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Auto-associative amphiphilic polysaccharides as drug delivery systems

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Self-assembly of amphiphilic polysaccharides provides a positive outlook for drug delivery systems without the need for solvents or surfactants. Various polymeric amphiphilic polysaccharides undergo intramolecular or intermolecular associations in water. This type of association, promoted by hydrophobic segments, led to the formation of various drug delivery systems such as micelles, nanoparticles, liposomes and hydrogels. Here, we review a selection of the most important amphiphilic polysaccharides used as drug delivery systems and their pharmaceutical applications. Attention focuses on amphiphilic chitosan owing to its unique properties such as excellent biocompatibility, non-toxicity and antimicrobial and bioadhesive properties.

Introduction

The hydrophilic chains of polysaccharides contain several groups of different molecular weights and chemical compositions. The nature of these groups can differentiate the polysaccharides from a structural point of view and leads to various physicochemical and biological properties [1,2]. Some polysaccharides such as dextran and cyclodextrins have a neutral charge, others such as chitosan are positively charged. Finally, polysaccharides such as alginate, heparin, hyaluronic acid and pectin are negatively charged (Fig. 1). The polysaccharides can be linear, for example dextran, chitosan and hyaluronic acid, or cyclic such as the cyclodextrins. In this review we will focus more specifically on linear polysaccharides.

Recently, there has been increased interest in the use of nanoparticles containing natural polysaccharides for drug delivery applications [2,3]. However, in most cases the need to incorporate organic solvents (for nanoprecipitation, emulsion solvent diffusion, emulsion evaporation, interfacial polycondensation combined with spontaneous emulsification methods) and/or highly acidic pH changes (e.g. for emulsion polymerization of alkylcyanoacrylates) represents an obstacle from a formulation point of view.

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To overcome these drawbacks, the polysaccharides can be chemically modified by grafting hydrophobic groups. Owing to intra- and/or inter-molecular hydrophobic interactions, the amphiphilic polysaccharides can self-associate in aqueous solution leading to different kinds of drug delivery systems such as micelles, nanoparticles, microspheres [4], liposomes [5–7] and hydrogels (Fig. 2). Basically, the structure of self-assembling polysaccharides can be chosen depending on the physicochemical properties of the drug to be loaded and the required route of administration.

By analogy with the phenomena of micelle formation of small surfactants or lipids, aggregation of amphiphilic polymers is controlled by the balance between the interaction of the hydrophobic groups and the hydrophilic chains. The concentration at which the polymer aggregation starts is usually called the critical aggregation concentration (CAC). At relatively high polymer concentrations intermolecular associations of polymers are induced through the association of hydrophobic groups, resulting in a remarkable increase in solution viscosity. For this reason, these types of polymers are called associating polymers and are used as thickeners to modify solution viscosity. Phase separation or gelation can be observed at higher polymer concentrations.

The hydrophobic core of these structures could be used to solubilize and encapsulate active ingredients with low solubility

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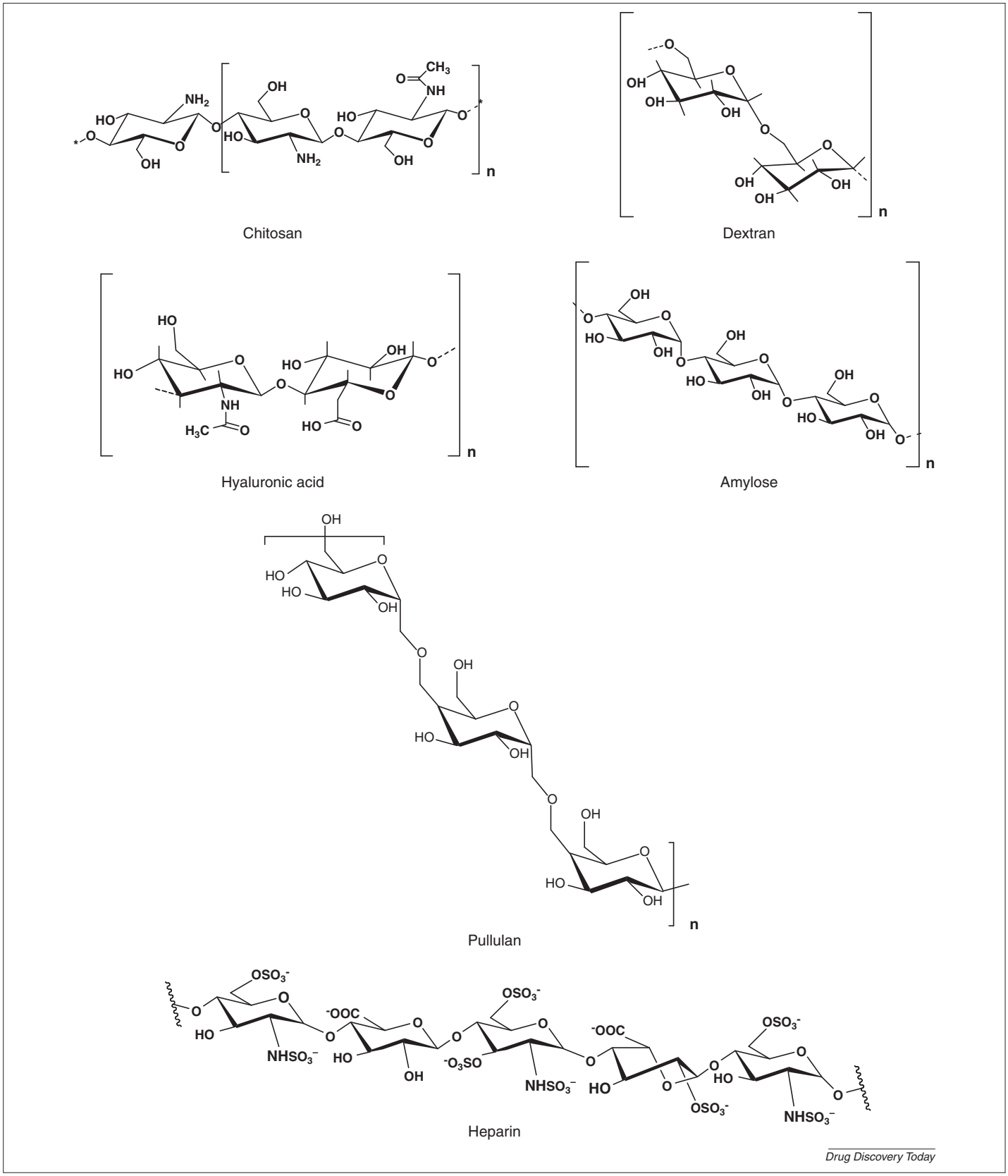

FIGURE 1

Chemical structure of some polysaccharides.

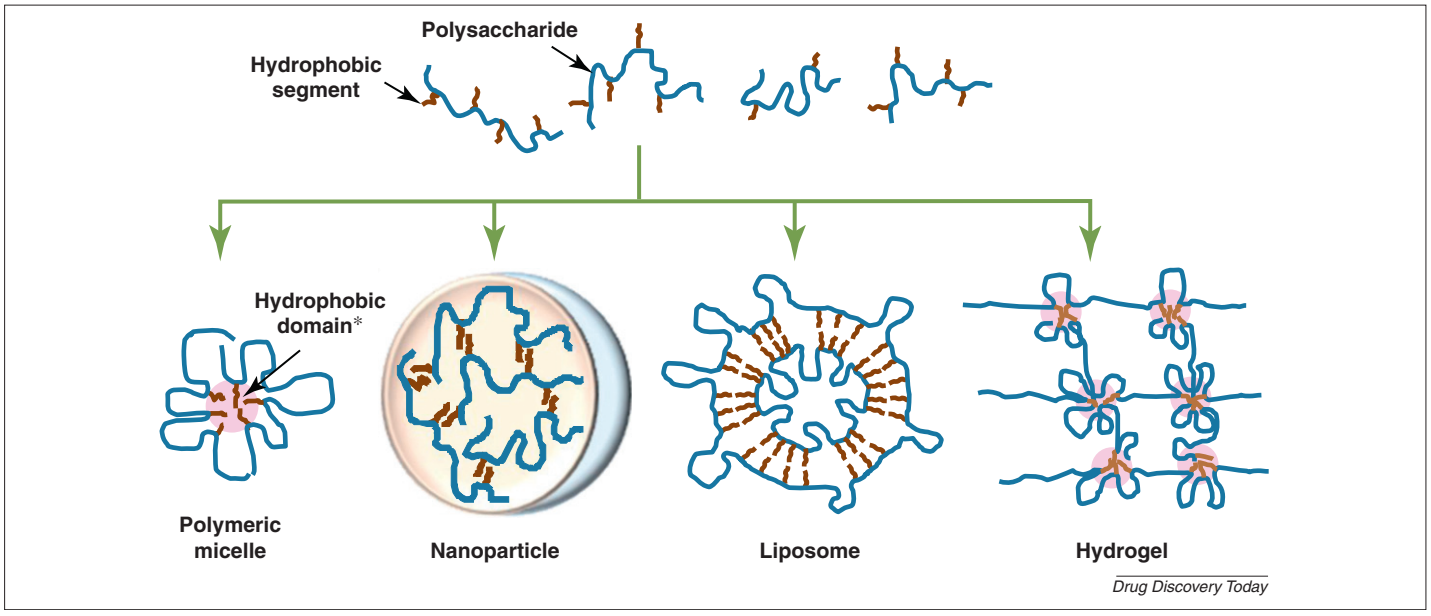

FIGURE 2

Schematic depiction of different drug delivery systems formed by self-association of amphiphilic polysaccharides in aqueous solution. *Hydrophobic domain due to the association of hydrophobic groups.

in aqueous media, whereas the hydrophilic shell would adsorb hydrophilic molecules through non-covalent interactions. Owing to a plethora of research works and review articles on amphiphilic nanoparticles obtained by the covalent linkage of polysaccharides with hydrophobic polymers such as poly(alkylcyanoacrylates) and poly(methyl methacrylates) (for review see Ref. [3]), we focus this paper on the drug delivery systems obtained when small hydrophobic segments (C_2–C_{18}) were grafted on polysaccharides.

This review attempts to give an overview of the major features concerning the amphiphilic polysaccharides used to form various high-ordered and nano-organized drug delivery systems. Special attention is paid to amphiphilic chitosan owing to its numerous interesting properties. Chitosan is widely used in a variety of applications, for example biomedical [8–12] pharmaceutical [13], metal chelation [14,15], food additive [16] and other industrial applications [17,18].

Hydrophobically modified chitosan

Chitosan is a linear heteropolymer of *N*-acetyl-D-glucosamine and D-glucosamine linked by β-(1–4)glycosidic bonds. This polysaccharide is obtained by the partial deacetylation of chitin which is the second largest and most abundant polysaccharide in nature after cellulose. Chitin is mainly derived from the shells of crustaceans such as lobster, shrimp or crab, and can also be found in fungi and insects.

The degree of acetylation (DA) is an essential characteristic of chitin and chitosan. It represents the fraction of *N*-acetyl-D-glucosamine relative to the total number of units. Chitin has a DA > 50%, whereas chitosan has a DA < 50%. Contrary to chitin, which is insoluble in aqueous and many organic solvents, chitosan is hydrophilic and soluble in acidic solutions by protonation of the amine groups present on the macromolecule. The solubility of chitosan is much higher than the DA and its molecular weight are low. Chitosan is biocompatible and can be biodegraded by enzymes such as lysozymes, some lipases and proteases [19].

Chitosan has the characteristic of being mucoadhesive through the interaction between the positive charges carried by the amine and the negative charges carried by membrane proteins [20]. For comparable viscosity hydrogels, when a formulation contains a mucoadhesive polymer it provides a persistence time greater than a simple viscosity agent such as carboxymethylcellulose or dextran [21]. It should be noted that, in addition to its mucoadhesive properties, chitosan (regarded for its excellent biocompatibility, non-toxicity and biodegradability) has antibacterial and antiparasitic activities that are well established [22].

Chitosan can be chemically modified by grafting hydrophobic groups. Owing to the presence of hydroxyl and amine groups, chitosan can be functionalized to obtain amphiphilic chitosan. However, because the amine of chitosan is more reactive than the hydroxyl groups, all the research works describing the formation of amphiphilic chitosan have been based on the chemical grafting of hydrophobic groups on the amine functional group by *N*-acylation reactions. Three different kinds of reactions could be used for the *N*-acylation of chitosan (Fig. 3).

(i) The *N*-acylation of chitosan was carried out in a mixture of pyridine and chloroform in the presence of oleoyl chloride [23] (Fig. 3a). The reaction of oleoyl chloride with chitosan was conducted at room temperature for 2 h and further refluxed for 10 h. The resulting product was poured into methanol, and the precipitated product was filtered and dried under vacuum for 24 h. The degree of *N*-acylation, which can be defined as the number of oleic acid groups per 100 *N*-acetyl-D-glucosamine units of chitosan, was evaluated by infrared spectroscopy [24]. The degree of substitution was calculated from the ratio of absorbance at 1655 cm^{-1} (ascribed to amide I band) and the hydroxyl band at 3450 cm^{-1} [24].

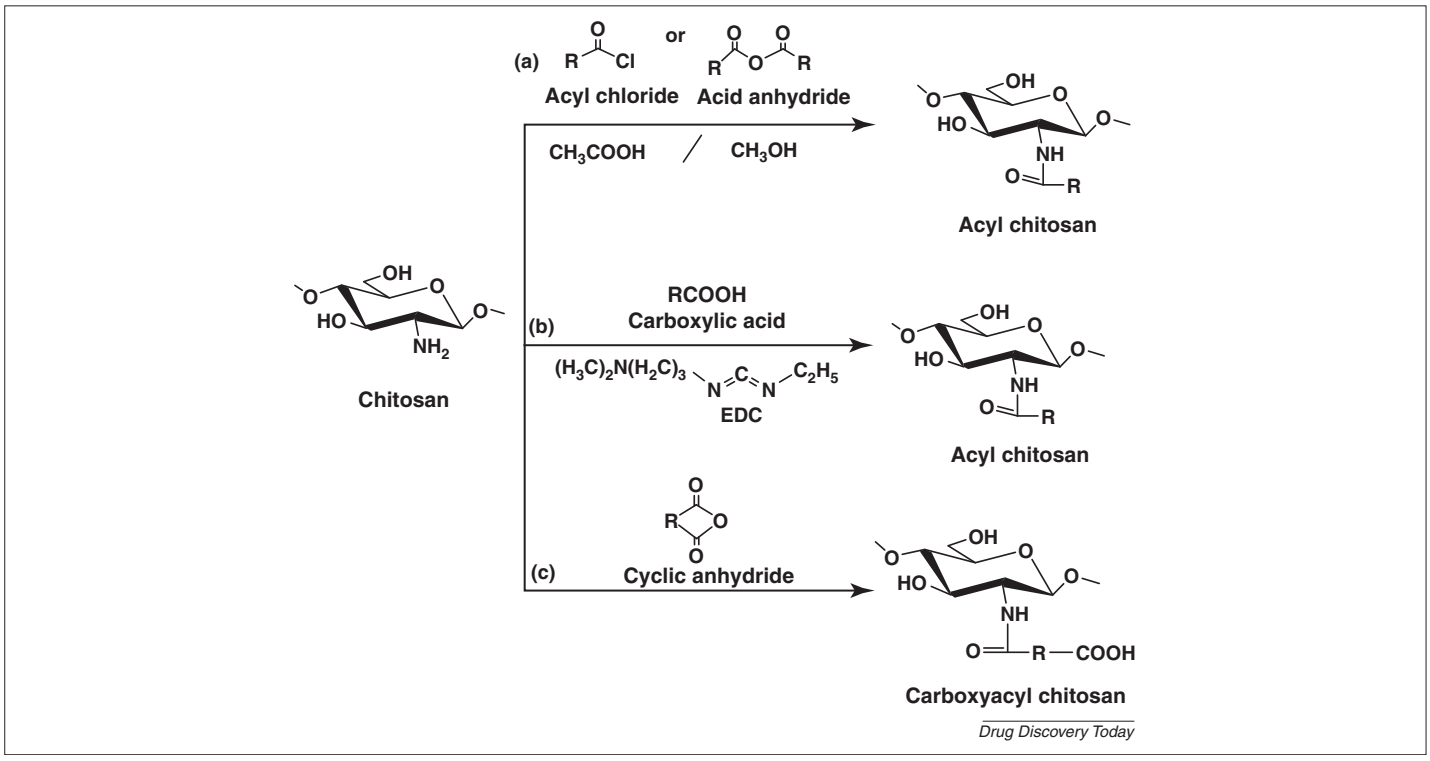

FIGURE 3

N-acylation chemical pathway of chitosan using **(a)** acyl chloride or linear acid anhydride, **(b)** carboxylic acid in presence of EDC and **(c)** cyclic acid anhydride. Abbreviation: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

(ii) The *N*-acylation of chitosan with carboxylic acid (much less reactive than the acyl chloride) required the use of coupling agents such as carbodiimide derivatives [25] (Fig. 3b). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) reacts with carboxyl groups of the fatty acid such as stearic ($C_{18}C_{36}O_2$) [26], linoleic ($C_{18}H_{32}O_2$) [27] and oleic ($C_{18}H_{34}O_2$) [28] acids, leading to a reactive intermediate ester for acylation of the primary amine groups of chitosan. For example, in the study by Lee *et al.* [29], fluids composed of superparamagnetic iron oxide nanocrystals (SPION), in contrast agents used in magnetic resonance imaging of the liver, have been incorporated into chitosan nanoparticles grafted with linoleic acid. Although another group [25,30] has shown that grafting deoxycholic acid on chitosan occurred in the presence of EDC, it was observed that the aggregates formed were able to load up 49.6% of doxorubicin (an anticancer agent inhibitor of topoisomerase II), and the nanoparticle size increased with the amount of active ingredient encapsulated.

(iii) *N*-carboxyacyl-chitosan derivates were obtained by reacting acid anhydrides [for example acetic (C_4), propionic (C_6), n-butyric (C_8), n-valeric (C_{10}), hexanoic (C_{12}), octanoic (C_{16}), lauric (C_{24}), palmitic (C_{32}) and stearic (C_{36}) anhydrides] in the presence of dimethyl sulfoxide (DMSO) [31–33] (Fig. 3c) [34,35]. All the obtained amphiphilic chitosans were soluble in water. However, the aqueous solubility of *N*-stearoyl derivates was controversial because Jiang and Quan [36] obtained highly water-soluble materials [degrees of substitution (DS) were 0.9, 8.2 and 13.5%], whereas Hirano *et al.* described water-insoluble derivates (DS ranges 0.42–0.82%) [37]. Water-insoluble *N*-long-chain saturated fatty acyl derivates were obtained by Hirano and used for affinity chromatography [38].

As with other polysaccharides, the grafting of hydrophobic groups on chitosan confers new physicochemical properties, including its ability to self-associate in water, buffers or in acetic acid solution (0.1 M) spontaneously [39] or under sonication [5,25,40–43] to form different types of drug delivery systems.

Micelles and nanoparticles

Micelles were formed by the auto-association of chitosan-bearing stearic acid (CAC ranged from 0.01 to 0.06 mg/ml) [26,44]. Other studies showed that chitosan was coupled with linoleic acid through an EDC-mediated reaction [27,45]. It was demonstrated that lower CAC values of *N*-stearoyl chitosan resulted in smaller sizes of the self-aggregates [36]. These low CACs indicated that micelles are more stable upon dilution than those based on other chitosan derivates such as deoxycholic acid-modified and 5β-cholanic acid-modified chitosans (from 1.7×10^{-2} to 26×10^{-2} mg/ml) [30,41,46,47]. The CACs of oleoyl-chitosans were too high (79.43, 31.6 and 10 mg/ml DS; 5, 11 and 27%, respectively) [23] and their solubility in HCl solution decreased when the DS was increased. These chitosans were insoluble at neutral pH. The surfaces of the micelles were further cross-linked by glutaraldehyde [26,27,43] or sodium tripolyphosphate [45] to form drug-loaded and shell cross-linked nanoparticles.

It is well known that the hydrophobic core of the micelles provides a loading possibility for water-insoluble drugs.

Amphiphilic chitosan-based micelles were used to encapsulate doxorubicin [44], paclitaxel [26,40,47,48], ibuprofen [39] and the amphiphilic adriamycin [49]. Furthermore, hydrophilic peptides, proteins and nucleic acids [25,50] were adsorbed onto chitosan-based micelles.

Hydrogels

Under certain conditions of chitosan molecular weight, concentrations and DS, hydrogels can be formed. Physically cross-linked chitosan hydrogels with lauric, myristic, palmitic or stearic acids were used for topical delivery of drugs. This study selected propranolol hydrochloride as a hydrophilic model drug to design a transdermal delivery system. The effect of the nature of the cross-linker on drug permeation through pig skin was evaluated and the main permeation parameters such as the diffusion coefficient, the flux and the lag time were calculated. All the chitosan hydrogels analyzed provided more cutaneous permeation of propranolol hydrochloride than the corresponding solution of the commercial drug.

The two most important parameters that have to be controlled for hydrogels are viscosity and water-absorbing capacity. Higher hydrogel viscosity, evaluated by using a viscosimeter, enables prolonged contact time at the site of application, whereas swelling properties are important for the drug loading and release. However, these parameters could be completed by rheological measurements to determine the elastic properties of the hydrogels. The hydrogel viscosity was increased and the swelling properties decreased by increasing the hydrophobic group chain length from laurate to stearate [51]. The hydrogel was probably formed through hydrophobic interactions of pendant groups and inter-chain H-bonding between chitosan units [52].

The release properties were controlled by the hydrophobicity of the hydrogels. For example, the release profiles of fluorescein isothiocyanate-dextran, gelucire 50/13 or d-α-tocopherol polyethylene glycol succinate as model macromolecules were faster and the gel porosity, as well as the swelling, were reduced when the hydrophobicity was low [52].

Hydrophobically modified dextran

In the study presented by Nichifor *et al.* [53], dextran molecular weight close to 30,000 g/mol was covalently bound to bile acids (cholic and deoxycholic acids) through ester linkages. Bile acids are natural products consisting of a facially amphiphilic steroid nucleus with a hydrophobic β-side and a hydrophilic α-side [54,55]. When these compounds are chemically bound to a water-soluble polymer the resulting amphiphilic polymer might exhibit a better compatibility with biological systems and interact favorably with proteins, enzymes or lipids [55,56].

The DS varied from 2 to 6% (mol bile acid/100 glucopyranose units) in the presence of a coupling agent: *N*,*N*′-dicyclohexylcarbodiimide and pyridine. After this reaction, amphiphilic dextran could self-associate for concentrations higher than 0.2 mg/ml and formed micelles of 130 nm for dextran grafted with cholic acid (DS 4%) and 150 nm for dextran grafted with deoxycholic acid (DS 3.6%). Viscosimetric measurements performed with aqueous solutions of amphiphilic dextrans showed that the hydrophobic interactions between bulky and rigid bile acid side chains occurred mainly intramolecularly. However, beyond a concentration of 6 mg/ml amphiphilic dextran cannot self-associate in the form of micelles but compact hydrophobic domains were formed under these conditions [53].

Dextran was also grafted with lauryl chains (C_{12}). Various DS were obtained (2.7, 4 and 7%). The amphiphilic dextran can self-associate spontaneously with poly-β-cyclodextrin to form stable supramolecular nanoparticles with a mean diameter of ~200 nm [57–60]. The cohesion of these stable structures is based upon a 'lock and key' mechanism by the formation of inclusion complexes between the hydrophobic alkyl chains (lauryl) on hydrophobized dextran (lock) and the molecular cavities contained in the poly-β-cyclodextrin (key) [61]. Numerous empty cyclodextrin units remained accessible for the inclusion of molecules used for cosmetic or phamaceutical applications such as benzophenone, tamoxifen [59], benzophenone-3 [60] and functionalized Gd^{3+} chelates [57]. The loading of the molecules into the nanoassemblies was performed at room temperature by mixing poly-β-cyclodextrin aqueous solutions containing the drug with hydrophobized dextran solutions.

Hydrophobically modified hyaluronic acid

Hyaluronic acid was chemically bonded to dioleoylphosphatidylethanolamine (DOPE) in the presence of EDC chloride as a coupling agent for 24 h at 37 °C. Ultrafiltration eliminates the coupling agent and the unreacted DOPE [62]. The resulting product was used in the preparation of cationic liposomes to form lipoplexes used in gene therapy [63,64].

Hydrophobically modified pullulan

Various cholesterol-bearing pullulans with different molecular weights from the parent pullulan and different DS from the cholesteryl moiety were synthesized by Akiyoshi *et al.* [65–67] and formed stable and monodisperse self-aggregates (20–30 nm) by intra- and/or inter-molecular self-aggregation in a diluted aqueous solution [65]. The cholesterol-bearing pullulan self-aggregates are regarded as a hydrogel nanoparticle, in which pullulan chains are cross-linked noncovalently by associating cholesteryl moieties. The sizes of the self-aggregates decreased with an increase in the DS of the cholesteryl moiety, whereas the aggregation number of cholesterol-bearing pullulans in one nanoparticle was almost independent of the DS [67].

Amphiphilic pullulan obtained is slightly soluble in water at a temperature of 50–60 °C for 12–24 h. No precipitation was observed even after heating. Interestingly, the formed nanoparticles are thermosensitive. The characteristic sol–gel temperature, determined by a fluorescence technique, decreased with an increase in the DS of cholesterol-bearing pullulans and the ionic strength of the medium [67]. The thermosensitivity of the nanoparticle hydrogel is related to the partial dehydration of the hydrophobized pullulan upon heating. Furthermore, temperature-sensitive hybrid nanoparticles were obtained by the self-assembly of two different polymers of cholesterol-bearing pullulans and a copolymer of *N*-isopropylacrylamide with *N*-[4-(1-pyrenyl)butyl]-*N*-n-octadecylacrylamide] (PNIPAM-C18Py) [68].

The cholesterol-bearing pullulans were able to bind various hydrophobic molecules such as porphyrin, bilirubin and antitumor adriamycin (for review see Ref. [66]). The binding constants became larger when the hydrophobicity of the probes was increased.

An interesting feature of hydrogel nanoparticles of cholesteryl-bearing pullulans is their ability to form complexes in water with various soluble proteins or enzymes, for example bovine serum albumin [66], insulin, cytochrome c, myoglobin and α-chymotrypsin which were complexed by the cholesterol-bearing pullulan nanoparticles [66]. In a more recent study, hexadecyl-group-bearing pullulans self-associated to form hydrogel nanoparticles. Above ~2% (w/w) the viscosity of the solutions drastically increased. At higher concentrations they formed macroscopic gels [69].

Hydrophobically modified amylose

Amylose was chemically grafted by linoleic acid [70] using two methods:

(i) Amylose solution was prepared in DMSO at 90 °C. Then, the amylose solution was brought to the crystallization temperatures: 90, 60 and 30 °C. Amylose–linoleic-acid complexes were prepared by adding linoleic acid to the amylose solution. After the linoleic acid had dissolved, water was added and the mixture was incubated at the crystallization temperature for 15 min, with vigorous stirring to complete the complex formation. Then, the suspension was cooled to 20 °C, and the amylose–linoleic-acid complexes were isolated by centrifugation. The wet pellet of the complexes was washed twice with an ethanol:water mixture, to remove residues of uncomplexed linoleic acid, and centrifuged as before. The complexes were then freeze-dried. The amylose grafted with the linoleic acid formed could self-associate to form spherical micelles with a diameter of 150 nm [70].

(ii) The second protocol designed to produce amylose–linoleic-acid complexes was conducted in KOH then HCl solution [71]. Amylose solution in 0.01 M KOH and a solution of linoleic acid in 0.01 M KOH preheated to 90 °C were mixed at different crystallization temperatures (90, 60 and 30 °C); the mixture was neutralized with 10 ml 0.1 M HCl. The complex was then precipitated by adjusting the pH to ~4.7 (by using 2 M HCl). The mixture was then held at the preset crystallization temperature for 24 h. All samples were then centrifuged, the supernatant was discarded and the precipitate was washed twice with an ethanol:water mixture (50:50, w/w) to remove residues of uncomplexed linoleic acid and to obtain salt-free complexes and centrifuged as before. The complexes were then freeze-dried. The product formed elongated nanoparticle structures with diameters ranging from 43 to 160 nm [70].

The micelles obtained by the first method demonstrated better stability to temperature, oxidation and pH changes [70].

Hydrophobically modified heparin

Heparin is a natural highly sulfated polysaccharide composed of units of sulfonated glucuronic acid and glucosamine derivatives. Heparin is used as an anticoagulant and is also being investigated as a possible agent to regulate complement activity and inflammation. Furthermore, heparins can interfere with the activity of growth factors such as beta fibroblast growth factor (βFGF) and vascular endothelial growth factor (VEGF), resulting in the inhibition of angiogenesis and tumor development. In view of these characteristics, spherical and monodisperse heparin-based nanoparticles that are chemically modified with deoxycholic acid were developed with different DS (6.2, 8 and 10%) [72]. Deoxycholic-acid-bearing heparin nanoparticles were covered with negatively charged heparin shells, exhibiting zeta potentials near −56 mV. Partition equilibrium constants for pyrene in the nanoparticles indicated that increasing DS enhanced the hydrophobicity of the nanoparticle core. The mean aggregation number of deoxycholic acid per hydrophobic microdomain, estimated by the fluorescence quenching methods using cetylpyridinium chloride, indicated that five to nine amphiphilic heparin chains comprised a hydrophobic domain in the conjugates [72].

Concluding remarks

This article discusses recent research on amphiphilic polysaccharides and their significance as drug delivery systems. Following this study, it is clear that the amphiphilic derivatives of chitosan have been extensively studied in comparison with other polysaccharides. The modified amphiphilic chitosans have the ability to self-associate in contact with aqueous media to form polymeric micelles, nanoparticles and hydrogels. Such structures can be used for the encapsulation and the release of active drugs.

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