading content increasing from 7.97% to 14.0%. Epirubicin release rate decreased with the pH increase of the release media. In PBS pH 7.4, the epirubicin release was very slow and the total release amount was about 24.9% in 48 h. Wang et al. [91] also prepared self-aggregated nanoparticles of cholesterol-modified O-carboxymethyl chitosan and investigated the interaction between bovine serum albumin and self-aggregated nanoparticles.

Akiyoshi et al. [92,93] synthesized various cholesterol-bearing pullulans with different MWs of the parent pullulan and DS of the cholesteryl moiety. Irrespective of the MW of the parent pullulan and the DS, all of cholesterol-pullulans provided unimodal and monodisperse self-aggregates in water. The size of the self-aggregate decreased with an increase in the DS of the cholesteryl moiety (hydrodynamic radius, 8.4–13.7 nm). However, the aggregation number of cholesterol-pullulans in one nanoparticle was almost independent of the DS. The polysaccharide density within the self-aggregate (0.13–0.50 g/ml) was affected by both the MW and the DS of cholesterol-pullulans. The mean aggregation number of the cholesteryl moiety (3.5–5.7) was almost the same for all the self-aggregates. The self-aggregate was regarded as a hydrogel nanoparticle, in which pullulan chains were cross-linked non-covalently by associating cholesteryl moieties. The characteristic temperature to cause a structural change of the nanoparticles decreased with an increase in the DS and the ionic strength of the medium. The thermo-responsiveness of the nanoparticles was related to the partial dehydration of the hydrophobized pullulan upon heating. Insulin was incorporated into the cholesterol-pullulans nanoparticles. The thermal denaturation and subsequent aggregation of insulin were effectively suppressed upon complexation onto the nanoparticles. The complexed insulin was significantly protected from enzymatic degradation. The original physiological activity of complexed insulin was preserved in vivo after i.v. injection [94]. Moreover, they also found that refolding of the heat-denatured enzyme effectively occurs with the nanoparticles and β-cyclodextrin according to a mechanism similar to that of a molecular chaperone. In particular, the irreversible aggregation of carbonic anhydrase B upon heating was completely prevented by complexation between the heat-denatured enzyme and the nanoparticles. The complexed carbonic anhydrase B was released by the dissociation of the self-aggregates upon the addition of β-cyclodextrin. The released carbonic anhydrase B refolded to the native form, and almost 100% recovery of the activity was achieved. The thermal stability of carbonic anhydrase B was drastically improved by capture of the unfolded form, which was then released to undergo refolding [95]. In addition, they also prepared thermo-responsive nanoparticles by self-assembly of two different hydrophobically modified polymers, namely, cholesterol-pullulan and a copolymer of N-isopropylacrylamide and N-[4-(1-pyrenyl)butyl]-N-n-octadecylacrylamide via their hydrophobic moieties [96], as well as hexadecyl group-bearing pullulan self-assembly nanoparticles [97].

Bile acids such as deoxycholic acid and 5β-cholanic acid are known to form micelles in water as a result of their amphiphilicity, which plays an important role in the emulsification, solubilization, and absorption of cholesterol, fats, and liphophilic vitamins in human body. Thus, it is expected that the introduction of deoxycholic acid or 5β-cholanic acid into chitosan would induce self-association to form self-aggregates. Lee et al. [98,99] covalently conjugated deoxycholic acid to chitosan via carbodiimide-mediated reaction to generate self-aggregated nanoparticles. Adriamycin was physically entrapped inside the self-aggregates. The maximum amount of entrapped adriamycin reached 16.5 wt.% of the self-aggregates, suggesting a loading efficiency of 49.6 wt.%. The size of adriamycin-loaded self-aggregates increased with increasing the loading content of adriamycin. Adriamycin was slowly released from the self-aggregates in pH7.2 PBS [100]. Chae et al. [101] chemically modified chitosan oligosaccharides with deoxycholic acid. Owing to the amphiphilic characters, the deoxycholic acid-chitosan formed self-aggregated nanoparticles in aqueous milieu. The particle size of the nanoparticles was in the range of 200–240 nm and the CMC was 0.012–0.046 g/L, depending on the DS. As efficient gene carriers, the nanoparticles showed superior gene condensation and protection of condensed gene from endonuclease attack than unmodified chitosan. Furthermore, deoxycholic acid-chitosan showed great potential for gene carrier with the high level of gene transfection efficiencies, even in the presence of serum. Park et al. [102] synthesized deoxycholic acid-heparin amphiphilic conjugates with different degree of substitution of deoxycholic acid, which provided monodispersed self-aggregates in water, with mean diameters (120–200 nm) decreasing with increasing DS. The aggregates were covered with negatively charged heparin shells, exhibiting ξ potentials near −56 mV. The CAC of the conjugates (0.02–0.003 mg/ml) depended upon the DS. Increasing DS enhanced the hydrophobicity of the self-aggregate inner core. The mean aggregation number of deoxycholic acid per hydrophobic microdomain indicated that five to nine of deoxycholic acid-heparin chains comprised a hydrophobic domain in the conjugates.

However, chitosan-based self-aggregates were difficult to be widely applied for drug delivery systems because chitosan aggregates are insoluble in biological solution (pH7.4) and they are readily precipitated within a few days. Recently, water-soluble chitosan derivatives have been used to increase their stability in biological solution and decrease the cytotoxicity induced by acidic solution, where chitosan is soluble. Of chitosan derivatives, glycol chitosan is emerging as a novel carrier of drugs because of its solubility in water and biocompatibility. Kim et al. [103] prepared deoxycholic acid covalently modified glycol chitosan self-aggregates as a new drug delivery system and investigated in detail the effect of deoxycholic acid attached to glycol chitosan on the formation, physicochemical characteristics, and stability of self-aggregates in aqueous media. The same group [104,105] covalently attached 5β-cholanic acid to glycol chitosan through amide formation using carbodiimide as catalyzer. The 5β-cholanic acid-glycol chitosan formed self-aggregates (210–859 nm in diameter) in an aqueous phase by intra- or intermolecular association between hydrophobic 5β-cholanic acids attached to glycol chitosan. The CMCs of 5β-cholanic acid-glycol chitosan (0.047–0.219 mg/ml in pH7.4) were dependent on the DS of 5β-cholanic acid and were significantly lower than those of low MW surfactants. The mean diameters of the self-aggregates decreased with the increase in the DS because of the formation of compact hydrophobic

inner cores. The increase in the DS enhances the hydrophobicity of inner core of self-aggregates. The aggregation number of 5β-cholanic acid per one hydrophobic microdomain increased with increasing the DS, which suggested that several 5β-cholanic acid-glycol chitosan chains were needed to form one hydrophobic domain. The 5β-cholanic acid-glycol chitosan self-aggregate nanoparticles were used to load Arg–Gly–Asp peptide. The peptide is considered to specifically bind to $\alpha_v\beta_3$ integrin expressed on endothelial cells in the angiogenic blood vessels, which provides a potential to inhibit tumor growth. The peptides were released from self-aggregates in a physiological solution (pH 7.4) for up to 1 day [106]. In addition, the 5β-cholanic acid-glycol chitosan was also used to spontaneously form self-assembled nanoparticle with DNA by hydrophobic interaction. As the 5β-cholanic acid-glycol chitosan content increased, the encapsulation efficiencies of DNA increased while the size of the nanoparticles decreased. Upon increasing 5β-cholanic acid-glycol chitosan contents, the nanoparticle became less cytotoxic. The increased 5β-cholanic acid-glycol chitosan contents also facilitated endocytic uptakes of the nanoparticles by COS-1 cells. The nanoparticles showed increasing in vitro transfection efficiencies in the presence of serum. In vivo results also showed that the nanoparticles had superior transfection efficiencies to naked DNA and a commercialized transfection agent [107]. The 5β-cholanic acid-glycol chitosan self-assembly nanoparticle were also used as a carrier for paclitaxel, an anticancer agent. Paclitaxel was efficiently loaded into the nanoparticles up to 10 wt.%. The paclitaxel-loaded nanoparticles were 400 nm in diameter and were stable in PBS for 10 days. These nanoparticles also showed sustained release of paclitaxel (80% of the loaded dose was released in 8 days at 37 °C in PBS). Owing to sustained release, the nanoparticles were less cytotoxic to B16F10 melanoma cells than free paclitaxel. Injection of paclitaxel-loaded nanoparticles into the tail vein of tumor-bearing mice prevented increases in tumor volume for 8 days [108].

FITC is a widely used hydrophobic fluorescein, the isothiocyanato of which can readily react with free amine to incorporate fluorescence labeling. Doxorubicin is an anti-tumor antibiotic, which can inhibit the synthesis of RNA and DNA and has a therapeutic effect on many tumors. FITC and doxorubicin themselves are hydrophobic cyclic molecules, which can be conjugated onto hydrophilic polysaccharides form amphiphilic copolymers. Park et al. [109,110] prepared hydrophobically modified glycol chitosans by chemical conjugation of FITC or doxorubicin to the backbone of glycol chitosan. Biodistribution of self-aggregates (300 nm in diameter) was evaluated using tissues obtained from tumor-bearing mice, to which self-aggregates were systemically administered via the tail vein. Irrespective of the dose, a negligible quantity of self-aggregates was found in heart and lung, whereas a small amount (3.6–3.8% of dose) was detected in liver for 3 days after intravenous injection of self-aggregates. The distributed amount of self-aggregates gradually increased in tumor as blood circulation time increased. The concentration of self-aggregates in blood was as high as 14% of dose at 1 day after intravenous injection and was still higher than 8% even at 3 days. When self-aggregates loaded with doxorubicin were administered into the tumor-bearing mice via the tail vein, they exhibited lower toxicity than but comparable anti-tumor activity to free doxorubicin. These results revealed the promising potential of self-aggregates on the basis of glycol chitosan as a carrier for hydrophobic anti-tumor agents [111]. The same group also chemically conjugated an anthracycline drug, adriamycin (i.e. doxrubicin), onto the backbone of glycol chitosan via an acid-labile cis-aconityl linkage. The conjugates were capable of forming nano-sized self-aggregates in an aqueous medium, when the adriamycin content in the conjugate was in the range of 2.0–5.0 wt.%. The self-aggregates were spherical in shape, and had mean diameters of 238–304 nm, depending on the adriamycin content. The CMCs of the conjugates were as low as $1.0–2.5\times10^{-2}$ mg/ml. The size of self-aggregates was not affected by the polymer concentration in the range from 50 to 2000 μg/ml, and was maintained up to 8 days in pH7.4 PBS, indicating high colloidal stability. The release of adriamycin from self-aggregates was significantly dependent on the pH of the medium due to the cis-aconityl linkage; e.g., the amount of adriamycin released for 4 days was 7.3 ± 0.3% at pH7, whereas it was 29.3 ± 1.9% at pH4. The cell viability results demonstrated that free adriamycin shows more potent cytotoxicity than the conjugates, primarily attributed to the sustained release of adriamycin from self-aggregates [112]. Cho et al. [113] studied in vivo tumor targeting and radionuclide imaging with FITC-conjugated glycol chitosan nanoparticles in terms of mechanisms, key factors, and their implications.

Polymeric nanoparticles can be delivered to specific sites by size-dependant passive targeting or by active targeting. Active targeting has been attempted by many investigators in order to gain a high degree of selectivity to a specific organ and to enhance the internalization of drug-loaded nanoparticles into the target cells. The internalization of nanoparticles is beneficial for more efficient drug therapy since the drug can be delivered directly to the target cells. Ligands such as sugar and vitamins have been introduced into the drug carriers in order to enhance the intracellular localization into the cancer cell. Vitamin H (biotin), one of the B complex vitamin families, is a growth promoter at the cellular level. Vitamin H and its derivatives have been used in cancer studies and in the tissue-engineering field. Vitamin H exists in the liver, kidney, pancreas, and in milk. In particular, the vitamin H content in cancerous tumors is higher than in normal tissue. Rapid proliferation of tumor cells may require extra vitamin H, and the cell surface receptors for vitamin H may be over expressed on tumor cells; although the vitamin H receptors in cancer cells have not been clearly defined. This hypothesis suggests a possible new approach for tumor targeting using vitamin H. To investigate this hypothesis, Na et al. [114] introduced vitamin H to pullulan acetate and prepared corresponding self-assembled nanoparticles (~100 nm) in order to improve their cancer-targeting activity and internalization. Three samples of biotinylated pullulan acetate, comprising 7, 20 and 39 vitamin H groups per 100 anhydroglucose units, were synthesized, with corresponding CMCs of $3.1\times10^{-3}$, $4.3\times10^{-3}$ and $6.8\times10^{-3}$ mg/ml in distilled water, respectively. Adriamycin was loaded into the nanoparticles as a model drug. The loading efficiencies and adriamycin content in the nanoparticles decreased with increasing vitamin H content due to a lower hydrophobicity. The rhodamine B isothiocyanate-labeled nanoparticles exhibited very strong adsorption to the HepG2 cells, while the rhodamine B isothiocyanate-labeled pullulan acetate nanoparticles did not show any significant interaction. The degree of the interaction increased with increasing vitamin H content. Confocal laser microscopy also revealed that the internalization of the nanoparticles into the cancer cells depended on the vitamin H content.

In addition, Zhu et al. [115] synthesized successfully N-succinyl-chitosan, which could be self-assembly of well-dispersed and stable nanospheres in distilled water with 50–100 nm in diameter. Experimental results indicated that a hydrophobic domain formed within these nanospheres. The assembly mechanisms were believed to be the intermolecular H-bonding of N-succinyl-chitosan and hydrophobic interaction among the hydrophobic moieties in N-succinyl-chitosan macromolecules. The in vitro cell culture indicated that N-succinyl-chitosan had non-toxicity and cell-compatibility. Park et al. [116] described N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles as a promising system for intracytoplasmic delivery of drugs.

### 3.4.3. Polyacrylate family molecules

Poly(methyl methacrylate) and poly(isobutyl cyanoacrylate) (PIBCA) all belong to polyacrylate family and are widely used for biomaterials. Containing carboxylic ester groups in their structures, they are hydrophobic. The efficient uptake of injected nanoparticles by cells of the mononuclear phagocyte system limits the development of long-circulating colloidal drug carriers. The complement system plays a major role in the opsonization and recognition processes of foreign materials. Since heparin is an inhibitor of complement activation, Passirani et al.

[117] prepared nanoparticles bearing heparin covalently bound to poly (methyl methacrylate) and evaluated their interactions with complement. The particles retained the complement-inhibiting properties of soluble heparin. Nanoparticles bearing covalently bound dextran instead of heparin were weak activators of complement as compared with crosslinked dextran or bare poly(methyl methacrylate) nanoparticles. In addition to the specific activity of bound heparin, the protective effect of both polysaccharides is hypothesized to be due to the presence of a dense brush-like layer on the surface of the particles. Such properties are expected to reduce the uptake by mononuclear phagocyte system in vivo. Additionally, the in vivo blood circulating time of the two kinds of nanoparticles were evaluated. After an initial phase of elimination from the blood with a half-life of 5 h, the remaining heparin nanoparticles circulated for more than 48 h and were still detectable in the plasma at 72 h. Dextran nanoparticles were also eliminated very slowly over 48 h. Bare poly(methyl methacrylate) nanoparticles were found to have a half-life of only 3 min. Both types of nanoparticles proved to be long-circulating. The potent capacity for opsonization of the poly(methyl methacrylate) core was hidden by the protective effect of either polysaccharide, probably due to a dense brush-like structure. In the case of heparin nanoparticles, the "stealth" effect was probably increased by its inhibiting properties against complement activation [118].

Yang et al. [119] prepared PIBCA-chitosan nanoparticles by emulsion polymerization of IBCA in the presence of chitosan as a polymeric stabilizer at low pH. Nimodipine as a model drug was successfully incorporated into the nanoparticles with mean particle diameter of 31.6 nm and a positive charge. Bertholon et al. [120] also prepared PIBCA-chitosan, PIBCA-dextran and PIBCA-dextran sulfate core-shell nanoparticles by redox radical or anionic polymerization of IBCA in the presence of chitosan, dextran or dextran sulfate. Moreover, complement activation induced by these nanoparticles was studied by evaluating the conversion of C3 into C3b in serum incubated with these nanoparticles. Cleavage of C3 increased with size of dextran bound in 'loops' configuration, whereas it decreased when dextran was bound in 'brush'. It was explained by an increasing steric repulsive effect of the brush, inducing poor accessibility to OH groups. The same trend was observed for chitosan-coated nanoparticles. Nanoparticles coated with a brush of chitosan activated the complement system lesser than nanoparticles coated with a brush of dextran. This was explained by an improved repelling effect. Dextran-sulfate-coated nanoparticles induced a low cleavage of C3 whereas it strongly enhanced protein adsorption. In conclusion, complement activation was highly sensitive to surface features of the nanoparticles. Bravo-Osuna et al. [121–124] developed PIBCA-thiolated chitosan nanoparticles by radical emulsion polymerization. The nanoparticles had mean hydrodynamic diameter around 200 nm and positive zeta potential values, indicating the presence of the cationic thiolated chitosan at the nanoparticle surface. Chauvierre et al. [125] synthesized polysaccharide-coated nanoparticles by radical emulsion polymerization of IBCA in the presence of various polysaccharides (dextran, dextran sulfate, heparin, chitosan, hyaluronic acid, pectin). They also measured the complement activation induced by different polysaccharide-coated nanoparticles and of the antithrombic activity of heparin. They applied heparin-PIBCA copolymers to carry hemoglobin. In water, these copolymers spontaneously formed nanoparticles with a ciliated surface of heparin. These nanoparticles maintained the heparin antithrombic properties and inhibited complement activation. This work demonstrated the hemoglobin loading on nanoparticle surface, rather than being encapsulated. With a size of 100 nm, these drug delivery systems made suitable tools in the treatment of thrombosis oxygen deprived pathologies [126]. In addition, they investigated for the first time the mobility of dextran chains on the PIBCA nanoparticles with electronic paramagnetic resonance. This technique opens an interesting prospect of investigating surface properties of polysaccharide-coated nanoparticles by a new physico-chemical approach to further correlate the mobility of the polysaccharide chains with the fate of the nanoparticles in biological systems [127]. They also examined the in vitro interactions of core-shell PIBCA-dextran or PIBCA-heparin nanoparticles with blood proteins [128].

## 4. Perspective

As reviewed above, so many nanoparticle drug delivery systems have been prepared. It can be predicted that, more nanoparticle drug delivery systems will emerge. Until now, these nanoparticles are generally investigated in terms of their physicochemical properties, drug-loading ability, in vitro toxicity, and comparatively simple in vivo tests. The more important issues, such as the specific interaction of these nanoparticles with human organs, tissues, cells, or biomolecules, the effect on human's metabolism brought by the nanoparticles, and the wider application of these nanoparticles for drug delivery, etc. await further deep study, which will be focused on in the near future.

## References

[1] T. Jung, W. Kamm, A. Breitenbach, E. Kaiserling, J.X. Xiao, T. Kissel, Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake? Eur. J. Pharm. Biopharm. 50 (2000) 147–160.
[2] C. Pinto Reis, R.J. Neufeld, A.J. Ribeiro, F. Veiga, Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles, Nanomedicine 2 (2006) 8–21.
[3] L. Illum, Nanoparticulate systems for nasal delivery of drugs: a real improvement over simple systems? J. Pharm. Sci. 96 (2007) 473–483.
[4] V.R. Sinha, R. Kumria, Polysaccharides in colon-specific drug delivery, Int. J. Pharm. 224 (2001) 19–38.
[5] J.W. Lee, J.H. Park, J.R. Robinson, Bioadhesive-based dosage forms: the next generation, J. Pharm. Sci. 89 (2000) 850–866.
[6] A. Rubinstein, Natural polysaccharides as targeting tools of drugs to the human colon, Drug Dev. Res. 50 (2000) 435–439.
[7] T.F. Vandamme, A. Lenourry, C. Charrueau, J. Chaumeil, The use of polysaccharides to target drugs to the colon, Carbohydr. Polym. 48 (2002) 219–231.
[8] C. Lemarchand, R. Gref, P. Couvreur, Polysaccharide-decorated nanoparticles, Eur. J. Pharm. Biopharm. 58 (2004) 327–341.
[9] K.A. Janes, P. Calvo, M.J. Alonso, Polysaccharide colloidal particles as delivery systems for macromolecules, Adv. Drug Deliv. Rev. 47 (2001) 83–97.
[10] M. Prabaharan, J.F. Mano, Chitosan-based particles as controlled drug delivery systems, Drug Deliv. 12 (2005) 41–57.
[11] J. Zhi, Y.J. Wang, G.S. Luo, Adsorption of diuretic furosemide onto chitosan nanoparticles prepared with a water-in-oil nanoemulsion system, React. Funct. Polym. 65 (2005) 249–257.
[12] H. Liu, B. Chen, Z.W. Mao, C.Y. Gao, Chitosan nanoparticles for loading of toothpaste actives and adhesion on tooth analogs, J. Appl. Polym. Sci. 106 (2007) 4248–4256.
[13] M. Bodnar, J.F. Hartmann, J. Borbely, Preparation and characterization of chitosan-based nanoparticles, Biomacromolecules 6 (2005) 2521–2527.
[14] M. Bodnar, J.F. Hartmann, J. Borbely, Nanoparticles from chitosan, Macromol. Symp. 227 (2005) 321–326.
[15] P. Calvo, C. RemunanLopez, J.L. VilaJato, M.J. Alonso, Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers, J. Appl. Polym. Sci. 63 (1997) 125–132.
[16] P. Calvo, C. RemunanLopez, J.L. VilaJato, M.J. Alonso, Chitosan and chitosan ethylene oxide propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines, Pharm. Res. 14 (1997) 1431–1436.
[17] D. Jain, R. Banerjee, Comparison of ciprofloxacin hydrochloride-loaded protein, lipid, and chitosan nanoparticles for drug delivery, J. Biomed. Mater. Res. 86B (2008) 105–112.
[18] B. Lu, S.B. Xiong, H. Yang, X.D. Yin, R.B. Zhao, Mitoxantrone-loaded BSA nanospheres and chitosan nanospheres for local injection against breast cancer and its lymph node metastases — I: formulation and in vitro characterization, Int. J. Pharm. 307 (2006) 168–174.
[19] B. Lu, S.B. Xiong, H. Yang, X.D. Yin, 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. 307 (2006) 175–181.
[20] H. Zhang, M. Oh, C. Allen, E. Kumacheva, Monodisperse chitosan nanoparticles for mucosal drug delivery, Biomacromolecules 5 (2004) 2461–2468.
[21] M. Luangtana-anan, P. Opanasopit, T. Ngawhirunpat, J. Nunthanid, P. Sriamornsak, S. Limmatvapirat, L.Y. Lim, Effect of chitosan salts and molecular weight on a nanoparticulate carrier for therapeutic protein, Pharm. Dev. Technol. 10 (2005) 189–196.
[22] A. Vila, A. Sanchez, K. Janes, I. Behrens, T. Kissel, J.L.V. Jato, M.J. Alonso, Low molecular weight chitosan nanoparticles as new carriers for nasal vaccine delivery in mice, Eur. J. Pharm. Biopharm. 57 (2004) 123–131.
[23] Y. Aktas, M. Yemisci, K. Andrieux, R.N. Gursoy, M.J. Alonso, E. Fernandez-Megia, R. Novoa-Carballal, E. Quinoa, R. Riguera, M.F. Sargon, H.H. Celik, A.S. Demir, A.A. Hincal, T. Dalkara, Y. Capan, P. Couvreur, Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26, Bioconjug. Chem. 16 (2005) 1503–1511.
[24] L.F. Qi, Z.R. Xu, X. Jiang, Y. Li, M.Q. Wang, Cytotoxic activities of chitosan nanoparticles and copper-loaded nanoparticles, Bioorg. Med. Chem. Lett. 15 (2005) 1397–1399.

[25] Y. Sun, A.J. Wan, Preparation of nanoparticles composed of chitosan and its derivatives as delivery systems for macromolecules, J. Appl. Polym. Sci. 105 (2007) 552–561.
[26] M.L. Tsai, S.W. Bai, R.H. Chen, Cavitation effects versus stretch effects resulted in different size and polydispersity of ionotropic gelation chitosan-sodium tripolyphosphate nanoparticle, Carbohydr. Polym. 71 (2008) 448–457.
[27] Y.Y. Zhang, Y. Yang, K. Tang, X. Hu, G.L. Zou, Physicochemical characterization and antioxidant activity of quercetin-loaded chitosan nanoparticles, J. Appl. Polym. Sci. 107 (2008) 891–897.
[28] Q. Gan, T. Wang, Chitosan nanoparticle as protein delivery carrier — systematic examination of fabrication conditions for efficient loading and release, Colloids Surf., B 59 (2007) 24–34.
[29] F. Maestrelli, M. Garcia-Fuentes, P. Mura, M.J. Alonso, A new drug nanocarrier consisting of chitosan and hydoxypropyleyclodextrin, Eur. J. Pharm. Biopharm. 63 (2006) 79–86.
[30] M. de la Fuente, B. Seijo, M.J. Alonso, Novel hyaluronan-based nanocarriers for transmucosal delivery of macromolecules, Macromol. Biosci. 8 (2008) 441–450.
[31] Y.M. Xu, Y.M. Du, R.H. Huang, L.P. Gao, Preparation and modification of N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride nanoparticle as a protein carrier, Biomaterials 24 (2003) 5015–5022.
[32] M. Amidi, S.G. Romeijn, G. Borchard, H.E. Junginger, W.E. Hennink, W. Jiskoot, Preparation and characterization of protein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system, J. Control. Release 111 (2006) 107–116.
[33] G. Sandri, M.C. Bonferoni, S. Rossi, F. Ferrari, S. Gibin, Y. Zambito, G. Di Colo, 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. 65 (2007) 68–77.
[34] J.O. You, C.A. Peng, Calcium-alginate nanoparticles formed by reverse microemulsion as gene carriers, Macromol. Symp. 219 (2004) 147–153.
[35] A. Zahoor, S. Sharma, G.K. Khuller, Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis, Int. J. Antimicrob. Agents 26 (2005) 298–303.
[36] D.G. Kim, Y.I. Jeong, C. Choi, S.H. Roh, S.K. Kang, M.K. Jang, J.W. Nah, Retinol-encapsulated low molecular water-soluble chitosan nanoparticles, Int. J. Pharm. 319 (2006) 130–138.
[37] Z.R. Cui, R.J. Mumper, Chitosan-based nanoparticles for topical genetic immunization, J. Control. Release 75 (2001) 409–419.
[38] Y. Chen, V.J. Mohanraj, J.E. Parkin, Chitosan-dextran sulfate nanoparticles for delivery of an anti-angiogenesis peptide, Lett. Pept. Sci. 10 (2003) 621–629.
[39] W. Tiyaboonchai, N. Limpeanchob, Formulation and characterization of amphotericin B-chitosan-dextran sulfate nanoparticles, Int. J. Pharm. 329 (2007) 142–149.
[40] B. Sarmento, S. Martins, A. Ribeiro, F. Veiga, R. Neufeld, D. Ferreira, Development and comparison of different nanoparticulate polyelectrolyte complexes as insulin carriers, Int. J. Pept. Res. Ther. 12 (2006) 131–138.
[41] B. Sarmento, A. Ribeiro, F. Veiga, D. Ferreira, Development and characterization of new insulin containing polysaccharide nanoparticles, Colloids Surf., B 53 (2006) 193–202.
[42] B. Sarmento, D. Ferreira, F. Veiga, A. Ribeiro, Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies, Carbohydr. Polym. 66 (2006) 1–7.
[43] B. Sarmento, D.C. Ferreira, L. Jorgensen, M. van de Weert, Probing insulin's secondary structure after entrapment into alginate/chitosan nanoparticles, Eur. J. Pharm. Biopharm. 65 (2007) 10–17.
[44] M. Alonso-Sande, M. Cuna, C. Remunan-Lopez, Formation of new glucomannan–chitosan nanoparticles and study of their ability to associate and deliver proteins, Macromolecules 39 (2006) 4152–4158.
[45] J. Du, R. Sun, S. Zhang, L.F. Zhang, C.D. Xiong, Y.X. Peng, Novel polyelectrolyte carboxymethyl konjac glucomannan-chitosan nanoparticles for drug delivery. I. Physicochemical characterization of the carboxymethyl konjac glucomannan–chitosan nanoparticles, Biopolymers 78 (2005) 1–8.
[46] J. Du, S. Zhang, R. Sun, L.F. Zhang, C.D. Xiong, Y.X. Peng, Novel polyelectrolyte carboxymethyl konjac glucomannan–chitosan nanoparticles for drug delivery. II. Release of albumin in vitro, J. Biomed. Mater. Res. Part B-Applied Biomaterials 72B (2005) 299–304.
[47] J. Du, R. Sun, S. Zhang, T. Govender, L.F. Zhang, C.D. Xiong, Y.X. Peng, Novel polyelectrolyte carboxymethyl konjac glucomannan–chitosan nanoparticles for drug delivery, Macromol. Rapid Commun. 25 (2004) 954–958.
[48] Z.H. Liu, Y.P. Jiao, F. Liu, Z.Y. Zhang, Heparin/chitosan nanoparticle carriers prepared by polyelectrolyte complexation, J. Biomed. Mater. Res. 83A (2007) 806–812.
[49] T. Li, X.W. Shi, Y.M. Du, Y.F. Tang, Quaternized chitosan/alginate nanoparticles for protein delivery, J. Biomed. Mater. Res. 83A (2007) 383–390.
[50] Y.H. Lin, C.K. Chung, C.T. Chen, H.F. Liang, S.C. Chen, H.W. Sung, Preparation of nanoparticles composed of chitosan/poly-gamma-glutamic acid and evaluation of their permeability through Caco-2 cells, Biomacromolecules 6 (2005) 1104–1112.
[51] P.W. Lee, S.F. Peng, C.J. Su, F.L. Mi, H.L. Chen, M.C. Wei, H.J. Lin, H.W. Sung, The use of biodegradable polymeric nanoparticles in combination with a low-pressure gene gun for transdermal DNA delivery, Biomaterials 29 (2008) 742–751.
[52] S. Sajeesh, C.P. Sharma, Novel pH responsive polymethacrylic acid-chitosan-polyethylene glycol nanoparticles for oral peptide delivery, J. Biomed. Mater. Res. 76B (2006) 298–305.
[53] Q. Chen, Y. Hu, Y. Chen, X.Q. Jiang, Y.H. Yang, Microstructure formation and property of chitosan-poly (acrylic acid) nanoparticles prepared by macromolecular complex, Macromol. Biosci. 5 (2005) 993–1000.
[54] Y. Hu, Y. Chen, Q. Chen, L.Y. Zhang, X.Q. Jiang, C.Z. Yang, Synthesis and stimuli-responsive properties of chitosan/poly (acrylic acid) hollow nanospheres, Polymer 46 (2005) 12703–12710.
[55] Y.L. Zheng, Y. Wu, W.L. Yang, C.C. Wang, S.K. Fu, X.Z. Shen, Preparation, characterization, and drug release in vitro of chitosan-glycyrrhetic acid nanoparticles, J. Pharm. Sci. 95 (2006) 181–191.
[56] O. Stoilova, N. Koseva, N. Manolova, I. Rashkov, Polyelectrolyte complex between chitosan and poly(2-acryloylamido-2-methylpropanesulfonic acid), Polym. Bull. 43 (1999) 67–73.
[57] Y.L. Zheng, W. Yang, C.C. Wang, J.H. Hu, S.K. Fu, L. Dong, L. Wu, X.Z. Shen, Nanoparticles based on the complex of chitosan and polyaspartic acid sodium salt: Preparation, characterization and the use for 5-fluorouracil delivery, Eur. J. Pharm. Biopharm. 67 (2007) 621–631.
[58] M. Thanou, B.I. Florea, M. Geldof, H.E. Junginger, G. Borchard, Quaternized chitosan oligomers as novel gene delivery vectors in epithelial cell lines, Biomaterials 23 (2002) 153–159.
[59] T. Kean, S. Roth, M. Thanou, Trimethylated chitosans as non-viral gene delivery vectors: cytotoxicity and transfection efficiency, J. Control. Release 103 (2005) 643–653.
[60] F. Zheng, X.W. Shi, G.F. Yang, L.L. Gong, H.Y. Yuan, Y.J. Cui, Y. Wang, Y.M. Du, Y. Li, Chitosan nanoparticle as gene therapy vector via gastrointestinal mucosa administration: results of an in vitro and in vivo study, Life Sci. 80 (2007) 388–396.
[61] H.L. Jiang, Y.K. Kim, R. Arote, J.W. Nah, M.H. Cho, Y.J. Choi, T. Akaike, C.S. Cho, Chitosan-graft-polyethylenimine as a gene carrier, J. Control. Release 117 (2007) 273–280.
[62] X.M. Xu, R.M. Capito, M. Spector, Plasmid size influences chitosan nanoparticle mediated gene transfer to chondrocytes, J. Biomed. Mater. Res. 84A (2008) 1038–1048.
[63] L. Jiang, F. Qian, X.W. He, F. Wang, D. Ren, Y. He, K. Li, S. Sun, C. Yin, Novel chitosan derivative nanopartictes enhance the immunogenicity of a DNA vaccine encoding hepatitis B virus core antigen in mice, J. Gene Med. 9 (2007) 253–264.
[64] X.D. Liu, K.A. Howard, M.D. Dong, M.O. Andersen, U.L. Rahbek, M.G. Johnsen, O.C. Hansen, F. Besenbacher, J. Kjems, The influence of polymeric properties on chitosan/siRNA nanoparticle formulation and gene silencing, Biomaterials 28 (2007) 1280–1288.
[65] Y. Yang, J.L. Chen, H. Li, Y.Y. Wang, Z. Xie, M. Wu, H. Zhang, Z.Z. Zhao, Q. Chen, M.L. Fu, K.Y. Wu, C. Chi, H.N. Wang, R. Gao, Porcine interleukin-2 gene encapsulated in chitosan nanoparticles enhances immune response of mice to piglet paratyphoid vaccine, Comp. Immunol. Microbiol. 30 (2007) 19–32.
[66] W.B. Tan, S. Jiang, Y. Zhang, Quantum-dot based nanoparticles for targeted silencing of HER2/neu gene via RNA interference, Biomaterials 28 (2007) 1565–1571.
[67] T.H. Kim, H.L. Jiang, D. Jere, I.K. Park, M.H. Cho, J.W. Nah, Y.J. Choi, T. Akaike, C.S. Cho, Chemical modification of chitosan as a gene carrier in vitro and in vivo, Prog. Polym. Sci. 32 (2007) 726–753.
[68] K. Letchford, H. Burt, A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes, Eur. J. Pharm. Biopharm. 65 (2007) 259–269.
[69] T. Ouchi, H. Nishizawa, Y. Ohya, Aggregation phenomenon of PEG-grafted chitosan in aqueous solution, Polymer 39 (1998) 5171–5175.
[70] R. Yoksan, M. Matsusaki, M. Akashi, S. Chirachanchai, Controlled hydrophobic/hydrophilic chitosan: colloidal phenomena and nanosphere formation, Colloid Polym. Sci. 282 (2004) 337–342.
[71] 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.G. Jin, K.K. Kim, K.Y. Ahn, S. Jung, Polyion complex micelles composed of all-trans retinoic acid and poly (ethylene glycol)-grafted-citosan, J. Pharm. Sci. 95 (2006) 2348–2360.
[72] J.S. Park, Y.S. Koh, J.Y. Bang, Y.I. Jeong, J.J. Lee, Antitumor effect of all-trans retinoic acid-encapsulated nanoparticles of methoxy poly(ethylene glycol)-conjugated chitosan against CT-26 colon carcinoma in vitro, J. Pharm. Sci. 97 (9) (2008) 4011–4019.
[73] X.D. Yang, Q.Q. Zhang, Y.S. Wang, H. Chen, H.Z. Zhang, F.P. Gao, L.R. Liu, Self-aggregated nanoparticles from methoxy poly(ethylene glycol)-modified chitosan: Synthesis; characterization; aggregation and methotrexate release in vitro, Colloids Surf., B 61 (2008) 125–131.
[74] P. Opanasopit, T. Ngawhirunpat, T. Rojanarata, C. Choochottiros, S. Chirachanchai, Camptothecin-incorporating N-phthaloylchitosan-g-mPEG self-assembly micellar system: effect of degree of deacetylation, Colloids Surf., B 60 (2007) 117–124.
[75] L. Choisnard, A. Geze, J.L. Putaux, Y.S. Wong, D. Wouessidjewe, Nanoparticles of beta-cyclodextrin esters obtained by self-assembling of biotransesterified beta-cyclodextrins, Biomacromolecules 7 (2006) 515–520.
[76] X.G. Chen, C.M. Lee, H.J. Park, OM emulsification for the self-aggregation and nanoparticle formation of linoleic acid-modified chitosan in the aqueous system, J. Agric. Food Chem. 51 (2003) 3135–3139.
[77] C.G. Liu, K.G.H. Desai, X.G. Chen, H.J. Park, Linolenic acid-modified chitosan for formation of self-assembled nanoparticles, J. Agric. Food Chem. 53 (2005) 437–441.
[78] C.G. Liu, K.G.H. Desai, X.G. Chen, H.J. Park, Preparation and characterization of nanoparticles containing trypsin based on hydrophobically modified chitosan, J. Agric. Food Chem. 53 (2005) 1728–1733.
[79] G.B. Jiang, D. Quan, K. Liao, H. Wang, Novel polymer micelles prepared from chitosan grafted hydrophobic palmitoyl groups for drug delivery, Mol. Pharmacol. 3 (2006) 152–160.
[80] F.Q. Hu, G.F. Ren, H. Yuan, Y.Z. Du, S. Zeng, Shell cross-linked stearic acid grafted chitosan oligosaccharide self-aggregated micelles for controlled release of paclitaxel, Colloids Surf., B 50 (2006) 97–103.
[81] J. Zhang, X.G. Chen, Y.Y. Li, C.S. Liu, Self-assembled nanoparticles based on hydrophobically modified chitosan as carriers for doxorubicin, Nanomed-Nanotechnol. 3 (2007) 258–265.

[82] I. Lalush, H. Bar, I. Zakaria, S. Eichler, E. Shimoni, Utilization of amylose-lipid complexes as molecular nanocapsules for conjugated linoleic acid, Biomacromolecules 6 (2005) 121–130.
[83] R. Gref, J. Rodrigues, P. Couvreur, Polysaccharides grafted with polyesters: novel amphiphilic copolymers for biomedical applications, Macromolecules 35 (2002) 9861–9867.
[84] C. Lemarchand, P. Couvreur, M. Besnard, D. Costantini, R. Gref, Novel polyester-polysaccharide nanoparticles, Pharm. Res. 20 (2003) 1284–1292.
[85] J.S. Rodrigues, N.S. Santos-Magalhaes, L.C.B.B. Coelho, P. Couvreur, G. Ponchel, R. Gref, Novel core (polyester)-shell(polysaccharide) nanoparticles: protein loading and surface modification with lectins, J. Control. Release 92 (2003) 103–112.
[86] C. Lemarchand, R. Gref, C. Passirani, E. Garcion, B. Petri, R. Muller, D. Costantini, P. Couvreur, Influence of polysaccharide coating on the interactions of nanoparticles with biological systems, Biomaterials 27 (2006) 108–118.
[87] H.J. Yu, W.S. Wang, X.S. Chen, C. Deng, X.B. Jing, Synthesis and characterization of the biodegradable polycaprolactone-graft-chitosan amphiphilic copolymers, Biopolymers 83 (2006) 233–242.
[88] S.H. Choi, J.H. Lee, S.M. Choi, T.G. Park, Thermally reversible pluronic/heparin nanocapsules exhibiting 1000-fold volume transition, Langmuir 22 (2006) 1758–1762.
[89] S.K. Han, J.H. Lee, D. Kim, S.H. Cho, S.H. Yuk, Hydrophilized poly(lactide-co-glycolide) nanoparticles with core/shell structure for protein delivery, Sci. Technol. Adv. Mater. 6 (2005) 468–474.
[90] Y.S. Wang, L.R. Liu, Q. Jiang, Q.Q. Zhang, Self-aggregated nanoparticles of cholesterol-modified chitosan conjugate as a novel carrier of epirubicin, Eur. Polym. J. 43 (2007) 43–51.
[91] Y.S. Wang, Q. Jiang, L.R. Liu, Q.Q. Zhang, The interaction between bovine serum albumin and the self-aggregated nanoparticles of cholesterol-modified O-carboxymethyl chitosan, Polymer 48 (2007) 4135–4142.
[92] T. Nishikawa, K. Akiyoshi, J. Sunamoto, Macromolecular complexation between bovine serum albumin and the self-assembled hydrogel nanoparticle of hydrophobized polysaccharides, J. Am. Chem. Soc. 118 (1996) 6110–6115.
[93] K. Akiyoshi, S. Deguchi, H. Tajima, T. Nishikawa, J. Sunamoto, Microscopic structure and thermoresponsiveness of a hydrogel nanoparticle by self-assembly of a hydrophobized polysaccharide, Macromolecules 30 (1997) 857–861.
[94] K. Akiyoshi, S. Kobayashi, S. Shichibe, D. Mix, M. Baudys, S.W. Kim, J. Sunamoto, Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin, J. Control. Release 54 (1998) 313–320.
[95] K. Akiyoshi, Y. Sasaki, J. Sunamoto, Molecular chaperone-like activity of hydrogel nanoparticles of hydrophobized pullulan: thermal stabilization with refolding of carbonic anhydrase B, Bioconjug. Chem. 10 (1999) 321–324.
[96] K. Akiyoshi, E.C. Kang, S. Kurumada, J. Sunamoto, T. Principi, F.M. Winnik, Controlled association of amphiphilic polymers in water: thermosensitive nanoparticles formed by self-assembly of hydrophobically modified pullulans and poly(N-isopropylacrylamides), Macromolecules 33 (2000) 3244–3249.
[97] K. Kuroda, K. Fujimoto, J. Sunamoto, K. Akiyoshi, Hierarchical self-assembly of hydrophobically modified pullulan in water: gelation by networks of nanoparticles, Langmuir 18 (2002) 3780–3786.
[98] K.Y. Lee, W.H. Jo, I.C. Kwon, Y.H. Kim, S.Y. Jeong, Structural determination and interior polarity of self-aggregates prepared from deoxycholic acid-modified chitosan in water, Macromolecules 31 (1998) 378–383.
[99] K.Y. Lee, I.C. Kwon, Y.H. Kim, W.H. Jo, S.Y. Jeong, Preparation of chitosan self-aggregates as a gene delivery system, J. Control. Release 51 (1998) 213–220.
[100] K.Y. Lee, J.H. Kim, I.C. Kwon, S.Y. Jeong, Self-aggregates of deoxycholic acid modified chitosan as a novel carrier of adriamycin, Colloid Polym. Sci. 278 (2000) 1216–1219.
[101] S.Y. Chae, S. Son, M. Lee, M.K. Jang, J.W. Nah, Deoxycholic acid-conjugated chitosan oligosaccharide nanoparticles for efficient gene carrier, J. Control. Release 109 (2005) 330–344.
[102] K. Park, K. Kim, I.C. Kwon, S.K. Kim, S. Lee, D.Y. Lee, Y. Byun, Preparation and characterization of self-assembled nanoparticles of heparin-deoxycholic acid conjugates, Langmuir 20 (2004) 11726–11731.
[103] K. Kim, S. Kwon, J.H. Park, H. Chung, S.Y. Jeong, I.C. Kwon, Physicochemical characterizations of self-assembled nanoparticles of glycol chitosan-deoxycholic acid conjugates, Biomacromolecules 6 (2005) 1154–1158.
[104] S. Kwon, J.H. Park, H. Chung, I.C. Kwon, S.Y. Jeong, I.S. Kim, Physicochemical characteristics of self-assembled nanoparticles based on glycol chitosan bearing 5 beta-cholanic acid, Langmuir 19 (2003) 10188–10193.
[105] 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, I.C. Kwon, Effect of polymer molecular weight on the tumor targeting characteristics of self-assembled glycol chitosan nanoparticles, J. Control. Release 122 (2007) 305–314.
[106] J.H. Park, S.G. Kwon, J.O. Nam, R.W. Park, H. Chung, S.B. Seo, I.S. Kim, I.C. Kwon, S.Y. Jeong, Self-assembled nanoparticles based on glycol chitosan bearing 5 beta-cholanic acid for RGD peptide delivery, J. Control. Release 95 (2004) 579–588.
[107] H.S. Yoo, J.E. Lee, H. Chung, I.C. Kwon, S.Y. Jeong, Self-assembled nanoparticles containing hydrophobically modified glycol chitosan for gene delivery, J. Control. Release 103 (2005) 235–243.
[108] 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, I.C. Kwon, Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel (Reprinted from Journal of Controlled Release, vol 109, pg 1, 2005), J. Control. Release 111 (2006) 228–234.
[109] M. Lee, Y.W. Cho, J.H. Park, H.S. Chung, S.Y. Jeong, K.W. Choi, D.H. Moon, S.Y. Kim, I. S. Kim, I.C. Kwon, Size control of self-assembled nanoparticles by an emulsion/solvent evaporation method, Colloid Polym. Sci. 284 (2006) 506–512.
[110] 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, S.Y. Jeong, Biodistribution and anti-tumor efficacy of doxorubicin loaded glycol-chitosan nanoaggregates by EPR effect, J. Control. Release 91 (2003) 135–145.
[111] 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, 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 27 (2006) 119–126.
[112] J.H. Park, Y.W. Cho, Y.J. Son, K. Kim, H. Chung, S.Y. Jeong, K. Choi, C.R. Park, R.W. Park, I.S. Kim, I.C. Kwon, Preparation and characterization of self-assembled nanoparticles based on glycol chitosan bearing adriamycin, Colloid Polym. Sci. 284 (2006) 763–770.
[113] Y.W. Cho, S.A. Park, T.H. Han, D.H. Son, J.S. Park, S.J. Oh, D.H. Moon, K.J. Cho, C.H. Ahn, Y. Byun, I.S. Kim, I.C. Kwon, S.Y. Kim, In vivo tumor targeting and radionuclide imaging with self-assembled nanoparticles: mechanisms, key factors, and their implications, Biomaterials 28 (2007) 1236–1247.
[114] K. Na, T.B. Lee, K.H. Park, E.K. Shin, Y.B. Lee, H.K. Cho, Self-assembled nanoparticles of hydrophobically-modified polysaccharide bearing vitamin H as a targeted anticancer drug delivery system, Eur. J. Pharm. Sci. 18 (2003) 165–173.
[115] A.P. Zhu, T. Chen, L.H. Yuan, H. Wu, P. Lu, Synthesis and characterization of N-succinyl-chitosan and its self-assembly of nanospheres, Carbohydr. Polym. 66 (2006) 274–279.
[116] J.S. Park, T.H. Han, K.Y. Lee, S.S. Han, J.J. Hwang, D.H. Moon, S.Y. Kim, Y.W. Cho, N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles for intracytoplasmic delivery of drugs: endocytosis, exocytosis and drug release, J. Control. Release 115 (2006) 37–45.
[117] C. Passirani, G. Barratt, J.P. Devissaguet, D. Labarre, Interactions of nanoparticles bearing heparin or dextran covalently bound to poly(methyl methacrylate) with the complement system, Life Sci. 62 (1998) 775–785.
[118] C. Passirani, G. Barratt, J.P. Devissaguet, D. Labarre, Long-circulating nanoparticles bearing heparin or dextran covalently bound to poly(methyl methacrylate), Pharm. Res. 15 (1998) 1046–1050.
[119] S.C. Yang, H.X. Ge, Y. Hu, X.Q. Jiang, C.Z. Yang, Formation of positively charged poly (butyl cyanoacrylate) nanoparticles stabilized with chitosan, Colloid Polym. Sci. 278 (2000) 285–292.
[120] I. Bertholon, C. Vauthier, D. Labarre, Complement activation by core-shell poly (isobutylcyanoacrylate)-polysaccharide nanoparticles: influences of surface morphology, length, and type of polysaccharide, Pharm. Res. 23 (2006) 1313–1323.
[121] I. Bravo-Osuna, T. Schmitz, A. Bernkop-Schnurch, C. Vauthier, G. Ponchel, Elaboration and characterization of thiolated chitosan-coated acrylic nanoparticles, Int. J. Pharm. 316 (2006) 170–175.
[122] I. Bravo-Osuna, G. Millotti, C. Vauthier, G. Ponchel, In vitro evaluation of calcium binding capacity of chitosan and thiolated chitosan poly(isobutyl cyanoacrylate) core-shell nanoparticles, Int. J. Pharm. 338 (2007) 284–290.
[123] I. Bravo-Osuna, C. Vauthier, A. Farabollini, G.F. Palmieri, G. Ponchel, Mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core-shell nanoparticles, Biomaterials 28 (2007) 2233–2243.
[124] I. Bravo-Osuna, G. Ponchel, C. Vauthier, Tuning of shell and core characteristics of chitosan-decorated acrylic nanoparticles, Eur. J. Pharm. Sci. 30 (2007) 143–154.
[125] C. Chauvierre, D. Labarre, P. Couvreur, C. Vauthier, Novel polysaccharide-decorated poly(isobutyl cyanoacrylate) nanoparticles, Pharm. Res. 20 (2003) 1786–1793.
[126] C. Chauvierre, M.C. Marden, C. Vauthier, D. Labarre, P. Couvreur, L. Leclerc, Heparin coated poly(alkylcyanoacrylate) nanoparticles coupled to hemoglobin: a new oxygen carrier, Biomaterials 25 (2004) 3081–3086.
[127] C. Chauvierre, C. Vauthier, D. Labarre, H. Hommel, Evaluation of the surface properties of dextran-coated poly(isobutylcyanoacrylate) nanoparticles by spin-labelling coupled with electron resonance spectroscopy, Colloid Polym. Sci. 282 (2004) 1016–1025.
[128] D. Labarre, C. Vauthier, U. Chauvierre, B. Petri, R. Muller, M.M. Chehimi, Interactions of blood proteins with poly(isobutylcyanoacrylate) nanoparticles decorated with a polysaccharidic brush, Biomaterials 26 (2005) 5075–5084.